UNIVERSITE DE STRASBOURG

Etudes des mécanismes physiopathologiques cognitifs des troubles addictifs

Habilitation à **D**iriger des **R**echerches

Spécialités : Psychologie et Neurosciences Cognitives

présentée et soutenue publiquement

par

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le 29 septembre 2016

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Remerciements

A Anne Giersch :

Pour toutes ces années que tu as passées à m'épauler et à m'accompagner dans la réalisation de mes travaux de thèse de sciences ainsi que pour avoir accepté d'être garante de ce travail. Merci encore de m'avoir donné le goût de la recherche ! Nos aventures tout au long de ces années nous ont amenées à développer une amitié et comme les aventures ne s'arrêtent jamais…… Merci !

A Brigitte Kieffer :

Pour cette année de stage postdoctoral passée dans votre laboratoire, nos échanges, notre collaboration, qui m'ont permis de me former à la recherche animale et à la biologie moléculaire.

A Monsieur Danion :

Pour votre soutien à ma carrière et au développement des projets cliniques et de recherche au CHU de Strasbourg.

A François Paille :

Pour l'aide et le soutien que vous m'apporter dans le développement des projets de recherche en addictologie et nos échanges sympathiques.

Aux membres du Jury, Madame Jenny Coull, Madame Sylvie Droit-Volet, Monsieur Kelche de me faire l'honneur d'avoir accepté d'évaluer ce travail.

Aux membres de l'unité INSERM, pour leur présence, leur soutien au quotidien et les rires partagés.

A mes amis

A ma famille

A mes parents

A mon mari, Frédéric

A mes enfants, Jules et Lisa

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Introduction

Le modèle neurocognitif de l'addiction permet de comprendre et de modéliser la mise en place de l'addiction et le maintien des comportements addictifs en lien avec les mécanismes neurobiologiques. Les troubles cognitifs liés à l'addiction sont de deux types, des troubles cognitifs induits par la toxicité des substances et par le sevrage, et des troubles cognitifs à l'origine de l'initiation et du maintien de la pathologie. Dans ce travail, nous proposons d'étudier, par des approches de psychologie expérimentale, les mécanismes physiopathologiques cognitifs de l'addiction.

Notre travail de thèse de sciences nous a permis d'étudier les altérations du codage des événements temporels chez les patients schizophrènes et les répercussions de ce trouble cognitif primaire sur d'autres fonctions cognitives secondaires impliquant une dimension temporelle. Ce travail nous a permis de nous former aux approches de psychologie expérimentale. Nous avons ensuite souhaité mettre en accord notre travail clinique d'addictologue avec notre travail de recherche. Nous avons utilisé les connaissances et les outils que ces expériences nous avaient apportés pour développer des projets de recherche. Dans ce travail, nous voulons explorer d'une part les troubles cognitifs liés à la toxicité spécifique des drogues, tels que les troubles de la perception visuelle et temporelle chez les consommateurs de cannabis. D'autre part, nous voulons étudier les troubles cognitifs qui sont responsables du maintien de la dépendance, comme les troubles exécutifs et surtout l'impulsivité chez les patients. Plus précisément, il s'agit de mieux comprendre le lien hiérarchique entre les troubles cognitifs présents dans l'addiction, c'est-à-dire entre des troubles de l'anticipation temporelle d'une récompense et l'impulsivité. Dans la mesure où les travaux initiaux sur la schizophrénie fournissent le cadre théorique de nos projets sur l'anticipation temporelle chez les consommateurs de cannabis, nous aborderons d'abord le contexte théorique dans lequel nous avons réalisé ces premières études et les résultats de ces travaux, puis nous définirons l'addiction et ses conséquences sur le plan cognitif afin de décrire nos projets dans une dernière partie.

Chapitre 1 : Contexte théorique

1) Clinique de la schizophrénie

Dans notre travail de thèse, nous nous sommes intéressés aux troubles de la perception temporelle dans la schizophrénie. La schizophrénie est une maladie qui concerne 1% de la population et est d'apparition précoce dans la vie du sujet, entre 20 et 30 ans. Cette maladie est caractérisée par la présence de troubles délirants, de troubles dissociatifs et de troubles cognitifs. Les troubles délirants sont polymorphes, de différents mécanismes (hallucinations, intuition délirante, interprétation….) et de différents thèmes (mystique, persécution, jalousie, fantastique….). Les troubles dissociatifs sont caractérisés par une perte de concordance entre la pensée, l'affectivité, l'expression verbale et le comportement. La dissociation se traduit notamment par une désorganisation de la pensée, des barrages, des troubles de la fluence verbale, une mimique inadaptée, un maniérisme, une autonomie de l'expression verbale par rapport aux contenus idéatifs, des troubles sémantiques et syntaxiques, une ambivalence surtout affective, des mouvements stéréotypés parasitaires, une bizarrerie, voire une impulsivité "gratuite" de certaines conduites. Enfin, si ces troubles sont particulièrement envahissants durant les premières années qui suivent la déclaration de la maladie, les troubles cognitifs sont eux à l'origine d'une diminution de l'autonomie des patients. Les troubles cognitifs présents dans la schizophrénie sont nombreux, tels que des troubles de la mémoire épisodique (Berna et al., 2016), des troubles de la méta-mémoire (Bacon et al.), des troubles de l'attention (Delevoye-Turrell et al., 2006), des troubles des fonctions exécutives (Barch, 2005, revue; Salamé et Danion, 2007), mais aussi des troubles de la perception temporelle subjective (Minkowski, 1927 ; Elvevag, 2003 ; Lewis, 1932; Freedman, 1974 ; Rabin, 1957; Rammsayer, 1990, Tracy et al., 1998). Les troubles de la

perception temporelle chez les patients schizophrènes concernent une rupture du sens de la continuité du temps chez les patients, comme si les patients n'étaient pas connectés de façon continue au monde extérieur. Ces troubles ont été décrits notamment par Minkowski dans son ouvrage Le temps vécu. Selon Minkowski la perception de la continuité temporelle chez le sujet sain s'opère de la façon suivante : « le temps se fractionne en éléments isolés, que tout naturellement, dans la vie normale, nous intégrons les uns aux autres » (Minkowski, 1927). Ainsi, l'homme serait capable de lier des évènements séparés dans le temps pour les mettre en lien dans une continuité temporelle. Pour Minkowski, la perturbation de ces mécanismes aurait un rôle central dans la schizophrénieElle induirait une perception fragmentée des évènements dans le temps générant ainsi une fragmentation du réel (Minkowski, 1927). La fragmentation du réel renvoie à la perte de réalité décrite par de nombreux psychiatres et notamment par Bleuler. Bleuler (1911) tente à cette époque de mieux comprendre l'hétérogénéité des symptômes de la schizophrénie. Selon lui, le symptôme le plus spécifique de la schizophrénie serait la schize, c'est à dire la fracture du psychisme dont souffrent les patients. La schize aurait pour conséquence directe les troubles dissociatifs eux-mêmes à l'origine de troubles secondaires délirants et déficitaires. Cette dissociation se caractérise notamment par une altération de la continuité de la logique et des idées générant ainsi une fragmentation de la réalité perçue. Cette description de la pathologie implique une hiérarchie entre les troubles. En 1999, reprenant les idées de Bleuler, Nancy Andreasen modélise la schizophrénie et la définit comme une maladie caractérisée par un trouble cognitif de base (lathéménologique) en lien avec des anomalies neurobiologiques et des troubles de la connectivité d'une part et générant des troubles cognitifs secondaires puis des symptômes cliniques tels que la dissociation d'autre part. Pour Nancy Andreasen, il existerait une hiérarchie entre les troubles cognitifs, certains

troubles cognitifs élémentaires pouvant ainsi générer des troubles cognitifs secondaires puis des symptômes cliniques. Par exemple, le temps étant impliqué dans de nombreuses activités mentales, comme le langage, toute altération de l'intégration temporelle des évènements pourra altérer le rythme et la séquence des phrases voire être à l'origine de barrages, typiquement rencontrés dans la schizophrénie : le patient s'arrête au milieu d'une phrase puis reprend plus tard exactement où il en était, comme si le temps ne s'était pas écoulé durant l'intervalle muet. Pour Nancy Andreasen, l'intégration des évènements dans le temps pourrait être un des troubles cognitifs primaires présent dans la schizophrénie. Ainsi, explorer et mieux comprendre les altérations de la continuité du temps chez les patients schizophrènes est un enjeu dans la compréhension des mécanismes sous-tendant les troubles psychiatriques.

2) Le concept de moment présent

La continuité du temps suppose qu'à partir d'évènements isolés le sujet perçoive une continuité entre les évènements juste passés et les évènements futurs anticipés, qu'il soit capable de les mettre en lien dans un moment imperceptible, indicible qu'est le moment présent. Certains phénoménologistes comme James en 1890 se sont intéressés à la question du moment présent. Dans « The Stream of consciousness », il postule que la conscience du temps présent serait en lien avec l'agrégation de parties substantielles qui renferment les contenus idéiques et de parties transitives qui assurent la transition entre les parties substantielles (James, 1890, p 609). Ces parties transitives génèrent à la fois un sentiment de continuité mais aussi, la sensation d'un changement dans une continuité. A la fin du XIXeme et au début du XXème siècle dans son ouvrage « Sur la phénoménologie de la conscience intime du temps », Husserl va plus loin dans la description de ce sentiment de continuité. Il propose un modèle pour rendre compte du sentiment de continuité temporelle qui émerge,

bien que nous percevions des évènements discontinus dans le temps.Il définit trois temps, celui juste passé ou rétention, le maintenant, et l'instant futur ou protention. La rétention retient le moment juste passé de la conscience, qui est dès lors présent dans l'expérience du moment. En d'autres mots, la rétention dépend d'un mécanisme qui permet de lier l'évènement passé avec le processus présent. La rétention ne retient pas des contenus réels mais 'intentionnels' c'est-à-dire le sens de ce qui vient juste de se passer consciemment. La protention correspond au processus permettant, à partir du présent et du passé, d'anticiper l'événement à venir. Pour Husserl, l'ensemble de ces trois temps, rétention, présent et protention, permettraient la création d'un sentiment de continuité à partir d'évènements perçus séparément. Ce sentiment de continuité serait à l'origine de la perception d'un temps subjectif présent : cette conscience du temps présent est le niveau explicite de la perception du temps. C'est ainsi que nous pouvons écouter une mélodie, en retenant les notes juste passées et en anticipant les prochaines. La mélodie entendue serait le résultat de l'intégration temporelle des notes isolées. C'est la différence entre le réel de l'objet, dans ce cas des notes isolées, et la mélodie perçue subjectivement à la suite de l'intégration temporelle. C'est aussi par ce processus que le sujet peut détecter une fausse note. La note perçue est alors différente de celle attendue par le sujet dans l'enchaînement musical : elle est détectée fausse par rapport à la note attendue et anticipée par le sujet.

Pour Husserl, le présent naît donc du lien entre les évènements tout juste perçus et ceux anticipés. Pour lui, ce lien est nécessaire pour percevoir la succession des évènements et le rapport qu'ils entretiennent les uns avec les autres. Par le biais de cette construction du décours temporel, l'homme est capable de leur donner une cohérence. Le lien de continuité permet de créer cette cohérence temporelle et ce flux de conscience. C'est donc ce lien,

cette continuité dans le temps qui constitue la clef de voûte de notre conscience du temps selon Husserl.

Si l'approche de Husserl permet de mieux se représenter phénoménologiquement la notion de continuité temporelle, des auteurs comme Von Baer et Pöppel, se sont intéressés à définir la durée de ce moment présent ou fenêtre temporelle à un niveau expérimental.

3) La fenêtre temporelle : mesure du temps présent

En 1865, le physicien von Baer, puis Exner (1875) se sont intéressés à la discrimination des intervalles de temps en modalité auditive. Ces auteurs ont montré à cette époque que des intervalles de temps d'une durée inférieure à 30 ms sont perçus comme des points dans le temps. Tous les événements durant cet intervalle sont perçus comme simultanés. Par exemple, Exner avait remarqué qu'en modalité auditive, pour ordonner 2 stimuli survenus à des moments différents, il est nécessaire qu'ils soient séparés d'un délai de 20 à 40 ms (Exner, 1875) De là nait l'idée d'une fenêtre temporelle élémentaire, dont la durée n'est pas perçue subjectivement par l'homme, mais qui, pour l'audition serait de 20 à 40 ms. . Cette fenêtre temporelle élémentaire reflète donc la capacité de discrimination entre deux évènements à un niveau conscient. En dehors de ces fenêtres temporelles élémentaires, d'autres fenêtres temporelles ont été décrites, plus proches des concepts de James et Husserl. Pöppel (1994,1997a) reprend les expériences menées par Vierordt (1868) qui s'est lui intéressé à la reproduction expérimentale d'intervalles de temps. Dans ces expériences, Vierordt observe que les sujets ont tendance à reproduire des intervalles de durée plus longue ou plus courte que la durée de l'intervalle de référence. Mais de temps en temps, les sujets reproduisent tous un intervalle de durée identique appelé intervalle d'indifférence : Vierordt montre en effet que, si les intervalles à reproduire sont choisis entre 1 s et quelques

secondes (par exemple 5 s), on observe un intervalle d'indifférence avec une certaine variabilité à environ 3 s. Selon Pöppel, cet intervalle d'indifférence pourrait être le reflet d'un processus neuronal spécifique responsable de l'intégration temporelle. Certaines expériences renforcent l'idée d'une intégration temporelle sur une fenêtre de 3 secondes. En effet, des expériences sur la structure temporelle d'un discours spontané chez l'adulte (Vollrath et al., 1992) et chez les enfants (Kowal et al., 1975) montrent que le langage est structuré selon des fenêtres temporelles de 3 secondes, correspondant à la durée d'une phrase, et donnant ainsi au discours son rythme. 10 à 14 syllabes forment habituellement une phrase dans un discours spontané, définissant ainsi une structure sémantique et syntaxique (Kowal et al., 1975 ; Vollrath et al., 1992). Dans les actes moteurs spontanés et intentionnels, Kolwalska et al., en 1998, ont montré qu'il existe chez les enfants une fenêtre temporelle de 1 à 3 secondes, c'est-à-dire une durée moyenne du mouvement. La fenêtre varie en fonction de la complexité du mouvement. Si le mouvement est simple et répété plusieurs fois, la fenêtre temporelle, c'est-à-dire la durée d'un mouvement, est de 1.1 sec. Si le mouvement est complexe, par exemple parce qu'il implique la perception d'une cible visuelle (déplacer sa tête et son regard vers une cible), la fenêtre temporelle du mouvement est de 2 à 3 secondes. (Kolwaska et al, 1998). Pöppel (1997a & b) suggère qu'une fenêtre temporelle de 3 secondes serait à la base de la représentation consciente du présent.

Cette hypothèse est en lien avec les idées de Husserl qui décrit la perception du présent comme un acte de conscience, ainsi qu'avec les idées de James (1890-1950), qui souligne que le présent subjectif est basé sur la perception du décours temporel des phénomènes. Pöppel suggère que le présent subjectif mesurerait environ 2 à 3 secondes. Mais, ce présent subjectif de 2 à 3 secondes ne rend pas compte de la découverte de von Baer et Exner sur l'existence d'une fenêtre temporelle de 30 ms. Pöppel propose l'existence de deux

processus cognitifs impliqués dans la perception temporelle : un processus à basse fréquence avec une fenêtre temporelle de 3 secondes et un processus à haute fréquence avec une fenêtre temporelle de 30 ms reliée à la perception de la succession des événements dans le temps (Pöppel, 1994). Cette unité de 30 ms d'intervalle temporel est le reflet d'une fenêtre temporelle élémentaire durant laquelle l'ensemble des données sont traitées comme simultanées (Pöppel, 1997b), alors que la fenêtre de 3 secondes est celle à l'intérieur de laquelle les événements élémentaires sont reliés les uns aux autres. Ces deux processus conditionneraient la perception subjective du temps.

D'autres auteurs se sont intéressés à la fenêtre d'intégration temporelle en condition multi sensorielle, plus particulièrement lors de l'intégration de signaux visuels et auditifs. L'étude de Van Wassenhove et al. (2007) se base sur l'effet Mac Gurck ou McGurk-MacDonald décrit en 1976 (McGurk & McDonald, 1976). L'effet McGurk est un phénomène perceptif qui montre une interférence entre l'audition et la vision lors de la perception de la parole. Pour mettre l'effet McGurk en évidence, une vidéo est présentée, qui montre une personne prononçant un phonème (p.ex. /ga/) alors que la bande sonore diffuse l'enregistrement d'un autre phonème (p.ex. /ba/). On a alors l'impression d'entendre un troisième phonème intermédiaire (p.ex. /da/) qui provient de la fusion intermodale (McGurk & McDonald, 1976). Dans une première expérience, McGurk & Mac Donald ont montré des stimuli de ce type pour lesquels les informations visuelles et auditives étaient séparées par des intervalles de temps variant de – 467 ms (le premier stimulus étant sonore) à + 467 ms (le premier stimulus étant alors visuel). La fusion des réponses survient de manière préférentielle sur un intervalle compris entre -30 ms et +170 ms. Dans cette expérience, la fusion est révélée par le type de syllabe entendue par le sujet ('da'). Ceci signifie que le sujet n'émet à aucun moment un jugement temporel. C'est l'intégration automatique des informations visuelles

et auditives qui permet la fusion. Ces jugements temporels étaient demandés dans une deuxième expérience, au cours de laquelle les auteurs ont montré les mêmes stimuli et ont demandé aux sujets de décider si les informations visuelles et auditives étaient synchrones ou non. L'asynchronie n'était perçue que quand les informations étaient séparées par d'au moins 200ms. Ceci suggère qu'il existe une fenêtre de l'intégration temporelle dans la modalité audio-visuelle de l'ordre de 200 ms (Van Wassenhove, 2007). La différence entre ces deux jugements (fusion vs. perception d'asynchronie) a été confirmée dans une étude ultérieure (Martin et al, 2013). V. Van Wassenhove fait la distinction entre temps implicite et temps explicite (2009). Il peut y avoir un traitement temporel neuronal de l'information sans accès à la conscience. Quels sont les mécanismes neuronaux sous-tendant la perception d'un temps explicite et d'un temps implicite ? A quoi la fenêtre temporelle d'intégration élémentaire correspondrait-elle? La similarité des fenêtres d'intégration entre modalités sensorielles a conduit différents auteurs à suggérer qu'il existe un mécanisme neuronal spécifique quelle que soit la modalité sensorielle. Dans l'étude de 2007, Van Wassenhove et al. ont étudié la fenêtre temporelle de l'intégration multi sensorielle, dans les modalités audio-visuelles. Les informations visuelles et auditives étant traitées dans des voies neuronales distinctes, la synchronisation temporelle de ces informations est critique. Les difficultés posées par cette synchronisation mènent à un élargissement considérable des fenêtres temporelles, qui rendent ce paradigme adéquat pour l'analyse des différents mécanismes d'intégration à l'intérieur de ces fenêtres. Les liens entre les mécanismes d'intégration neuronale des évènements temporels et la perception du temps explicite restent néanmoins incompris. Récemment, Northoff s'est intéressé au lien entre l'activité cérébrale de repos et la conscience du temps présent. Selon lui, il n'existerait pas de réelle différence entre le temps neuronal et le sentiment de continuité temporelle. Dans son

« hypothèse neuro-phénoménale », Northoff propose que les caractéristiques des variations de fréquence (basse versus haute fréquence) dans l'activité cérébrale de repos dans les régions corticales médianes et notamment thalamo-corticales puissent générer un sentiment de continuité temporelle (Northoff, 2013). Comme James, il postule que cette activité serait associée à des contenus idéiques. Northoff se base sur l'observation d'un isomorphisme entre l'activité cérébrale de repos et l'activité cérébrale d'un sujet lors d'un voyage mental (D'Argembeau et al., 2010 a et b). Dans ce voyage mental, le sujet se déplace aisément dans le temps, du passé vers le futur. Ainsi, il y aurait un lien direct entre l'activité neuronale et la représentation temporelle subjective. Enfin, selon Northoff, les parties transitives décrites par James entre les contenus idéiques seraient le résultat de variations importantes de l'activité cérébrale en lien avec des stimuli externes ou internes. Ainsi, pour Northoff, la perception consciente du temps serait en lien avec l'activité neuronale à l'échelle de la milliseconde et cette perception consciente serait le résultat d'une agrégation des évènements présentés à l'échelle de la milliseconde (Northoff, 2013). L'hypothèse de Northoff, bien que séduisante, est basée sur le seul constat d'un isomorphisme entre deux activités cérébrales, celle de repos et celle liée au voyage mental ; ceci ne permet donc pas de conclure. Par ailleurs, si Northoff tente d'expliciter la perception consciente du temps, il n'explique pas comment des évènements temporels très rapprochés (séparés de moins de 40 ms) peuvent être à la fois séparés dans une succession temporelle, mais aussi intégrés dans une continuité. La question des liens entre succession et continuité temporelles est donc loin d'être résolue. Afin d'avancer sur cette question, dans notre travail, nous avons souhaité mieux comprendre le traitement de la succession des informations temporelles chez le patient schizophrène mais aussi chez le sujet sain, en distinguant les niveaux explicite et implicite.

4) Chez le patient schizophrène : l'altération de la temporalité comme noyau commun à l'hétérogénéité des symptômes dans la schizophrénie ?

De nombreux psychiatres se sont intéressés à la perception du temps chez les patients schizophrènes. En effet, les patients présentent sur le plan clinique un rapport au temps perturbé dans de multiples dimensions. Ils sont perdus dans les saisons, les dates, les heures parfois. Selon Northoff, si les caractéristiques des variations de fréquence dans l'activité cérébrale de repos dans les régions thalamo-corticales peuvent générer un sentiment de continuité temporelle chez les sujets sains, des altérations dans cette activité pourraient engendrer des altérations de la continuité temporelle chez les patients schizophrènes (Northoff, 2015). Des études EEG de l'activité cérébrale au repos ont montré qu'il existe une augmentation de l'activité dans les bandes alpha, theta, delta, gamma, que ce soit dans le même hémisphère ou bien entre les hémisphères cérébraux (Hanslmayr et al., 2013 ; Kam et al., 2013). Selon Northoff, la fragmentation de la pensée serait en lien avec des altérations des variations de l'activité cérébrale au repos dans les régions corticales médianes et particulièrement thalamo-corticales chez les patients, ces mêmes régions étant impliquées dans le voyage mental (Northoff, 2015). Pour Northoff, il existerait un lien direct entre les modifications neuronales et les symptômes cliniques, la fragmentation de la pensée. S'il est possible que les altérations à l'EEG, surtout en fréquences spatiales basses, rendent compte d'anomalies du voyage mental décrites par ailleurs chez les patients schizophrènes, rien ne prouve actuellement que l'on puisse établir un lien direct et causal entre les deux. On peut tenir le même raisonnement pour des phénomènes à des fréquences plus élevées. Des enregistrements électro-encéphalographiques et magnéto-encéphalographiques ont en effet permis d'évaluer les activités synchrones à haute fréquence dans la bande gamma (20-

70 Hz), bande associée aux activités cognitives, notamment les mécanismes d'intégration ou de liaison entre les informations. Les auteurs ont montré une anomalie de la puissance de l'activité gamma cérébrale et un profil de connectivité anormal dans la schizophrénie (Fletcher et al., 1999; Haig et al., 2000; Kwon et al., 1999; Lee et al., 2003). De telles anomalies pourraient avoir un impact sur perception du sentiment de continuité temporelle chez les patients, dans la mesure où les fréquences concernées pourraient être en rapport avec des anomalies des fenêtres temporelles élémentaires. Par ailleurs, cette anomalie de la connectivité entre les aires corticales serait reliée à des anomalies de la connectivité anatomique (Andreasen 1999; Fletcher et al., 1999; Foucher et al., 2007; Tononi & Edelman 2000). Nancy Andreasen propose, dans son hypothèse néobleulérienne (1999), l'existence d'un déficit cognitif fondamental, qui serait le résultat de la dysconnectivité neuronale, et serait à l'origine d'autres troubles cognitifs qui impliquent la perception temporelle comme le langage, le séquençage moteur qui sont altérés chez nos patients. Chez le sujet normal, les fonctions cérébrales seraient sous-tendues par des activations neuronales en réseau, soit une connectivité anatomique entre les aires cérébrales. L'hypothèse formulée par Andreasen repose sur l'existence chez le sujet schizophrène d'une anomalie de la connectivité entre les diverses aires anatomiques, et notamment dans un réseau qui inclut les cortex préfrontal et pariétal (à droite), le thalamus, les ganglions de la base et le cervelet. De façon intéressante, ces structures ont notamment été impliquées dans la perception du temps (Gibbon et al., 1997; Ivry and Spencer, 2004; Matell & Meck, 2004). Le dysfonctionnement neuro-anatomique et fonctionnel de ce réseau aboutirait, selon Nancy Andreasen, à un déficit cognitif fondamental, 'lathéménologique', nommé dysmétrie cognitive. La dysmétrie cognitive n'est pas définie précisément mais recouvre notamment, selon les propositions d'Andreasen, la notion de perception du temps. Là encore les données

empiriques manquent, mais ces modèles suggèrent que mieux comprendre les anomalies du codage des évènements dans le temps chez les patients schizophrènes nous permettrait d'avancer dans notre compréhension de la maladie.

Seules quelques données expérimentales concernaient la perception du temps chez ces patients quand nous avons commencé notre travail. Des études se sont intéressées à la perception de la durée chez les patients schizophrènes et montrent que les patients ont tendance à surestimer les intervalles de temps et rapportent une sensation subjective d'un temps perçu plus long que le temps passé objectivement. Ces différentes études se sont intéressées à des intervalles de temps allant de la ms à l'heure (Rabin, 1957; Pearl & Berg, 1963; Rammsayer, 1990). Par exemple, dans l'étude d'Elvevag et al. (2003), les sujets (patients versus contrôles) devaient dire si les intervalles de durée (variant de 200 à 800 ms) présentés étaient différents ou non d'un intervalle de référence d'une durée de 500ms. Les patients étaient moins précis que les contrôles pour reconnaître les intervalles d'une durée similaire à celle de l'intervalle de référence. D'autres études comme celle de Todd en 2006 et celle Lee et al. en 2009 ont montré que les patients présentent des altérations lors de jugements de durée. Cependant, ces études montrent que les troubles de la sensibilité temporelle sont corrélés aux troubles en mémoire de travail et aux déficits en attention soutenue chez les patients et plus généralement à la sévérité des symptômes négatifs. Les résultats de ces trois études ne permettent pas de conclure quel processus, du jugement ou de la perception ou bien de la mémoire, est altéré chez les patients.

Ainsi, ces études montrent à quel point il est difficile d'étudier la perception du temps en dehors d'autres facultés cognitives telles que la mémoire et l'attention, qui sont connus pour être altérées chez les patients schizophrènes. Par ailleurs, la perception d'une durée est un

jugement temporel explicite et ne permet ainsi pas de différencier le jugement temporel explicite de l'implicite. De notre côté, nous avons abordé la question de la perception temporelle sous un angle différent, celui de la perception d'une asynchronie .

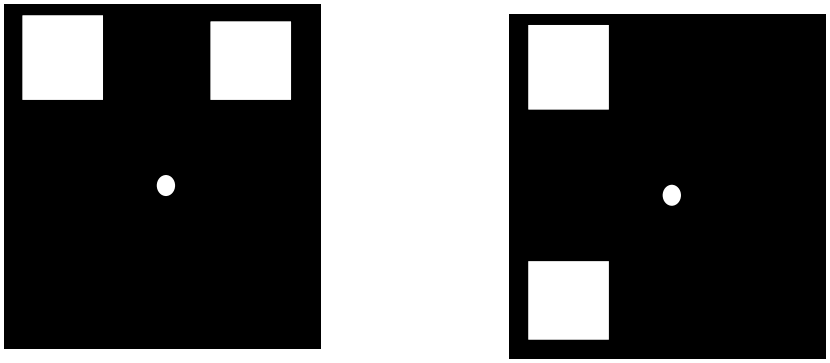
Chapitre 2 : Travaux antérieurs : Etude du codage des évènements dans le temps chez les patients schizophrènes

Dans nos études, nous avons examiné la fenêtre temporelle en modalité visuelle. Dans chaque étude, nous avons recruté un groupe de patients schizophrènes appariés individuellement en âge, sexe et niveau d'études à un groupe de sujets volontaires sains. En nous appuyant sur des expériences réalisées au préalable sur la perception de simultanéité chez le sujet sain (Elliott et al., 2006), nous avons réalisé une première expérience dans laquelle deux barres apparaissaient sur un écran et dans laquelle le sujet devait répondre si elles étaient apparues simultanément ou non. Les barres étaient présentées de part et d'autre d'un point de fixation central, dans deux hémichamps différents. Nous faisions varier le SOA (stimulus onset asynchronie), c'est-à-dire l'intervalle de temps qui séparait l'apparition de la première barre de l'apparition de la deuxième. Ceci nous permettait de mesurer le seuil de perception d'asynchronie qui correspond au SOA pour lequel le sujet perçoit l'asynchronie d'apparition des barres dans 50% des cas. Nous avions montré que le seuil de perception d'asynchronie des contrôles est de 50 ms. En cela, nous avions reproduit les résultats de Von Baer. Par ailleurs, nous avions montré que les patients avaient un seuil de perception d'asynchronie beaucoup plus important que les contrôles, aux environ de 100 ms (Giersch et al., 2009). Nous nous sommes alors posé deux questions. Les barres étant présentées dans des hémichamps différents et des altérations du transfert inter-

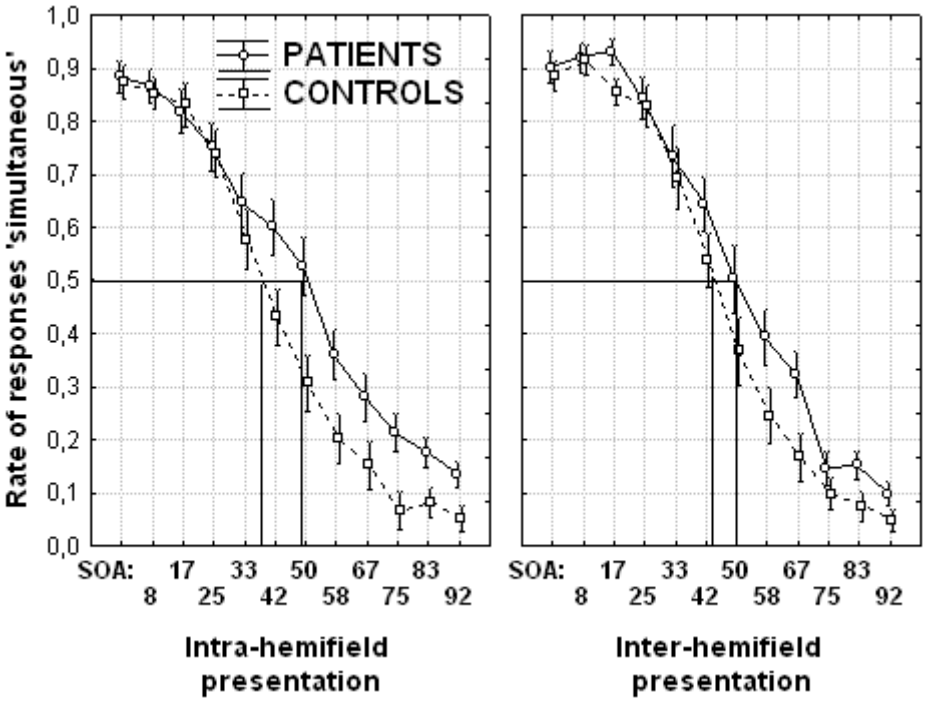
hémisphérique ayant été décrites chez les patients (Endrass et al., 2002 ; Mohr et al., 2000), des troubles du transfert inter-hémisphérique pourraient-ils rendre compte des altérations présentes chez les patients dans cette première étude ? Par ailleurs, si l'élargissement de la fenêtre temporelle persiste quand les stimuli sont présentés dans le même hémichamp, quels sont les mécanismes à l'origine de l'élargissement de la fenêtre temporelle chez les patients ?

1) Etude 1

Pour répondre à notre première question, dans notre première étude, nous avons souhaité comparer la présentation de carrés synchrones versus asynchrones dans le même hémichamp versus dans des hémichamps différents.



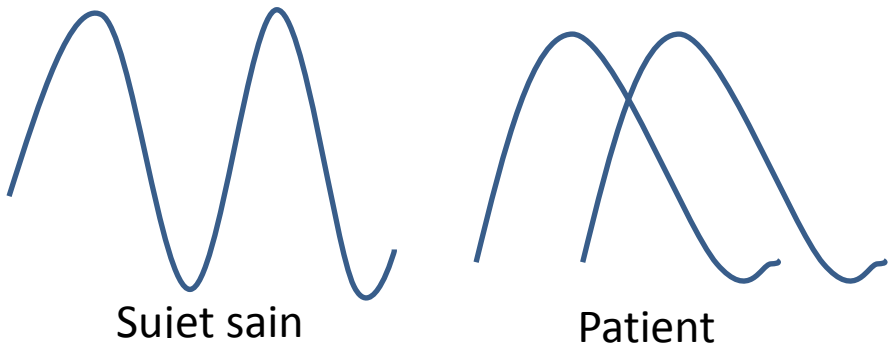
Nous avons pu montrer qu'il existe un élargissement de la fenêtre temporelle chez les patients schizophrènes indépendamment du transfert interhémisphérique : les patients présentent un élargissement de la fenêtre temporelle que les stimuli soient présentés dans le même ou bien dans deux hémichamps différents.



Sur ce graphique, sont présentés les taux de réponses synchrones des sujets en fonction des SOA (stimulus onset asynchrony, c'est-à-dire l'intervalle de temps qui sépare l'apparition du premier carré de l'apparition du deuxième. A gauche, les stimuli sont présentés dans le même hémichamp et à droite dans des hémichamps différents. Les patients (courbe pleine) présentent une augmentation du seuil de perception d'asynchronie par rapport aux contrôles (courbe pointillée) quelle que soit la présentation des stimuli, dans le même ou bien dans deux hémichamps.

Par ailleurs, nous avons souhaité comprendre les mécanismes à l'origine de l'élargissement de la fenêtre temporelle chez les patients. Nous avons souhaité vérifier deux hypothèses :

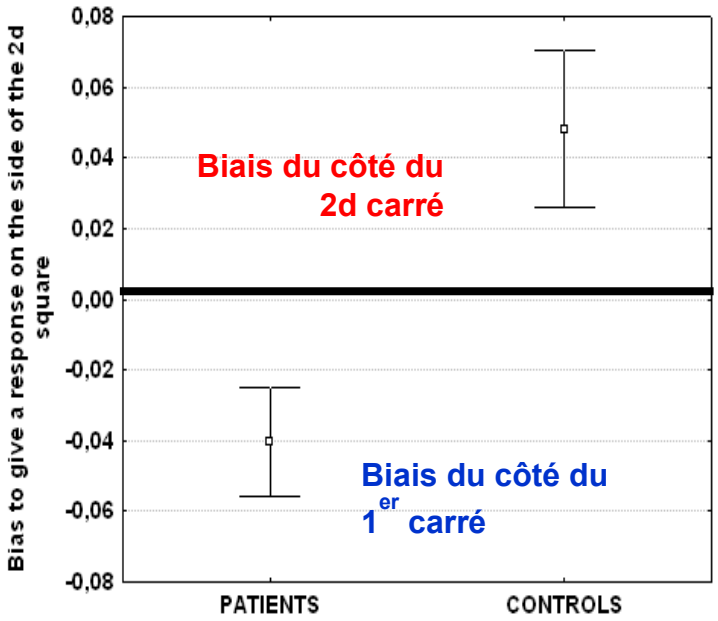
- Premièrement, une fusion excessive des stimuli dans le temps pourrait être à l'origine d'un codage neuronal bruité des stimuli, ce qui rendrait difficile leur distinction



- Deuxièmement, l'augmentation de la fenêtre temporelle pourrait être en lien avec un trouble de la perception de la succession des stimuli chez les patients.

Pour répondre à cette question, nous nous sommes intéressés à l'effet Simon. Pour rappel, l'effet Simon (Simon, 1969) reflète la tendance du sujet à donner une réponse manuelle du côté du stimulus (Simon, 1969). Dans notre tâche, lorsque deux stimuli sont présentés dans le même hémichamp, il existe un effet Simon classique. Par exemple, si les deux stimuli sont présentés à gauche du centre de l'écran, les sujets ont tendance à répondre à gauche. Lorsque les stimuli sont présentés dans des hémichamps différents et de manière synchrone, il n'y a pas d'effet Simon. En effet, dans ce cas, l'information est parfaitement symétrique dans les deux hémichamps, et la réponse ne peut pas être biaisée d'un côté ou de l'autre. Par contre, lorsque les stimuli sont présentés de manière asynchrone dans deux hémichamps, l'asymétrie temporelle induit à nouveau un effet Simon. Chez les volontaires sains, notre équipe a confirmé l'existence d'un biais du côté du deuxième stimulus (Poncelet & Giersch, 2015) : à partir de 17 ms, les réponses des volontaires sont plus fréquentes du côté du deuxième stimulus. A ces asynchronies très courtes, les volontaires sains ne perçoivent pas consciemment l'asynchronie. Cela signifie que même pour ces SOA imperceptibles, l'asynchronie est traitée. Nous avons appliqué cette méthode aux patients pour vérifier dans quelle mesure l'asynchronie est traitée automatiquement. Les prédictions étaient les suivantes : si les patients fusionnent les évènements dans le temps, ils ne devraient pas présenter d'effet Simon pour des SOA très courts. Si par contre leur traitement implicite diffère de leur traitement explicite des évènements temporels, nous devrions pouvoir mesurer un effet Simon chez les patients.

Les résultats ont montré que pour des délais très courts d'asynchronie, les patients ont bien un effet Simon (Lalanne et al., 2012a). A ces délais très courts, l'asynchronie n'est pas rapportée explicitement par les sujets. Si les stimuli étaient réellement fusionnés dans le temps, ils auraient dû être traités comme synchrones par les voies visuelles, et aucun effet Simon n'aurait dû être observé. Le fait que la réponse des patients soit biaisée montre que l'asynchronie a été traitée. Ce premier résultat nous permet d'exclure l'hypothèse d'une fusion excessive des stimuli dans le temps chez les patients. Par contre, aux SOA les plus courts, les patients avaient tendance à répondre du côté du premier stimulus, alors qu'aux SOA les plus longs, les patients présentaient comme les contrôles un biais du côté du deuxième stimulus. Il existe donc chez les patients une dissociation en fonction de la durée des SOA : ils ont un biais du côté du premier stimulus pour des SOA très courts et un biais du côté du deuxième pour des SOA longs. De telles altérations confirment que les patients ne fusionnent pas les stimuli dans le temps et suggèrent qu'il existe un trouble de la comparaison des informations dans le temps (Lalanne et al., 2012a).



Ce graphique représente les biais de réponse des patients et des contrôles. Pour de courtes asynchronies entre 8 et 17 ms, les patients présentent un biais à répondre du côté du

premier carré tandis que les témoins présentent un biais à répondre du côté du second carré.

Néanmoins, la limite de cette étude était d'avoir présenté les stimuli non seulement séparés dans le temps mais aussi séparés dans l'espace. La littérature montre en effet que les patients fragmentent excessivement les informations dans l'espace (Silverstein et al., 2006; Uhlhaas et al., 2006; Van Assche & Giersch, 2011). Le biais du côté du premier carré chez les patients aux asynchronies les plus courtes pouvait donc être en relation avec une fragmentation excessive dans l'espace plutôt que dans le temps.

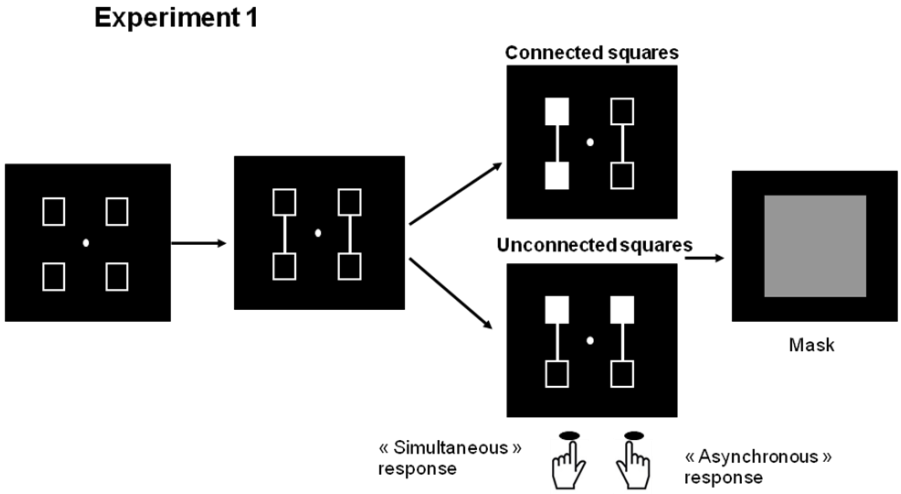
Dans une deuxième étude, nous avons souhaité examiner si les troubles de la perception d'asynchronie observés chez les patients pour des délais très brefs pouvaient être en lien avec la séparation des stimuli dans l'espace. Dans nos deux études précédentes, en cas de présentation asynchrone, les stimuli étaient séparés à la fois dans l'espace et dans le temps. Pour éviter que les stimuli soient séparés dans l'espace, nous avons manipulé le groupement des stimuli par le biais de connecteurs. Les travaux de l'équipe ont en effet montré que les patients sont tout aussi bien capables de grouper des informations dans l'espace lorsque ces informations sont connectées spatialement : ils n'ont pas d'altération du groupement par connecteurs (Van Assche & Giersch , 2011).

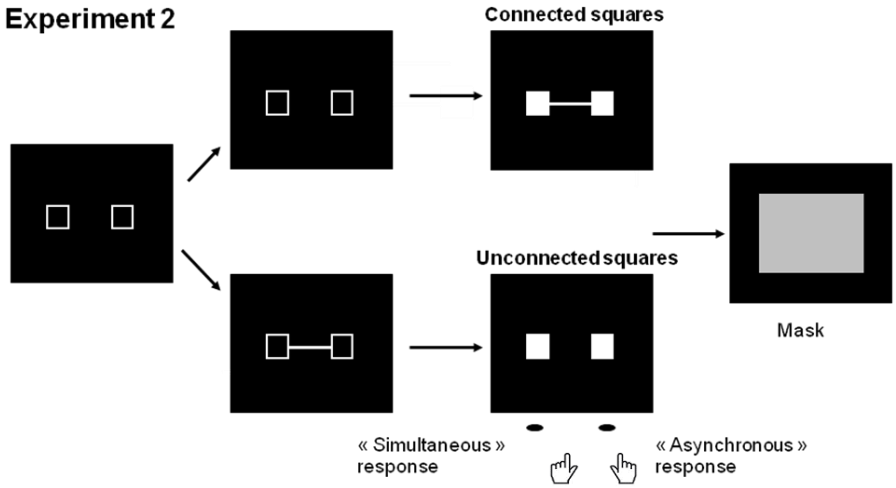
2) Etude 2

Nous avons présenté deux stimuli, connectés ou non, de manière synchrone ou asynchrone soit dans le même hémichamp soit dans deux hemichamps. Nous avons demandé aux sujets de déterminer si la présentation des cibles était synchrone ou non. Il y avait quatre localisations possibles pour les stimuli cibles. Le fait qu'il y ait 4 localisations possibles engendre néanmoins une difficulté concernant la localisation du deuxième stimulus (2

localisations sont possibles après l'apparition du premier stimulus, soit dans le même hémichamp, soit dans deux hémichamps différents). Pour compléter cette expérience et contourner le problème de l'incertitude concernant la localisation de la deuxième cible, nous avons réalisé une deuxième expérience similaire mais dans laquelle seulement deux cibles sont présentées.

Dans les deux expériences (4 versus 2 localisations possibles), nous avons donc vérifié l'effet du groupement des ciblesLa persistance de l'effet Simon en présence de connecteurs signerait donc bien la persistance d'un trouble temporel et non spatial.





Pour des asynchronies très brèves, nous avons montré que les patients présentent un biais du côté du premier carré alors que les contrôles présentent un biais du côté du deuxième carré (Lalanne et al., 2012b). Nos résultats reproduisent les résultats de l'étude 1. Ce biais du côté du premier carré est présent chez les patients quand les carrés sont connectés et quand il n'y a aucune incertitude spatiale. Ce résultat permet d'exclure une difficulté spatiale à l'origine de la fragmentation des stimuli observée à un niveau implicite. Les altérations observées chez les patients seraient donc en lien avec une fragmentation des évènements dans le temps (Lalanne et al., 2012b)

3) Conclusion générale de ces deux études

Notre travail nous a permis de montrer l'existence de deux types de traitement temporel, un traitement temporel implicite et non conscient (entre 0 et 30 ms) et un traitement temporel explicite (> 30 ms) chez les sujets sains. Chez les patients, nous avons montré une augmentation de la fenêtre temporelle, c'est-à-dire une altération du jugement temporel explicite. Cette augmentation de la fenêtre temporelle n'est pas liée à un excès de fusion des stimuli dans le temps chez les patients. Dans notre dernière étude, nous avons montré que même si les évènements sont connectés dans l'espace, ce qui devrait aider nos patients à grouper les informations dans l'espace, il persiste un trouble de la perception de la succession des évènements temporels pour des évènements séparés de 8 à 17 ms. Contrairement aux volontaires sains, les patients ne suivent ou ne prédisent pas automatiquement les deux stimuli dans le temps. Dès lors, on peut se demander si les patients codent le premier stimulus isolément sans prendre en compte de manière systématique l'évènement suivant. S'il n'est pas possible de répondre à cette question sur la base de ces résultats, nous avons montré qu'il existe un codage implicite des évènements

dans le temps chez les sujets sains et que ce codage est déficient chez les patients. Ce résultat est particulièrement important puisqu'il rejoint les descriptions cliniques, c'est-à-dire la fragmentation temporelle chez les patients schizophrènes décrite par Minkowski (1927). De telles altérations pourraient être en relation avec des altérations des capacités de prédiction temporelles chez les patients. Suivre deux stimuli sur des échelles de temps aussi courtes suppose que lorsqu'un premier stimulus est traité par le système neuronal, un deuxième stimulus entrant puisse être traité même si le traitement du premier n'est pas fini. Pour que le système puisse traiter le deuxième évènement, il est nécessaire d'anticiper/ de prédire la survenue de ce deuxième évènement. De tels phénomènes ont été observés pour des durées de 40 ms (Correa et al. 2006a et b), c'est-à-dire qu'il a été démontré que le sujet oriente consciemment son attention vers un évènement futur. Dans nos études, nous mettons en évidence chez les sujets sains un traitement implicite et non conscient d'une séquence d'événements séparés par des durées de 8 à 17 ms : ce résultat suggère l'existence d'un traitement automatisé, c'est-à-dire non conscient, du codage des évènements dans le temps, avec probablement une composante prédictive.

De tels troubles sont susceptibles d'avoir des conséquences importantes sur d'autres fonctions cognitives, dans la mesure où toutes les fonctions cognitives se déroulent dans le temps. Dans une dernière étude, nous avons examiné si de telles altérations pourraient être à l'origine d'autres troubles cognitifs présents dans la schizophrénie. Par exemple, des troubles de la prédiction temporelle pourraient avoir un impact sur le langage, le contrôle moteur, le séquençage des informations sensorielles quelles qu'elles soient. De manière plus générale, un tel trouble pourrait rendre compte de nombreux déficits cognitifs secondaires chez nos patients schizophrènes. A un niveau implicite, l'absence de perception de continuité d'une séquence d'informations peut entrainer des erreurs d'interprétation des

signaux sensoriels de même qu'à un niveau explicite, un élargissement de la fenêtre temporelle peut conduire à détecter des informations comme simultanées alors qu'elles sont bel et bien séparées dans le temps. Ainsi, les troubles de la perception temporels, qu'ils soient implicites ou explicites pourraient être à l'origine d'interprétations délirantes sur la base soit d'erreurs dans d'intégration des signaux dans une continuité et/ou d'erreurs de séquençage des informations. Le trouble de la prédiction temporelle pourrait être un déficit lathéménologique au sens où l'entend Nancy Andreasen, c'est-à-dire un déficit cognitif primaire ayant pour conséquence des déficits cognitifs secondaires et enfin un retentissement clinique.

4) Etude 3

Dans notre dernière étude, nous avons souhaité examiner si les troubles de la prédiction temporelle pouvaient être à l'origine des altérations retrouvées dans les tâches de masquage chez les patients. Dans ces tâches, une cible est présentée avant ou après l'apparition d'un masque et le sujet doit localiser ou identifier la cible. La difficulté de la tâche varie avec l'intervalle de temps entre le masque et la cible. Plus la cible et le masque sont rapprochés, plus la discrimination de la cible est difficile. De nombreuses publications montrent que les effets de masquage sont amplifiés dans la schizophrénie et ce indépendamment des médicaments antipsychotiques (Braff, 1981 ; Butler et al., 1996 ; Butler et al., 2002), ou du niveau intellectuel (Koelkebeck et al., 2005). Les altérations semblent par contre corrélées aux symptômes négatifs (Green & Walker, 1986 ; Slaghuis & Curran, 1999 ; Weiner et al., 1990). Le mécanisme de ces troubles est en revanche bien moins certain. Pour Van Essen, les performances dans les tâches de masquage visuel sont interprétées en terme d'interaction entre deux systèmes visuels, le système magnocellulaire

et parvocellulaire, qui sont à la base du traitement de l'information visuelle (Van Essen et al., 1992). Le système magnocellulaire code les informations de grande taille et brèves tandis que le système parvocellulaire code les informations fines et statiques nécessaires pour la reconnaissance des objets en perception visuelle. Comme de nombreux auteurs Van Essen fait l'hypothèse d'un système magnocellulaire hyperactif qui serait à l'origine d'une interruption du traitement de l'information par le système parvocellulaire et donc à l'origine d'une augmentation des effets du masque sur la cible (Bedwell et al ., 2003 ; Breitmeyer, 1984; Green et al., 1994b ; Schuck & Lee 1989).

Cependant un fonctionnement excessif du système magno-cellulaire n'a pas été observé (Laprévote et al., 2013), et l'explication proposée actuellement repose sur une interaction complexe entre système magno- et parvo-cellulaire. Le système magno-cellulaire permettrait normalement d'inhiber le système parvo-cellulaire qui a la particularité de fonctionner de manière tonique plutôt que de manière transitoire (Breitmeyer & Granz, 1976). Ainsi dans la tâche de masquage rétro-actif, le premier stimulus provoque une stimulation tonique du système parvo-cellulaire qui est interrompue quand le masque active le système magno-cellulaire. Chez les patients, un système magno-cellulaire déficient n'interromprait pas l'activation tonique du système parvocellulaire induite par le premier stimulus, ce qui provoquerait une fusion de la cible et du masque, et une plus grande difficulté à distinguer le premier stimulus. Tout se passerait comme si la persistance du premier stimulus était rallongée. Slaghuis (1999) propose que l'augmentation des effets de masquage chez les patients soit la résultante d'effets de persistance augmentés.

Ces hypothèses ne permettent néanmoins pas de rendre compte des déficits observés chez les patients. L'équipe de Green et al. (Green et al., 1994 a, 1994b, 2002 , 2003, 2006) s'est

intéressée aux tâches de masquage pro-actif, dans lesquelles la cible est présentée après le masque. Le traitement de la cible ne peut donc pas être interrompu par le traitement du masque. Pourtant, dans ces tâches, les patients présentent aussi des altérations (Green et al., 1994 a, 1994b, 2002 , 2003, 2006). L'équipe de Green propose d'interpréter les résultats observés chez les patients à la lumière de l'hypothèse de Di Lollo et al. Selon Di Lollo et al. (2000), les effets observés sont liés à une substitution d'objet. Cette théorie a été élaborée pour rendre compte des phénomènes de masquage observés quand le masque n'est pas superposé à la cible, mais présenté à côté (métacontraste ou paracontraste). Le masque est efficace, mais là non plus le phénomène ne peut pas s'expliquer par une fusion des stimuli dans le temps. Selon di Lollo (2000), l'identification d'un objet n'est pas seulement le résultat d'un traitement de la cible par les voies visuelles ascendantes, c'est-à-dire d'un traitement automatique. L'identification d'un objet est aussi le résultat d'un traitement par des voies descendantes qui permettent de résoudre l'ambiguïté entre les différentes interprétations possibles de la cible (Di Lollo et al., 2000). Dans les tâches de masquage, un conflit surviendrait lorsque le traitement de l'information visuelle par les voies ascendantes et le traitement de l'information par les voies descendantes se superposent dans le temps, l'un correspondant à la cible et l'autre au masque. On assiste alors à une substitution d'objet, de la cible par le masque, avant que le contrôle descendant n'ait permis de lever les ambiguïtés pour l'identification de la cible. Le traitement des informations relatives à une cible active la région latérale occipitale chez les volontaires sains, région qui serait impliquée dans l'organisation de l'information (Grill- Spector et al. 2000; Bar et al. 2001; Green et al. 2005; Carlson et al. 2007). Chez les patients schizophrènes, l'activation de cette région est diminuée durant les tâches de masquage et quel que soit l'intervalle entre la cible et le masque (Green et al., 2009). Selon Green et al. (2010), lla théorie de la substitution d'objet

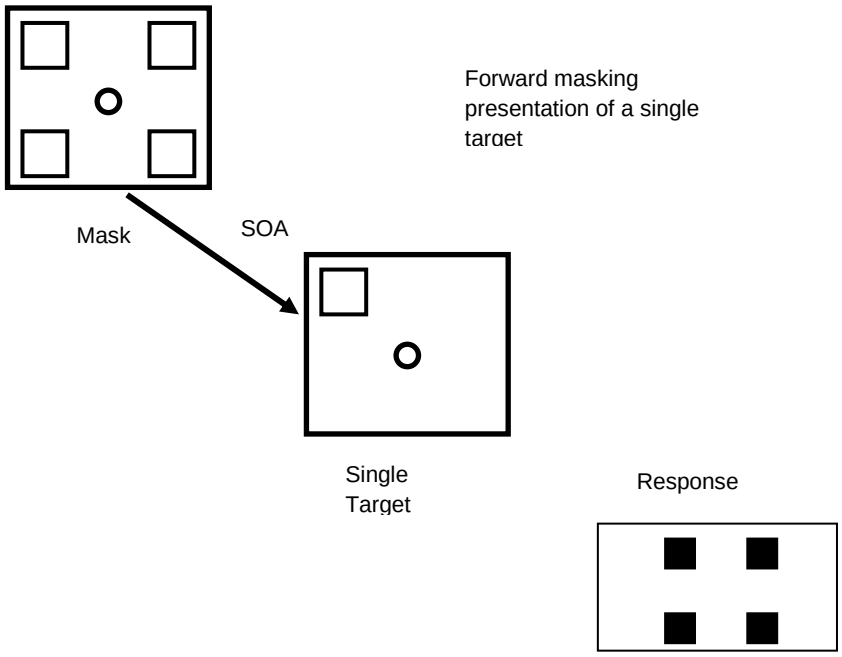
permettrait de rendre compte de l'augmentation des effets de masquage chez les patients (Green et al., 2010). Cependant les mécanismes exacts d'altération chez les patients ne sont pas définis pour autant, et le masquage était compris comme un test explorant des mécanismes perceptifs précoces.Or, si la théorie de la substitution souligne l'importance des mécanismes descendants dans les tâches de masquage (Di Lollo et al., 2000), peu d'études se sont proposées d'étudier les altérations des mécanismes attentionnels dans les tâches de masquage chez les patients schizophrènes. Granholm et al. (2009) ont évalué les ressources attentionnelles engagées durant une tâche de masquage rétro-actif en mesurant la dilatation pupillaire. Ils ont montré que les patients n'allouent pas correctement leurs ressources attentionnelles ou bien allouent préférentiellement leur attention au masque au lieu de l'allouer à la cible. Si dans les tâches de masquage, l'attention doit être allouée dans le temps et dans l'espace, on peut imaginer que les patients aient des difficultés à allouer leur attention sur la cible, comme le suggère Granholm et al. (Granholm et al., 2009). Un trouble de l'allocation attentionnelle dans le temps pourrait donc être à l'origine de déficits dans les tâches de masquage chez les patients schizophrènes.

Dans notre étude, nous avons vérifié si les troubles de codage des événements dans le temps pouvaient expliquer certaines des altérations dans les tâches de masquage. En effet, ces tâches impliquent une discrimination de la cible et du masque dans le temps. Nous avons réalisé la tâche de masquage et de jugement de simultanéité/asynchronie dans le même protocole.

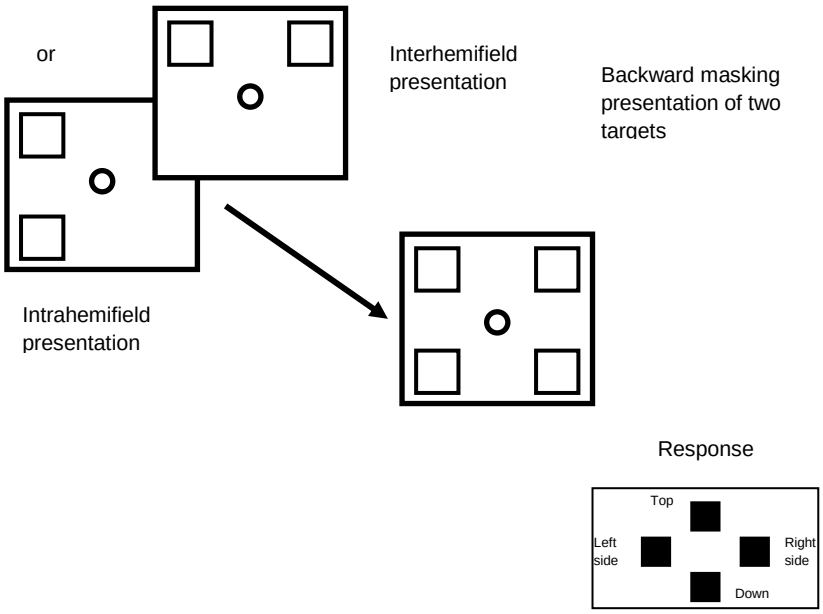
Nous avons testé les sujets inclus dans l'étude 2 avec des tâches de masquage pro-actif et de masquage rétro-actif (dans le masquage rétro-actif, la cible est présentée avant le masque, alors que dans le masquage pro-actif, le masque précède la cible ; dans les deux cas, le sujet

doit localiser la cible). Nous avons comparé les résultats dans une tâche de masquage classique, c'est-à-dire une cible unique à localiser parmi 4 localisations possibles, et une tâche de masquage avec une cible composée de deux carrés. Cette dernière tâche a été utilisée parce que les cibles étaient similaires à celles utilisées dans la tâche de discrimination de simultanéité versus asynchronie. Par ailleurs, nous avons souhaité examiner deux conditions attentionnelles différentes, c'est-à-dire l'attention divisée versus l'attention focalisée. Pour cela, nous avons présenté une cible composée de deux éléments (deux cibles présentées dans deux hémichamps en haut, ou en bas ; cibles présentées dans le même hémichamp à droite ou à gauche) versus une cible unique (en haut à droite, en haut à gauche, en bas à droite, en bas à gauche) dans 4 localisations possibles. La présentation d'une cible unique permet de focaliser l'attention alors que la présence de deux cibles amène les sujets à diviser leur attention.

Dans les tâches de masquage, la cible est présentée avant ou après l'apparition d'un masque. La difficulté de la tâche varie avec l'intervalle de temps entre le masque et la cible qui varie de -250 ms à 0 ms (masque et cible sont alors synchrones) à +250 ms. Le sujet doit discriminer la cible du masque quel que soit l'intervalle de temps les séparant.



Les sujets



Nos résultats ne nous ont pas permis de mettre en évidence de corrélation claire entre la discrimination des évènements dans le temps et les effets de masquage. Par contre, les résultats suggèrent des effets majeurs de la focalisation de l'attention (Lalanne et al., 2012c).

En effet, nos résultats ont montré que les contrôles bénéficient de la focalisation attentionnelle dans la tâche de masquage classique alors que les patients n'en bénéficient pas. Ainsi, les performances des sujets contrôles s'améliorent sensiblement lorsqu'ils doivent localiser une cible plutôt que deux, ce qui n'est pas le cas des patients. Les résultats mènent à une altération typique de la performance chez les patients en cas de cible unique. Par contre, et de façon remarquable, les patients ne présentent plus d'altération significative en cas de cibles doubles (Lalanne et al., 2012c).

Figure 1

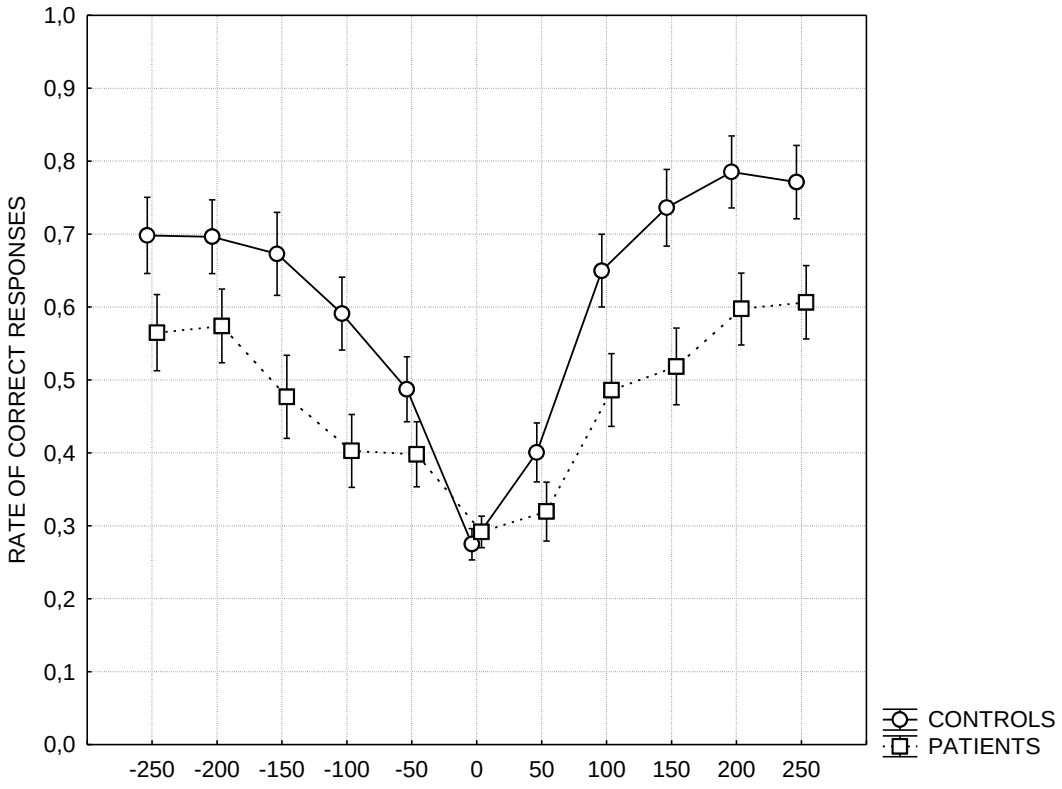
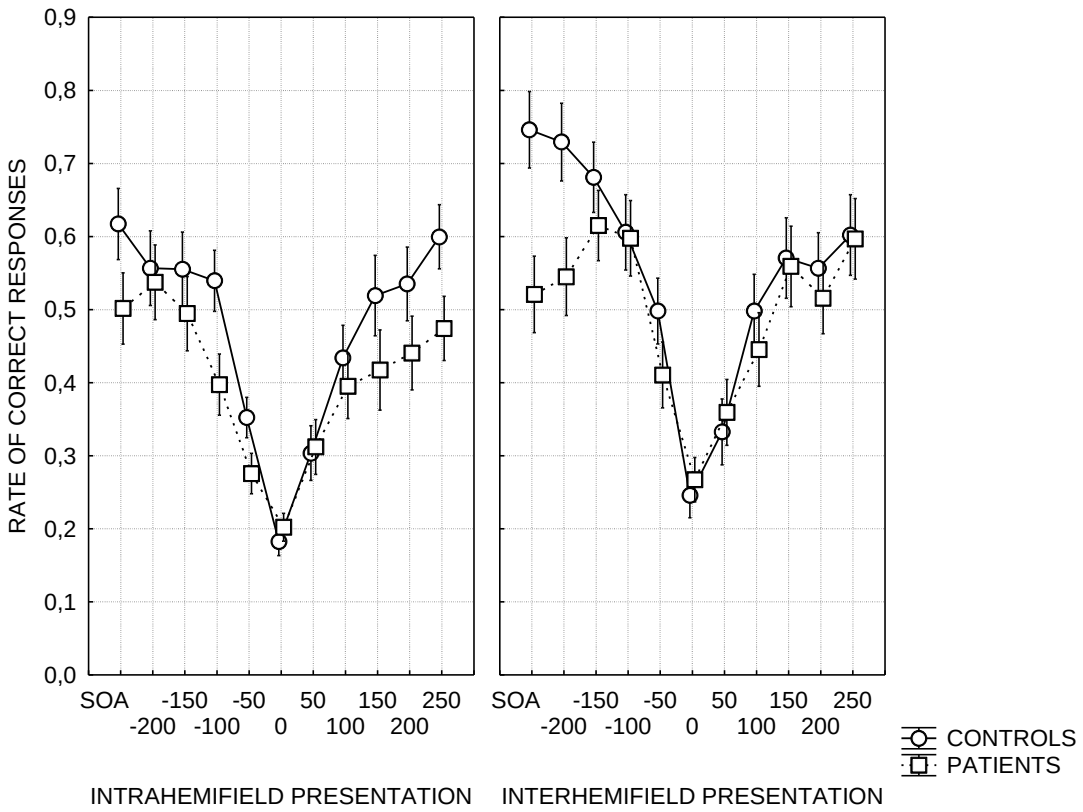


Figure 2



Figures 1 et 2 : Ces graphiques représentent le taux de réponses correctes en fonction de l'intervalle qui sépare l'apparition de la cible de celle du masque (0 à 250 ms). Lorsque la cible apparait avant le masque, on parle de masquage rétroactif (SOA supérieurs à 0 ms) ; lorsque la cible apparait après le masque on parle de masque pro-actif (SOA inférieur à 0 ms). La figure 1 présente les résultats du masquage en présence d'une seule cible. La figure 2 présente les résultats du masquage en présence de deux cibles soit disposées dans le même hémichamp (figure de droite) soit dans des hémichamps différents (figure de gauche). Les patients présentent un déficit important lorsqu'une seule cible est présentée mais rattrapent ce déficit lorsque deux cibles sont présentées et ce particulièrement dans le cadre du masquage rétroactif en présentation inter-hémisphérique des cibles.

L'absence d'altération chez les patients en cas de cibles doubles est remarquable dans la condition de masquage rétroactif (masque après la cible), condition dans laquelle ils présentent systématiquement une altération en cas de cible unique. En cas de cibles unique, les patients doivent sélectionner la cible dans le temps et la séparer du masque. La littérature montre que les patients présentent des troubles de la sélection attentionnelle (Granholm et al., 2009 ; Luck and Gold, 2008). En présence de deux cibles, le rôle de la

sélection attentionnelle serait moindre dans la tâche, ce qui permettrait aux patients de rattraper leur performance et d'égaliser celles des contrôles.

Nos résultats suggèrent qu'il existe des troubles de la sélection attentionnelle chez nos patients. Cette sélection attentionnelle s'opérant dans un temps très court, il est possible que les patients présentent des troubles de la sélection attentionnelle dans le temps. Nos résultats ne permettent pour l'instant pas de trancher cette question.

5) Synthèse et hypothèses

L'ensemble des résultats convergent vers un trouble du codage des évènements dans le temps chez les patients et cela à deux niveaux, explicite et implicite. Dans une revue récente (Giersch et al., 2015), nous avons montré que plus la fenêtre temporelle des patients était élargie, plus ils fragmentaient les évènements dans le temps lorsque les évènements sont séparés de 8 à 17 ms. Ainsi, il y aurait un lien entre l'intensité des troubles à un niveau implicite et explicite. Le déficit explicite rendrait compte de difficultés à compenser des troubles du codage des évènements à un niveau implicite, neuronal. Selon la théorie du codage prédictif développée par Friston en 2009, il existe un rafraichissement régulier de l'information au niveau neuronal : lorsqu'une information est traitée par le système nerveux, ce dernier est en attente de l'information suivante. Ce codage prédictif se déroule dans le temps. C'est comme si le traitement de l'information entrante était rafraîchi avec une certaine fréquence. Le fait d'anticiper la survenue d'une deuxième information après la première pourrait permettre de mettre en lien ces deux informations, si, comme les résultats plus récents de l'équipe le suggèrent, c'est une séquence de deux événements qui est prédite plutôt qu'un stimulus après l'autre. Ces mécanismes de prédiction pourraient

sous-tendre la continuité temporelle, dans la mesure où ils permettent de suivre l'information à une fréquence élevée : si les sujets ne peuvent distinguer consciemment deux événements en-deça de 30 ms, ils y arrivent à un niveau implicite, et restent ainsi en contact avec l'environnement plus efficacement à un niveau implicite qu'à un niveau explicite. Les mécanismes de prédiction permettraient aussi de pouvoir détecter que l'information reçue n'arrive pas au bon moment dans le temps par rapport à la prédiction réalisée et qu'il existe une erreur, comme le suggère la théorie du codage prédictif. Si de nombreux auteurs proposent une altération du codage prédictif chez les patients schizophrènes en lien avec des troubles de la connectivité neuronale (Uhlhaas & Singer, 2010), nos résultats suggèrent que ce n'est pas au niveau de la détection de l'erreur (Fogelson et al., 2014; Ford et al., 2014; NotreDame et al., 2014) que le problème se pose chez les patients mais avant, dans la capacité à prédire et coder la recherche/l'apparition d'une deuxième information après la première. Cela pourrait expliquer les altérations retrouvées chez les patients dans les tâches de tapping. Dans cette tâche, on demande au sujet de répéter une séquence de tapping sur une surface, c'est-à-dire qu'ils doivent taper avec l'index sur une surface lisse à une fréquence donnée et donc enchainer une séquence motrice. Lorsque le doigt entre en contact avec la surface, il existe un retour sensoriel qui signe cette rencontre et que l'on appelle retour haptique. Les volontaires présentent un temps de contact très faible avec la surface tandis que les patients présentent un temps de contact bien supérieur (Delevoye-Turrell et al., 2012). Selon la littérature (Kilner et al., 2007), les volontaires prédiraient implicitement l'ensemble de la séquence motrice (baisser l'index, aller à la rencontre de la surface et lever l'index) à l'avance et ce qui permettrait au sujet d'anticiper les évènements de la séquence dans le temps et de se passer du retour haptique prédit. Ceci conduirait à de très faibles temps de contact. Par contre, les patients seraient

incapables de prédire la séquence motrice et auraient besoin du retour haptique pour pouvoir enchainer une autre séquence (Delevoye-Turrell et al., 2012). Ceci les conduirait à augmenter le temps de contact. Ainsi, les patients sont donc beaucoup plus dépendants des informations sensorielles réelles que les volontaires sains. Les patients seraient donc plus en difficultés pour se détacher des informations sensorielles réelles, qui en cas d'anomalies d'intégration des informations dans le temps, les conduiraient à des erreurs d'interprétation et des idées délirantes. C'est ainsi que le délire de persécution par exemple, est particulièrement centré sur les indices provenant du réel qui alimentent la persécution sur la base d'interprétations erronées.

En conclusion, notre travail permet d'apporter des hypothèses plus précises concernant les liens hiérarchiques entre un trouble cognitif primaire, la fragmentation temporelle, et des troubles cognitifs secondaires comme le trouble du jugement temporel, des troubles du contrôle moteur et enfin, des symptômes cliniques corrélés à ces troubles que sont les troubles dissociatifs. Ainsi, nous avançons dans la compréhension de la schizophrénie et avons enrichi le modèle proposé par Nancy Andreasen. D'autres études doivent permettre de comprendre le lien entre la fragmentation temporelle et les troubles de la connectivité chez nos patients d'une part, mais aussi le lien hiérarchique entre les troubles du codage des évènements dans le temps et d'autres troubles cognitifs comme les troubles exécutifs, les troubles de la mémoire qui sont particulièrement présents dans la maladie.

Mon travail clinique s'est orienté en 2012 sur la prise en charge des addictions chez l'adulte et l'adolescent. J'ai donc été amenée à développer un axe de recherche sur l'étude des troubles cognitifs dans la maladie addictive, en accord avec mon travail clinique. Je me suis particulièrement intéressée aux effets cognitifs liés au cannabis, dont on sait qu'il est un

facteur précipitant de l'entrée en psychose. Par ailleurs, pour améliorer ma formation sur les troubles neurobiologiques induits par l'addiction, j'ai réalisé un stage postdoctoral dans le laboratoire du Professeur Brigitte Kieffer (IGBMC-UMR 7104-U964) grâce à un financement ANR pour l'étude des mécanismes moléculaires des troubles dysphoriques induits par l'abstinence prolongée à l'héroïne. La littérature montre en effet que les systèmes sérotoninergique et kappa/dynorphinergique sont impliqués dans la régulation des émotions et de l'humeur. L'activation du système sérotoninergique est impliquée dans la régulation positive de l'humeur (Chilmonczyk et al., 2015). Par contre, l'activation du système kappa/dynorphinergique entraine une diminution de la synthèse de dopamine au niveau méso-limbique, une augmentation de la sécrétion de cortisol, à l'origine de troubles dysphoriques (Lutz et Kieffer, 2013). L'idée était de vérifier que les troubles dysphoriques induits par l'abstinence prolongée à l'héroïne chez la souris, dépendent d'interactions entre ces deux grands systèmes, le système sérotoninergique et le système kappa/dynorphinergique. Sur un plan comportemental, j'ai examiné les effets dysphoriques et les déficits d'interactions sociales induits par l'abstinence à l'héroïne. J'ai montré que l'abstinence prolongée à l'héroïne est à l'origine de déficits d'interactions sociales chez les souris. En cela, j'ai reproduit les travaux de l'équipe publiés à plusieurs reprises sur plusieurs souches de souris (première publication dans Psychopharmacology). Dans un deuxième temps, j'ai montré que de tels déficits peuvent être prévenus à la fois par un traitement antidépresseur sérotoninergique (fluoxetine) mais aussi et de manière égale par un traitement de type antagoniste des récepteurs opiacés kappa (norBNI). Enfin, j'ai montré que ces deux traitements réversent de manière identique les troubles une fois installés. Sur le plan moléculaire, j'ai montré que dans les régions cérébrales impliquées dans l'addiction, à savoir l'amygdale, le nucleus accumbens, le cortex préfrontal et le noyau dorsal du raphé,

l'abstinence prolongée à l'héroïne entraine une augmentation de la régulation de la transcription du gène de la dynorphine (peptide endogène du récepteur kappa). Par ailleurs, elle entraîne une diminution de la régulation de la transcription du gène SERT (transporteur de la sérotonine) dans le noyau dorsal du raphé, structure principalement impliquée dans la synthèse de la sérotonine. Ces altérations induites par l'abstinence à l'héroïne, sont réversées par les deux traitements, fluoxetine et norBNI, mais ces résultats seront à confirmer. Cette étude a été publiée récemment (Lalanne L, Ayranci G, Filliol D, Lutz PE, Kieffer B. Low sociability in a mouse model of protracted heroin abstinence is both prevented and treated by fluoxetine and the kappa antagonist NorBNI, Addiction Biology, revision). Ces résultats permettent d'envisager de nouvelles voies thérapeutiques chez l'homme pour traiter les deficits émotionnels associés à l'abstinence à l'héroine, facteurs majeurs de rechute des conduites addictives.

Chapitre 3: L'addiction et ses conséquences cognitives

1) Généralités

Le terme 'addiction' vient de l'anglais « addiction » ou « to be addict to » qui signifie « s'adonner à ».

De nos jours, on parle plus volontiers de troubles addictifs que d'addictions proprement dites.

Qui est concerné ?

Les troubles addictifs sont nombreux et variés avec l'émergence de nouvelles addictions et touchent des catégories différentes de population selon la forme d'addiction. Il existe des addictions avec et sans substances. Parmi les addictions aux substances, il faut citer l'addiction aux produits illicites tels que le cannabis, la cocaïne, l'héroïne et l'addiction aux produits licites tels que le tabac et l'alcool. Si la consommation de cannabis est particulièrement banalisée aujourd'hui, elle est pourvoyeuse de troubles cognitifs, psychiques et physiques chez 1% des sujets qui sont des consommateurs réguliers et de fait répondent aux critères de dépendance. Parmi les addictions comportementales, on peut citer le jeu pathologique avec le développement des jeux d'argent en ligne, les jeux vidéo, les achats compulsifs, l'addiction à l'exercice physique etc...

Les « Chiffres Clés 2012 » sur l'usage des drogues établis par l'OFDT (Observatoire Français des Drogues et Toxicomanies) révèlent que :

- 3,8 millions de consommateurs d'alcool sont des consommateurs dits à risque - dépendants ou non - parmi les adultes âgés de 18 à 75 ans.
  - 2,1 millions de fumeurs ont eu recours à des médicaments d'aide à l'arrêt du tabac au cours de l'année 2010.
- Les usagers réguliers de cannabis représenteraient 1,2 millions de personnes dans la population des 11-75 ans en France (au moins 10 consommations de cannabis dans le mois).

L'OMS (CIM-10) définit l'addiction comme « un état de dépendance périodique ou chronique à des substances ou à des comportements ».

Plus précisément, on définit la personne souffrant d'addiction comme « toute personne dont l'existence entière est tournée vers la recherche des effets produits sur son corps et son esprit par une substance plus ou moins toxique (drogue tolérée, interdite ou prescrite) ou une conduite (jeu, conduite alimentaire, achat...), sous peine d'éprouver un intense malaise physique et/ou psychologique ».

L'addiction se caractérise par l'utilisation d'une substance psychoactive ou la pratique d'une activité nocive pour la santé qui créée des perturbations de la conscience, des facultés cognitives, de la perception, de l'affect ou du comportement. Elle s'accompagne d'un syndrome de dépendance et d'un syndrome de sevrage à l'arrêt de la consommation ou de l'activité.

Le psychiatre américain Aviel Goodman dans son article de 1990 « Addiction: definition and implications» in British Journal of Addictions (Vol. 85, 1990) propose une définition de l'addiction qui va être reprise très couramment en psychiatrie.

Selon lui, l'addiction est un processus par lequel un comportement, qui peut fonctionner à la fois pour produire du plaisir et pour soulager un malaise intérieur, est utilisé sous un mode caractérisé par :

1) l'échec répété dans le contrôle de ce comportement ;

2) la persistance de ce comportement en dépit de conséquences négatives significatives.

Il propose un ensemble de critères diagnostiques des addictions encore utilisées aujourd'hui en psychiatrie.

Les critères sont les suivants :

-impossibilité de résister aux impulsions à réaliser ce type de comportement ;

-sensation croissante de tension précédant immédiatement le début du comportement ;

-plaisir ou soulagement pendant sa durée ;

-sensation de perte de contrôle pendant le comportement ;

-certains éléments du syndrome ont duré plus d'un mois ou se sont répétés pendant une période plus longue.

Si depuis l'émergence du DSM-IV (1980), l'addiction était diagnostiquée de manière catégorielle selon l'abus de substance et la dépendance à une substance, récemment le DSM-5 a introduit des critères dimensionnels afin de mieux rendre compte des différences interindividuelles et de pouvoir définir la sévérité du comportement. En effet, les dernières

années, de nombreux addictologues s'étaient aperçus qu'un patient pouvait répondre aux critères de l'abus et de la dépendance en même temps et ainsi être particulièrement difficile à classer dans l'une ou l'autre catégorie ou bien passer régulièrement d'une catégorie à l'autre. Les critères du DSM-5 regroupent donc les critères de l'abus et de la dépendance. Plus le nombre de critères est important, plus l'addiction ou trouble de l'usage est sévère. L'expression « troubles liés à l'usage de substances » est définie par l'existence d'au moins deux des critères suivants sur une période d'un an :

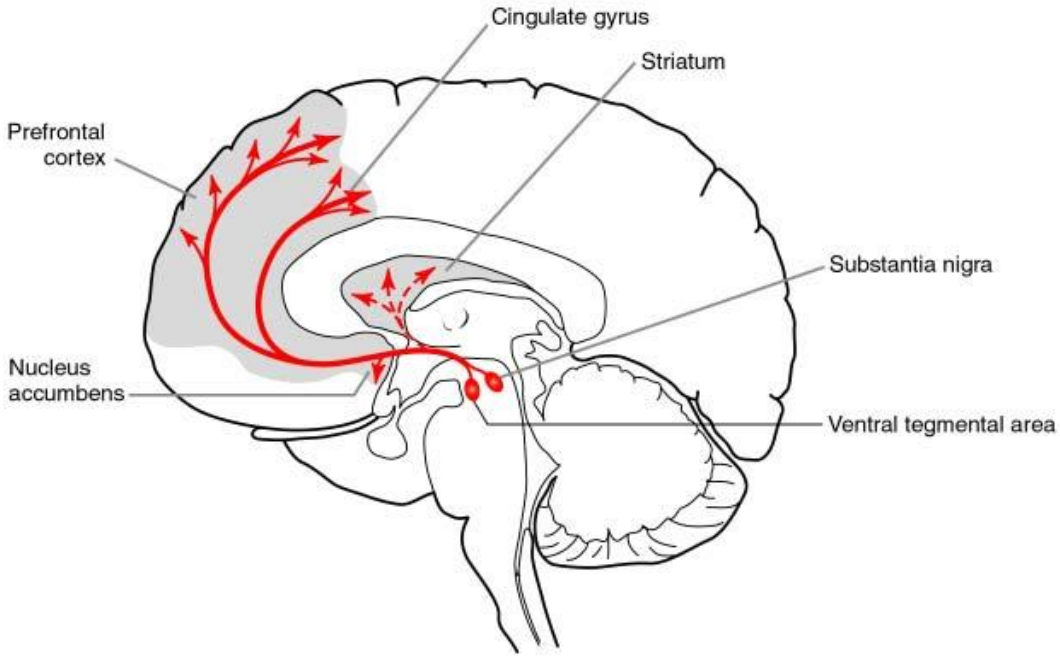
- consommation importante ou sur période plus longue que prévue ;
- efforts infructueux d'arrêt ;
- chronophagie ;
- craving (envie irrépressible de consommer);
- altération des obligations ;
- persistance d'usage malgré problème qu'il cause ;
- abandon des autres activités ;
- situations dangereuses ;
- tolérance
- sevrage

La sévérité se cote par tranches : légère si 2-3 symptômes, modérée si 4-5 symptômes, sévère au-delà.

2) Les troubles cognitifs liés aux addictions

La consommation de substances psychoactives est pourvoyeuse de troubles cognitifs tels que des troubles de la mémoire, des fonctions exécutives, de l'attention, de la perception

temporelle, etc….. Ces troubles cognitifs sont de deux types : il existe des troubles cognitifs spécifiques de la toxicité de la substance et donc qui varient d'une substance à l'autre ; par ailleurs, il existe des troubles cognitifs communs aux addictions avec et sans substances qui sont à l'origine même du maintien des consommations et des rechutes, comme les troubles mnésiques mais surtout les troubles exécutifs (Lalanne et al., 2016). Ces troubles cognitifs se mettent en place lors d'une consommation régulière et chronique de drogues (ou de non drogues, jeux, achats etc…), et persistent d'autant plus après l'arrêt des consommations que les sujets ont consommé précocement et que leur consommation a été importante. Ils sont sous-tendus par des anomalies neurobiologiques au niveau du système de récompense composé par l'aire tegmentale ventrale et le nucleus accumbens qui sont les aires de la récompense proprement dite, l'amygdale et l'hippocampe qui constituent le système d'apprentissage [24], et le cortex préfrontal qui est la région d'auto-contrôle, de planification et de prise de décision [3].



Hyman SE, et al. 2006.
Annu. Rev. Neurosci. 29:565–98

Voie dopaminergique : meso-corticale, meso-limbique et nigro-strié.

Ces altérations neurobiologiques se traduisent in fine par des remaniements de la plasticité synaptiques d'autant plus importants que les troubles de l'inhibition et la consommation compulsive sont importants (Koob et Le Moal, 2008). Elles se traduisent par la présence de troubles cognitifs mnésiques, exécutifs, attentionnels et motivationnels. De telles altérations rendent la capacité des patients à suivre leur traitement, à adhérer à leur suivi et à s'insérer dans la société, beaucoup plus compliquée et constituent une entrave à leur prise en charge. En effet, la capacité du sujet à arrêter un produit suit plusieurs stades qui engagent les fonctions cognitives. Ces stades ont été décrits par Le Berre en 2012 et sont au nombre de trois :

1) Le stade de précontemplation : le consommateur heureux
2) Le stade de contemplation : durant lequel le sujet prend conscience des difficultés liées à ses consommations mais n'est pas prêt à arrêter
3) Le stade de prise de décision d'arrêt des substances.

Ces stades mettent en jeu les capacités mnésiques, motivationnelles et surtout exécutives qui toutes sont altérées par la consommation de substances (Lalanne et al. 2015, in press). La capacité du sujet à prendre conscience des conséquences négatives de ses consommations, se souvenir des situations à risque de consommer, identifier les moments les plus difficiles en terme de craving (envie irrépressible de consommer) font intervenir les facultés mnésiques. Par ailleurs, la capacité du sujet à inhiber son envie de consommer, à mettre en place des stratégies efficaces pour contourner les lieux qui rappellent et motivent la consommation de substance font intervenir les capacités exécutives. Le sujet doit par ailleurs faire preuve de flexibilité mentale pour s'adapter et changer de stratégie lorsqu'il se rend compte qu'il est dans une situation à risque pour lui. Enfin, il lui faut prendre des décisions, notamment pour arrêter les consommations, rompre avec les amis de

consommation, éviter les lieux dans lesquels le sujet est amené à consommer. Dans nos projets nous nous intéressons aux effets cognitifs spécifiques et communs des substances afin de mieux comprendre la hiérarchie entre ces troubles cognitifs.

La consommation de cannabis a des effets aigus et chroniques sur le plan cognitif et peut avoir des conséquences importantes tant sur le plan individuel que social. Par exemple, le cannabis est fréquemment retrouvé chez les auteurs d'accidents de la voie publique. La littérature montre qu'en aigu, il existe une toxicité du cannabis sur le plan cognitif, c'est-à-dire des troubles de l'attention, de la mémoire et des difficultés à réaliser des tâches complexes et des troubles de la vigilence. L'ensemble de ces troubles concourreraient à augmenter les accidents de la route (Soderstom et al., 1995; Laumon et al., 2011; Asbridge et al., 2012 for review). Bien que la littérature ait mis en évidence des effets du cannabis sur la vision (Dawson, 1977 ; Russo et al., 2004), aucune étude ne s'est intéressée aux effets spécifiques du cannabis sur les fonctions visuelles et notamment sur la sensibilité au contraste, c'est-à-dire la capacité d'un sujet à discriminer le contraste d'un objet à différentes fréquences spatiales et temporelles (statique versus en mouvement). Cette fonction est en effet particulièrement engagée dans la conduite automobile de jour comme de nuit, lorsqu'un sujet doit repérer les obstacles voire éviter un objet en mouvement. Nous voulons donc tester les fonctions de sensibilité au contraste des sujets consommateurs de cannabis versus des sujets non-fumeurs, et des fumeurs de tabac, puisque les consommateurs de cannabis sont aussi consommateurs de tabac.

D'autres troubles cognitifs liés spécifiquement au cannabis, comme les troubles de la perception temporelle, sont très probablement impliqués dans les accidents de la route. Des altérations de la perception du temps ont été d'ailleurs mises en évidence chez les

consommateurs de cannabis. De telles altérations sont aussi présentes dans d'autres maladies mentales comme c'est le cas dans la schizophrénie, et ceci est d'autant plus intéressant que le cannabis est un facteur d'entrée en schizophrénie (McLoughlin et al., 2014, Revue Cochrane). En prise aigue, le cannabis est pourvoyeur de troubles délirants, de troubles dissociatifs et de troubles cognitifs, qui peuvent persister plusieurs jours après la consommation et sont alors identifiés sous le terme de pharmaco-psychose. La pharmaco-psychose n'est pas spécifique de la consommation cannabis, elle peut se rencontrer lors de la consommation d'autres toxiques comme la cocaïne ou l'héroïne. Néanmoins, la pharmaco-psychose cannabique peut être le prélude d'entrée en schizophrénie ou bien, et plus rarement, dans une maladie bipolaire (Casadio et al., 2011 ; Feingold et al., 2014). Les sujets ayant consommé de manière quotidienne depuis l'âge de 15-16 ans sont particulièrement à risque d'entrée en psychose (Dervaux et al., 2015 ; Di Forti et al., 2014). Par ailleurs, ce sont les mêmes qui présentent des déficits cognitifs persistants longtemps après l'arrêt de leur consommation (Brook et al., 2008 ; Meier et al., 2012). Nous voulons donc examiner si l'on retrouve des altérations du codage des évènements dans le temps chez ces patients, tels que ces altérations ont pu être décrites chez les patients schizophrènes (Lalanne et al., 2012a et b). A partir d'un protocole récemment élaboré dans l'unité (Poncelet & Giersch, 2015), nous voulons vérifier si les sujets consommateurs de cannabis fragmentent les évènements dans le temps à la manière des patients schizophrènes.

Par ailleurs, l'étude des troubles cognitifs communs aux addictions pourrait nous permettre de mieux comprendre le lien entre les troubles cognitifs primaires, comme les troubles de la perception temporelle, et des troubles cognitifs qui impactent la vie quotidienne des patients souffrant d'addiction, que sont les troubles impulsifs, se caractérisant par des

troubles de l'inhibition, des troubles de la planification, des troubles de la capacité à prendre une décision. Cette question rejoint la question posée par Nancy Andreasen dans son modèle de schizophrénie à savoir, le lien hiérarchique entre les troubles cognitifs primaires et secondaires. Chez les consommateurs précoces de cannabis (avant 15-16 ans), il persiste une impulsivité quand bien même le sujet a arrêté depuis longtemps sa consommation. Ce constat clinique est d'une importance capitale dans l'addiction, puisque le passage d'une consommation contrôlée vers une consommation compulsive de drogues est favorisé par la présence de traits impulsifs. Les troubles cognitifs primaires qui sous-tendent ces traits impulsifs sont méconnus. Récemment, McGuire et Kable (2013) ont proposé que l'impulsivité des patients souffrant d'addiction les conduirait à surestimer la durée des évènements dans le temps et à choisir des récompenses immédiates aux récompenses différées mais préférées. Cette hypothèse nous conduisent à questionner le rôle de troubles de l'anticipation temporelle d'une récompense dans les choix impulsifs des sujets. Pour examiner les liens entre anticipation temporelle et impulsivité, nous testerons les capacités d'anticipation temporelle de volontaires sains et de fumeurs de cannabis dont nous déterminerons le niveau d'impulsivité à l'aide d'échelles comportementales (BIS11). Pour examiner l'anticipation temporelle, nous utiliserons une approche comportementale, la « Hazard function task », qui permet de mesurer la capacité d'un sujet à anticiper la survenue d'un événement sur la base d'un indice temporel. Pour déterminer l'effet de la récompense sur la tâche, la « Hazard function task » sera réalisée dans une situation récompensante versus non récompensante. Ainsi, mieux comprendre les liens entre impulsivité et anticipation temporelle permettra de développer des perspectives de remédiation cognitive adaptée. Dans un premier temps, pour des raisons méthodologiques, nous voulons étudier la remédiation de troubles cognitifs exécutifs, chez des patients

souffrant de trouble de l'usage d'alcool et ce pour des aspects méthodologiques. En effet, les patients souffrant d'alcoolo-dépendance sont assez aisés à recruter et sont volontiers en demande de sevrage alcool contrairement aux sujets consommateurs chroniques de cannabis. Or, il nous apparait impossible de mettre en évidence les effets d'une remédiation cognitive chez des sujets poursuivant l'usage du toxique quel qu'il soit.

Enfin, dans un dernier projet, nous voulons avancer dans la compréhension de l'articulation entre les troubles cognitifs et les anomalies neurobiologiques. Ainsi, nous voulons mieux comprendre les liens entre les polymorphismes de certains gènes et les troubles cognitifs persistants tels que l'impulsivité, chez les consommateurs précoces de cannabis.

Chapitre 4- Projets

1) Projet 1 : Evaluation des troubles cognitifs visuels et temporels chez les consommateurs chroniques de cannabis

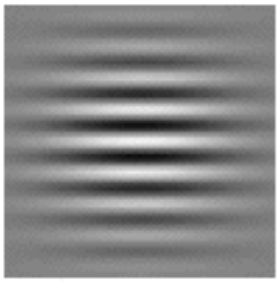
1.1 Etude de la sensibilité au contraste chez les consommateurs de cannabis

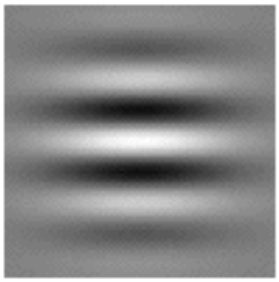
Les dernières années, de nombreuses études ont mis en évidence un lien entre les accidents de la route et la consommation de cannabis (SAM) avec une augmentation du risque d'accident multiplié par 1.8 (Laumon et al., 2011). Ces études montrent l'implication de la consommation aigue de cannabis, dont le produit psychoactif étudié ici particulièrement est le THC (tétrahydrocannabinol). Néanmoins, aucune n'a examiné l'effet d'une consommation chronique sur la conduite automobile (Laumon et al, 2011 ; Soderstrom et al., 1995 ; Asbridge et al., 2012). Pourtant la consommation chronique de cannabis impacte de nombreuses fonctions cognitives comme les capacités

attentionnelles et la prise de décision qui sont particulièrement engagées lors de la conduite automobile. Cependant, étant donné le mode d'action du cannabis, il est aussi susceptible d'affecter des mécanismes plus élémentaires que l'attention c'est-à-dire la vision. Nous faisons l'hypothèse qu'une consommation chronique de cannabis pourrait avoir un impact sur la sensibilité au contraste mais aussi la perception temporelle.

En effet, d'un point de vue neurobiologique, plusieurs études évoquent un lien entre les cannabinoïdes et le système visuel. Tout d'abord, une revue de la littérature de 2015 reprend de nombreuses références ayant montré la présence des récepteurs cannabinoïdes au niveau des voies visuelles (Schwitzer et al., 2015). Ensuite, Leroy et coll. (Leroy et al., 2012 ; Tomasi et al., 2015) ont aussi montré que le cannabis entraîne une diminution globale de la disponibilité des transporteurs de Dopamine (DAT) sur l'ensemble des circuits dopaminergiques chez les consommateurs chroniques de cannabis. Or, la dopamine intervient également sur le système visuel et ce dès la rétine, puisque l'exposition à la lumière augmente sa sécrétion par certaines cellules de la rétine, les cellules amacrines (Luvone et al., 1978). De plus, concernant les circuits dopaminergiques, des études ont montré une baisse de la sensibilité au contraste chez des sujets atteints de la Maladie de Parkinson (MP) (Skrandies et al., 1986)), c'est-à-dire chez des patients présentant une baisse de leur taux de dopamine cérébrale. L'implication de la dopamine est confirmée par le fait que les altérations de la sensibilité au contraste chez les patients atteints de MP sont partiellement améliorées après administration de Levodopa (Hutton et al., 1993). La littérature suggérant que le cannabis induit des troubles de la régulation dopaminergique (Leroy et al., 2012; Tomasi et al., 2015) qui eux-mêmes ont un impact sur la sensibilité au contraste, nous voulons examiner la sensibilité au contraste chez des consommateurs chroniques de cannabis. A

notre connaissance, cette question n'a jamais été explorée. Nous contrôlerons les effets du tabac qui impacte notamment la régulation de la dopamine au niveau du système de récompense (Swan et al., 2007). Tester la sensibilité au contraste consiste à évaluer la capacité de discrimination des contrastes des objets statiques et dynamiques. Elle est normée (Ginsburg, 2003). Nous ferons la mesure de la sensibilité au contraste chez des sujets consommateurs de cannabis, des sujets consommateurs de tabac et des non-fumeurs. Le stimulus est constitué d'un réseau sinusoïdal, c'est-à-dire une alternance de bandes verticales claires et sombres, qui changent progressivement de contraste, du blanc vers le noir et vice versa.





Les sujets consommant du cannabis, comme les consommateurs de drogues en général, ont tendance à augmenter leur prise de risque et donc sont reconnus comme plus audacieux dans leur réponse. De ce fait, leur tendance à répondre qu'ils perçoivent un stimulus alors qu'aucun stimulus n'est présent pourrait être augmentée. Pour pouvoir mesurer ce biais de réponse, nous évaluerons la sensibilité au contraste des sujets par une méthode constante, dans laquelle les sujets doivent dire s'ils perçoivent le stimulus présenté ou non. Dans cette méthode, nous pouvons calculer le biais de décision à partir du taux de fausses alarmes, soit le nombre de fois où le sujet répond que le stimulus est présent en l'absence de stimulus.

Ceci nous permet de déterminer sa stratégie de réponse dans ce cas précis. Cette méthode permet de déterminer un seuil de sensibilité au contraste pour une fréquence spatiale donnée en dynamique puis en statique mais ne permet d'évaluer que peu de fréquences spatiales du fait du nombre d'essais requis pour chaque condition. Afin d'examiner la sensibilité au contraste pour des fréquences spatiales plus nombreuses, nous examinerons la sensibilité au contraste par une méthode d'ajustement, qui est moins objective que la première et à l'origine de biais. Dans cette méthode, le sujet diminue le contraste du stimulus jusqu'à ce qu'il ne le distingue plus du fond : il peut y avoir un biais comme dans la méthode constante, mais cette fois on ne peut pas le mesurer.

Enfin, ces expériences sont associées à un enregistrement du signal rétinien par électrorétinogramme qui permettra de rendre compte de son fonctionnement et d'anomalies neurobiologiques sous-jacentes (protocole ISCEV (International Society for Clinical Electrophysiology of Vision)). Des financements ont été obtenus en 2012 pour de tels projets et les résultats sont en cours d'investigations (APJ 2012, dont je suis la coordonnatrice ; ANR SAMENTA dont la coordination est réalisée à Nancy par le Dr Laprévote).

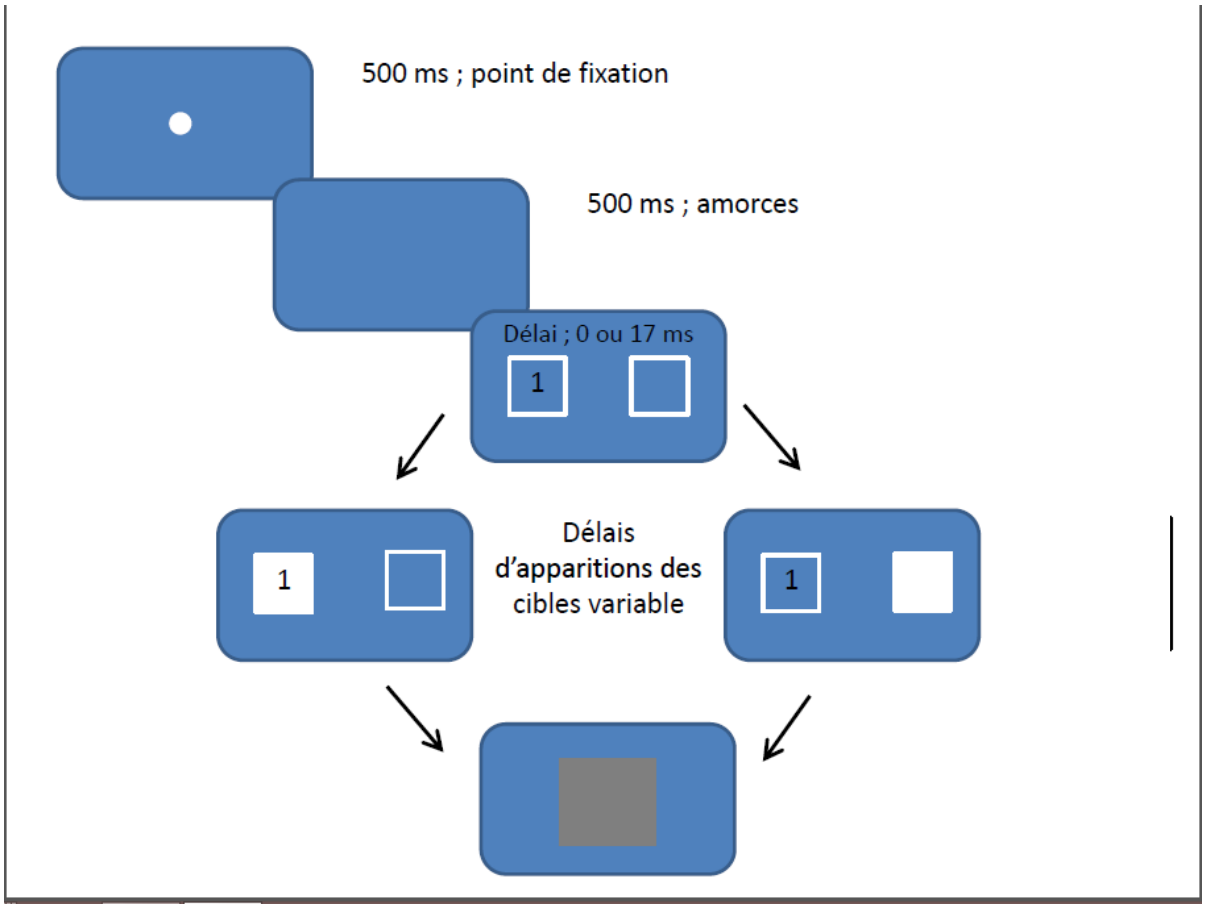
1.2 Etude du codage des évènements temporels chez les consommateurs de cannabis

Les consommateurs chroniques de cannabis ont tendance à surestimer la durée d'intervalles de temps par rapport à des sujets non-fumeurs (Atakan et al., 2012 ; Anderson et al., 2010). Par ailleurs, lorsqu'on leur demande de reproduire un intervalle de temps, les fumeurs ont

tendance à reproduire des intervalles de temps plus courts que ceux présentés. Ces altérations varient en fonction de la consommation aigue ou chronique de cannabis, de la tâche utilisée et de la méthodologie. Il est reconnu que les patients dépendant du cannabis et consommateurs depuis de nombreuses années (> 9 ans), présentent des déficits dopaminergiques au niveau du circuit striato-thalamo-cortical (Leroy et al., 2012 ; Tomasi et al., 2015). Or certaines études ont montré qu'un tel circuit est aussi impliqué dans le traitement des informations temporelles (Coull, et al., 2004, 2008, 2011). Par ailleurs, il existe un lien entre une consommation précoce et sévère de cannabis (consommation déjà avant l'âge de 15-16 ans) et le risque d'entrée en psychose (Dervaux et al., 2015 ; Di Forte et al., 2014) mais aussi la persistance de troubles cognitifs après même l'arrêt des consommations (Brook et al., 2008 ; Meier et al., 2012). Nous avons pu montrer qu'il existe une fragmentation temporelle chez les patients schizophrènes. Il est donc pertinent d'examiner s'il existe des troubles de la fragmentation temporelle chez les fumeurs de cannabis et ce d'autant plus que leur consommation a été précoce. Nous examinerons les capacités de codage des évènements dans le temps de fumeurs de cannabis ayant débuté précocement la consommation, avant 16 ans, versus un groupe de sujets ayant débuté plus tardivement la consommation, après 16 ans, versus un groupe de sujets non-fumeurs. Les consommateurs de cannabis étant aussi consommateurs de tabac, et le tabac ayant aussi une action sur la sécrétion de dopamine cérébrale, nous recruterons un groupe témoin de fumeurs de tabac, (Swan et al., 2007). Pour tester les troubles du codage des évènements dans le temps chez nos patients, nous reprendrons un paradigme développé par Anne Giersch et Patrick Poncelet chez les sujets sains (Poncelet et Giersch, 2015). Dans ce protocole, deux cadres sont présentés dans deux hémichamps différents. Les cadres apparaissent soit de manière synchrone, soit de manière asynchrone et séparés de 17 ms.

Ensuite, un flash apparait soit dans le carré de droite ou celui de gauche. Le sujet doit répondre manuellement du côté du flash grâce à un boitier réponse. Les temps de réponse et les erreurs sont enregistrés, et l'influence de l'asynchronie des amorces sur la détection de la cible est examinée. Lorsque les deux amorces sont synchrones, quel que soit le côté de l'apparition de la cible: il s'agit de la condition neutre. Lorsque les amorces apparaissent de manière asynchrone, il existe un effet d'amorçage du côté de la seconde amorce chez les volontaires sains, comme cela a été montré par Poncelet et Giersch en 2015. Ainsi, les sujets sont plus rapides quand la cible apparait du côté de la deuxième plutôt que du côté de la première amorce. Nous examinerons cet effet chez les consommateurs de cannabis et vérifierons si les sujets consommateurs de cannabis fragmentent les évènements dans le temps et autrement dit présentent un effet d'amorçage du côté de la première amorce. Ce protocole permettra donc l'étude de l'impact de la fragmentation temporelle à un niveau implicite sur un jugement explicite non temporel.

Schéma



Ainsi, nous pourrons déterminer s'il existe des troubles neurocognitifs communs, telle que la fragmentation temporelle, chez les fumeurs précoces de cannabis et les patients schizophrènes. Un tel trouble pourrait-il être considéré comme un trouble cognitif primaire ?

Dans le cadre du modèle de Nancy Andreasen, il est particulièrement important de comprendre mieux les liens hiérarchiques entre les troubles cognitifs. Comme nous l'avons souligné plus haut, un ou plusieurs troubles cognitifs primaires pourraient être à l'origine de troubles cognitifs secondaires, comme des troubles exécutifs particulièrement invalidants et qui sont à l'origine de la persistance des comportements addictifs et de la marginalisation des patients. Nous proposons donc d'examiner le lien éventuel entre de tels troubles et les altérations de la prédiction dans le temps.

2) Liens entre impulsivité et troubles de la prédiction temporelle

Si la consommation de cannabis est particulièrement banalisée aujourd'hui, elle est néanmoins pourvoyeuse de troubles cognitifs, psychiques et physiques chez 1% des sujets qui sont des consommateurs réguliers et répondent aux critères de dépendance. Les fumeurs de cannabis dépendants sont particulièrement à risque de développer des troubles cognitifs et ce d'autant plus que leur consommation est précoce. En effet, les patients fumeurs de cannabis ayant eu un usage précoce (avant 15-16 ans) présentent une impulsivité marquée (Brook et al., 2008 ; Meier et al., 2012). De nombreuses études chez l'homme et chez l'animal ont montré qu'il existe un lien entre l'impulsivité et le risque de développer une consommation compulsive de produit et ainsi de devenir dépendant. Il existe trois types d'impulsivité, l'impulsivité motrice qui correspond à une incapacité à inhiber/contrôler une réponse motrice, l'impulsivité exécutive, qui correspond à une incapacité à contrôler la prise de décision conduisant le sujet à des décisions immatures et l'impulsivité temporelle, qui correspond à l'incapacité à différer une réponse dans le temps (Caswell et al., 2015). Ces trois types d'impulsivité sont peu explorés et à notre connaissance, aucune étude ne s'est attachée à distinguer les différents types d'impulsivité, plus particulièrement l'impulsivité temporelle des deux autres types, chez les patients souffrant d'addiction. Il est donc particulièrement important de mieux comprendre les mécanismes physiopathologiques et neurobiologiques de l'impulsivité dans l'addiction afin de développer de nouvelles stratégies de remédiation cognitive.

En 2007, Wittmann et al. ont montré que des consommateurs chroniques de psychostimulants présentent des troubles du jugement de durée et ont des difficultés à reproduire des intervalles de temps : ils surestiment systématiquement la durée de l'intervalle de temps présenté par rapport à des volontaires sains ; par ailleurs, lorsqu'ils

doivent reproduire un intervalle de temps, ils reproduisent systématiquement des intervalles de durée plus courte que ceux présentés. De telles altérations ont été corrélées au niveau d'impulsivité présenté par les sujets (échelles BIS, ZTIP) (Wittmann et al., 2011). Ainsi, il semble exister un lien entre un trouble de l'estimation de la durée et l'impulsivité des sujets. Néanmoins, les mécanismes de ce lien ne sont pas vraiment éclaircis. D'une part, il reste à confirmer que l'impulsivité en lien avec la surestimation de la durée est de nature temporelle c'est-à-dire ni motrice, ni décisionnelle. D'autre part, il reste à comprendre si ce sont les troubles de l'estimation de durée qui expliquent l'impulsivité temporelle, ou si ce sont plutôt des altérations sur le circuit de la récompense qui rend compte des troubles temporels. La revue de la littérature montre que les patients impulsifs préfèrent systématiquement les récompenses immédiates à des récompenses différées mais préférées par les sujets. Ces résultats pourraient suggérer un déficit primaire de l'attente temporelle. Cependant plusieurs études suggèrent également des perturbations des mécanismes d'anticipation de la récompense elle-même chez les patients. Certains auteurs se sont intéressés à ces mécanismes d'anticipation par des tâches de « monetary incentive delay task ». Dans ces tâches, un indice est présenté avant la cible. L'indice indique au sujet ce qui surviendra en cas de bonne réponse, à savoir un gain ou une perte d'argent. Le délai entre la cible et l'indice varie entre 2000 et 2500 ms, ce qui permet d'enregistrer les activations cérébrales liées à l'anticipation. Les sujets doivent répondre aussi vite que possible à la cible. Ces différentes études se sont surtout intéressées à l'activation du striatum ventral en IRMf durant la période d'anticipation du gain ou de la perte chez les patients souffrant de troubles addictifs (alcool (Beck et al., 2009 ; Bjork et al., 2008), tabac (Rose et al., 2013), cocaine (Jia et al., 2011 ; Bustamante et al., 2013 ; Patel et al., 2013), jeu pathologique (Balodis et al., 2012 ; Choi et al., 2012). Deux études menées chez des patients alcoolo-dépendants comme

celle de Bjork et al (2008) et Beck et al (2009) ont examiné la corrélation entre le niveau d'activation du striatum ventral et l'impulsivité (BIS 10 ou 11). Bjork et al (2008) montrent une corrélation positive entre le niveau d'impulsivité et l'activation du striatum ventral dans la période d'anticipation de la récompense tandis que Beck et al (2009) trouvent une relation inverse. On retrouve des résultats contrastés dans l'addiction au jeu de hazard et d'argent (Balodis et al., 2012 ; Choi et al., 2012). Enfin, Pine et al., (2010) ont montré qu'en augmentant de manière pharmacologique la sécrétion de dopamine cérébrale, neurotransmetteur dont la sécrétion striatale est impliqué à la fois dans les phénomènes de récompense (Koob et Volkow, 2010) mais aussi dans la perception temporelle (Coull et al., 2004, 2008, 2011), les sujets choisissaient une récompense plus immédiate mais moins substantielle à une récompense plus importante mais différée (Pine et al., 2010). Cet effet était corrélé à leur impulsivité. Ainsi, même si la relation entre l'anticipation d'une récompense et l'impulsivité est hétérogène et encore mal comprise, elle semble bel et bien exister. La dimension temporelle est également présente, dans la mesure où le gain ou la perte surviennent après un délai, le plus souvent constant. Cette dimension n'est cependant pas testée, ou n'est pas dissociée de la récompense. On peut se demander si les anomalies observées persisteraient en l'absence de récompense.

Nous distinguerons tout d'abord si c'est l'impulsivité temporelle et/ou bien l'impulsivité motrice/exécutive, qui est/sont altérée(s) lorsqu'un sujet doit anticiper une récompense dans le temps. Pour cela, les sujets devront passer une tâche de « hazard function », dans laquelle deux conditions sont présentées : dans la première, un indice temporel permet de prédire l'intervalle qui sépare l'indice de la cible, dans la deuxième condition, l'indice est neutre et ne renseigne pas sur l'intervalle séparant l'indice et la cible. Les sujets impulsifs présentant une hypersensibilité à la récompense (Matin et Potts, 2004), la tâche sera

réalisée dans deux situations, récompensante versus non récompensante. Dans tous les cas, les sujets doivent répondre le plus rapidement possible.

En cas d'impulsivité motrice ou exécutive, plus l'intervalle entre la cible et l'indice sera long, plus le sujet montrera des réponses précoces, c'est-à-dire avant l'arrivée de la cible. Si cette tendance est primaire, la question est de savoir si elle peut être compensée par l'attention. La condition attentionnelle au cours de laquelle les sujets seront avertis du délai entre indice et cible permettra de vérifier si l'attention permet de diminuer la proportion de réponses précoces.

Dans la dernière hypothèse, les prédictions dépendent du mécanisme physiopathologique sous-jacent à l'impulsivité temporelle :

- soit l'impulsivité temporelle est un déficit fondamental et endogène, c'est-à-dire une préférence pour une récompense précoce, et on peut s'attendre à des temps de réactions normaux à intervalle court mais allongés pour les intervalles longs, et ceci uniquement en cas de récompense.
- Soit l'impulsivité temporelle est un trouble de l'anticipation temporelle. Dans ce cas le sujet aura des difficultés à anticiper l'arrivée de la cible dans le temps, ce qui entrainera une plus grande variabilité des temps de réponses quel que soit l'intervalle.

Par ailleurs, nous voulons explorer les corrélats neurobiologiques des liens entre anticipation temporelle et impulsivité. Récemment, McGuire et Kable ont proposé que des troubles de l'anticipation dans le temps puissent être à l'origine de l'impulsivité c'est-à-dire de l'incapacité à différer une réponse immédiate (Caswell et al., 2015). Deux hypothèses permettraient alors de rendre compte de la relation entre anticipation temporelle et

impulsivité. D'une part, il est possible que l'impulsivité soit un déficit endogène responsable de troubles de l'anticipation temporelle. Dans ce cas, les troubles devraient être observés surtout quand c'est une récompense qui est anticipée, les sujets impulsifs présentant une hypersensibilité à la récompense (Martin et Potts, 2004). Alternativement, il est possible qu'un trouble de l'anticipation temporelle soit à l'origine de l'impulsivité des sujets, et dans ce cas, il s'agit d'un déficit temporel fondamental présent que la situation soit récompensante ou non. Pour avancer sur cette hypothèse, nous ferons un enregistrement électro-encéphalographie de la composante de variation négative ou CNV durant la tâche d'anticipation temporelle, la « hazard function task ». La CNV a été décrite pour la première fois par Walter, Cooper, Aldridge, McCallum et Winter en 1964. C'est un potentiel évoqué négatif mesuré à la surface du scalp qui comprend deux composantes, une composante cognitive et une composante motrice. Deux sous-composantes cognitives sont distinguées, une composante précoce qui survient 0.7 sec à 1 sec après le stimulus et est associée à l'orientation de l'attention vers le stimulus. Une seconde composante, plus tardive est associée aux attentes temporelles et à la préparation de la réponse (Loveless & Sanford, 1974; McCallum, 1988; Rohrbaugh & Gaillard,1983). Deux études ont mis en évidence un lien entre l'augmentation de l'amplitude de la composante tardive de l'onde CNV et l'impulsivité lors d'une tâche de go/nogo chez des patients dépressifs (Pierson et al., 1993; Hansenne and Ansseau, 2001). Nous ferons donc un enregistrement EEG de la contingente de variation négative qui reflète les attentes temporelles et dont l'amplitude est modifiée en cas d'impulsivité. L'enregistrement se fera durant une tâche de « hazard function » simplifiée, dans une situation non récompensante puis dans une situation récompensante. Afin que l'enregistrement EEG ne soit pas parasité par des indices attentionnels et de façon à ce que le signal soit le moins bruité possible, l'expérience de « hazard function task » sera

réduite au bloc neutre, dans lequel aucun indice attentionnel ne permet de prédire l'intervalle entre l'indice et la cible. Le sujet devra répondre le plus rapidement possible après l'apparition de la cible. Nos prédictions sont les suivantes :

Si l'impulsivité est indépendante des mécanismes d'anticipation et de décision temporelle, la CNV ne devrait pas être altérée (van Rijn et al., 2011).

En cas d'impulsivité 'temporelle' liée à la récompense, seule la situation récompensante amplifiera l'onde 'CNV'. En cas de trouble de l'anticipation temporelle, quelle que soit la situation (récompensante ou non), les temps de réaction liée à l'incertitude concernant l'apparition de la cible seront plus variables, et l'amplitude de la CNV diminuera: les sujets ayant des difficultés à anticiper l'apparition de la cible, ils auront aussi des difficultés à préparer leur réponse dans le temps, ce qui diminuera l'amplitude de l'onde « CNV ».

Pour examiner ces questions, nous recruterons des fumeurs précoces de cannabis, dont la consommation a débuté précocement (15-16 ans), versus des non-fumeurs. En effet, les patients fumeurs de cannabis ayant eu un usage précoce (avant 15-16 ans) présentent une impulsivité marquée et persistante longtemps après l'arrêt de la consommation (Brook et al., 2008 ; Meier et al., 2012).

Ce projet a pour but d'améliorer la compréhension de la physiopathologie cognitive de l'impulsivité afin de développer au mieux des projets de remédiation cognitive adaptée

a. Perspectives de cette recherche :

En cas de trouble de l'anticipation temporelle en lien avec l'impulsivité, une remédiation cognitive spécifique des troubles exécutifs permettrait d'aider les patients à différer leur choix dans le temps. Pour ce faire, nous devrons développer de nouveaux outils. Si ce projet est à envisager dans le futur, un autre projet de remédiation cognitive chez des usagers de substances et en particulier l'alcool est en cours de développement. En effet, il nous est plus aisé de développer des protocoles chez ce type de patients pour plusieurs raisons :

- comme nous le verrons ci-dessous, il existe une très bonne documentation des troubles cognitifs exécutifs chez ces patients

-l'accès à ces patients est facile en consultation d'addictologie

-ces patients sont plus fréquemment en demande de sevrage, et il est nécessaire pour la réalisation de projets de remédiation cognitive (RC) de recruter des patients sevrés, afin d'évaluer correctement l'impact de la RC.

3) Impact d'un programme de remédiation cognitive des troubles de l'inhibition sur le nombre de jours d'abstinence cumulés chez des patients alcoolo-dépendants

L'alcoolo-dépendance est une pathologie chronique, caractérisée par une perte de contrôle et nombreuses rechutes après sevrage. Elle s'accompagne de troubles cognitifs croissants avec la durée de la maladie, la gravité et le nombre des intoxications et des sevrages. Selon Ihara et al. (2000), les troubles cognitifs induits par la consommation chronique et excessive d'alcool sont de trois types : des troubles exécutifs, des troubles exécutifs avec une atteinte mnésique et enfin, une atteinte cognitive globale. Les troubles exécutifs et en particulier de

l'inhibition entravent la réduction de la consommation et à fortiori le maintien de l'abstinence. En effet, la consommation impulsive des patients est majorée par le manque d'inhibition (Allsop et al. 2000). Houben et al., (2011, 2012) ont montré que des programmes de remédiation cognitive des fonctions exécutives permettent aux sujets de réduire leur consommation d'alcool jusqu'à 1 à 2 mois après le traitement par remédiation cognitive. Néanmoins, 2 mois de maintien de réduction de la consommation d'alcool paraît très peu au regard de la chronicité de la maladie alcoolique, les risques de rechute en cas d'abstinence étant particulièrement élevés les trois premiers mois. Par ailleurs, dans ces études (Houben, 2011, 2012), les programmes de remédiation étaient informatisés et délivrés à l'identique à tous les patients quelle que soit la gravité de leurs troubles cognitifs.

C'est pourquoi nous développerons un programme de remédiation cognitive centré sur les fonctions d'inhibition adaptés aux déficits du patient sur une période de 3 mois.

Dans notre étude, nous voulons examiner chez des patients alcoolo-dépendants présentant une consommation à haut risque d'alcool selon les critères de l'OMS (consommation supérieure à 6 unités d'alcool /jour pour un homme et 4 pour une femme), et poursuivant un objectif d'abstinence après le sevrage, les effets d'une remédiation cognitive groupale et spécifique sur leur capacité à augmenter leur nombre de jours d'abstinence cumulés à 3 mois. Nous évaluerons la persistance de ces effets après l'arrêt de la remédiation cognitive c'est-à-dire à 8 mois, sur le nombre de jours d'abstinence cumulés. Enfin, nous examinerons le transfert des acquis sur la vie quotidienne des patients. A notre connaissance, ces deux derniers points n'ont jamais été étudiés dans les précédentes études.

Nous recruterons deux groupes de patients après évaluation de leur consommation d'alcool (TLFB : questionnaire Alcohol Timeline Followback) antérieure au sevrage. Comme tout

patient hospitalisé, il bénéficiera d'une évaluation psychiatrique, somatique et neuropsychologique de ses troubles permettant de déterminer la gravité de l'altération sur le plan exécutif (test de stroop (z-scores entre –1.65 (seuil à partir duquel un sujet est altéré) et –2.5 (seuil de non inclusion)). Enfin, une évaluation du retentissement de la dépendance à l 'alcool sur les activités basales, les activités instrumentales et la vie sociale (NADL) sera réalisée. A la fin de cette visite, en l'absence de contre-indication, le sujet signera un consentement éclairé et sera inclus dans l'étude.

Les patients seront randomisés soit dans le groupe expérimental, soit dans le groupe contrôle et appariés en fonction de l'âge, du sexe et du niveau d'étude.

Dans le premier groupe, les patients bénéficieront d'une prise en charge psychosociale de type BRENDA (une évaluation Biopsychosociale et Restitution au patient, une compréhension Empathique du patient, une identification des besoins du patients avec les soignants (Needs), des conseils Directs au patient sur ses besoins, une évaluation (Assess) des réactions du patient à ces conseils) et des ateliers à raison de 2 fois 1h30 par semaine (groupes de parole, réduction des risques, séances d'information thérapeutique).

Dans le deuxième groupe, les patients bénéficieront d'une prise en charge d'une prise en charge psychosociale de type BRENDA et d'une remédiation cognitive des troubles de l'inhibition sur une période de 3 mois à raison de 2 séances de 1 heure 30 par semaine. Le protocole de remédiation cognitive a été développé par Isabelle Offerlin Meyer (Annexe 1).

Deux visites d'évaluation à 3 mois et 8 mois pour les deux groupes sont prévues à l'issue de la prise en charge par remédiation cognitive versus psychosociale pour tous les patients :

Ces visites comporteront un bilan addictologique (TLFB) somatique et psychiatrique, neuropsychologique et une évaluation du retentissement sur les activités basales, les activités instrumentales et la vie sociale (NADL).

Tous les patients bénéficieront d'un suivi addictologique durant la période de l'étude soit 8 mois.

4) Etude des liens entre le polymorphisme génétique du récepteur CB1 des sujets consommateurs précoces de cannabis et leurs déficits cognitifs

Pour améliorer mes connaissances en neurobiologie sur les mécanismes de l'addiction j'ai réalisé un stage post-doctoral dans le laboratoire de Brigitte Kieffer. Ce travail de recherche fondamentale a été l'occasion de me former au travail de recherche chez l'animal mais aussi de me former aux approches en biologie moléculaire et d'envisager des projets translationnels génétique-cognition.

La littérature a mis en évidence qu'une consommation chronique et excessive à l'adolescence est un facteur précipitant de l'entrée en schizophrénie (Dervaux et al.2015, Di Forti et al., 2014). Par ailleurs, une consommation chronique et excessive est aussi à l'origine de la persistance de troubles cognitifs, en particulier l'impulsivité (Brook et al., 2008 ; Meier et al., 2012) chez les consommateurs de cannabis et ce malgré l'arrêt des consommations. L'impulsivité est un trouble cognitif commun chez nos sujets consommateurs précoce de cannabis mais aussi chez nos patients schizophrènes. Pour avancer dans la compréhension de l'articulation entre les troubles cognitifs et les troubles neurobiologiques, nous voulons examiner les liens entre les polymorphismes de certains gènes et les troubles cognitifs persistants chez les consommateurs pércoces de cannabis. Le polymorphisme du gène CNR1

localisé sur le chromosome 6 (6q 14-15) codant pour le récepteur cannabinoide CB1 est à cet égard un candidat fort intéressant. En effet, il a été montré que certains polymorphismes de ce gène sont associés à la dépendance aux substances (Suárez-Pinilla et al., 2015), et que d'autres polymorphismes de ce gène tel que rs1535255, rs202329 et rs1049353 sont plus fortement associés aux comportements impulsifs et constitue un facteur de vulnérabilité neurobiologique pour les comportements addictifs au cannabis (Bidwell et al., 2013 ; Ehlers et al., 2007). Par ailleurs, certains polymorphismes de ce gène sont associés au développement de troubles psychotiques de type schizophréniques (Ujike et al., 2002 ; Dinu et al., 2009). Nous voulons donc mesurer de façon répétée l'impact du cannabis sur les fonctions cognitives telles que l'impulsivité par des échelles (BIS, ZTPI) et des tests classiques (CPTAx), l'anticipation temporelle dans une situation récompensante versus non récompensante mais aussi la fragmentation temporelle chez des consommateurs précoces de cannabis sur 5 ans. Nous examinerons s'il existe un lien entre le polymorphisme génétique qu'ils présentent, et la sévérité, et la persistance des troubles cognitifs mis en évidence dans ces tâches. Cette étude implique un suivi longitudinal d'adolescents et de jeunes adultes, ce qui est rendu possible par le développement récent de consultations d'addictologie (Dr Lalanne-Tongio) au Centre d'Accueil Médico-Psychologique pour Adolescents (CAMPA) à Strasbourg. Si les troubles cognitifs peuvent être facilement examinés dans notre unité, nous demanderons à Katia Befort son aide pour la partie génétique.

Conclusion et Perspectives

Nos projets ont pour but d'étudier les mécanismes physiopathologiques cognitifs de l'addiction. L'expertise que nous avons acquise en psychologie expérimentale sur l'étude du codage des évènements temporels dans la schizophrénie, nous permet d'examiner les troubles cognitifs liés à la toxicité du cannabis en particulier sur les voies visuelles et sur la perception temporelle. De plus, nous voulons examiner les troubles cognitifs responsables du maintien de l'addiction et plus particulièrement l'impulsivité. L'impulsivité est en effet un facteur de risque pour l'initiation et le maintien de l'addiction, que ce soit en préclinique comme en clinique. Notre projet est d'examiner les liens entre l'anticipation temporelle d'une récompense et l'impulsivité. Nous voulons ainsi pouvoir élaborer des traitements de réhabilitation cognitive adaptés de l'impulsivité, pas seulement dans le domaine de l'addiction à l'alcool mais aussi dans celui de l'addiction au cannabis. Enfin, nous proposons d'explorer le lien entre une consommation précoce et sévère de cannabis, c'est-à-dire avant l'age de 16 ans, les altérations cognitives induites par ce type de consommation tels que la fragmentation temporelle et l'impulsivité, et les modifications génétiques associées. La consommation précoce et sévère de cannabis étant associée à un surrisque d'entrée en schizophrénie, ce questionnement parait fondamental pour mieux comprendre les mécanismes associés à la transition vers cette pathologie. De tels projets translationnels sont en cours d'élaboration.

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Annexe 1- Programme de remediation cognitive

I. Prise en charge des troubles de l'inhibition :

Ce premier volet de la prise en charge comporte deux tâches (une Tâche d'Association Implicite – IAT, et une tâche de lecture de texte. Pour une description plus exhaustive des tâches, voir Annexe 3).

Fréquence : hebdomadaire

Durée : 60 minutes

Nombre de séances : 12

Modalité d'intervention : pour l'**IAT** → en groupe [3-4 personnes] ; les sujets travaillent sur un ordinateur ; les consignes sont données à l'ensemble du groupe. Les participants s'entraînent à cette tâche à chaque séance. L'évolution progressive des différents scores donnera une indication sur le degré de préférence implicite pour l'alcool (forte versus modérée ou légère).
Pour la tâche de **lecture de texte** → en individuel (les textes diffèrent à chaque session (n = 12), mais à l'intérieur d'une session, sont identiques pour tous les participants).

Selon Laloyau et al. (2012), les comportements de consommation d'alcool ont tendance à devenir automatiques, ne demandent pas d'efforts, et sont réalisés sans intention explicite. A contrario, le maintien de comportements d'abstinence n'est pas automatique, nécessite un contrôle conscient, implique un réel effort, en ce sens qu'il est orienté contre l'exécution automatique des schémas de consommation d'alcool. En outre, le contrôle des pensées et comportements liés à l'alcool impliquent l'intégrité des capacités d'inhibition et de flexibilité cognitive, dans la mesure où l'intégrité du fonctionnement exécutif semble être un élément central dans l'évitement de la rechute (Noël et al., 2008).

Notre revue de la littérature, nous a en outre permis d'observer qu'une des équipes qui a le plus publié dans le domaine de la prise en charge par remédiation cognitive proposée aux sujets patients ayant un trouble de l'usage d'alcool, mettait assez systématiquement en

œuvre un **Test d'Association Implicite** (**IAT**; Greenwald, McGhee, & Schwartz, 1998) pour évaluer et travailler les pulsions automatiques à consommer de l'alcool. Ces auteurs ont mesuré les préférences automatiques (implicites) pour l'alcool à l'aide du Test d'Association Implicite (IAT - Houben et Wiers, 2006, 2007a,b, 2009 ; Houben et al., 2011 ; Houben et al., 2011a ; Houben et al., 2012, Wiers et al., 2002 - pour une description exhaustive, se reporter en Annexe 3).

Aussi, nous proposons d'utiliser une tâche identique à celle utilisée par Houben et al. (2011a), en ce qui concerne la tâche d'association implicite. En revanche, nous adaptons la méthodologie en proposant :

- Une tâche écologique de lecture d'un texte (décrite ci-dessous), ainsi que
- d'autres tâches de MDT (voir partie II ci-dessous et Annexe 4 pour le détail de la tâche), et
- un volet métacognitif et psychoéducationnel (voir partie III ci-dessous et Annexe 5 pour le détail de la tâche).

B – En effet, les mêmes auteurs avaient également soumis leurs participants à une tâche de Stroop informatisée. Nous proposons une autre tâche, inspirée d'une étude de Levine et al., (2000), que nous adapterons à la problématique spécifique de nos participants.

Ecologique et contextualisée, la **tâche** que nous proposons consiste, à partir de la **lecture d'un texte** que nous aurons rédigé (environ 140 mots) et qui tournera autour de la problématique de l'alcool, à barrer tous les termes relatifs à l'alcool à l'aide d'un stylo rouge, d'encercler ceux relatifs à des noms de fruits et légumes à l'aide d'un stylo vert, et de surligner ceux relatifs à des nombres, à l'aide d'un surligneur jaune, et à inhiber un certain

nombre de distracteurs (pour une description exhaustive de la tâche, se reporter en Annexe 3).

II. Entraînement de la composante Mise à Jour de la MDT

Ce second volet de la prise en charge globale comporte une seule tâche qui se subdivise néanmoins en deux parties : une partie cognitive (exercices classiques) et une partie dite écologique qui se fera à partir d'analyses de scénarios et de mises en situation réelle (pour une description détaillée, et le principe des exercices se reporter en Annexe 4) :

Fréquence : hebdomadaire
Durée : de 60 à 90 minutes (en fonction du degré de fatigabilité des sujets)
Nombre de séances : 12
Modalité d'intervention : en individuel.

Les données de la littérature (Houben et Wiers, 2011a ; Laloyau et al., 2012) sont unanimes sur le fait qu'un trouble de l'usage d'alcool d'alcool perturbe les fonctions exécutives de base, comme la Mémoire de Travail (MDT), fonction dont l'intégrité permet le maintien en mémoire et la manipulation des buts et informations pertinent(e)s. Aussi, dès lors que ce type de fonctions exécutives (MDT) est affaibli ou altéré, les sujets semblent ne plus être en mesure de contrôler leurs habitudes de consommation, et davantage guidés par des pulsions automatiques.

Afin de travailler cette fonction particulière qu'est la MDT, nous proposons des exercices type « papier-crayon », qui impliquent notamment la fonction « Mise à Jour » (MàJ), fonction qui correspond à des modifications permanentes du contenu de la MDT basées sur les dernières informations reçues (remplacer les informations mémorisées l'instant d'avant, afin de les actualiser), et est affectée chez les patients ayant un trouble de l'usage d'alcool.

Nous nous focaliserons sur les aspects impulsifs qui sous-tendent cette fonction, avec un but précis, à savoir : entraver les tendances impulsives qui animent les participants, en les entraînant à se focaliser sur un autre aspect de l'interaction et être ainsi en mesure (progressivement) de changer d'avis à la suite d'un contrôle conscient.

Inspirés des travaux initiaux de Duval et al. (2005, 2008), les exercices proposés seront néanmoins adaptés, pour le volet écologique, à la problématique particulière des participants de l'étude.

Basés sur des stratégies de réorganisation, le but de ces exercices est de transmettre aux sujets trois types de stratégies de réorganisation qu'ils n'utilisent pas, ou non systématiquement (Seron & Van der Linden, 2000) que sont : (a) le double codage (verbal et visuel), (b) le traitement en série et (c) la réduction (ou l'adaptation) de la vitesse de traitement, afin de garantir un transfert des acquis aux situations de la vie quotidienne. Le but qui sous-tend ces stratégies est double : il s'agit d'une part de consolider les étapes d'encodage avant de procéder à la transformation de l'information et, d'autre part, de réduire la charge mentale qui pèse sur l'Administrateur Central. En outre, l'adaptation de la vitesse de traitement de l'information a également pour fonction de réduire l'impulsivité « naturelle » des sujets.

III. Prise en charge métacognitive restauratrice du contrôle inhibiteur

Ce troisième et dernier volet de la prise en charge globale comporte deux tâches :

1. Le **Goal Management Training** classique (GMT - Levine et al., 2000, 2007, 2011) qui s'incrit dans la catégorie des interventions métacognitives et combine la psychoéducation et l'entraînement à la résolution de problèmes à travers la prise de conscience et

l'intégration des échecs (et succès) relatifs aux tâches personnelles des participants, plutôt qu'à partir d'une pratique décontextualisée et répétitive d'une tâche automatique sans rapport avec les préoccupations/difficultés des sujets. En ce sens, il est donc parfaitement adapté à la problématique spécifique des bénéficiaires de ce type de prise en charge, et intrinsèquement attrayant, ce qui constitue une dimension essentielle pour l'assiduité aux séances, l'observance des préconisations transmises et la réussite.

2. Tenue d'un **Journal de bord.**

Fréquence : hebdomadaire Durée : 60 minutes Nombre de séances : 12 Modalité d'intervention : (i) <u>en groupe</u> pour le **GMT**, chaque sujet exposant des situations qui lui sont propres à l'ensemble des membres du groupe [3-4 personnes], qui interagissent avec lui et entre eux, et livrent un feed back immédiat. (ii) <u>en individuel</u> pour le travail effectué sur le **journal de bord**.

1. Pour notre étude, nous proposons une version modifiée du **GMT**, qu'il s'agit d'adapter à la problématique propre aux personnes souffrant d'un trouble modéré à sévère de l'usage d'alcool. En effet, selon Houben et Wiers (2009), le trouble de l'usage d'alcool se développerait en raison de dysfonctionnements du système impulsif, qui génère alors des pulsions automatiques à consommer de l'alcool, et des perturbations dans le système de réflexion, qui n'est plus en mesure d'inhiber l'influence de ces pulsions automatiques. Aussi, le rôle de la version modifiée du GMT que nous proposerons sera d'une part, à partir de situations très concrètes et personnelles à chaque sujet, de restaurer leur contrôle inhibiteur, et d'autre part de promouvoir progressivement la généralisation des acquis à d'autres domaines que ceux travaillés en séances.

2. Dans notre étude, cette promotion de la généralisation sera en outre renforcée en invitant les participants à tenir un **journal de bord** (hors des séances de prise en charge) dans lequel ils noteront les tentations concrètes (pas uniquement en lien avec l'abus d'alcool) auxquelles ils sont exposés dans leur vie quotidienne et sociale (pendant la durée de la thérapie, et au-delà), les circonstances dans lesquelles surviennent ces tentations, et les stratégies qu'ils mettent (ou non) en œuvre afin de ne pas y succomber. Ce journal constitue le moyen d'appliquer concrètement, dans la vie de tous les jours, ce qui est travaillé en séance à partir de scénarios qui, bien qu'inspirés du réel, sont exclusivement centrés sur les problématiques en relation avec l'abus d'alcool, et restent fictifs dans le sens où ils ne sont pas en train d'advenir, au moment précis où ils sont analysés.

En effet, nous partons d'une expérience antérieure (Offerlin-Meyer, I. 2012 – Etude N°3 in Thèse) qui nous a permis de mettre en évidence toute l'importance d'aménager, dans les prises en charge proposées aux sujets, des situations spécialement dédiées au transfert et au maintien des acquis afin d'augmenter la sensiblisation des sujets à l'emploi des stratégies apprises en séances, à d'autres contextes que ceux travaillés « en laboratoire ». Enfin, les participants seront invités à continuer à se servir de leur journal et à le compléter régulièrement au terme de la thérapie, ce qui a pour but de promouvoir et renforcer le maintien des acquis à long terme.

Ce journal sera rapporté lors de chaque séance de thérapie, et son contenu sera examiné en fin de séance de GMT afin, d'une part de prévenir/exclure toute difficulté dans la prise de notes et/ou la synthétisation des données. Par ailleurs, ce « contrôle » permettra également de valoriser l'effort fourni à travers un feed back positif et encourageant. Les évolutions observées à travers la progression de ces récits en termes de diminution de tentations et/ou

de restauration *ad integrum* du contrôle inhibiteur, seront autant d'arguments d'efficacité de la prise en charge proposée, et d'acquisition de capacités de transfert et de généralisation de la part des participants.

Pour l'ensemble des trois volets que comporte cette prise en charge, l'inter-action sera favorisée, le feed-back systématique, constructif et bienveillant. Le thérapeute a un rôle actif ; il est le garant du respect de chacun et d'un environnement favorable à l'accès sécurisant et motivant à l'autonomie.

Annexe 2- Curriculum Vitae

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2 enfants, Jules Tongio né le 05/06/2008 et Lisa née le 09/05/2012

Psychiatre-Addictologue au CHU de Strasbourg
Chercheur à l'Unité INSERM 1114 « Neuropsychologie cognitive et physiopathologie de la Schizophrénie

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Pôle de Psychiatrie et Santé Mentale
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Cursus de Formation

Diplômes et Titres Universitaires

1995 : Baccalauréat scientifique, option Mathématique, mention Assez-Bien
1997 : Concours PCEM1 : (14/1150)
1999 : Certificat de Biochimie Générale
2002 : Lauréate du concours d'internat. Rang de classement : nord 569 sud :1114
2006 : Certificat de Psychobiologie du comportement
2006 : Master 1 de Neurosciences à Strasbourg
2006 : Thèse de médecine, mention Très honorable
2006 : DES de psychiatrie, mention Très bien
2006 : Diplôme Universitaire des « Bases Conceptuelles des Psychothérapies Analytiques »
2007 : Master 2 Neuropsychologie, mention Bien (Toulouse)
2009 : Diplôme Universitaire de « Pratique et Théorie de l'électro-convulsivothérapie et de la rTMS » (Bordeaux)
2010 : Diplôme Interuniversitaire « Addictions, Psychiatrie, VIH et Hépatites Virales » (Strasbourg)
2011 : Thèse de sciences (Strasbourg)

2013 : DESC d'Addictologie (Nancy)

Fonctions et Titres Hospitaliers

Septembre 1995 à octobre 2002 : Etudiante en médecine (Premier et deuxième cycles)
Novembre 2002 à mai 2007 : Internat de Spécialité en Psychiatrie à Strasbourg
- Novembre 2002 à avril 2003 : Service de psychiatrie II du Professeur Patris (CHU, secteur G09)
- Mai 2003 à octobre 2003 : Service de psychiatrie I du Professeur Danion- Unité mère-nourrisson (CHU, secteur G09)
- Novembre 2003 à octobre 2004: Service de psychiatrie I du Professeur Danion- Unité de consultations (CHU, secteur G09)
- Novembre 2004 à avril 2005 : Service de psychiatrie du Dr Bart (Etablissement Public de Santé Alsace Nord (EPSAN) à Brumath, secteur G07)
- Mai 2005- octobre 2005 : Service de psychiatrie du Dr Patris (EPSAN Brumath, secteur G02)
- Novembre 2005 à octobre 2006 : service de pédopsychiatrie du Dr Pomes (Mulhouse, Hasenrain, pavillon 6)
- Novembre 2006 à mai 2007 : Année de Médaille d'Or au CHU de Strasbourg en psychiatrie (Interrompue en cours par nomination à un poste de Chef de Clinique)

Mai 2007 à avril 2011 : Chef de Clinique –Assistant des Hôpitaux Universitaires de Strasbourg (CHU de Strasbourg)
Mai 2011 à février 2013 : Praticien Hospitalier de psychiatrie I service du professeur Jean-Marie Danion aux CHU de Strasbourg et chercheur à l'unité INSERM 1114
Mars 2013 à février 2014 : Stage postdoctoral dans le laboratoire du Pr Brigitte Kieffer à l'Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC-UMR 7104-U964)
Depuis février 2014: Praticien Hospitalier de psychiatrie I, service du professeur Jean-Marie Danion au CHU de Strasbourg et chercheur à l'unité INSERM 1114

Stages Postdoctoral

Au titre de la Mobilité (mars 2013 à février 2014) : Stage postdoctoral dans le laboratoire du Pr Brigitte Kieffer à l'Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC-UMR 7104-U964)

Qualification Ordinale :

Inscrite à de l'Ordre des Médecins depuis 2007

Sociétés Savantes et collèges

Membre de la Psychiatrie de l'EST
Membre de l'AFPBN
Membre de la SFA
Membre de la COPAAH

Organismes scientifiques

Fonctions :

Chercheur dans l'unité INSERM 1114 pour l'étude de la « Neuropsychologie Cognitive et Physiophatologie de la schizophrénie » dirigée par le Dr Anne Giersch.

Projets dotés d'un financement public :
-Appel à Projet Jeune chercheur obtenu en 2012 pour « L'étude de la sensibilité au contraste chez les consommateurs chroniques de cannabis » (investigateur principal).
- ANR SAMENTA obtenue en 2012 pour le projet « CAUSA MAP (Evaluation du traitement magnocellulaire chez les fumeurs chroniques de cannabis) » (investigateur associé en collaboration avec le Dr Laprevote Vincent et le Professeur Raymund Schwan (Nancy)).
- Projet COSINUS (COhorte pour l'évaluation des facteurs Structurels et INdividuels de l'USage de drogues) financé par la Mildeca. (Investigateur en collaboration avec Dr Marie Jauffret-Roustide (Paris), Dr Perrine Roux (Marseille) et Pr Marc Auriacombe (Bordeaux)).

Participation à d'autres projets de recherche
- PHRC-I obtenu par Benoit Trojak (CHU de Dijon) en 2014 sur l'efficacité de la rTMS haute fréquence dans la diminution de la consommation d'alcool chez des patients souffrant d'un trouble de l'utilisation de l'alcool: étude multicentrique randomisée, contrôlée.
- PREPS obtenu par Benjamin Rolland (CHU de Lille) en 2014 pour l'étude SAMBA (Sevrage Ambulatoire d'Alcool : évaluation d'un dispositif de pratiques infirmières avancées comparé à une prise en charge de référence en médecine générale)
- PRI obtenu par Valérie Wolff en 2013 au CHU de Strasbourg sur « La prévalence des sténoses intracrâniennes chez les consommateurs réguliers de résine de cannabis comparée à celle d'un groupe de sujets contrôles. Etude préliminaire de faisabilité. »

Distinctions
Deuxième Prix de lauréat du concours de médecine et spécialités médicales pour une année supplémentaire (Médaille d'Or), juillet 2006
Prix de thèse Paul Reiss : Université Louis Pasteur, novembre 2007

Activités de soins

Responsabilités
- Consultations psychiatrie-addictologie pour adultes et adolescents
- Hospitalisations des patients souffrant de la comorbidité psychiatrique et addictologique
- Consultations de secteur pour des patients souffrant de troubles psychiatriques et de troubles addictifs et nécessitant une prise en charge de secteur

Activités de Recours et de référence (depuis novembre 2014)
- Consultations psychiatrie-addictologie pour adultes et adolescents
- Consultations pour addictions aux jeux de hasard et d'argent
- Consultations pour addictions aux jeux vidéo

Développement de la filière de prise en charge des addictions : unité de sevrages complexes et hôpital de jour

Activités Pédagogiques

Enseignement (2007-2011) : durant le clinicat

En deuxième cycle des études médicales

-Sémiologie des pathologies cérébrales organiques : états confusionnels, démences, états maniaques (cours 2h, DCEM1, au total 18h)
-Ethique en psychiatrie et Hospitalisation sous contrainte (TD 1h, DCEM3, au total 2h)
-Ethique en Psychiatrie et information du patient (TD 1h, DCEM3, au total 4h)
-Schizophrénie (TD 2h, DCEM3, au total 26h)
-Démence et confusion (TD 2h, DCEM3, au total 26h)
-Les psychotropes (TD 2h, DCEM3, au total 2h)
- Sauvegarde de justice, tutelle, curatelle (TD 2h, DCEM3, au total 2h)

En troisième cycle des études médicales

- Théorie et Pratique de l'électro-convulsivothérapie (2 h)
- Aspects pratiques de l'ECT (2 h)
- Effets secondaires, médications et ECT (2h)
- Participation régulière aux Conférences des Chefs de Cliniques de Préparation à L'ECN (6h)

Ecole de Masso-kinésithérapie

- Les névroses (1h)
- Les psychotropes (1h)

Faculté de Psychologie en master 1 de Psychologie **:**

- Clinique de la schizophrénie (2h)

Enseignement de 2011 à 2013 et de 2014 jusqu'à nos jours

- Enseignement en deuxième cycle :

- Physiopathologie générale des addictions (DFGSM3, 1h)
- Introduction aux Addictions : généralités (DFASM2, 1h)
- Addictions sans substance (DFASM2, 1h)
- Douleur et Santé Mentale (DFASM1, 1h)

- **Direction/Organisation** depuis octobre 2014 sous la responsabilité du Pr P Vidailhet de l'enseignement en DFASM2 sur « Les addictions »

- Enseignement en troisième cycle (internes DES):

- Séminaire Urgences Psychiatriques
Cours : les intoxications aigues et syndrome de sevrage (2h)
- Séminaire de Psychiatrie Médico-Légale
Cours : Législation et Addiction : substances licites et illicites (2h)
- Séminaire Recherche en Psychiatrie (2h)
- Séminaire « ECT et rTMS : approche théorique et pratique »
Cours : Aspects pratiques l'électroconvulsivothérapie (ECT) et ECT d'entretien (2h) ; Effets secondaires et indésirables ECT et médicaments (2h)

- **Direction/Organisation** sous la responsabilité du Pr P Vidailhet du séminaire inter-régional sur les Addictions avec et sans produits (2h de cours assurées dans ce séminaire).
- **Direction/Organisation** du séminaire « ECT et rTMS : approche théorique et pratique »

- **Direction/Organisation** de la Session Interrégionale du DESC d'addictologie à Strasbourg (2 h de cours assurée dans cette session)

- Capacité et Diplômes Universitaires
- DIU de Psychiatrie Médico-Légale coordonné par le Pr M Patris à Strasbourg
Cours : Criminalité et Addictions » (2h).
- DIU de Tabacologie et aide au sevrage tabagique coordonné par le Pr E Quoix sur Strasbourg
Cours : Troubles cognitifs et tabac

- **Direction/Organisation** sous la responsabilité du Pr JM Danion de la Capacité d'Addictologie Clinique de Strasbourg depuis janvier 2012 (14 heures de cours assurées dans cet enseignement)
- **Co-organisation** avec le Dr JP Lang sous la responsabilité du Pr M Doffoël de la session strasbourgeoise du DIU « Addictions, Psychiatrie, VIH et Hépatites virales » (8 h de cours assurées dans cet enseignement)

- Enseignement en Master
- Faculté de Psychologie en Master 1 de psychologie
Cours : Addictions et schizophrénie (2h)
- Faculté de Psychologie en Master 2 Neuropsychologie
Cours : Modèle cognitif des Addictions (1h)
Cours : Troubles cognitifs des addictions (1h)
- Faculté de Psychologie en Master 2 Neurosciences
Cours : Clinique des addictions (2h)

- Ecole de Masso-kinésithérapie
Les Addictions (1h)

- Faculté Dentaire
Les produits illicites (1h)

- Formation médicale continue
Travail et schizophrénie (DEPULP, 2010)
Addictologie Psychiatrie et troubles neurocognitifs (Plateforme ETP Alsace, 2015)

- Autres

Formation SIDES à l'Université de Strasbourg et certification obtenu en novembre 2014

Travaux scientifiques

Thème de recherche

Etude des troubles cognitifs, et plus particulièrement des troubles de la perception temporelle et visuelle chez les sujets consommateurs de cannabis au moyen d'outils développés pour la recherche des troubles de la perception visuelle et temporelle dans la schizophrénie.

Publications Périodiques

Articles Originaux

1. Anne Giersch, Laurence Lalanne, Caroline Corves, Janina Seubert, Zhuanghua Shi, Jack Foucher and Mark A. Elliott. Extended visual simultaneity thresholds in patients with schizophrenia Schizophr Bull. 2009 jul;35(4):816-25
2. Lalanne L, van Assche M, Giersch A. When Predictive Mechanisms Go Wrong: Disordered Visual Synchrony Thresholds in Schizophrenia. Schizophr Bull. 2012 May;38(3):506-13.
3. Lalanne L, Dufour A, Giersch A: Attention and Masking in Schizophrenia. Biol Psy. 2012 Jan 15;71(2):162-8.
4. Lalanne L, Van Assche M, Giersch A : Is time fragmented in Schizophrenia? Neuropsychologia. 2012 Oct;50(12):2736-44.
5. Ayranci G, Befort K, Lalanne L, Kieffer B, Lutz PE. Dissociation of heroin-induced emotional dysfunction from psychomotor activation and physical dependence among inbred mouse strains. Psychopharmacology (Berl). 2015 Jun;232(11):1957-71.
6. Benoit Trojak, Vincent Meille, Laurence Lalanne, Sophia Achab, Hélène Poquet, Eddy Ponavoy, Emilie Blaise, Bernard Bonin, Jean-Christophe Chauvet-Gelinier. Transcranial Magnetic Stimulation combined with Nicotine Replacement Therapy for smoking cessation: a randomized controlled trial. Brain Stimul. 2015 Nov-Dec;8(6):1168-74.
7. Lalanne L, Ayranci G, Filliol D, Befort K, Glaveriaux C, Kieffer BL, Lutz PE. Kappa opioid receptor antagonism and chronic antidepressant treatment have beneficial activities on social interactions and grooming deficits during heroin abstinence. Addiction Biology, Addict Biol. 2016 Mar 22. doi: 10.1111/adb.12392.
8. Vincent Meille, Bruno Vergès, Laurence Lalanne, Lysiane Jonval, Laurence Duvilllard, Jean-Christophe Chauvet-Gelinier, Bernard Bonin, Benoit Trojak.Effects of transcranial magnetic stimulation on the hypothalamic-pituitary axis in depression: Additional data. Journal of Neuropsychiatry and Clinical Neurosciences. Accepted

Rapport de cas:

9. Ruppert E, Lalanne L, Foucher J, Zimmermann MA, Hirsch E, Vidailhet P. Electroconvulsive therapy for psychosis in a patient with epilepsy related to hypothalamic hamartoma.Epileptic Disorder. 2013 Sep;15(3):347-51
10. Lalanne L, Weiner L, Trojak B, Berna F, Bertschy G. Substance-use disorder in high-functioning autism: clinical and neurocognitive insights from two case reports. BMC Psychiatry. 2015 Jul 7;15:149.

11. Weibel S, Lalanne L, Riegert M, Bertschy G. High-dose baclofen efficacy on alcoholism, but inefficacy on comorbid bulimia: A case report. Journal of Dual Diagnosis. 2015 Oct 12:0.
12. Lalanne L, Meriot ME, Ruppert E, Zimmermann MA, Danion JM, PhD1,2, Vidailhet P. Attempted infanticide and suicide inaugurating catatonia associated with Hashimoto's encephalopathy: a case report. BMC psychiatry, 2016 Jan 19;16(1):13.

Revues générales

1) Sur proposition

Revues Professionnelles sur proposition

13. Thomas Schwitzer, Raymund Schwan; Karine Angioi; Isabelle Ingster-Moati; Laurence Lalanne; Anne Giersch; Vincent Laprevote. The cannabinoid system and visual processing: a review on experimental findings and clinical Presumptions. Eur Neuropsychopharmacol. 2015 Jan;25(1):100-12.
14. Lalanne L, Lutz PE, Trojak B, Lang JP, Kieffer BL, Bacon E. Medications between psychiatric and addictive disorders.Prog Neuropsychopharmacol Biol Psychiatry. 2015 Oct 27;65:215-223. doi: 10.1016/j.pnpbp.2015.10.009.

Revue Pédagogique sur proposition

15. Lalanne L, Laprevote V, Danion JM, Bacon E. Theorical reflection on the place of memory and temporal cognitive mechanisms in Addictive disorders. Encephale. 2016 Feb 11. pii: S0013-7006(15)00229-8.

2) Sur invitation

Revues Professionnelles sur invitation

16. Giersch A, Lalanne L, van Assche M, Elliott MA.On disturbed time continuity in schizophrenia: an elementary impairment in visual perception? Front Psychol. 2013;4:281.
17. Giersch A, Poncelet P, Capa RL, Martin B, Duval CZ, Curzietti M, Hoonakker M, van Assche M, Lalanne L. Disruption of information processing in schizophrenia: the time perspective. Schizophrenia Research: Cognition. Volume 2, Issue 2, June 2015, 78–83.
18. Lalanne L, Ayranci G, Kieffer BL, Lutz PE. The kappa opioid receptor: from addiction to depression, and back Front Psychiatry. 2014 Dec 8;5:170.
19. Giersch A, Lalanne L, Isope P. Implicit timing as the missing link between neurobiological and self disorders in schizophrenia? Time, cerebellum, self and schizophrenia. Frontiers in Human Neurosciences.

Revue Pédagogique sur invitation

20. Lang JP, Bonnewitz ML, Kusterer M, Lalanne-Tongio L. Alcohol consumption in patients with psychiatric disorders: Assessment and treatment. Encephale. 2014 Sep;40(4):301-7.

Lettre à la Rédaction

21. Lalanne L, Elowe J, Danion JM, Vidailhet P, Foucher J. A suicide attempt in a context of bipolar symptoms leading to a diagnosis of Sneddon syndrome. Journal of Neuropsychiatry and Clinical Neurosciences. 2013 Jul 1;25(3):E11-2.
22. Lalanne L, Jantzi C, Zimmermann MA, Danion JM, Foucher J. Melancholia associated with severe cognitive disorders as the expression of late onset postpartum anti-NMDA receptor limbic encephalitis. Journal of Neuropsychiatry and Clinical Neurosciences 2015;27(2):e168-9.

Articles en révision

Revue en révision

Laurence Lalanne, Vincent Laprevote, Benoit Trojak and Elisabeth Bacon. Cognitive mechanisms in addictive disorders: Cognitive contributions to the initiation and maintenance of addictive disorders.

Trojak B, Sauvaget A, Fecteau S, Lalanne L, Koch S, Bulteau S, Zullino D, Achab S. Outcome of Non-Invasive Brain Stimulation in Substance-Related and Addictive Disorders: a review of randomized sham-controlled clinical trials Drug and Alcohol Dependence

Case report en révision

Laurence Lalanne, Nicot C, Lang JP, Bertschy G, Salvat E. Ketamine: a new way to manage opioid withdrawal?

Communications

Congrès internationaux

Communications orales sur proposition avec résumé

-Communication orale le 2/4/2011 à « l'International Congress on Schizophrenia Research » à Colorado Springs : « Time versus space organization in Schizophrenia », Anne Giersch, Laurence Lalanne-Tongio, Mitsouko Van Assche

- Communication orale le 5/05/2014 au Congrès International d'Addictologie de l'Albatros à Paris: Déficits émotionnels dans l'abstinence prolongée à l'héroïne : implication des systèmes Kappa/dynorphinergique et sérotoninergique, Laurence Lalanne-Tongio, Gulebru Ayranci, Dominique Filliol, Pierre-Eric Lutz, Brigitte Kieffer

-Communication Orale le 10/06/2015 au Congrès International d'Addictologie de l'Albatros à Paris: Médications croisées dans les doubles diagnostics, Laurence Lalanne-Tongio, Elisabeth Bacon

Communications orales sur proposition sans résumé

-Communication orale le 4/11/2005 au Symposium Neurex (Réseau transfrontalier de trois pays européens Angleterre, France Allemagne dans le domaine des neurosciences) à Otrott, Alsace: « How do schizophrenic patients perceive simultaneity ? », Laurence Lalanne-Tongio et Anne Giersch

-Communication orale le 6/11/2010 au Symposium Neurex à Strasbourg: « Time versus space organization in patients with schizophrenia », Laurence Lalanne-Tongio, Mitsouko Van Assche, Anne Giersch
-Communication orale le 30/05/2011 au « 10th World Congress of Biological Psychiatry » à Pragues: « Time versus space organization in schizophrenia », Laurence Lalanne-Tongio, Anne Giersch

Communications affichées sur proposition avec résumé

- Communications affichées du 13 au 15/12/2007 au second meeting de « West European Societies of Biological Psychiatry » à Strasbourg:
« The abnormalities of functional integration in schizophrenia: FMRI and MEG imaging are pointing to the same issues. » Jack R Foucher, Laurence Lalanne-Tongio, Anne Giersch, Mark A Elliott, Bich Thuy Pham.
« Synchrony detection in patients with schizophrenia», Laurence Lalanne-Tongio, Mark A Elliott, Jack R Foucher, Anne Giersch
- Communication affichée du 21 au 25/06/2008 à l' « International Congress on Schizophrenia Research » à Venise: « Dissociating time and spatial event discrimination in schizophrenia », Anne Giersch A, Laurence Lalanne-Tongio, Jack R Foucher, Mark A Elliott
- Communication affichée du 28/03 au 1/04/2009 à l' « International Congress on Schizophrenia Research » à San Diego: « Difficult comparison of events onsets independent of interhemispheric transfer », Laurence Lalanne-Tongio, Jack R Foucher, Mark A Elliott, Anne Giersch
- Communication affichée du 7 au 12 mai 2010 à « the 10th annual meeting of the Vision Sciences Society» à Naples en Floride: Temporal and spatial grouping: questions derived from studies in patients with schizophrenia, Laurence Lalanne-Tongio, Anne Giersch
- Communication affichée du 2 au 6/04/2011 à l' « International Congress on Schizophrenia Research » à Colorado Springs : Temporal Fragmentation in schizophrenia?, Laurence Lalanne-Tongio, Mark A Elliott, Jack R Foucher, Anne Giersch
- Communication affichée du 1 au 3 juin Congrès International d'Addictologie de l'Albatros à Paris: Médications croisées dans les doubles diagnostics, Laurence Lalanne-Tongio, Elisabeth Bacon

Communications affichées sur proposition sans résumé

- Communication affichée du 15 au 17/07/2009 au RPPW12 (« Rhythm Production and Perception Workshop ») à Lille: « Time and planning in patients with schizophrenia », Laurence Lalanne-Tongio, Anne Giersch, Mitsouko van Assche, Jack R Foucher, Yvonne Delevoye-Turrell, Mark A Elliott

Congrès nationaux

Communications Orales sur invitation

-Communication orale le 20/11/2005 au Congrès du Grand Est à Gérardmer: Relation entre 'l'expérience du présent' et la schizophrénie : une approche psychophysique, Laurence Lalanne-Tongio
-Communication orale le 7/07/2005 à Megève: Relation entre l'expérience du présent et les mécanismes neuronaux : Approche psychophysique et neurophysiologique chez des patients souffrants de schizophrénie, et chez des sujets sains, Laurence Lalanne-Tongio

- Communication Orale le 16/11/2012 aux 3ème s journées d'Addictologie à Strasbourg: Place du psychiatre dans la prise en charge des addictions, Laurence Lalanne-Tongio
- Communication Orale le 13/12/2013 aux Journées d'Addictologie du Grand Est sur le thème Actualités et controverses: L'addiction, une maladie primaire chronique du cerveau, Laurence Lalanne-Tongio
-Communication Orale le 20/12/2013 à la deuxième journée de Psycho-Addictologie: Les médications croisées entre addictions et troubles psychiatriques, Laurence Lalanne-Tongio
- Communication Orale le 16/04/2014 à Strasbourg: Dépendance à l'alcool : actualité et perspectives, Laurence Lalanne-Tongio
- Communication Orale le 20/06/2014 aux Journées d' « Actualités en Alcoologie : vers les neurosciences cliniques» à Reims: Psychopharmacologie : systèmes opiacés, Laurence Lalanne-Tongio
-Communication orale le 17/11/2014 aux Journées d'Addictologie Du grand Est: Dépénalisation du cannabis en France en 2015 ?, Laurence Lalanne-Tongio
-Communication orale le 13/02/2015 dans le cadre des Journées du Congrès Français de Psychiatrie à Strasbourg: Alcool : vers quelles perspectives en 2015, Laurence Lalanne-Tongio
- Communication Orale le 5/06/2015 lors des « Ateliers en Addictologie » organisé par Arthur Kaladjan à Reims: L'addiction une maladie neurobiologique et cognitive, Laurence Lalanne-Tongio
-Communication orale le 18/06/2015 au CPNLF à Tours : Les indices associés au désir de consommer de l'alcool, Laurence Lalanne-Tongio
- Communication orale le 4/12/2015 au JADE à Châlons-en-Champagne : Transfert de dépendance et éducation thérapeutique, Laurence Lalanne-Tongio
-Communication Orale le 29/09/2015 à Strasbourg au congrès INSIDE : Addiction et troubles schizophréniques, Laurence Lalanne-Tongio

-Communication Orale le 9 janvier 2016 au congrès du GEPS : D'une tentative de défenestration à l'encéphalopathie d'Hashimoto : histoire d'une patiente catatonique, Marie-Emmanuelle Meriot, Pierre Vidailhet, Laurence Lalanne-Tongio

Communications Affichées sur proposition avec résumé

- Communication affichée du 21 au 23/01/2010 au Congrès de l'Encéphale à Paris: A suicide attempt in a context of bipolar symptoms leading to a diagnosis of Sneddon syndrome, Julien Elowe, Jean-Marie Danion, Pierre Vidailhet, Jack R Foucher, Laurence Lalanne -Tongio
- Communication affichée 19 au 21/01/2011 au congrès de l'Encéphale à Paris: Catatonie et encéphalopathie d'Hashimoto, Marie-Emmanuelle Meriot, Jean-Marie Danion, Marie-Agathe Zimmermann, Pierre Vidailhet, Laurence Lalanne-Tongio
- Communication affichée du 21 au 24/01/2014 au Congrès de l'Encéphale à Paris: Mécanismes cognitifs de l'addiction: place des processus mnésiques et temporels, Laurence Lalanne-Tongio, Vincent Laprevote, Jean-Marie Danion, Elisabeth Bacon
- Communication affichée du 3 au 5/10/2012 au Congrès de la Société Marcé Francophone à Paris: Place of the perinatal care in prevention of infanticide in the post-partum period, Negro Hortense, Lalanne-Tongio Laurence, Zimmermann Marie-Agathe, Danion-Grilliat Anne

Réunions régionales

- Communication orale le 15/12/2011 à l'association Ithaque à Strasbourg: Codage des événements dans le temps : perturbations chez les patients schizophrènes, Laurence Lalanne-Tongio
- Communication orale le 12/09/2012 au Séminaire Urgences au CHRU de Strasbourg: Urgences et psychiatrie: Pathologies intriquées, somato-psychiques : comment s'y retrouver? A propos d'un cas: Madame H, Laurence Lalanne-Tongio
- Communication orale le 13/06/2013 aux journées de la Fédération de Médecine Translationnelle à Strasbourg: Le temps et la Schizophrénie : L'Histoire d'un dialogue entre la Clinique et la psychophysique. Anne Giersch (Service de Psychiatrie, UMR_S 1114) - Laurence Lalanne-Tongio (UMR 7104; UMR S_964).
- Communications orales le 27/09/2014 et le 8/10/2014 à Strasbourg : Neurobiologie de la dépendance à l'alcool, Laurence Lalanne-Tongio
-Communication orale le 20/01/2015 à Strasbourg: Comment aborder la question de la dépendance à l'alcool en médecine générale ?, Laurence Lalanne-Tongio
-Communication Orale le 23/06/2015 à Strasbourg : Usage d'alcool: nouveaux paradigmes de soins et nouvelles prises en soins thérapeutiques, Laurence Lalanne-Tongio et Jean-Philippe Lang
-Communication Orale le 17/12/2015 à Brumath au Club Neuropsy sur le thème « Addictions et troubles psychiatriques : médications dans les doubles diagnostics », Laurence Lalanne-Tongio
-Communication orale au CSAPA d'ITHAQUE le 4 février 2016 sur le thème « Approche neurobiologique et cognitive des addictions », Laurence Lalanne-Tongio
- Communication orale à Strasbourg le 25 avril 2016 sur le thème « Alcool et Double diagnositics», Laurence Lalanne-Tongio
- Communication orale à Thann le 19 mai 2016 sur le thème « Approche neurobiologique et cognitive des addictions », Laurence Lalanne-Tongio
- Communication orale à Comar le 4 juin 2016 sur le thème « Addictions et omorbidités psychiatriques», Laurence Lalanne-Tongio

Conférences grand public

Participation le 19/03/2015 à une table ronde « Dialogues à cœurs ouverts, les adolescents face aux risques » dans le cadre Les Semaines d'Information sur la Santé Mentale sur « Etre adolescent aujourd'hui » au Centre Administratif de Strasbourg.

Interview

- Interview en janvier 2012 pour l'émission de radio « la Tête au carré » sur RTL : Les boissons énergisantes, bénéfices et danger, Laurence Lalanne-Tongio (Diffusé en mai 2012)

Organisation de Congrès

- Organisation des 3èmes journées d'addictologie le 16 novembre 2012 à Strasbourg, sur le thème de : Place de la Psychiatrie dans le domaine des addictions
- Participation à l'organisation du congrès de la Société de Psychiatrie de l'Est du 29 novembre 2014, à Strasbourg sur le thème : Alcool : Nouvelles Pratiques- Consommations-Diagnostics-Thérapeutiques
- Participation à l'organisation des journées du Congrès Français de Psychiatrie du 13/02/2015 à Strasbourg pour la session addictologie de l'après-midi
-Participation à l'organisation du congrès du GEPS à Strasbourg du 7 au 9 janvier 2016.
- Participation à l'organisation du congrès soutenu par neurex des 29 au 31 mars 2016 à Strasbourg sur le thème : « Self disorders in schizophrenia: which pathophysiological roles for cognitive impairments, from the retina to the cerebellum.

- Organisation d'une journée de congrès le 10 mai 2016 à Strasbourg sur le thème : « Point sur l'addiction au jeu de hasard et d'argent ».

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- Revue canadienne : Drogues, Santé et Société
- Revue : Gériatrie psychologie neuropsychiatrie du vieillissement

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- Mémoire de Capacité clinique d'addictologie :

- Addiction au travail : Réflexion théorique et approche Clinique, Vanessa Slimani 2013
- Traitements de substitution en milieu pénitentiaire, Elise Gaugler, 2013
- Addictions aux jeux : Jeux de Hasard et d'Argent chez les patients du CSAPA Le Cap : Résultats d'enquête, Sophie Odermatt, 2013
- Alcool et risque suicidaire : à propos d'un cas, Mohamed Ali Tounzani, 2014
- Addiction et troubles de la personnalité Boderline, à propos d'un patient, Dr Olivier Meyer, 2014
- Nouveautés dans le traitement médicamenteux de l'alcoolo-dépendance ?, Dr Christophe Roegel, 2014.

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- Traitements pharmacologiques utilisés dans les paraphilies, Hélène Kieffer, 2008
- Utilisation du Seroquel lors de la Grossesse et l'allaitement, Pauline Chauffer, 2009

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- Troubles psychotiques et pathologies auto-immune, Julien Elowe, 2009 (publication en 2013, Julien Elowe est deuxième auteur)
- Hypothermie liée aux neuroleptiques, Raoul Steiner, 2009
- L'Homicide Altruiste: à propos d'un cas de Mélancolie, Hortense Negro, 2010

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- A propos d'un cas atypique de dépression majeure, Camille Jantzi, 2015 (publication en 2014, Camille Jantzi est deuxième auteur)

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- Contributions à l'étude de l'infanticide dans ses rapports à la psychiatrie, Hortense Negro, 2011
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- Rapport Master 1

- Etude déficits émotionnels induits par l'abstinence à l'héroïne chez la souris, Matthias Vienne, 2013
- Etude du codage temporel chez les consommateurs chronique de cannabis, Sébastien Kirchherr, 2014

- Mémoire de Master 2 (sous la responsabilité du Dr Anne Giersch)

-Etude de la sensibilité au contraste chez les sujets consommateurs chroniques de cannabis, Eglantine Ferrand-Devouge, 2013
- Etude du codage des évènements temporels chez les consommateurs chroniques de cannabis, Sébastien Kirscherr, juin 2015 reporté à juin 2016 pour raison de santé
-Etude des liens entre impulsivité et anticipation temporelle, Lucie Rauch, pour juin 2016

- Thèse de Sciences

Participation à l'encadrement de thèse de Gulebru Ayranci (mars 2013 à mars 2014) sous la responsabilité du Pr Brigitte Kieffer et membre du jury de la thèse le 23 septembre 2015: Gulebru Ayranci a publié un papier en premier co-auteur, un papier en premier auteur, et un papier en deuxième auteur.

Annexe 3- Principales Publications

Schizophrenia Bulletin Advance Access published September 27, 2010

Schizophrenia Bulletin
doi:10.1093/schbul/sbq107

When Predictive Mechanisms Go Wrong: Disordered Visual Synchrony Thresholds in Schizophrenia

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Patients with schizophrenia display an impaired sense of temporal continuity, and we showed that they judge events as being simultaneous even in case of large onset asynchronies. We check here whether this means a fusion of events in time, or on the contrary, a segregation of events and a deficit in coding time-event structure. Subjects decided whether 2 squares were displayed simultaneously or asynchronously on the screen and gave their response by hitting a left or right response key. The implicit processing of asynchrony was explored by means of the Simon effect, which refers to the finding that manual responses are biased to the side of the stimulus. We checked whether responses were biased to the side of the first or second square, when squares were asynchronous and displayed on opposite sides. Results revealed an enlarged time window in patients irrespective of the squares' position (intra- vs interhemispheric presentation). But for asynchronies eliciting "synchronous" judgments, patients' responses were biased to the side of the first square. In contrast, controls were biased in all cases to the side of the second square. The inverse effects observed below thresholds in patients and controls cannot be attributed to a generalized deficit. In controls, elementary predictive mechanisms would allow anticipation of upcoming events, whereas patients appear to process squares as if isolated rather than following each other. Predictive mechanisms would be impaired in patients, who would rather rely on reactive mechanisms in order to perceive asynchrony.

Key words: schizophrenia/prediction/synchrony/time/interhemispheric transfer/Simon effect

Introduction

A disturbed sense of continuity has been reported in patients with schizophrenia.[1,2] Patient's own reports illustrate this disturbance "Time splits up and doesn't run forward anymore. These arise uncountable disparate now, now, now, all crazy and without rule or order."[3] This alteration might be related with patients' impairments in a range of tasks regarding time processing at different timescales, ie, duration evaluation[4–9] or discrimination of successive events in time.[10,11] Impairment in the sense of continuity might be especially related to difficulties in coding time-event structure, as suggested in recent experimental studies.[12] However, it is still unclear how impairments observed in experimental studies relate with clinical evidence. In fact, experimental studies usually suggest that patients fuse events even though they are separated by a large delay, whereas clinical evidence suggests a fragmentation of events in time. In order to resolve this conundrum, we dissociate here effects at implicit and explicit levels in both patients with schizophrenia and controls.

The fact that the sense of continuity is related to the coding of time-event structure is based on ideas stemming from phenomenology. Even the simplest conscious perceptual experiences are embedded in a continuous flow of conscious experience. For example, when at a concert where several instruments are being played all at once, the musicians sometimes play a note at the same time and sometimes the notes are played successively. When played in succession, the note that has just been played is not only remembered but also phenomenologically perceived, even as it is "retained" in its being-past. "Its being-past is something now, something present itself, something perceived."[13(p219)] Similarly, the "yet-to-come" tone is perceived, as it is "protained" in its being-future. For Husserl,[13] the experience of continuity requires thus the integration of past, present, and future moments. Especially important for the present study, "now" includes an expectation regarding future moments.[13] As suggested by Varela,[14] this might result from neural constraints. In fact, the processing of information, even the simple display of a square on a computer

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screen, requires time, thus implying that, rather than being coded as single points in time, events have a duration. This means different events overlap in time even if their onset is shifted in time. The result would be a sense of continuity rather than the perception of discrete moments.

The idea that events overlap and are fused in time is also supported experimentally. Brecher,[15] eg, showed that subjects need a delay of 30–100 ms between 2 event onsets to distinguish the first from the second. This delay might represent "the subjective present" described by Husserl.[13] We recently showed that patients with schizophrenia needed larger onset asynchronies than controls to correctly judge that 2 bars were presented one after another rather than simultaneously,[16] independent of a response bias effect.[12] At a phenomenological level, this would imply less smoothness in the flow of conscious experience, with a reduced ability to perceive a recent event as passed and a to-come event as future. However, a difficulty to report an asynchrony explicitly does not necessarily mean that events are fused together at an implicit level. Even if patients do not consciously discriminate asynchronous stimuli for short stimulus onset asynchronies (SOAs), they may nonetheless perceive a difference implicitly. Our question is whether, in the case of patients, all events included within the same "simultaneity window" are fused together into one single percept. In order to evaluate the implicit processing of asynchronies, we used the Simon effect.

The Simon effect refers to the finding that performance is faster and more accurate when the stimulus appears on the same side as the responding hand, even if stimulus location is irrelevant to the task.[17,18] The Simon effect thus reflects a tendency to press the button on the side of the stimulus but requires no explicit judgment. The visuomotor Simon effect is indeed believed to rely on direct activation of the manual response through visual stimulation.[19] In our task, 2 stimuli were presented simultaneously or asynchronously. The stimuli were both on the left or right side of the screen or one was on the left and one on the right. Subjects had to hit a left-hand response key with the left hand when the stimuli were judged to be simultaneous and a right-hand response key with the right hand when they were judged to be asynchronous. When the 2 stimuli are on the same side, subjects' response should be biased to the side of the stimuli, thus reflecting the classical Simon effect. When the 2 stimuli are on opposite sides, however, a classical Simon effect cannot be expected, and in particular, no Simon effect can occur in case of 2 simultaneous stimuli. It is only when the 2 stimuli are asynchronous that a Simon effect may be observed again. The first square indeed appears by itself on the screen for a short duration, until the second square is displayed. If this short-duration stimulus is subconsciously detected as such, ie, an isolated stimulus on one side of the screen, a Simon effect should be observed on the side of this first square. In contrast, if the second square is expected, the Simon effect could be observed on the side of this second square even in the absence of a conscious perception of asynchrony. Exploring the Simon effect will thus yield information about the implicit coding of time-event structure.

Table 1. Demographic and Clinical Data of the Participants

	Patients	Controls
Gender (M/F)	9/9	9/9
Age (mean ± SD)	35.7 ± 6.3	34.3 ± 6.4
Years of education (mean ± SD)	11.8 ± 2	12.5 ± 1.8
Medication (typical/atypical/no medication)	3/14/1	
Dose of chlorpromazine equivalents	275 mg/day	
PANSS positive symptoms (mean ± SD)	15.4 ± 4	
PANSS negative symptoms (mean ± SD)	19 ± 7.3	
PANSS general symptoms (mean ± SD)	35.8 ± 12.8	
PANSS total (mean ± SD)	70.3 ± 21.2	

Note: PANSS, Positive and Negative Syndrome Scale.

Our predictions are the following. First, patients are expected to require larger asynchronies than controls to report them. The question is whether this means a real fusion of events in time. It is explored by measuring the Simon effect when the 2 stimuli are on opposite sides. In case of fusion, events are perceived as identical on both sides. There cannot be any bias to either side, and no Simon effect should be observed. In contrast, if events are segregated below threshold, a Simon effect might be observed. In that case, the type of bias (on the side of the first or the second stimulus) will give indications regarding how information has been processed, eg, with or without an expectation regarding the second stimulus. Most importantly, it will indicate whether patients and controls process asynchronies in the same way or not at an implicit level, ie, when they explicitly judge stimuli as simultaneous.

Methods

Subjects

Eighteen stabilized outpatients with schizophrenia took part in the study. They were diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* criteria.[20] Symptoms were assessed with the help of the Positive and Negative Syndrome Scale.[21]

The control group matched the patients' group in terms of gender, age, and level of education (Fs < 1) (table 1).

The project was approved by the local ethics committee. All subjects gave their informed written consent prior

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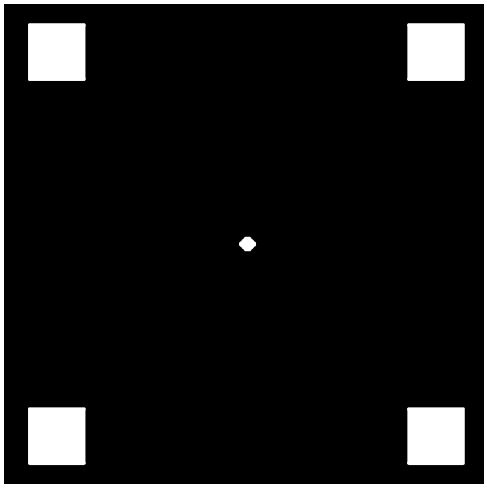

Fig. 1. Illustration of the 4 Possible Target Locations. Two squares are presented at the same time or asynchronously in 1 of 4 possible locations: upper, lower, right, or left.

to testing, in accordance with the recommendations laid down in the Helsinki Declaration.

Details concerning exclusion criteria and the equipment (computer, 120 Hz monitor and 50 Hz eye tracking) can be found in online supplementary material.

Stimuli

Stimuli were 2 squares (0.8° × 0.8°) displayed at 1 of the 4 corners of a virtual square (5.5° × 5.5°) located in the middle of the screen. They were thus presented in 2 of 4 possible locations. When presented at the top or bottom of the virtual square, they were interhemifields, whereas when they were located on the right or left of the virtual square, they were in the same hemifield (figure 1).

Contrast levels were chosen to be as similar as possible to those used in our previous experiment.[12] To reduce the influence of a transient response, squares increased gradually in luminance from 0.02 to 12 cd/m^2, over a presentation interval of 75 ms.

Procedure

Subjects had to decide whether the 2 squares displayed on the screen appeared at the same time or not. Each trial began with the presentation of the central fixation point, which remained on the screen throughout the trial. Subjects had to fixate this point for 500 ms, this being checked by continuous eye tracking. Stimuli then appeared either simultaneously (SOA = 0 ms) or asynchronously. Twelve levels of SOA were used (from 0 to 92 ms in steps of 8.3 ms). Squares stayed on the screen until subjects had responded. The stimuli and fixation point were then removed from the screen, and the next trial started after a delay of 1000 ms. Subjects were instructed to hit a left response key with the left hand in the case of simultaneous squares and a right response key with the right hand in the case of asynchronous squares. No emphasis was put on response speed.

Each target location (upper, lower, right, or left) was equally represented. Each combination of position (same or different hemifields) and SOA (12 levels) was tested 20 times in random order, yielding a total of 480 trials.

Threshold Evaluation

To control for a tendency to give asynchronous responses, the individual data were subjected to the following probability-based correction:

$$P_{\mathrm{adj}}(x) = \frac{P(x)}{P(0)},$$

where $P(0)$ is the percentage of "simultaneity response" for simultaneous squares (SOA = 0). This correction ensures that "asynchronous responses" taken into account in the following analysis cannot be attributed to false alarms. The "thresholds" were then derived from a linear adjustment between the SOAs and the corrected rate of "simultaneous" responses (rate of "simultaneous" responses = $a \times \mathrm{SOA} + b$) for each subject. They were calculated as the SOA corresponding to equivalent rates of simultaneous and asynchronous responses (50% for both). In other words, the threshold corresponds to the "point of subjective equality."

Results

There was a similar false alarm rate before data correction in patients (22.4%) and controls (24%) when squares were synchronous ($F < 1$). Although high and possibly related to the fact that there was uncertainty regarding the location of the target squares, these false alarm rates were clearly lower than in our preceding study (around 40%).

An ANOVA was conducted on thresholds with the group (patients vs controls) as between-group variable and with the type of presentation (intra- vs interhemifield) as within-group variable. The threshold was significantly higher in patients (50.1 ms) than controls (41.7 ms): $F[1, 34] = 4.8$; $P < .05$. Although the difference between patients and controls is slightly more apparent when the squares are displayed in the same hemifield (49.7 ms in patients vs 39.8 ms in controls, $F[1, 34] = 4.5$, $P < .05$) rather than interhemifield (50.6 ms in patients vs 43.7 ms in controls, $F[1, 34] = 3.8$, $P = .059$), there was no interaction between presentation (intra- or interhemifield) and group ($F < 1$) (figure 2). These data were confirmed in the analyses on response times (RTs) (see online supplementary material).

An effect of presentation (intra- vs interhemifield presentation) became apparent, however, when the rates of "synchronous" responses observed for all SOAs were

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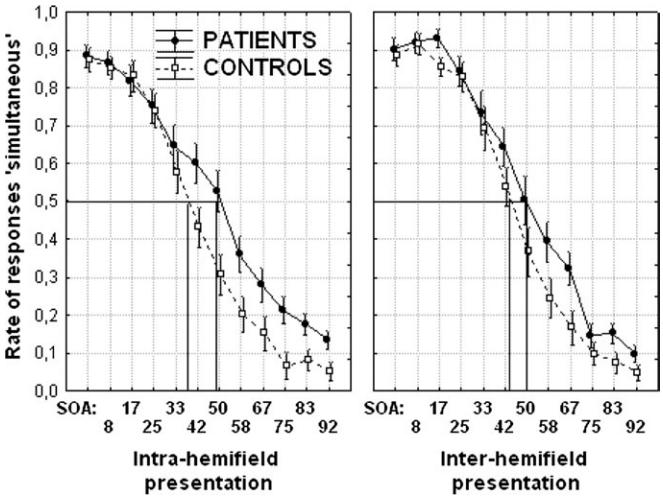

Fig. 2. Rates of Responses "Simultaneous" as a Function of the SOAs and Targets' Location, Intrahemifield (Left Panel) vs Interhemifield (Right Panel) in Patients (Continuous Line) vs Controls (Dotted Line). Thresholds are indicated by the straight lines: 49.7 ms in patients vs 39.8 ms in controls when targets are presented in the same hemifield (left panel) and 50.6 ms in patients vs 43.7 ms in controls when they are presented interhemifields (right panel).

taken into consideration. We performed an ANOVA with the group as between-group variable (patients vs controls) and with type of presentation (intra- vs interhemifield) and the SOAs (from 0 to 92 ms) as within-group variables.

There was an effect of intra- vs interhemifield presentation, ($F[1, 34] = 7.8$, $P < .01$), with subjects overall making 3.6% more errors, ie, more "simultaneous" responses, in the case of presentation interhemifield than in case of squares presented within the same hemifield. This effect interacts significantly with the SOA ($F[11, 374] = 2.7$, $P < .005$) but not with group (Fs < 1; there was neither any significant 3-way interaction between group, type of presentation, and SOA). Whereas there was no effect of intra- vs interhemifield presentation for SOA = 0 ms ($F < 1$), a significant effect of presentation was found for SOAs ranging from as little as 8.3 ms (difference of 5.8%, $F[1, 34] = 5.7$, $P < .05$), right up to 41.7 ms (differences between 5% and 10%, Fs > 4.4, Ps $< .05$). These results show a disadvantage for interhemifield presentation, which is expected since it requires an interhemispheric transfer of information. More surprisingly, this effect is observed at very short asynchronies, which yield simultaneous judgments. If this had reflected a true absence of asynchrony perception, then performance should have been equivalent whatever the target conditions.

A sensitivity to short asynchronies was also supported by analyses on RTs, at least in patients (see online supplementary material). The Simon effect was used to examine this further.

As emphasized in the Introduction, the Simon effect reflects the tendency to respond with the hand that is on the same side as the stimulus. A sensitivity to short asynchronies may be due to 2 different mechanisms, and these possibilities can be disentangled by exploring the Simon effect occurring when the squares are displayed on opposite sides. First, subjects' responses might be influenced by a sense of direction and by expectation regarding the second square. In that case, their response should be biased on the side of the second square onset. Alternatively, they might be sensitive to the first square's onset, which remains on the screen by itself for a duration equivalent to the SOA. In that case, the response should be biased on the side of the first square.

Due to the sensitivity to short asynchronies revealed by the advantage for intrahemifield presentation, we were especially interested in the Simon effect observed below threshold. Because the threshold varies between subjects, we averaged the percentage of "simultaneous" responses for SOAs below and above individual thresholds. The number of SOAs taken into account was thus adapted to each subject, with the highest SOA considered below threshold being at least 12 ms below the subject's threshold.

It should be noted that we checked that the basic Simon effect was not altered in patients relative to controls, by comparing rates of "simultaneous" responses when the 2 squares were displayed on the left or on the right (see the online supplementary material).

The critical analysis, though, concerned the Simon effect in the case of squares displayed in 2 different hemifields (first square on the left, second on the right, or vice versa). This analysis showed a significant interaction between group, SOAs (sub- vs suprathreshold), and presentation sides ($F[1, 34] = 5.7$, $P < .05$). Regarding SOAs below threshold, patients gave 4.7% more responses using the left response key ("simultaneous" responses), when the first square was on the left and the second on the right, than when the locations were reversed ($F[1, 17] = 5.5$, $P < .05$). This bias to the side of the first square was consistent across patients, being reversed in 3 patients among 18 only, and the Simon effect was still significant when using the nonparametric Wilcoxon test ($T = 20$, $z = 2.04$, $P < .05$). In contrast with patients, the responses of control subjects were biased to the side of the second square (figure 3). Below threshold, controls gave 5.7% more responses with the left-hand key when the second square was on the left than when it was on the right ($F[1, 17] = 4.9$, $P < .05$). The effect was reversed in 5 among 18 controls and tended to be significant with the Wilcoxon test ($T = 35$, $z = 1.7$, $P = 0.08$).

The profile was similar in both groups for SOAs above threshold, with a bias to the side of the second square. There was a higher percentage of responses with the left-hand key ("simultaneous" responses) when the second square was on the left rather than on the right (by 6% in patients, $F[1, 17] = 6.8$, $P < .05$

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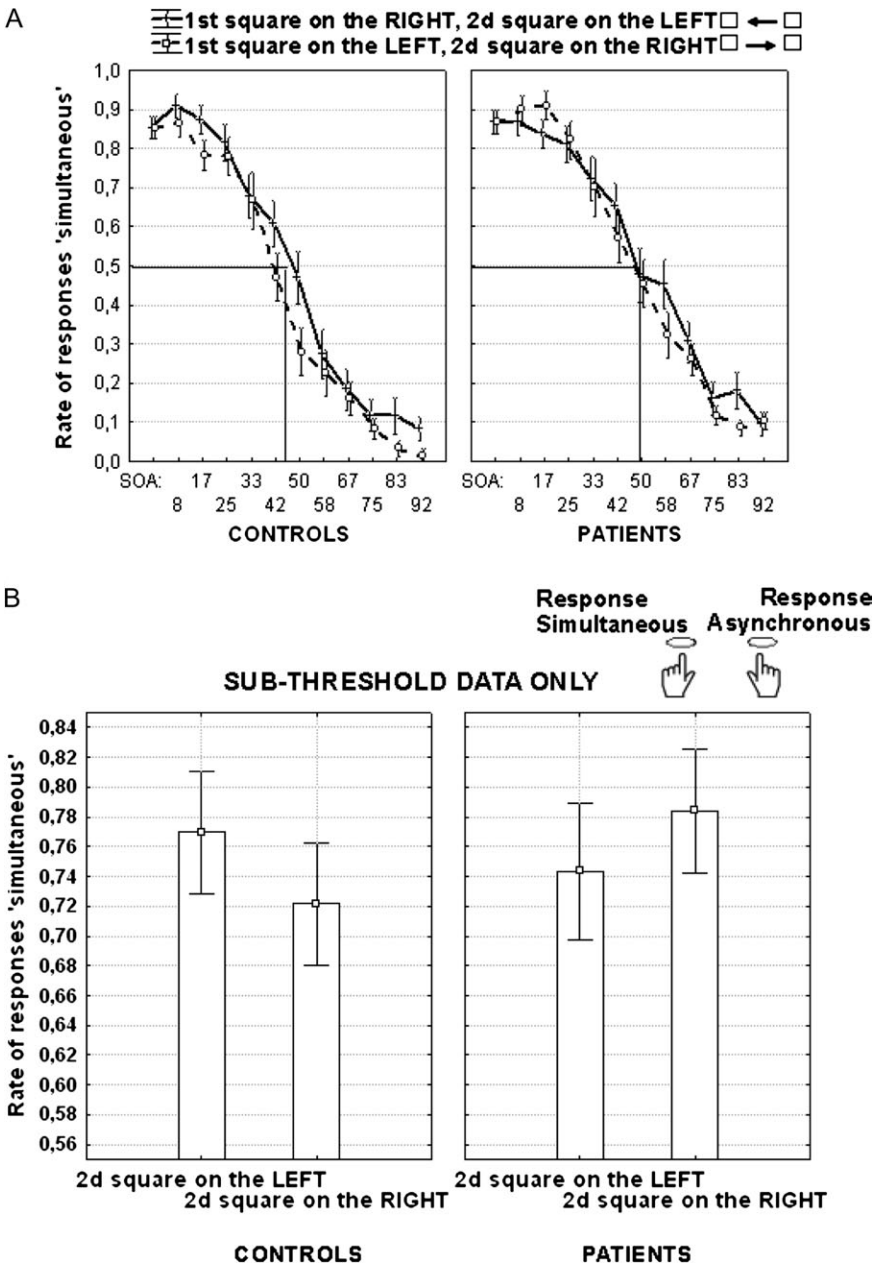

Fig. 3. (A) Rate of responses "simultaneous" as a function of SOAs when squares are presented in 2 different hemifields, either the first square on the right side and the second on the left (continuous line) or vice versa (dotted line) in controls (left panel) and patients (right panel). Thresholds for interhemifield presentation are indicated by the straight lines. The mean rate of responses "simultaneous" below threshold, calculated as a function of individual responses, is illustrated in (B) as a function of the side of the second square in patients (left panel) and controls (right panel).

and by 7.4 % in controls, $F[1, 17] = 12.9$, $P < .005$). In patients, the inverted profile below and above threshold produced a significant interaction between SOA (below vs suprathreshold) and the side of the second square, ($F[1, 17] = 15.2$, $P < .005$).

Correlations

There was no correlation between the medication dose in chlorpromazine equivalents and their intrahemifield threshold ($r = -.03$) or interhemifield threshold ($r = .04$). In patients but not in controls, there was a correlation between the mean threshold of asynchrony detection and the amplitude of the Simon effects observed both below the threshold ($r = -.56$, $N = 18$, $P < .05$) and above threshold ($r = .57$, $N = 18$, $P < .05$). The larger the bias on the side of the first square below threshold, the lower the threshold in patients. In contrast, the larger the bias on the side of the second square above threshold, the higher the threshold.

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Discussion

Our results replicate previous results showing that the threshold of asynchrony detection is higher in patients than in controls.[12,16] Despite the simplicity of the test, there was still a threshold difference between patients and controls. The extent of the threshold difference may seem small (around 10 ms) but represents an increase of about 20% compared with the threshold of controls. Given the temporal precision of most cognitive functions (language and motor control), this difference may constitute an important drawback for patients, especially as it appears to increase in the case of more complex tasks[12] and because the results show impairments even when stimuli are processed in the same hemisphere, thus generalizing previous results. Coupled with results showing similar disturbances in the auditory modality[16] and reduced sensitivity to onset asynchrony,[22] the results add to the literature showing impairments related to time in patients. They suggest deficits at an additional and more elementary level than duration perception, which also involves memory[5–8] or critical flicker fusion and masking, which involves spatial fusion.[10,11]

Our main question was whether the extended window of synchrony perception observed in patients implies, or not, a fusion of the events occurring within the same temporal window. The results not only suggest that this is not the case but also reveal a qualitative difference in the way patients and controls detect asynchronies. The Simon effect analysis indeed suggests that in the present paradigm implicit processing differs qualitatively in patients and controls. The Simon effect produces a tendency to hit response keys on the same side as the stimulus. In case of 2 stimuli displayed in different hemifields, the Simon effect shows whether responses are biased to the side of the first or second square. The results show patients and controls are biased by the second square when the SOA is large enough. For short SOAs, however, and contrary to controls, patients were biased to the side of the first square. This suggests that the first square is detected at least implicitly, even though its duration is very short. This is consistent with past studies that showed patients' sensitivity to short-duration stimuli.[23] That this response profile does not persist above threshold shows patients are performing the task correctly and do not mistakenly give a response after the first square. If that had happened, the influence of the first square should have increased when it stayed by itself on the screen for longer, ie, for longer SOAs. Such was not the case. In addition, the RTs increase around the threshold (see online supplementary material) shows that patients do not answer in an impulsive way after the first stimulus. It seems thus that both patients and controls follow instructions and are influenced by the last event for large SOAs.

Despite this, the mechanisms at play clearly differ between groups below threshold. The results in patients are consistent with a simple feed-forward processing of information. The first displayed square is necessarily processed in a feed-forward way. The display of this square represents the only event occurring on the screen, at least for a short period. The fact that it is displayed alone on the screen should elicit an automatic "visuomotor" Simon effect.[19] This should occur even if the first stimulus is not consciously perceived as isolated. This effect fades slowly with time,[19,24] and the Simon effect elicited by the first stimulus thus disappears as the SOA between the 2 consecutive squares increases. When the SOA is large enough and when the second square is clearly dissociated from the first one, its appearance can then elicit a Simon effect on its side. All effects observed in patients might thus occur in a feed-forward way and independently from a perception of succession. This hypothesis is further supported by the correlation between the threshold of simultaneity/asynchrony discrimination and the amplitude of the Simon effect on the side of the first square at short SOAs. This correlation suggests that patients' judgment of asynchrony at threshold is facilitated by the perception of an isolated square. What seems to lack in patients is thus the comparison between the onsets of the stimuli. It is as if they would rather process 2 isolated stimuli, thus making it difficult to compare their onsets. Controls, on the contrary, appear to be sensitive to the succession of the 2 squares even below threshold. This is consistent with the fact that thresholds derived from temporal order judgments are usually lower than those derived from simultaneity/asynchrony judgments.[25] It is likely that this relies on top-down processes, at least in our paradigm. The results in patients certainly suggest that the perception of succession is not automatic. As a matter of fact, the perception of succession depends on the active comparison of the squares' onsets because it cannot rely on a passive perception of motion (the perception of illusory motion was indeed prevented by the fact that stimuli stayed on the screen once displayed). This means that due to the task controls direct their attention toward the expected second stimulus. This might explain the lack of Simon effect on the side of the first stimulus, and the fact that this effect occurs rather on the side of the second stimulus.

The theory of predictive coding is especially suited to explain the effects observed here. According to predictive coding, the representation activated in visual perception by sensory information would be permanently compared with following upcoming information in order to correct interpretation.[26,27] If this is true, it means that processing is dynamic and inherently codes time succession. It might thus provide the basis for an implicit coding of time processing. According to the present results, however, an additional step would be required to compare the onsets of 2 separate stimuli.[28] This might involve the configuration of early predictive mechanisms to attend not only to 1 but also to 2 successive events that are separated in space.

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As many models, predictive coding can be adapted to include the possibility of early predictive coding to be guided by higher level expectations.[29] This means that following the instructions explicitly would lead to the configuration of early predictive mechanisms at an implicit level. In the framework of this model, patients might be impaired due to 2 possible impairments. They may have a difficulty to configure early predictive mechanisms according to the instructions. Else the early predictive mechanisms might themselves be impaired. Either possibility is consistent with the literature, inasmuch as both types of mechanisms are supposed to rely on feedback connections from high-level structures to earlier levels, which have been suggested to be impaired in schizophrenia both at a neurobiological level[30–32] and at a functional level.[33–35] An impairment of predictive mechanisms would be consistent with difficulties observed in the motor domain[36–38] and with a proposal from Gallagher and Varela.[39] A difficulty to configure task sets might also be related to decision-making processes that are known to be impaired in patients.[40–42]

One of the limitations of our study is that most patients were treated with antipsychotic medication. An effect of treatment cannot be ruled out, although no positive correlation was found between the increase in patients' threshold and treatment in chlorpromazine equivalent. A recent study has also shown that even first-episode patients having been treated for less than 6 weeks are impaired at detecting asynchronies between 2 rectangles onsets.[43] In any case, the present study confirms the existence of enlarged temporal windows at least in treated patients. This impairment at an explicit level is not associated with a fusion of events but rather a paradoxical fragmentation of information displayed within the temporal window, as suggested by implicit motor responses. We propose that patients are unable to anticipate immediate upcoming events, consistent with earlier clinical descriptions[1,44] and recent proposals.[39] Within the framework of Husserl's work, this would mean a deficit in the integration of future moments in the subjective present, ie, a deficit in protention. Inasmuch this integration underlies the sense of continuity, such an impairment might be involved in the patients' disturbance of the sense of continuity. Even though an elementary disturbance, it might rely on impaired anteroposterior connectivity and might impact on a wide range of everyday life activities, such as language, reasoning, or motor control, which require anticipating events on a very short timescale.

Funding

French National Institute for Health and Medical Research (INSERM); Centre Hospitalier Régional Universitaire of Strasbourg (Inner project grant from the University Hospital of Strasbourg, API- HUS n°3494); the Medicine Faculty of Strasbourg.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

Acknowledgments

The Authors have declared that there are no conflicts of interest in relation to the subject of this study.

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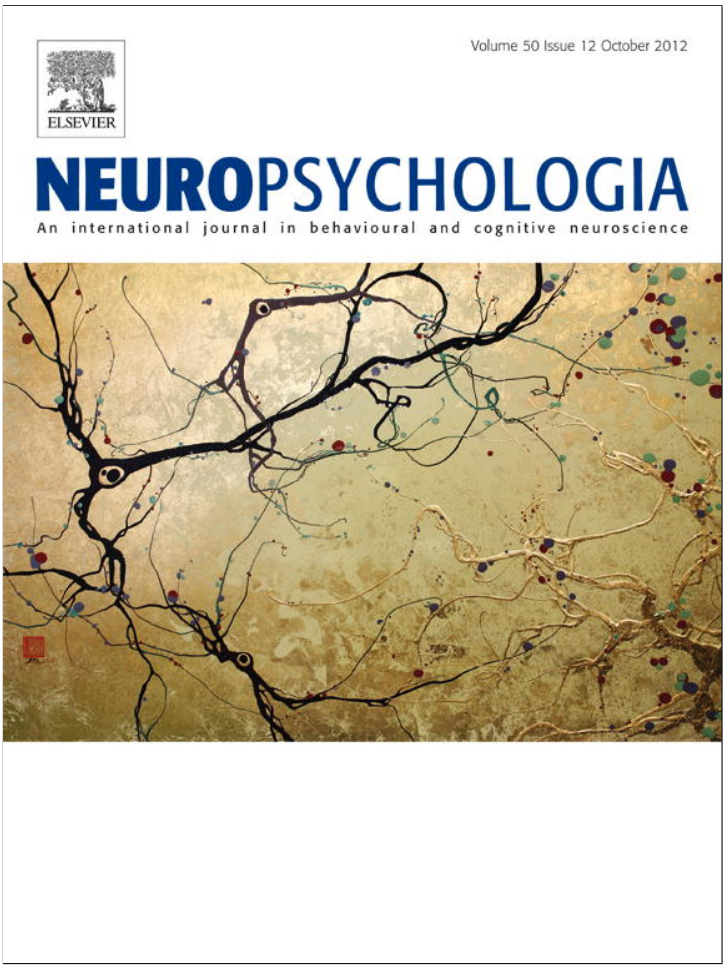

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Neuropsychologia 50 (2012) 2736–2744

Contents lists available at SciVerse ScienceDirect

Neuropsychologia

journal homepage: www.elsevier.com/locate/neuropsychologia

Looking forward: An impaired ability in patients with schizophrenia?

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ARTICLE INFO

Article history:
Received 28 December 2011
Received in revised form
1 July 2012
Accepted 15 July 2012
Available online 25 July 2012

Keywords:
Synchrony
Simon effect
Time
Schizophrenia
Anticipation
Implicit processing

ABSTRACT

When two visual stimuli occur within 8 to 17 ms of one another, subjects cannot tell they are asynchronous, yet recent results show they are not processed as simultaneous. Two spatially separate squares were presented at an interval ranging from 0 to 92 ms and remained on the screen until subjects responded. Subjects pressed a right or left response key according to the judged simultaneity/asynchrony of the stimuli. We evaluated the Simon effect, i.e., the tendency to press the key on the same side as the stimulus. We found an effect even when the squares were displayed on opposite sides of the screen, with their onsets separated by less than 20 ms. Controls were biased towards the last stimulus, whereas patients with schizophrenia were biased towards the first. We investigate here whether the results are related to spatial or temporal processing. Using the same paradigm, we explored the impact of spatial grouping by comparing connected vs. unconnected stimuli and manipulating the predictability of the second stimulus location. We tested different groups of mildly symptomatic patients and matched controls in two studies.

Under 20 ms, when stimuli were connected and the 2nd square location was predictable, patients tended to press the key to the side of the 1st square, whereas controls displayed the opposite tendency. The results suggest that controls put more emphasis on the last occurring event, but not patients with schizophrenia. This impairment is observed when spatial difficulties are removed, suggesting it is related to time rather than space.

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1. Introduction

No task explores time selectively without being associated with the coding of other properties. For example, estimating the duration of a sound is associated with the coding of this sound. Similarly, judging whether two visual stimuli are simultaneous or asynchronous is associated with the coding of their visual properties. Results in the literature suggest there is usually a coherence between the organization of events in time and space. In fact, perceptual grouping in space promotes the perception of synchrony in time (Nicol & Shore, 2007). Since there is no clear evidence that the coding of time properties is independent of the coding of spatial properties, it could mean that time properties are derived only secondarily. Here, we explore this question by testing patients with schizophrenia, who are impaired at organizing information in both space and time. We examine whether impairments observed in previous studies are related to difficulties with spatial processing or time-event structure coding. Spatial grouping cues are used to manipulate the organization of simultaneous and asynchronous stimuli: stimuli were either grouped by means of a line segment connecting them, or were unconnected (Palmer & Rock, 1994). If the coding of the time-event structure is secondary to spatial processing, abnormalities in patients should be reduced when spatial difficulties are alleviated. The opposite pattern of results would suggest impairments related to time in patients and, more generally, the existence of mechanisms selectively related to time, independent of space. This study aims therefore both to resolve a fundamental issue, i.e., the relationship between spatial processing and time-event structure coding, and to further our understanding of the disturbances observed in schizophrenia.

1.1. Simultaneity/asynchrony discrimination in patients with schizophrenia

Several studies show that patients with schizophrenia experience difficulties with time perception at a subjective level (Davalos, Kisley, & Freedman, 2005; Elvevag, McCormack, Gilbert, & Brown, 2003; Volz et al., 2001). In particular, impairments in discriminating between simultaneous and asynchronous stimuli has been replicated several times (Foucher, Lacambre, Pham, Giersch, & Elliott, 2007; Giersch et al., 2009; Schmidt, McFarland, Ahmed, McDonald, & Elliott, 2011). Patients usually require larger asynchronies than controls to report an asynchrony. The impairment can be considered explicit

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http://dx.doi.org/10.1016/j.neuropsychologia.2012.07.023

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insofar as subjects are explicitly asked to decide whether or not stimuli are simultaneous. However, this means the impairment may be connected with the need for an explicit judgement rather than with time itself. Subjects may be unable to report an asynchrony but may still code it at an implicit level. An effect is considered implicit if it is not the result of a direct question put to the subject. For example, implicit processing is thought to play an important part in sensorimotor timing (Repp, 1999; Van Wassenhove, 2009) and is not necessarily equated with explicit judgments (Van Wassenhove, 2009). Impaired motor sequencing has been observed in patients with schizophrenia and is compatible with timing impairments at an implicit level (Delevoye-Turrell, Giersch, Wing, & Danion, 2007). Aside from motor control, the few studies that have explored implicit effects of time perception have also shown impairments in patients with schizophrenia (Haggard, Martin, Taylor-Clarke, Jeannerod, & Franck, 2003; Lalanne, van Assche, & Giersch, 2012a; Voss et al., 2010). Most importantly, visual perception studies have shown a dissociation between impairments at the explicit and implicit level (Del Cul, Dehaene, Reyes, Bravo, & Slachevsky, 2009; Herzog, Kopmann, & Brand, 2004), hence the need to explore both implicit and explicit time-event coding.

1.2. Exploring the implicit processing of asynchronies with the Simon effect

We used an original approach to explore the implicit processing of asynchronies (Lalanne et al., 2012a), with the help of the Simon effect, which refers to the finding that performance is faster and more accurate when the stimulus appears on the same side as the responding hand, even if stimulus location is irrelevant to the task (Hommel, 2011a; Simon, 1969). The Simon effect involves the coding of the spatial location of the stimulus and an impact on response selection. The mechanisms of this impact are discussed, and may involve a shift of attention. Alternatively, it would be based on all available information being weighted according to criteria that may vary from task to task (see reviews and discussions on the Simon effect in Hommel, 2011a, b; Van der Lubbe & Abrahamse, 2011). For the sake of simplicity we will refer to these mechanisms as a shift of emphasis, without presuming about the possible involvement of attention. In our task (Lalanne et al., 2012a), two stimuli (2 squares), were presented simultaneously or asynchronously. Subjects responded with their left hand when the stimuli were judged to be simultaneous and their right hand when they were judged to be asynchronous. In half of the trials, both stimuli were on the same side of the screen, either to the left or right, in which case subjects' responses were biased to the side of the stimuli, reflecting the classical Simon effect. In our study, this effect was not impaired in patients relative to controls (see Lalanne et al., 2012a for a discussion on this point), which meant we were able to use the Simon effect as a tool when the two stimuli were on opposite sides. The use of the Simon effect in this configuration is new, but our previous results (Lalanne et al., 2012a) suggest it usefully complements the collection of explicit judgments.

Of course, no Simon effect can be observed with two simultaneous right and left stimuli, since in such a case identical information is displayed on both sides of the screen. When the two stimuli are asynchronous, however, a Simon effect can be observed once more. We made a distinction between the Simon effect observed with short and long asynchronies, since different mechanisms may be at play. According to our results, all subjects tend to press the response key to the side of the 2nd square with large asynchronies (Lalanne et al., 2012a). At least a partial explanation for this might be the conscious perception of a temporal order of appearance, which might cause subjects to put more emphasis on the last occurring stimulus. Such a shift in emphasis makes all the more sense given the prior entry, which has been evidenced in many studies. It shows that when two stimuli are displayed in quick succession, subjects are better at discriminating the order or asynchrony of the two stimuli when the first one has been spatially cued (review in Spencer & Parise, 2010). Prior entry thus shows the importance of focusing on the location of the first occurring stimulus *before* the sequence is displayed. The Simon effect occurs at the response level, and complements prior entry data by showing what happens *after* stimuli have been displayed. A Simon effect to the side of the 2nd square suggests the emphasis placed on stimuli shifts from the first to the last occurring event between the start and finish of the events sequence.

The most important results, however, were observed with short asynchronies. With short SOAs, an explicit influence of the stimuli order is impossible, since the asynchrony is too short for subjects to report it. This does not rule out a Simon effect, however, since, since Treccani, Umiltà, and Tagliabue (2006) have described a Simon effect when the stimulus triggering the effect is not consciously perceived. In our own paradigm, when two stimuli are displayed with a short and unnoticed asynchrony, the Simon effect is necessarily triggered implicitly. We showed that in control subjects a Simon effect was observed to the side of the 2nd square even when subjects were not aware of an asynchrony, suggesting more emphasis on the second stimulus. Conversely, with short asynchronies patients were biased towards the side of the 1st square. The results with patients show that the first, short stimulus is sub-consciously detected, and that patients do not merge stimuli in time. Had squares been merged in time, they would have been processed as simultaneous. Nothing could have possibly triggered a Simon effect to the side of the first or second stimulus, since there would have been no first or second stimulus anymore: the first stimulus was equally often on the right and left side, and no right or left bias could explain the results. The existence of the Simon effect thus indicates that squares had been distinguished in time at short SOAs. The results further suggest that, on an implicit level, patients do not put emphasis on the second stimulus like controls. It is to be noted that all subjects responded after the 2nd square was displayed. No anticipated responses were observed. Regarding patients, the normalization of the bias with large SOAs rules out response impulsiveness as an explanation for the Simon effect to the side of the 1st stimulus.

1.3. Time or space?

All in all, the results showed that the Simon effect is useful for obtaining information about the implicit coding of the time-event structure. The results to date have shown significant differences between patients and controls with very short SOAs, suggesting a qualitative difference in the implicit coding of the time-event structure. However, it is unclear whether this effect is related to space or time. As suggested above, the Simon effect to the side of the second stimulus might reveal a shift of emphasis from the first to the second stimulus. The Simon effect to the side of the first stimulus might thus be due to a lack of emphasis shift in patients. The difficulty here is that the shift occurs in space and not only in time. Spatial organization, which is known to be disrupted in schizophrenia, may be at the root of the difficulties patients experience with short asynchronies. It has frequently been reported that patients' perception is fragmented in space (Silverstein & Keane, 2011; Uhlhaas, Phillips, Mitchell, & Silverstein, 2006; Van Assche & Giersch, 2011). If they spatially segregate stimuli, it might explain why they isolate the 1st square from the second and have difficulty shifting the emphasis to the last stimulus. They would have an initial difficulty to shift in

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space rather than in time. Here, we attempt to tease apart spatial and temporal effects.

1.4. Paradigm and predictions

To explore the relationship between spatial and time processing, we conducted two experiments. First, we presented 2 squares among four possible locations as in the previous study, and subjects had to perform the same simultaneity/asynchrony discrimination task. This time, however, we manipulated spatial organization by adding connectors, and the target squares were either connected or not. Targets were separated both in space and time when they were unconnected, and presented asynchronously. By contrast in the case of connected squares, connectors reduced their separation in space by promoting their grouping. If anything, the grouping by connectors should promote the perception of synchrony, by yielding the perception of one group of stimuli rather than two separate items (Nicol & Shore, 2007). The influence of connectors should be evidenced by an overall reduction of the Simon effects, whatever the SOA. We also considered an additional spatial difficulty in the case of four possible locations for the stimuli. In this configuration the location of the 2nd square is uncertain. For example, if the 1st square appears in the upper right location, the second can appear either in the lower right or the upper left location. Consequently, patients may have experienced a difficulty linked to this spatial uncertainty. The second experiment was conducted to eliminate any difficulty relating to space, with only two possible locations for the stimuli, i.e., to the right and left of the centre of the screen. In this case the location of the 2nd square was always predictable.

We made a number of predictions. The critical one concerned the Simon effect to the side of the first stimulus at short asynchronies, as previously observed in patients. If spatial impairments are the cause of this alteration, connectors and the use of 2 squares should reduce the Simon effect to the side of the 1st square in patients. On the other hand, if this abnormality is independent of spatial impairments, it might be observed even when targets are connected and when there is no uncertainty about the spatial location of the second stimulus. Such a result would suggest the existence of mechanisms dedicated to coding the time-event structure. We also checked the overall Simon effect occurring with all SOAs (including large SOAs), as a control for the efficiency of our spatial time-event coding manipulations. As emphasized above, connectors were expected to reduce the Simon effect overall, including therefore the Simon effect to the side of the 2nd square. In addition, spatial predictability was expected to result in increased expectancy regarding the 2nd square, producing a greater Simon effect to the side of the 2nd square, at least in the absence of connectors.

2. Method

2.1. Subjects

There were two experiments, and we detail demographic characteristics in Table 1. In Experiment 1, 24 patients with schizophrenia and 22 controls took part but five patients and 3 controls were excluded because they were unable to do the task, as confirmed by the rate of synchronous responses which was constant across all SOAs. This did not occur in Experiment 2.

All patients were diagnosed by two senior psychiatrists and using the mini international neuropsychiatric interview, according to the criteria laid down in the diagnostic and statistical manual of mental disorders (4th ed.; American Psychiatric Association, 1994). Symptoms were assessed with the help of the positive and negative syndrome scale (PANSS, Kay, Opler, & Fiszbein, 1987).

Exclusion criteria for patients and controls were: a history of alcohol or drug dependency, neurological or medical pathology, disabling sensory disorder and general anaesthesia within the past 3 months prior to testing. An additional exclusion criterion for controls was psychotropic medication within the 3 weeks prior to testing. All subjects had normal or corrected-to-normal visual acuity.

The project was approved by the local ethics committee. All subjects gave their informed written consent prior to testing, in accordance with the recommendations laid down in the Helsinki Declaration.

Table 1
Demographic and clinical data of the participants.

	Patients	Controls
Experiment 1		
Gender (M/F)	12/7	12/7
Age (mean ± SD)	37 ± 6.5	38 ± 7
Years of education (mean ± SD)	12 ± 1.6	12 ± 2
Medication (typical/atypical/no medication)	6/12/1	–
Dose of chlorpromazine equivalents	259 mg/day	–
PANSS positive symptoms (mean ± SD)	16 ± 2.8	–
PANSS negative symptoms (mean ± SD)	20 ± 8	–
PANSS general symptoms (mean ± SD)	37 ± 12	–
PANSS total (mean ± SD)	73 ± 20	–
Experiment 2		
Gender (M/F)	11/7	11/7
Age (mean ± SD)	38 ± 6	38 ± 7.5
Years of education (mean ± SD)	12.5 ± 2	12 ± 2
Medication (typical/atypical/no medication)	5/12/1	–
Dose of chlorpromazine equivalents	240 mg/day	–
PANSS positive symptoms (mean ± SD)	16 ± 2	–
PANSS negative symptoms (mean ± SD)	23 ± 7	–
PANSS general symptoms (mean ± SD)	39 ± 13	–
PANSS total (mean ± SD)	78 ± 19	–

2.2. Apparatus

The experiments were run on a Pentium 4 PC equipped with a Cambridge Research System stimuli generator (Rochester, Kent, UK), and programmed with Matlab 7.0.1 (Mathworks, 1984–2004). Stimuli were displayed on an EIZO monitor (21 in., 120 Hz refresh rate). Ocular coordinates were monitored using a CRS video eye tracker (50 Hz), mounted on a rigid headrest and held at a constant distance of 114 cm from the monitor. The eye tracker output was recorded via the analog-to-digital converter input of the visual stimulus generator, which also controlled the visual display. Participants answered by hitting one of two response keys connected to the computer.

2.3. Stimuli

There were two different experiments (Fig. 1).

In Experiment 1, stimuli were 2 squares ($0.8^\circ \times 0.8^\circ$) which were displayed in two among four possible locations, at the bottom, top, left or right side of the screen centre. In Experiment 2, stimuli were 2 squares displayed on opposite sides of the screen centre (right and left).

In each experiment, squares were connected or not. Two connectors ($4.5^\circ \times 0.03^\circ$) were displayed horizontally or vertically in the first experiment, and related squares by pairs. In the second experiment, 2 squares were connected, or not, by a connector displayed horizontally (Fig. 1). Stimuli and connectors were drawn with a line width of 0.06°.

Luminance of squares increased according to a sigmoid function in 10 steps of luminance levels from 0.02 (background luminance) to 12 cd/m^2, over a presentation interval of 75 ms (8.3 ms per step). The luminance increased gradually to avoid magno-cellular pathway activation, as in our previous experiments (Giersch et al., 2009; Lalanne et al., 2012a).

In both experiments, the whole display was masked after the subjects' response by means of a grey square of 6.9° displayed in the centre of the screen. It was used to avoid persistence effects. The luminance of this mask was 0.1 cd/m^2.

2.4. Procedure

2.4.1. Procedure in Experiment 1

At the start of each trial the central fixation point was displayed along with four empty squares ($0.8^\circ \times 0.8^\circ$) in each corner of a virtual square ($5.5^\circ \times 5.5^\circ$) in the middle of the screen. Empty squares were linked by two connectors, both presented either horizontally or vertically.

Subjects first had to fixate the central fixation point, as checked by continuous eye tracking. The trial did not start until after a continuous 500 ms fixation period, following which two adjacent squares were shaded gray, either simultaneously (SOA = 0 ms) or asynchronously. Shaded squares at the top or bottom of the virtual

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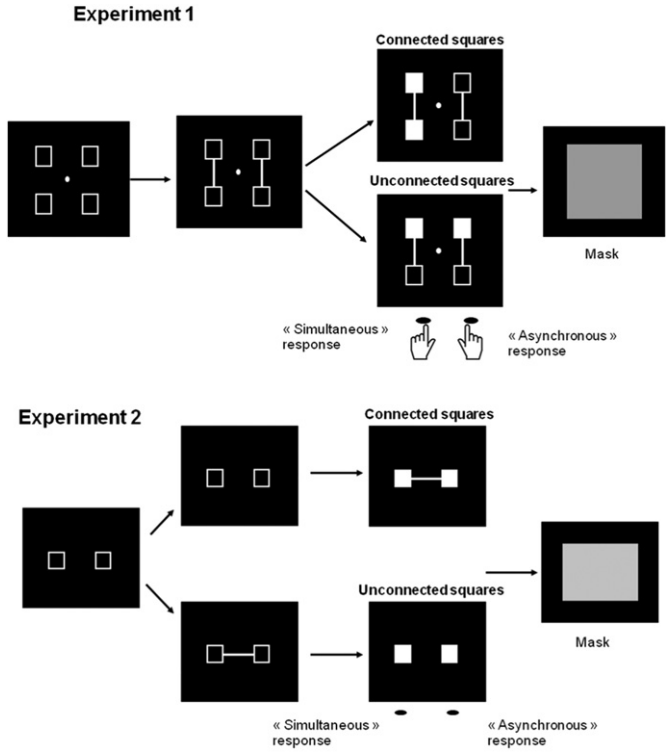

Fig. 1. Illustration of the procedure: four connected or unconnected squares are presented in Experiment 1 (upper panel) and only two in Experiment 2 (lower panel). In both experiments, 2 squares are shaded in, in gray, either simultaneously or asynchronously. These 2 squares are either connected or not. Subjects are instructed to hit the right key when they think the squares are shaded in asynchronously and the left key when they think the shading occurs simultaneously. Once subjects have responded, a mask is presented.

square were across hemifields (*interhemifield* condition), whereas shaded squares to the left or right of the virtual square were in the same hemifield (*intrahemifield* condition). In each condition, shaded squares were either connected or unconnected, depending on the horizontal or vertical orientation of the connectors. Twelve levels of stimuli onset asynchrony (SOA) were used (from 0 to 92 ms in 8.3 ms steps). Filled squares remained on the screen until a response had been given. Subjects were instructed to respond by hitting a left response key in the case of squares judged to be shaded in at the same time (synchronously) and a right response key in the case of squares judged to be shaded in at different times (asynchronously).

Each target location (upper, lower, right or left) was equally represented. Each combination of target square location (same or different hemifields), connector condition (connected or unconnected) and SOA (12 levels: 0, 8, 17, 25, 33, 41, 50, 58, 67, 75, 83, and 92 ms) was tested 16 times in random order, yielding a total of 768 trials.

2.4.2. Procedure in Experiment 2

The procedure was identical to that of Experiment 1, except that only two empty squares were first displayed and then filled. To limit the duration of the experiment, we used only six SOA levels from 0 to 83 ms. In the case of asynchronous shading, each combination of connector condition (connected or unconnected), order of squares (first on the right side and second on the left and vice versa) and SOA (17, 33, 50, 67, and 83 ms) was tested 10 times. In the case of simultaneity, each connector condition was tested 20 times. There were thus 240 trials.

2.5. Statistical analyses

For each task, we conducted an analysis of variance (ANOVA) on the rate of simultaneous responses with group as a between-group variable and with experimental conditions as within-group variables. We further report the partial eta2 (effect size). We decomposed interactions by ANOVAs on a restricted number of conditions—for example, on each SOA.

Two patients and two controls were left-handers. Excluding them did not change the results, and the present results are pooled across right- and left-handers. It should be noted that the Simon effect refers to the tendency to press to the side of the 1st or the 2nd square, and whatever the direction of this tendency, this reflects key presses to the right side in half trials and to the left side in half trials. It is unlikely that lateralization plays a role.

3. Results

We started by calculating the false alarm rate, i.e., the rate of 'asynchronous' responses when squares appeared simultaneously. The false alarm rate with the 4-squares presentation was 21.2% in patients and 11.5% in controls. This difference only tended towards significance ($F[1, 36]=3.7$, $p=0.062$, partial $\eta^2=0.09$) and was due to a very high false alarm rate from a few patients. The following results were the same with or without these patients, and results are reported for the entire patient sample.

With the 2-squares presentation, the false alarm rate was approximately 27.6% in patients and 28% in controls ($F[1, 34]<1$, partial $\eta^2<0.001$). Although high, false alarm rates with the 4-squares presentation were clearly lower than in our previous studies (Giersch et al., 2009) and in Experiment 2 when only 2 squares were presented. The reason for this may be that the difficulty of Experiment 1 encouraged subjects to give fewer 'asynchronous' responses. To control for this confounding factor, we corrected data by dividing synchronous response rates for each SOA by the highest rate of synchronous responses in each subject (see Giersch et al., 2009 for details). The following analyses were all performed on corrected data, and focused on the Simon effect.

3.1. Results with 4 squares (Experiment 1)

The Simon effect reflects the tendency to respond with the hand on the same side as the stimulus. We first checked the classic Simon effect when squares are presented in the same hemifield.

3.1.1. Intrahemifield presentation

When squares were presented on the right side, subjects pressed the right key more often and hence gave 4.5% more asynchronous responses than when the squares were presented on the left side (averaged over all SOAs and groups, $F[1, 36]=20.9$, $p<.001$, partial $\eta^2=0.37$). There was no interaction with the group ($F[1, 36]=1.9$, ns, partial $\eta^2=0.05$). This suggests the classic Simon effect is similar in both groups, as in our previous study, and cannot account for abnormalities with less conventional measurements (Lalanne et al., 2012a).

3.1.2. Interhemifield presentation

We analysed the Simon effect when target squares were displayed in different hemifields (1st square on the left, 2nd on the right, or vice versa). Results are illustrated in Fig. 2. Of course, there can be no Simon effect in the case of simultaneity. With asynchrony, a Simon effect related to the 1st square should increase the 'synchronous' response rate when the 1st square is on the left and the 'asynchronous' response rate when the first stimulus is on the right. This pattern is reversed in the case of a Simon effect related to the 2nd square. We thus evaluated the Simon effect by examining the rate of 'simultaneous' responses as a function of the order of the squares. Crucial effects are those concerning short SOAs that produce the same rate of 'simultaneous' responses as perfect synchrony, because these Simon effects indicate how the asynchronies are processed at an implicit level. By contrast, it can be expected that the Simon effect with longer SOAs is influenced by the conscious perception of asynchrony. It is important to evaluate Simon effects with larger asynchronies to check for the possibility of impulsive responses by patients and to control for the impact of connectors.

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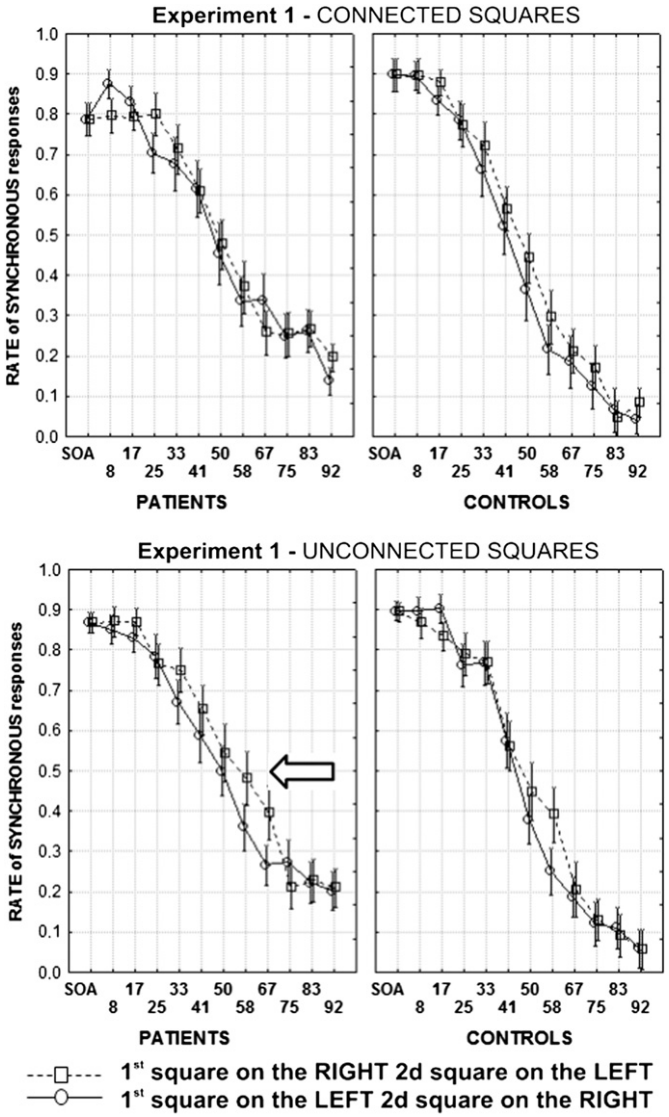

Fig. 2. Rate of synchronous responses in Experiment 1 (four possible locations for the squares) in the case of two connected (upper panel) or unconnected shaded squares (lower panel) as a function of the SOA (0 to 92 ms) in patients (left side) and controls (right side). The dotted line represents the rate of synchronous responses when the 1st square is on the right and the 2nd square on the left, whereas the continuous line shows the rate of synchronous responses when the order is reversed. The arrows denote significant Simon effects. There is also a small bias to the side of the 1st square with short SOAs in patients when squares are connected. This bias is similar to that observed in Experiment 2.

We conducted an ANOVA on the rate of synchronous responses with the connector condition (connected vs. unconnected), order of squares (1st square on left, 2nd on right, or vice *versa*) and SOAs as within-group variables and the group as between-group variable. An interaction was found between the order of the squares, connector condition and group ($F[1, 36]=4{,}3$, $p<.05$, partial $\eta^2=0.1$). We used sub-analyses to break down this interaction and looked for a Simon effect in each group (patients vs. controls) and for each condition (connected vs. unconnected). It is important to note that the Simon effect was measured across all SOAs. Included therefore was the Simon effect deriving from an explicit perception of asynchrony. There was a Simon effect, i.e., an effect of the order of squares, in patients only, and only when the squares were unconnected ($F[1, 18]=4.9$, $<.05$, partial $\eta^2=0.2$), in which case their responses were biased to the side of the 2nd square (by 4%). There was no significant effect in controls. The effect in patients is observed in the unconnected condition, but the stimuli differ from those used in previous experiments. This result suggests patients and controls reacted differently to the addition of connectors in the experiment, which may be in keeping with the visual organization impairments observed in patients (Van Assche & Giersch, 2011).

The graph also suggests a Simon effect to the side of the first stimulus in patients in the case of connected squares for the SOAs of 8 and 17 ms (Fig. 2). This was confirmed in a sub-analysis based on those two SOAs alone (the extent of the bias is 6%, $F[1, 18]=4.8$, $p<.05$, partial $\eta^2=0.21$). The effect was present in almost all patients, with a reversal in only 4 patients (out of 19). Controls displayed a non-significant bias to the side of the second stimulus for the same SOAs (2.4%, $F<1$, partial $\eta^2=0.03$), which differed significantly from the one observed in patients ($F[1, 36]=4.3$, $p<05$, partial $\eta^2=0.1$). These effects can be regarded as implicit since the rate of 'simultaneous' responses at 8 and 17 ms was the same as the rate observed at perfect synchrony ($F[2, 72]=1.2$, ns, partial $\eta^2=0.033$). However, this effect yielded no significant interaction with SOAs in the overall analysis.

3.2. Results in the case of 2 squares (Experiment 2)

In Experiment 2 we analysed the Simon effect in the same way as in Experiment 1, except that there were only 6 SOAs, not 12. The overall analysis showed an interaction between connector condition, order of squares, group and SOA ($F[5, 170]=3.1$, $p<.05$, partial $\eta^2=0.08$), and a tendency towards a significant interaction between condition, order of squares and group ($F[1, 34]=3$, $p=.09$, partial $\eta^2=0.08$). We looked at these results separately in controls and in patients, as illustrated in Fig. 3. As with Experiment 1, we looked for the Simon effect, i.e., the effect of the order of squares, in each condition (connected vs. unconnected) and each group. Again, the crucial effect is the Simon effect observed with short SOAs. As in Experiment 1, the rate of 'simultaneous' responses at SOA 17 ms (the SOA of 8 ms was not tested in Experiment 2) did not differ significantly from the rate observed at perfect synchrony ($F[1, 34]<1$, partial $\eta^2=0.01$).

First, we analysed the results obtained with controls. They did not vary with SOAs. There was an interaction between the order of squares and the connector condition ($F[1, 17]=6.1$, $p<.05$, partial $\eta^2=0.26$). An effect of the order of squares was found only in the unconnected condition, with a bias to the side of the 2nd square (amplitude of 7.2%, $F[1, 17]=10$, $p<.01$, partial $\eta^2=0.37$). This means there was a Simon effect to the side of the 2nd square when all SOAs were pooled, and when connectors were absent.

Second, we looked at the patients' results. There was an interaction between connector condition, order of squares and SOAs ($F[5, 85]=3.5$, $p<.01$, partial $\eta^2=0.17$). There was an interaction between the order of squares and SOAs only in the connected condition ($F[5, 85]=3$, $p<.05$, partial $\eta^2=0.15$), with a bias of 15.3% to the side of the 1st square exclusively for the SOA of 17 ms ($F[1, 17]=10$, $p<.01$, partial $\eta^2=0.37$). Only 3 out of 18 patients displayed a reverse Simon effect (towards the second stimulus).

3.3. Comparison of the two experiments

Since the two experiments were run in distinct blocks and with different groups of subjects, "Experiment" was taken as a between-group variable, and analyses of variance were conducted in the same way as above, with only 6 SOAs (0, 17, 33, 50, 67, and 83 ms). We started by comparing the Simon effect observed to the side of the 1st square for the 17 ms SOA in the connected condition. An interaction was found between the group and the order of squares ($F[1, 70]=10.2$, $p<.005$, partial $\eta^2=0.13$). Based on the average over the two experiments, patients showed a

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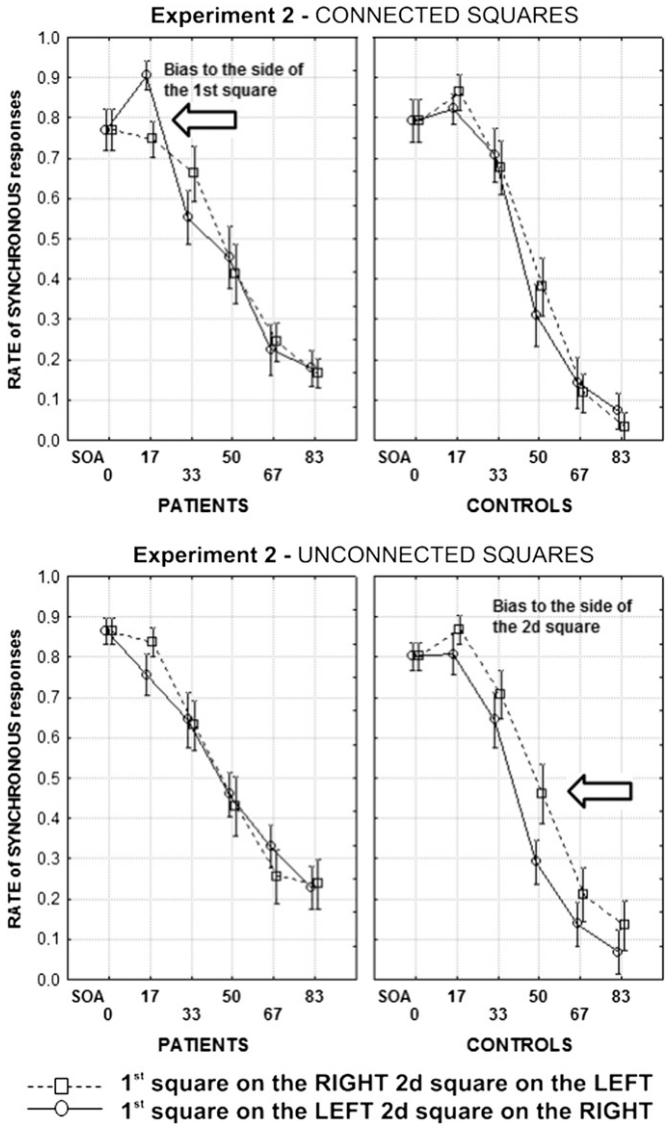

Fig. 3. Rate of synchronous responses in Experiment 2 (two possible locations for the squares) in the case of two connected (upper panel) or unconnected squares (lower panel) as a function of the SOA (0 to 83 ms) in patients (left side) and controls (right side). The dotted line represents the rate of synchronous responses when the 1st square is on the right and the 2nd square on the left, whereas the continuous line represents the rate of synchronous responses when the order is reversed. Arrows denote significant Simon effects.

global bias of 9.9% to the side of the 1st square ($F[1, 35]=11.3$, $p<.005$, partial $\eta^2=0.24$) compared with a bias of 4.3% to the side of the 2nd square ($F[1, 35]=1.7$, ns, partial $\eta^2=0.04$) for controls. There was no effect of Experiments (2 vs. 4 squares).

We then compared biases to the side of the 2nd square in the unconnected condition (this time with all SOAs). The analysis revealed an interaction between the order of squares, group and experiments ($F[1, 70]=11{,}7$, $p<.005$, partial $\eta^2=0.14$). The profile of patients and controls was inversed. Patients were biased (4% bias) to the side of the 2nd square in Experiment 1 (4 squares) but showed no bias in Experiment 2 (2 squares). This produced a significant interaction between the order of squares and experiments ($F[1, 35]=6.1$, $p<.05$, partial $\eta^2=0.15$). Controls showed the opposite results. They were biased (7% bias) to the side of the 2nd square in Experiment 2 (2 squares) but showed no bias in Experiment 1 (4 squares). This effect in controls also resulted in a significant interaction between the order of squares and experiments ($F[1, 35]=5.9$, $p<.05$, partial $\eta^2=0.14$).

3.4. Correlations

The patients were treated and displayed clinical symptoms as evaluated with the PANSS. We looked for correlations between Simon effects and treatment (in equivalent chlorpromazine), or PANSS scores, but found none.

4. Discussion

In our study, we explored the implicit coding of time properties by using 2 squares appearing simultaneously or asynchronously, and by analysing the Simon effect, i.e., the automatic tendency to hit the key located on the same side as the stimulus displayed on the computer screen. We were interested in the Simon effect in the case of asynchronous presentation across hemifields, because it enables us to check whether subjects' responses are biased to the side of the 1st or 2nd square. Of particular interest is the Simon effect occurring with short asynchronies. With these, subjects give the same rate of 'simultaneous' responses as with synchronous stimuli, showing they do not perceive the asynchrony explicitly. If the asynchrony is not processed at all, the squares presented on both sides would be strictly identical, and no Simon effect should be observed. Conversely, the existence of a Simon effect indicates an implicit processing of the asynchrony. The type of Simon effect, i.e., a bias for the 1st or 2nd square, also allows us to ascertain how asynchrony is implicitly processed.

In both experiments and when targets were connected, patients showed a bias to the side of the 1st square, whereas controls showed no bias, in keeping with previous results (Lalanne et al., 2012a). This effect has been reproduced three times in patients, but has never been observed in controls. This confirms patients process short asynchronies and distinguish stimuli in time at a subconscious level. It is striking that the present result was observed when the stimuli were connected, and when there was no uncertainty about the location of the 2nd square. In other words, the bias was observed when all spatial ambiguities were removed. The presence of connectors did not reduce the bias towards the side of the 1st square in patients. On the contrary, it was greater in the case of connectors. This shows that the abnormalities observed in patients are independent of spatial difficulties, and suggests an impairment concerning time selectively. A Simon effect relating to the 1st square suggests subjects give their response as if the 1st square is isolated and without taking account of the 2nd square, i.e., without shifting emphasis onto the last occurring event. Alternative explanations should be considered first, though.

4.1. Mechanisms underlying the Simon effect with short asynchronies

A Simon effect at delays under 20 ms is surprising, and its mechanisms warrant discussion. There are a number of different explanations. One is that the motion perception system codes a signal akin to an apparent motion between the two stimuli, leading to a Simon effect to the side of the 2nd square. In patients with schizophrenia a disturbance at the level of motion perception has been described (review in Chen, 2011) and could give rise to the Simon effect to the side of the 1st square. It is unlikely, however, that apparent motion is involved in the case of short asynchronies. The distance between the two stimuli and the short amount of time that elapses between their display at short asynchronies make the involvement of apparent motion unlikely. Several authors have systematically manipulated the spacing and delay between stimuli and checked how this affected apparent

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motion (Baker & Braddick, 1985; Frederiksen, Verstraten, & Van de Grind, 1993). They used random patterns of dots moving in a coherent way, either right-left, or up-down, and subjects were instructed to discriminate the direction of the motion. Those studies show that, across different exposure durations, inter-stimulus intervals and eccentricity, the optimal displacement allowing apparent motion to be perceived does not exceed 1°, way below the distance we used in our experiment. Strybel, Manligas, Chan, and Perrott (1990), with a setting closer to our own, i.e., two lights displayed at various distances and with varying SOAs, also observed a reduction in perceived apparent motion with separations greater than 2°. In addition, they show that larger separations require larger SOAs for apparent motion to emerge (see Gepshtein and Kubovy (2007), for a more exhaustive discussion on the relationship between space and time in apparent motion). Hence apparent motion is an unlikely explanation for our results.

1. Another proposal might have been that the results are related to conflict monitoring. The Simon effect produces a conflict between the key-press elicited by the location of a stimulus and the key-press elicited by the response to the task. Inasmuch as conflict monitoring is believed to be impaired in patients with schizophrenia (Van Veen & Carter, 2002; Yücel et al., 2002), the question might have been whether such an impairment affected the results, especially at short asynchronies. Such an explanation is unlikely to explain all the data, however, even if an involvement of conflict monitoring cannot be formally excluded. First, it should be noted that the conflict is not self-evident when a stimulus is displayed on each side of the screen. If any, the conflict should be stronger when all stimuli are displayed on one side, thus being closer to the classic Simon effect's configuration. The results showed no evidence of a stronger Simon effect in patients, even in this configuration, neither at short nor at long asynchronies. Second, previous work has suggested that conflict monitoring is normal in patients with schizophrenia when conflicting information is processed at an implicit level (Dehaene et al., 2003), which is the case here when asynchronies are below 20 ms. Most importantly however, conflict monitoring impairments in patients are believed to result from an inability to suppress non pertinent information (review in Melcher, Falkai, and Gruber (2008)). This should result in heightened conflict effects. In other words, the Simon effect's amplitude should increase in patients with schizophrenia relative to controls. What was observed, however, was a qualitative alteration of the Simon effect, and not a quantitative modification. A conflict monitoring effect would not explain why patients are biased to the side of the first rather than the second stimulus in the sequence.
2. A better explanation for the Simon effect to the side of the 1st square is thus a deficit of the mechanisms usually identified as explaining this effect, namely an attention shift or an increase in the weight assigned to the second stimulus (Hommel, 2011a, b). Based on our results it is possible to refine our interpretation by suggesting the effect observed in patients is independent of spatial difficulties. This argument is backed up by the results showing the expected effects of the spatial manipulations in controls. At least with high asynchronies, both patients and controls showed a clear bias to the side of the 2nd square, either in Experiment 1 or 2 and when squares were unconnected. The loss of this effect in the case of connectors is consistent with the literature suggesting that connectors promote the perception of synchrony and make it more difficult to perceive an asynchrony between the first and 2nd square (Nicol & Shore, 2007). The impact of the predictability of the location of the 2nd stimulus also confirms the efficiency of this manipulation. In controls, the Simon effect to the side of the 2nd square was observed mainly in Experiment 2, consistent with our anticipation that the predictability of the second stimulus location would increase the weight of the second stimulus. Even the heightened Simon effect in patients in Experiment 1 can be explained by spatial organization effects.

Patients displayed a larger bias than controls when squares were not only unconnected but belonged to different groups of squares (Experiment 1). We have already shown that this configuration is difficult for patients, because it requires not only the re-grouping of separate items but also their segregation from the groups to which they belong (Giersch & Rhein, 2008; Van Assche & Giersch, 2011). Nevertheless, when the task provides subjects with a strong incentive to re-group items, patients do so (Giersch, van Assche, Huron, & Luck, 2011), although the task then demands a great deal of their attention (Giersch et al., 2011). In the present task, the deployment of attention associated with re-grouping may help patients to attend to the 2nd square in the case of a large asynchrony, thus explaining why patients display the Simon effect to the side of the second stimulus mainly in Experiment 1.

4.2. A deficit at looking forward?

All the results at high asynchronies show the influence of the spatial and attention manipulations on the Simon effects, in both controls and patients. It is in contrast to the bias to the side of the 1st stimulus with short asynchronies, which is clearest once spatial difficulties have been removed. If this particular effect cannot be explained by spatial impairments, it may reveal specific mechanisms dedicated to temporal information processing. As the prior entry effect has shown (Spence & Parise, 2010), attention is first expected to be directed towards the first stimulus when 2 squares are displayed in succession. By the time the response is given, however, our results indicate that more weight is attributed to the last occurring stimulus. These results thus reflect a shift in emphasis in both time and space. Since the Simon effect to the side of the 1st square is observed mainly when spatial difficulties are removed, the results suggest a deficit in patients as regards shifting in time. In addition, this effect occurs even when the stimuli are not consciously distinguished in time, and when they are separated by a very short time interval. This means that even if the Simon effect relies on an attention shift, it is driven by a sub-conscious effect. Given that patients show attention shifts with larger SOAs, the suggestion is that the Simon effect to the side of the 1st square is due to an impairment at the level of the mechanisms that allow for this sub-conscious drive. Moving in time towards the last occurring stimulus and an impaired ability for patients to do so may have to do with elementary neural mechanisms, one of which may be related to the theory of predictive coding. According to this theory (Friston, 2008), the brain constantly predicts the next sensory entry. This may occur at very short timescales, since it is related not to the ability to attend consciously to the next event but, rather, to the ability for the neuronal networks to encode successive events (Varela, 1999). This would make it possible to detect unexpected events and to adjust to an ever-changing environment. Our results may be the consequence of a system that naturally places more emphasis on the last occurring event because it is wired to look sub-consciously for the next occurring event. Delays under 20 ms are compatible with a weight shift between competing neuronal networks. The Simon effect at short asynchronies may thus reflect these elementary mechanisms.

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4.3. Impact of the time-event structure coding impairment

Regarding the deficit in patients, an impaired ability to look for the next event may explain why patients appear to be 'stuck' on the first occurring stimulus. They would be able to compensate for this difficulty, however, when the task conditions allow them to shift their attention towards the last occurring event, like in Experiment 1 at large asynchronies. This shows once again that the deficit affects an elementary mechanism in a very selective way. It is still unclear to what extent this deficit accounts for other deficits encountered in this pathology, in other cognitive domains or on a larger timescale. For example, patients with schizophrenia show impaired sensory predictions in the case of motor control (Turgeon, Giersch, Delevoye-Turrell, & Wing, 2012), which may be related to the deficits described here. They are also impaired when it comes to discriminating events displayed successively in the same location (Green, Lee, Wynn, & Mathis, 2011; Schechter, Butler, Silipo, Zemon & Javitt, 2003; Slaghuis & Bishop, 2001). In these paradigms, there is a dissociation between explicit judgements and implicit effects, like in the present study (Del Cul et al., 2009; Herzog et al., 2004). We recently proposed that these effects may be related at least in part to a difficulty with shifting attention in time (Lalanne, Dufour, Després, & Giersch, 2012b; see also Granholm, Fish & Verney, 2009). More generally, we may ask whether the deficits described in our work account for the disturbed sense-of-time continuity described at a clinical level (Fuchs, 2007; Kimura, 1994; Vogeley & Kupke, 2007). Husserl (1928) proposed that the sense-of-time continuity relies on integrating past, present and future moments. Difficulty with moving in time, or predicting the next event may disrupt the feeling of continuity. This may play a key role in the cognitive impairments observed in patients with schizophrenia, in keeping with the idea that time impairments have a central role to play in the pathophysiology of schizophrenia (Andreasen, 1999; Minkowski, 1933). Time continuity is inherent to our psychic life, and the stability it ensures may be necessary for spotting discontinuous events efficiently. Conversely, a disruption of this continuity would de-stabilize our perception of the environment, as described by the patients with schizophrenia themselves: 'Time splits up and doesn't run forward anymore. These arise uncountable disparate now, now, now, all crazy and without rule or order' (quoted in Kimura (1994)).

4.4. Conclusion and limits

All in all, patients and controls are both sensitive to the effect of connectors and to spatial grouping, consistent with data suggesting that the processing of temporal properties follows spatial processing, and is influenced by spatial grouping. The results also suggest attention may be involved. In contrast to this, the abnormal bias to the side of the first stimulus in the case of two connected targets in Experiment 2 can hardly be related to grouping impairments and suggests a dissociation of the coding of temporal properties at short and longer asynchronies. In fact, the dissociation between the Simon effects observed in patients with short and long SOAs suggests that mechanisms underlying implicit and explicit time-event coding differ qualitatively. Implicit and automatic coding of events in time have already been dissociated, (review in Rohenkuhl, Coull, and Nobre (2011), but only rarely at asynchronies as short as observed here (Efron, 1973). Our proposal is that the effects observed at short asynchronies in patients with schizophrenia are revealing elementary mechanisms of time-event structure coding, possibly related to those involved in predictive coding, i.e., to the expectation of the next event in time.

One of the limitations of our study was that most patients were on antipsychotic medication. An effect of treatment could not be ruled out, although no positive correlation was found with the Simon effects. Whatever the case, the results would still be relevant as regards the dissociation between spatial and time effects with short asynchronies.

Acknowledgments

This research was financed by the French National Institute for Health and Medical Research (INSERM), the Centre Hospitalier Régional Universitaire of Strasbourg (API-HUS no. 3494), and the Medicine Faculty of Strasbourg.

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Attention and Masking in Schizophrenia

Laurence Lalanne, André Dufour, Olivier Després, and Anne Giersch

Background: Patients with schizophrenia are known to be impaired in masking tasks, but the mechanisms underlying their deficits are still elusive. Our study was intended to examine attentional effects, which have a known impact on masking in healthy volunteers but have only rarely been explored in relation to masking in patients.

Methods: We compared focused versus divided attention in 18 control subjects and 18 patients using forward and backward masking tasks. In the conventional masking task, subjects had to locate one target among four possible locations. Presentation of one target allows attention to be focused, in contrast with the divided attention task in which two targets were presented either in the same hemifield or different hemifields.

Results: Our results reproduce patients' deficits in forward and backward masking tasks but only when one target is presented. We show that control subjects benefit from focused attention, much more so than patients. Furthermore, patients' performance is identical to that of control subjects in backward masking when targets are presented across hemifields. This performance equalization was checked to ensure it was not due solely to the redundancy of signals (two vs. one). We achieved this by comparing performance when two targets were presented in the same vs. across hemifields, the latter yielding a greater redundancy gain.

Conclusions: From the results, it is unlikely that redundancy can account for the whole pattern of results, which suggest instead that attention deficits play a role in backward masking impairments in patients.

Key Words: Backward masking, divided attention, focused attention, forward masking, redundancy gain, schizophrenia

Patients with schizophrenia consistently display deficits in visual masking tasks (1–4). This particular deficit is related neither to intellectual deterioration (5) nor to neuroleptics (6,7). Several authors have proposed that it reflects vulnerability to schizophrenia (8,9) and has an impact on social outcome (10). These results suggest it reveals a key impairment in patients, hence the need to gain a better understanding of its underlying mechanisms and significance. The role of attention has been shown repeatedly in respect of backward masking (11), and attention impairments have been widely described in patients with schizophrenia (12,13). However, the impact of attention on masking paradigms has rarely been explored in patients. In our study, we wished to determine the extent to which patients with schizophrenia were still impaired when their attention could not be focused on a single target, and we controlled for a possible confounding factor, the redundancy gain.

In visual masking paradigms, a target (usually a letter or symbol) is presented briefly in quick succession with a mask. The subject is instructed to identify or localize the target, the visibility of which is lessened by the mask. The mask is displayed either before or after the target (forward or backward masking tasks). Mask and target are separated by a stimulus onset asynchrony (SOA), the duration of which is manipulated. Patients with schizophrenia need larger asynchronies between target and mask to reach a performance level equivalent to control subjects (1–4). Mechanisms that are proposed as an explanation for the impairment in patients are derived from theories that account for masking in healthy volunteers. Masking is a phenomenon traditionally attributed to interactions between neural visual channels known as "transient" and "sustained" channels (14–18). The transient channel responds to stimulation quickly and briefly and conveys information of low spatial and high temporal frequency, that is, transient and global information. In contrast, the sustained channel responds more slowly and to high spatial frequencies and underlies stimulus identification and fine-scale analysis. These systems correspond, respectively, to the magnocellular and parvocellular visual pathways and interact in complex ways, depending on the type of masking procedure. Backward masking in healthy volunteers is usually believed to involve both integration and interruption mechanisms. Integration is defined as a fusion between the target and the mask relying on the integration of the sustained activities elicited by both stimuli, and it occurs primarily at short SOAs (maximal between 0 and 30 msec). Interruption, on the other hand, is believed to occur at longer SOAs (maximal between 50 to 100 msec), when the transient information conveyed by the mask "interrupts" the sustained processing of the target, thus reducing its visibility (11,14).

On this basis, several hypotheses, not mutually exclusive, have been proposed to explain schizophrenia patients' impairments in backward masking. One influential hypothesis, based on studies that evaluated the detection of low versus high spatial frequency information, suggests a deficit at the level of the transient channel (6,19), which, if impaired, would mean target processing would not be interrupted as efficiently. Consequently, the first stimulus would persist longer and would be merged with the mask via integration mechanisms (20). However, this hypothesis alone may not be enough to explain the entire pattern of deficits observed in patients during masking tasks. Rassovsky *et al.* (21) used meta- and paracontrast to show that masking deficits are still observed in patients even when the mask location does not overlap with the target location, such that mask and target cannot be fused in space. Effects observed in metacontrast and paracontrast are explained by "object substitution," which might also account for the deficits observed in patients. It is a theory based on common views of visual perception, in which object identification requires not only feedfor-

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Received May 27, 2011; revised Sep 1, 2011; accepted Sep 10, 2011.

0006-3223/$36.00
doi:10.1016/j.biopsych.2011.09.018

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ward processing but also feedback connections to overcome any ambiguity between the different interpretations that may be derived from the initial feedforward processing (22). With masking, both the target and the mask would elicit feedforward and feedback processing in turn. However, the target and the mask are presented in close spatiotemporal proximity. This means a possible mismatch between feedforward and feedback processing. For example, in backward masking, when the target has disappeared and the mask is displayed, the feedforward processing would correspond to the mask, whereas the feedback would correspond to the target. It would lead to the target being substituted by the mask, before all ambiguities about the target have been overcome. It might account for patients' deficits if the target processing is too weak or the mask too strong. However, like the hypothesis with respect to excessive integration, this theory must also account for results in implicit tasks that show patients are very sensitive to short duration information, even when efficiently masked. Herzog *et al.* (23) explored the incidental influence of short-duration stimuli on performance in a masking task. They used a 20-msec prime and found that patients were as sensitive to such short-duration stimuli as control subjects. Del Cul *et al.* (24) also showed that the implicit processing of the target was preserved. On the whole, the results of the latter studies show the typical increase in backward masking in patients, which, however, does not seem to be systematically associated with the target and the mask fusing at an early level of processing and still allows the target to be processed efficiently.

As proposed in several models (25), attention is important for enabling information to reach consciousness. Furthermore, the latest "object-substitution" theory stresses the importance of feedback connections that may be reinforced through attention. In fact, studies on object substitution have been particularly helpful in highlighting how attention influences metacontrast (26,27) and even backward masking (28). In addition, attention effects have frequently been described in patients with schizophrenia (29–31), especially with respect to visual perception (13,32), although few studies have confirmed its role in masking deficits in patients with schizophrenia. Granholm *et al.* (33) assessed how attentional resources are allocated during backward masking tasks by measuring pupil dilation. They suggested that patients either failed to allocate sufficient resources or that they mistakenly allocated them toward the mask instead of the target. This would be consistent with the literature suggesting selection control deficits (34). Rassovsky *et al.* (35) manipulated attention by warning subjects before a subset of trials that they would receive a monetary reward for a good response. Although this manipulation improved patient performance, the effect was fairly modest. In all experiments, patients remained impaired in relation to control subjects in all conditions. The hypothesis that attention influences backward masking in patients warrants further examination. We made no attempt in our study to improve masking performance by manipulating attention or to measure attention alongside the masking task. Rather, we tried to minimize the impact of attention in the masking procedure to see whether the performance of patients and control subjects would then be equated. We reasoned that attention allocation should be efficient, at least in healthy control subjects, when focused on a single target but not when divided between two locations. If attention has a role to play in the masking deficit found in patients, their performance should be more similar to that of control subjects when attention is divided. We therefore checked the impact of attention by comparing performance when attention is focused on a single target versus when it is divided between two targets. In the divided-attention condition, two targets were presented, which means a possible redundancy gain in which detection is facilitated by comparison of two identical stimuli with a single one (36,37). Despite this, attention focalization predicted better performance when there was only one target. We predicted less attention focalization efficiency in patients, with similar performance for one versus two targets. However, another explanation for this could be an effect on the redundancy gain, and we wanted to determine whether this gain was affected in patients. Our approach was based on recent studies suggesting that the redundancy gain might be affected in a paradoxical way in presentation across hemifields. Indeed, several studies have demonstrated that the redundancy gain is greater in detection tasks when signals are displayed across hemispheres than when the same number of signals is displayed within hemifields (38,39). Underlying this at least in part is the fact that bilateral stimuli activate both hemispheres in parallel: bilateral activation would help detection and motor response more than unilateral activation (38). This means that in addition to the redundancy gain due to the presentation of two targets instead of only one, the presentation of the two targets in different hemifields should result in even further detection facilitation (38, 39). This effect might be paradoxically enlarged in patients with schizophrenia because of interhemispheric transfer disturbances. When interhemispheric transfer is disturbed, as in cases of commissurotomy or callosal agenesis (40,41), redundancy gain is increased. This is also the case in schizophrenia (42).

This might have a number of implications for the masking paradigm because in the typical paradigm, the mask includes information displayed in both hemifields, implying the activation of both hemispheres. If the redundancy gain was increased in patients, it would mean a higher impact for the mask in patients than in control subjects. However, the presentation of two rather than one target should help patients more than control subjects. This should be especially when the two targets are presented in different hemifields. To confirm this, we contrasted a condition in which both targets are presented in the same hemifield with one in which they are displayed in different hemifields. If the redundancy gain is higher in patients than control subjects and accounts for performance variations across tasks, patients' performance should improve more than that of control subjects when targets are presented across hemifields rather than within the same hemifield.

Our predictions were as follows. First, in healthy control subjects, the possibility of focusing attention in the single target presentation should lead to better performance than when there are two targets. Focused attention may help to amplify the signal conveyed by the target, thus helping to avoid substitution or interruption of target processing by the mask. Inasmuch as these effects have been observed mainly in metacontrast and backward masking, we expected them to be less pronounced in forward masking (11,28). If patients have difficulty related to focused attention, the advantage of having a single target should be lessened, especially with backward masking. Furthermore, by comparing performance as a function of within-hemifield and between-hemifield presentation, we were also able to check the advantage provided by the redundancy of signals across hemifields and to establish whether there was a differential effect of mask and target redundancy between groups. If patients benefit from an increased redundancy effect, their performance should improve more than that of controls when targets are presented in different hemifields as opposed to the same hemifield. This should be true in both backward and forward masking.

Methods and Materials

Demographic characteristics of the 18 patients and 18 control subjects are displayed Table 1. It should be noted that the patients'

Table 1. Demographic and Clinical Data of the Participants

	Patients	Control Subjects
Sex (Male/Female)	11/7	11/7
Age (mean ± SD)	35.7 ± 6.3	34.3 ± 6.4
Education (years, mean ± SD)	11.8 ± 2	12.5 ± 1.8
Medication (typical/atypical)	4/14	—
Dose of Chlorpromazine Equivalents	253 mg/day	—
PANSS Positive Symptoms (mean ± SD)	15.4 ± 4	—
PANSS Negative Symptoms (mean ± SD)	19 ± 7.3	—
PANSS General Symptoms (mean ± SD)	35.8 ± 12.8	—
PANSS Total (mean ± SD)	70.3 ± 21.2	—

PANSS, Positive and Negative Syndrome Scale.

group is fairly asymptomatic. Details on participants (diagnosis, exclusion criteria, and ethics) and on the apparatus can be found in Supplement 1.

Stimuli

In all tasks subjects were instructed to locate target stimuli (squares .8° × .8°, luminance 3.26 cd/m^2), which could be displayed in four possible locations (in the four corners of a virtual square (5.5° × 5.5°) around a central fixation point. The single masking task corresponded to the traditional masking experiment in which a single target is presented for 33 msec and randomly assigned to one of the four locations (top right or left, or bottom right or left).

In the double masking task, two target stimuli were presented for 33 msec either in different hemifields or within the same hemifield. The pairs of squares were displayed either at the top, bottom, or left or right side of the center of the screen. Stimuli were followed or preceded by a mask, defining backward and forward masking, respectively. The mask was presented for 33 msec at a luminance of 3.26 cd/m^2 on a black background of .02 cd/m^2, with four white squares superimposed on the four target locations.

Procedure

The order of the two masking tasks (single vs. two target stimuli) was counterbalanced across subjects in each group. The procedure is illustrated in Figures 1 and 2 and is detailed in Supplement 1. Eleven SOAs were used with values between −250 msec (forward masking) and +250 msec (backward masking) and including 0 msec (in which case, only the mask was visible).

Results

We first analyzed separately the results in the different masking tasks. This was done to check whether we replicate the typical results from the literature. For each task, we conducted an analysis of variance (ANOVA) on correct responses with group as a between-group variable and with type of masking (backward vs. forward) and SOAs as within-group variables. We add the partial eta2 (effect size). Note that we had to exclude the SOA = 0 when comparing forward and backward masking. We decomposed interactions by means of Tukey post hoc analyses, completed in some cases by ANOVAs on a restricted number of conditions—for example, on each SOA.

Single Masking Task

The global analysis of variance showed a group effect [$F(1,34) = 8.9$, $p < .01$, partial $\eta^2 = .21$), with patients making globally 15.5% more errors than control subjects, but no interaction with SOA or type of masking (Figure 3).

Double Masking Task

Squares Presented in the Same Hemifield. There was an effect of the type of masking presentation [$F(1,34) = 6$, $p < .05$, partial $\eta^2 = .15$], with an increase of errors by 4% in backward compared with forward presentation (Figure 4, left panel). There was no significant effect of group even when considering forward and backward masking separately [$F(1,34) = 2.7$, ns, partial $\eta^2 = .07$ for forward masking and $F(1,34) = 1.8$, ns, partial $\eta^2 = .05$ for backward masking).

Squares Presented in Different Hemifields. The ANOVA showed an interaction between group and masking type (backward vs. forward) [$F(1,34) = 6.1$, $p < .05$, partial $\eta^2 = .15$; Figure 4, right panel]. Performance was then analyzed separately in backward and forward masking. Patients and control subjects had similar performance in backward masking task ($F < 1$, partial $\eta^2 = .003$). In forward masking, however, the analysis showed a significant interaction between SOA and group [$F(4,136) = 3.9$, $p < .005$, partial $\eta^2 = .1$). Decomposing this interaction by means of the

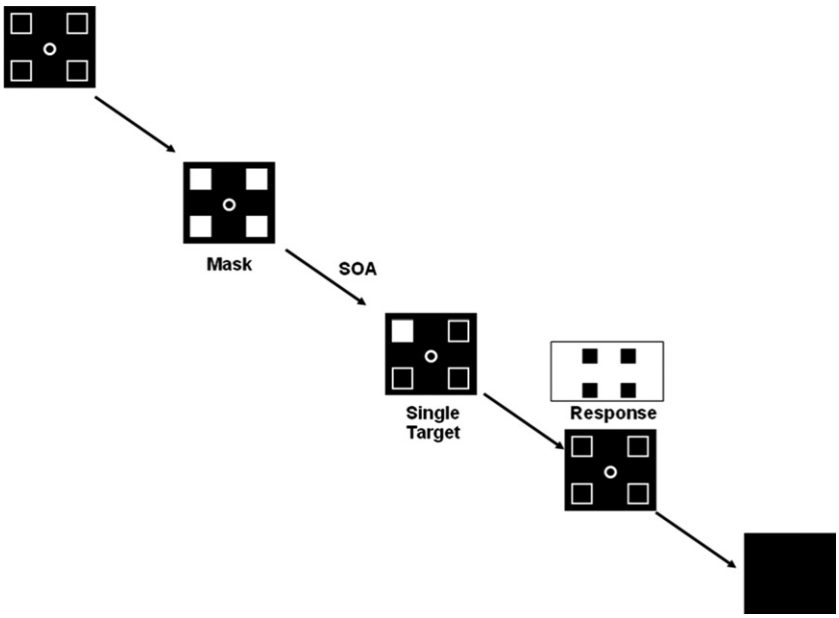

Figure 1. This figure depicts the time course of a forward masking task in which subjects locate the single target, which is presented after the mask. Each trial began with the presentation of the central fixation point and four empty squares. Subjects first had to fixate the central point for 500 msec, as checked by continuous eye tracking. The mask was displayed first, followed by the target, which was represented by the filling in of one square. After the mask and targets had disappeared, empty squares remained on the screen until subjects responded by hitting one of four keys arranged to match the target locations: top left, top right, bottom left, and bottom right. The procedure is the same in backward masking, except that the mask is presented after the target instead of before. SOA, stimulus onset asynchrony.

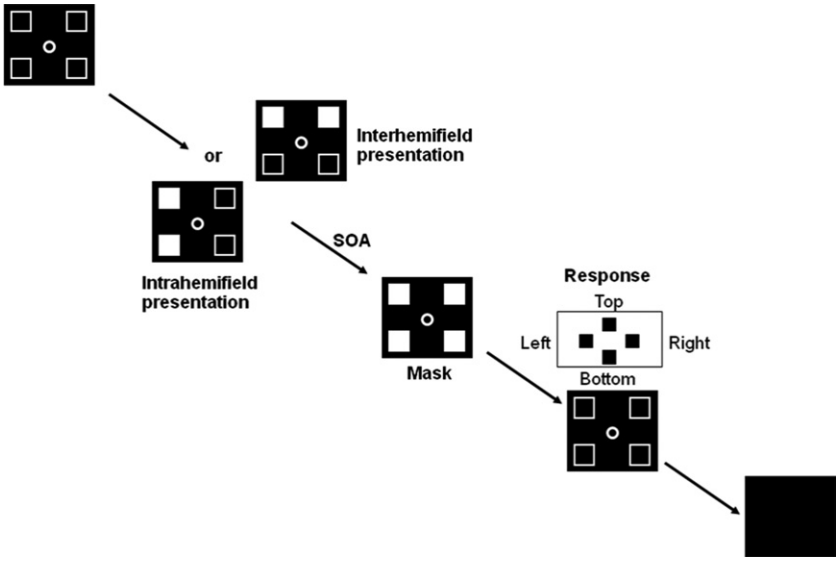

Figure 2. This figure depicts the time course of a backward masking task in which two targets are presented at the top, bottom, or left or right side of the screen center. The procedure is identical to the one used with one single target, except that subjects have to locate a pair of targets rather than one single target. They respond by hitting one of four keys arranged to match the locations of the targets. This description holds for forward masking, except that the mask follows rather than precedes the targets. SOA, stimulus onset asynchrony.

Tukey post hoc analysis did not show any significant effect. Only ANOVAs performed on each SOA showed an impairment for the two largest SOAs at 200 msec and 250 msec [by 18%, $F(1,34) = 5.99$, $p < .05$, partial $\eta^2 = .15$ at 200 msec, and by 21.7%, $F(1,34) = 9.2$, $p < .01$, partial $\eta^2 = .21$ at 250 msec].

Comparison Between Masking Conditions

The differences in performance observed in the various masking conditions were confirmed in a global analysis with group as between-group variable and the type of task (double masking intrahemifield, double masking interhemifield, single masking), the type of masking (forward vs. backward), and SOAs as within-group variables (Figure 5). The analysis showed a significant interaction between the four variables [$F(8,272) = 2$, $p < .05$, partial $\eta^2 = .06$).

Backward Masking

The analysis restricted to data on backward masking showed a significant interaction between the type of task and group [$F(2,68) = 5.2$, $p < .01$, partial $\eta^2 = .13$), and there was a significant effect of the type of task in each group [$F(2,34) = 25$, $p < .001$, partial $\eta^2 = .6$ in controls and $F(2,34) = 6.6$, $p < .005$, partial $\eta^2 = .28$ in patients].

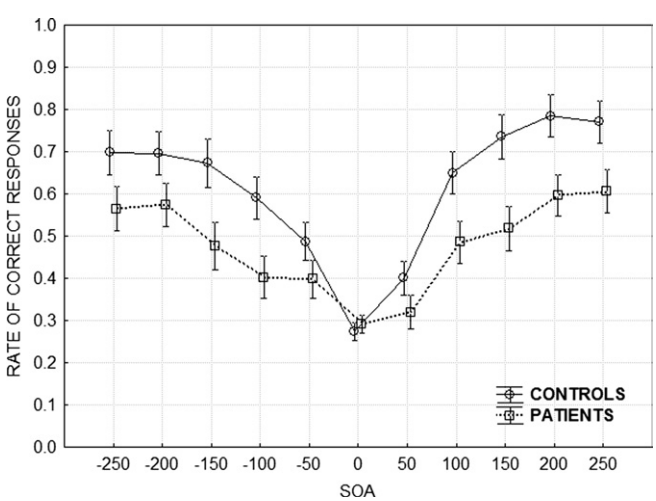

Figure 3. Rate of correct responses as a function of stimulus onset asynchronies (SOAs; msec) when only one square is presented in forward (SOAs below 0 msec) and backward (SOAs > 0 mecs) masking condition.

We first compared performance in tasks with one versus two targets to check the effects of attention focalization. We then compared performance in tasks with two targets in the same versus in two hemifields to check the effect of redundancy gain.

One Target Versus Two: Attention Focalization. Following the effect of the type of task in control subjects (discussed earlier), Tukey post hoc analyses showed that performance was better with a single target, compared with when there were two targets 1) in two hemifields (by 15.7%, $p < .001$) or 2) within the same hemifield (by 19%, $p < .001$; Figure 5, left panel). Similar results were observed with ANOVAs comparing performance with one target and two targets (either in the same or in different hemifields).

The advantage provided by the single target was not as clear in patients (Figure 5, right panel). Tukey post hoc analyses showed that performance improved significantly in patients when there was a single target rather than two targets in the same hemifield, but only by 9.8% ($p < .05$). This improvement was larger in control subjects than in patients, as confirmed by a sub-ANOVA showing an interaction between type of task (one single target vs. two targets in the same hemifield) and group [$F(1,34) = 4.2$, $p < .05$, partial $\eta^2 = .1$].

When comparing performance in the task with a single target and two targets in two different hemifields with a Tukey post hoc analysis, there was no difference in patients (45.7% vs. 46.9%, $p > .7$). This again differed significantly from the improvement observed in controls, as shown by a sub-ANOVA showing an interaction between type of task (one single target vs. two targets in two hemifields) and group [$F(1,34) = 9.4$, $p < .005$, partial $\eta^2 = .22$).

Two Targets in Two Hemifields Versus One: Redundancy Gain. Both patients and control subjects' performance improved when the two targets were displayed in two hemifields rather than in one unique hemifield (Figure 5), although this effect was significant only in patients [$F(1,17) = 8.5$, $p < .01$, partial $\eta^2 = .33$) and not in control subjects [$F(1,17) = 2.6$, ns, partial $\eta^2 = .13$]. This did not yield any interaction between type of task and group [$F(1,34) = 1.4$, ns, partial $\eta^2 = .04$).

Forward Masking

The subanalysis restricted to forward masking showed a significant interaction among the type of task, SOAs, and group [$F(10,340) = 2.2$, $p < .05$, partial $\eta^2 = .06$).

Controls' performance globally varied with the task [$F(2,34] = 8.6$, $p < .001$, partial $\eta^2 = .33$] but not with SOAs ($F < 1$, partial $\eta^2 =$

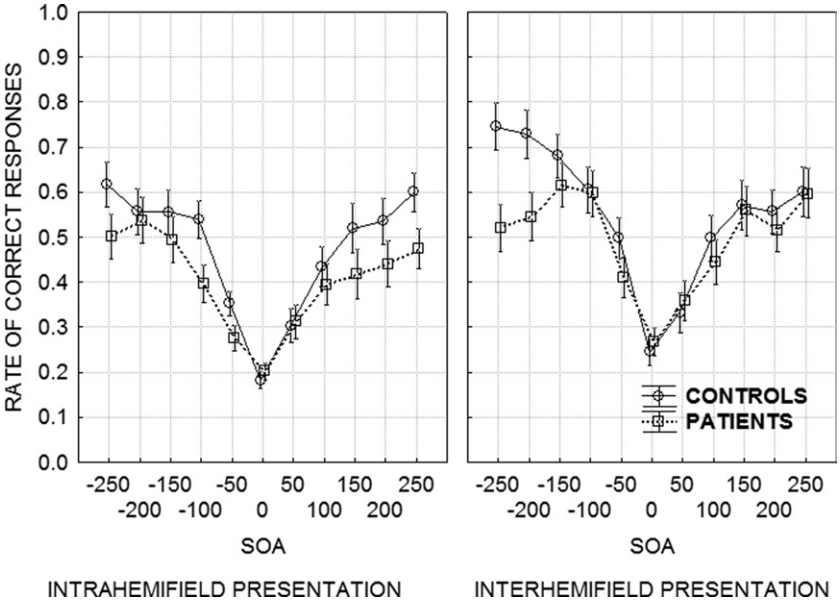

Figure 4. Rate of correct responses when two squares are presented in forward (stimulus onset asynchrony [SOAs] < 0 msec) and backward (SOAs > 0 msec) masking condition. Patients' (continuous line) and control subjects' (dotted line) responses are represented on the left side for intrahemifield presentation of the squares and on the right side for interhemifield presentation.

.04, Figure 5 left panel). Tukey post hoc analyses showed that performance was identical in the masking tasks with a single target and with two targets presented in different hemifields (35% vs. 37% errors, $p = .9$). Performance dropped significantly when two targets were displayed in the same hemifield (by 12% compared with two targets displayed in different hemifields $p < .005$), and by 11% compared with one single target, $p < .01$). These results suggest an effect of redundancy gain with targets presented in different hemifields.

In patients, performance also varied according to the task, but only for some SOAs (Figure 5, right panel), as suggested by a significant interaction between type of task and SOA [$F(10,170) = 3$, $p < .005$, partial $\eta^2 = .15$). Tukey post hoc analyses showed that performance improved when two targets were presented in different hemifields compared with all other target conditions but only for the SOA of 100 msec (by 20%, $p < .005$).

Impact of Treatment on Performance

To determine the impact of treatment on patients' impairments in masking tasks, we conducted the same ANOVA as described earlier on correct responses in patients but with treatment in chlorpromazine equivalent as covariate. We found the same effects as described above.

Discussion

First, the results we obtained in our study reproduce deficits in masking tasks in schizophrenia patients already broadly described in the literature (35,43–45). With a single target, we found an impairment in both backward and forward masking. Although some studies suggest a selective impairment in backward masking, the results in the literature are mixed (46), and several studies have shown results close to ours (47). Most important, how-

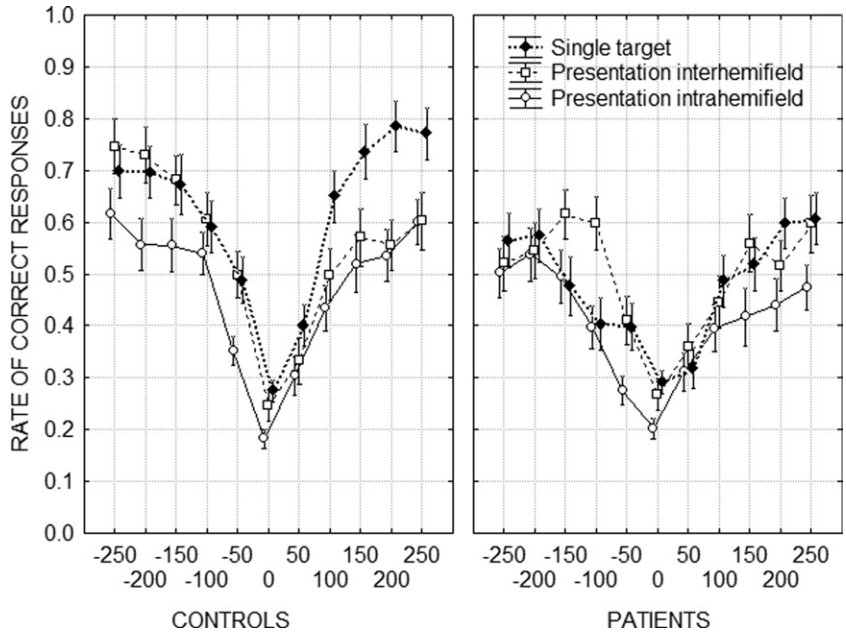

Figure 5. Comparison of correct response rates for presentation of two targets (intrahemifield, empty circles versus interhemifield, empty squares) versus one target (dotted curve with diamond) as a function of stimulus onset asynchronies (SOAs) in forward (SOAs < 0 msec) and backward (SOAs > 0 msec) masking condition.

ever, our results enabled us to explore both the advantage of detecting a single target compared with two targets, at least in backward masking, as well as the advantage provided by the redundancy of signals across hemifields, especially in forward masking.

The most important result of this study is that the performance of patients and control subjects was similar in backward masking when there were two targets rather than one, but they differed in terms of their ability to benefit from focusing on a single target. Control subjects' performance improved with presentation of one target compared with two. Patients' performance, by contrast, either remained the same or improved less than that of control subjects. These results suggest that an impairment in terms of focused attention may account, at least in part, for the masking deficits observed in patients. The results cannot be explained in terms of a generalized deficit, which would have led to a larger impairment in the most difficult condition, that is, with two targets. There are alternative explanations, however, in particular, the impact of redundancy, which ought to be considered first.

When presenting two targets instead of one, there is a redundancy gain. If patients benefit more than control subjects from redundancy, this should be revealed when targets are displayed across hemispheres because of interhemispheric transfer disturbances (42). It would explain why performance of patients and control subjects are equated when two targets are presented across hemifields. Disturbed attention focalization and enhanced redundancy both lead to a flattening of the performance difference between the conditions with a single target and with two targets presented across hemifields. We bypassed this difficulty by comparing the presentation of two targets within and across hemifields. A susceptibility to redundancy was expected to result in better performance for across- than for within-hemifield presentation, for both backward and forward masking. This was not fully confirmed. Although patients improved for across- compared with within-hemifield presentation in backward masking, this was not statistically larger in patients than in control subjects. Furthermore, in forward masking, performance improvement was somewhat smaller in patients than control subjects, and better performance for two targets displayed across rather than within hemifields was significant only for one SOA in patients. All in all, redundancy across hemifields might be an interesting avenue to explore but does not seem to explain fully why patients match control subjects' performance in presentation of two targets rather than one. The lack of advantage provided by the presentation of a single target therefore has more to do with altered attention focalization. It might be suggested that such alteration is related to impaired attentional capture in the single target presentation. Two targets are better detected because of the redundancy gain and thus might better capture attention. However, such a hypothesis would lead to the same inconsistencies as the one related to the redundancy gain. We thus propose that the patients' impairment is related instead to difficulties in allocating attention to the target rather than the mask (33), that is, mechanisms requiring precise control of attention selection. These mechanisms have been suggested to be selectively impaired in schizophrenia (34). Difficulties with allocating attention accurately in time and space may also account for the difficulties observed in patients in forward masking, two targets, and large SOAs. These conditions are characterized by the fact that presenting the mask first should help subjects to expect the target. This is certainly possible when the SOAs are large enough (48,49). This hypothesis would be consistent with earlier results but requires further study (33,50).

One of the limitations of our study is that most patients were treated, and an effect of treatment cannot be ruled out, although using the drug dosage as covariate did not change the results of the analyses of variance. Also, the similarity of the results obtained with patients and control subjects in the presentation of two targets seems to preclude an explanation in terms of treatment. It should be confirmed, however, whether identical results would be found in a more symptomatic group and whether the matching on education level led to an underestimation of the impairment in patients relative to control subjects (51). However, our results do point to a possible impact of attention impairments in masking deficits in patients with schizophrenia. Our paradigm might be of use in event-related potential studies to determine for the effect of attention.

This research was financed by the French National Institute for Health and Medical Research, the University Hospital of Strasbourg (API-HUS n° 3494: Internal Project Grant from the University Hospital of Strasbourg), and the Medicine Faculty of Strasbourg.

The authors reported no biomedical financial interests or potential conflicts of interest.

Supplementary material cited in this article is available online.

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PSYCHIATRY

REVIEW ARTICLE
published: 08 December 2014
doi: 10.3389/fpsyt.2014.00170

The kappa opioid receptor: from addiction to depression, and back

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Comorbidity is a major issue in psychiatry that notably associates with more severe symptoms, longer illness duration, and higher service utilization. Therefore, identifying key clusters of comorbidity and exploring the underlying pathophysiological mechanisms represent important steps toward improving mental health care. In the present review, we focus on the frequent association between addiction and depression. In particular, we summarize the large body of evidence from preclinical models indicating that the kappa opioid receptor (KOR), a member of the opioid neuromodulatory system, represents a central player in the regulation of both reward and mood processes. Current data suggest that the KOR modulates overlapping neuronal networks linking brainstem monoaminergic nuclei with forebrain limbic structures. Rewarding properties of both drugs of abuse and natural stimuli, as well as the neurobiological effects of stressful experiences, strongly interact at the level of KOR signaling. In addiction models, activity of the KOR is potentiated by stressors and critically controls drug-seeking and relapse. In depression paradigms, KOR signaling is responsive to a variety of stressors, and mediates despair-like responses. Altogether, the KOR represents a prototypical substrate of comorbidity, whereby life experiences converge upon common brain mechanisms to trigger behavioral dysregulation and increased risk for distinct but interacting psychopathologies.

Keywords: kappa opioid receptor, place conditioning, reward, addiction, anhedonia, depression, comorbidity, animal models

INTRODUCTION

Addiction and depression are chronic relapsing disorders with devastating consequences for individuals and their social environment (1). Chronic exposure to drugs of abuse, as well as prolonged abstinence from these drugs, is strongly associated with lowered mood and a negative affective state. Conversely, in some individuals, depressed mood potently drives the consumption of euphoric psychoactive substances, a process referred to as self-medication. Accordingly, epidemiological studies have clearly demonstrated a marked comorbidity between addiction and depression (2, 3). This comorbidity is accompanied by greater functional disability, longer illness duration, less social competence, and higher service utilization. Therefore, understanding pathophysiological mechanisms underlying comorbidity has important therapeutic implications.

The present review will discuss numerous lines of evidence that have accumulated to document the kappa opioid receptor (KOR) as an important substrate in comorbidity between addictive and depressive disorders. The KOR belongs to the opioid system, a neuromodulatory system that is widely expressed throughout the central and peripheral nervous systems. The opioid system is composed of three G protein-coupled opioid receptors: mu (MOR), delta (DOR), and kappa (KOR), which under physiological conditions are activated by a family of endogenous peptides to inhibit neuronal activity. Among opioid peptides, dynorphins (encoded by the *Pdyn* gene) primarily activate the KOR and have very low affinity for MOR or DOR. Conversely, the other opioid peptides (endorphin and enkephalins) poorly interact with the KOR. Therefore, the dynorphin/KOR signaling pathway forms a distinct process within the opioid system (4, 5).

Opioid receptors tightly regulate motivational processes, and are identified as important players in psychiatric disorders characterized by reward dysfunction, such as addiction and depression (6, 7). Several exhaustive reviews have recently summarized data on the role of MOR and DOR in these disorders, and will be briefly mentioned when appropriate (6, 8–11). Our goal is to provide the reader with a historical and neuro-anatomical perspective on where, when, and how KORs are recruited in rodent models of addiction and stress-related psychopathology (12–16). First, we will summarize how the KOR progressively emerged as an anti-reward system that encodes dysphoria and limits motivational properties of drugs of abuse. Secondly, we will show that the KOR is recruited and activated during stressful experiences, thereby contributing to the emergence of depressive states (10, 17, 18). Finally, we will discuss two main aspects of how these roles of KOR in addiction, stress-related behaviors and depression have important implications for the understanding of comorbidity. On one hand, we will show that stress-induced recruitment of KOR

signaling is a potent trigger of drug-seeking and relapsing behaviors. On the other hand, we will summarize data on KOR function in the particular context of depressive-like behaviors that emerge during chronic exposure to drugs of abuse, as well as during drug abstinence (19–21).

As a pathophysiological substrate of comorbidity, the KOR represents a promising pharmacological target (10, 18). Clinical trials are currently on-going to assess KOR antagonists as a treatment for depression (22), in particular in the context of addicted patients suffering from comorbid depressive conditions (23, 24). Building on rodent studies, we will discuss the potential of therapeutic strategies targeting the KOR.

KAPPA OPIOID RECEPTOR: AN ANTI-REWARD, DYSPHORIC SYSTEM

Interest in KOR pharmacology historically stemmed from the hope of developing analgesic compounds devoid of the classical abuse potential of MOR agonists, such as morphine. Unfortunately, early human studies exploring properties of KOR agonists reported potent dysphoric and psychomimetic effects (25, 26). While these results clearly decreased the therapeutic potential of KOR in the treatment of pain, they also urged preclinical researchers to explore these intriguing dysphoric effects.

Activation of the MOR is known to induce euphoria in human, and to produce reinforcement in animal models. Therefore, researchers hypothesized that MOR and KOR may have opposite effects in the regulation of motivational processes, potentially through the modulation of common neuronal pathways. This framework was initially explored using conditioned place preference (CPP) or conditioned place avoidance (CPA). In this Pavlovian conditioning paradigm, a drug is repeatedly paired with a set of environmental stimuli that progressively acquire positive (CPP) or negative (CPA) motivational properties. Following repeated conditioning sessions, the animal subsequently exhibits preference or avoidance on re-exposure to the environmental stimuli (in the absence of the drug), a behavior that depends on learning, motivational, and hedonic mechanisms. The seminal rat study by Shippenberg and Hertz (27) reported that, as hypothesized, systemic administration of the KOR agonist U69593 or morphine yielded opposite effects, respectively producing CPA and CPP. While morphine-induced CPP reflects its reinforcing properties, KOR-induced CPA suggested that this receptor might be an anti-reward mechanism that contributes to a bi-directional regulation of motivation and hedonic tone.

The next step was to investigate underlying neurochemical substrates, with early studies exploring how the KOR may regulate the mesolimbic pathway (**Figure 1**). This pathway is composed of dopaminergic (DA) neurons that are located in the midbrain ventral tegmental area (VTA) and project to forebrain limbic structures, including the ventral striatum [or nucleus accumbens (NAc)] and prefrontal cortex (PFC). Animal and human data have clearly demonstrated that drug addiction (and mood disorders, see Part 2) associate with major disruptions of the brain's DA reward circuitry (28), which normally acts to predict and encode the salience of environmental stimuli and natural rewards. The now classical "unitary" theory of addiction postulates that essentially all drugs of abuse enhance DA transmission in the NAc, an

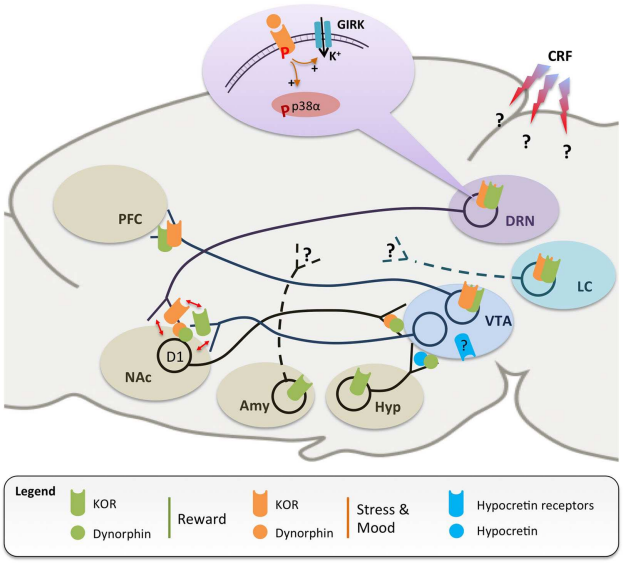

FIGURE 1 | A simplified scheme of neuronal circuits implicated in the regulation of reward (green) and stress (orange), which are both modulated by dynorphins and the kappa opioid receptor (KOR). KOR-mediated inhibition of ventral tegmental area (VTA) dopaminergic neurons projecting to the prefrontal cortex (PFC) is responsible for dysphoria and conditioned place aversion (13, 27, 33). Dynorphinergic medium spiny neurons, located in the nucleus accumbens (NAc) and expressing D1 dopamine receptors, send axonal projections back to the VTA (36), further supporting the importance of KOR in dopamine modulation and as an anti-reward agent. In addition, stressful experiences trigger widespread corticotropin releasing factor (CRF) release in the central nervous system (37), leading to dynorphin release and KOR phosphorylation, notably in the dorsal raphe nucleus (DRN) (38) and locus coeruleus (LC) (39). Stress-induced signaling events have been extensively characterized in the DRN, where activation of KOR stimulates G protein-coupled inwardly rectifying potassium channels [GIRK, see in Ref. (40)] and phosphorylation of the p38α kinase, in turn leading to translocation of the serotonin reuptake transporter to the plasma membrane and increased 5-HT reuptake (14). Similar stress-induced activation of KOR has also been documented at the level of the NAc, which appears to be the site where SERT translocation occurs (16). Available evidence also suggests that KOR regulation of 5-HT and DA neurotransmissions converge at the level of the NAc (red arrows), with important implications for comorbidity (see text for details). Further, recruitment of KOR signaling during stressful experiences has been shown: (i) in the amygdala, to potentiate conditioned place preference for drugs of abuse (20), and (ii) in the DRN (14) and LC (39), to mediate reinstatement of drug-seeking. KOR-dependent modulation of monoaminergic pathways has important implications for mood regulation. Systemic treatments with KOR agonist and antagonist have pro- and antidepressant-like effects, respectively. KOR activation locally in the NAc is sufficient to achieve a prodepressant-like effect (41–45), while knock-down of dynorphins in the NAc has opposite effect (46). Recently, hypocretin (blue) and dynorphin/KOR systems in the hypothalamus (Hyp) have been shown to stimulate and inhibit VTA DA neurons, respectively (47, 48). Avenues for future investigations include the identification of: (i) the signaling events following KOR activation in the LC and VTA; (ii) the brain regions receiving innervation from amygdala (Amy) and LC KOR-positive neurons; (iii) the brain sites where CRF acts to stimulate dynorphinergic neurons, and (iv) the neurochemical identity and projections targets of VTA neurons expressing hypocretin receptors. Altogether, data indicate that the KOR inhibits the activity of all three monoaminergic centers at multiple sites, thereby critically controlling their interactions in rodent models of addiction, depression, and dual diagnosis.

effect that is central to their rewarding properties (29). Within this line, many studies have consistently shown that acute reinforcing effects of morphine rely on disinhibition, i.e., the activation of DA neurons. This disinhibition occurs through the activation of MOR expressed by GABAergic interneurons located mainly in the tail of the VTA [tVTA, or RMTg, see in Ref. (30, 31)], but also in the VTA and NAc (32). In contrast, decreased DA signaling was hypothesized to be responsible for the encoding of KOR-mediated aversion. Using microdialysis, Spanagel and colleagues (33) showed that DA release in the NAc was decreased by infusion of a KOR agonist into the NAc, but not into the VTA (pharmacological agents used in every study discussed in the present review are summarized in **Tables 1–3**). In addition, infusion of a KOR antagonist (nor-BNI) into the NAc increased DA release, suggesting that dynorphins tonically reduce DA neurotransmission in this region. The most compelling evidence implicating DA neurons in KOR-induced aversion came recently (12) from the use of genetically modified mice using the Cre-lox recombination system (34). Bals-Kubik et al. took advantage of a knockin mouse expressing the Cre-recombinase under transcriptional control of the endogenous promoter of the DA transporter [DAT, a specific marker of DA neurons (35)]. These mice were bred with another knockin mouse harboring a conditional "floxed" KOR allele, thereby achieving the specific deletion of KOR in DA neurons (DAT KOR-cKO). At the behavioral level, KOR-induced CPA was abolished in DAT KOR-cKO mice, and restored upon virally mediated KOR re-expression in the VTA (12).

In parallel, investigators undertook a brain-wide analysis of regions where recruitment of the KOR may potentially encode aversion. The effect of local KOR activation was assessed in several areas using the CPA paradigm (49). Infusion of the KOR agonist U50,488H in the NAc was sufficient to induce a robust CPA, consistent with the notion that KOR activation in this region decreases DA release. Surprisingly, infusions in the PFC, lateral hypothalamus, and VTA (but not in the substantia nigra and dorsal striatum) had similar effects, suggesting that multiple KOR pools may regulate motivation and hedonic tone. These results also indicate that activation of VTA KOR induces CPA in the absence of any change in NAc DA release [see aforementioned neurochemical data (33)], implying the involvement of another brain region receiving DA innervation (i.e., the PFC, see below). In addition to the regulation of DA transmission, KOR expression and function is now under investigation in many other brain regions using rodent assays relevant to reward and mood [e.g., bed nucleus of the stria terminalis, BNST, amygdala, locus coeruleus (LC), see below].

An important next goal was to identify which neuronal cell types are controlled by the KOR. Electron microscopy approaches (50, 51) found that in the NAc, half of the axons that were KOR-immunoreactive also expressed DAT. Interestingly, this study found that almost one-third (29%) of these KOR-immunoreactive axons were DAT-negative but contacted pre-synaptic terminals of DAT-positive neurons, suggesting that the mesolimbic pathway is regulated at the level of the NAc by afferent neurons expressing the KOR. Based on recent evidence (16), it is likely that non-DAergic KOR-positive neurons are, at least in part, serotonergic (5-HT). Possibly, KORs expressed by 5-HT neurons may mediate DA/5-HT crosstalk in the NAc, and represent a mechanism that contributes to interactions between mood and reward, as well as between addiction and depression (see below). At the level of the NAc (52), there is also evidence for KOR-dependent modulation of glutamate release, suggesting that this receptor may be expressed pre-synaptically by glutamatergic cortical neurons that densely innervate the NAc. To our knowledge, the behavioral relevance of the latter KOR pool has not been addressed. Finally, in the PFC (53), the KOR was mainly located on pre-synaptic terminals, likely corresponding to DAergic inputs, although the neurochemical identity of these neurons was not assessed.

Other investigators used electrophysiology and immunohistochemistry to identify KOR-expressing neurons. Application of a KOR-selective agonist in the VTA decreased spontaneous firing activity of a sub-group of neurons (54). This KOR-mediated inhibition only occurred in DA cells, as indicated by immunoreactivity for tyrosine hydroxylase (the rate limiting enzyme for DA synthesis, and another marker of DA neurons). Electrophysiology, retrograde tracing and microdialysis were then combined to assess whether DA neurons projecting either to the NAc or to the PFC are differentially regulated by the KOR (55). These elegant experiments revealed that local KOR activation in the VTA hyperpolarized PFC-targeting DA neurons, but had no effect on NAc-targeting DA neurons. Accordingly, DA release was reduced in the PFC, but not in the NAc, upon VTA KOR activation.

Overall these results are consistent with previous CPA and microdialysis studies, and suggest a model whereby VTA KORs do not control NAc DA tone but rather modulate DA release in the PFC to produce CPA. The previously described DAT KOR-cKO mice recently provided strong evidence for the latter hypothesis. Tejeda et al. found that infusion of a KOR agonist in the PFC decreased DA overflow in wildtype (WT) but not in DAT KOR-cKO mice (13), confirming KOR-mediated control of DA transmission in the PFC. Importantly, the authors then directly tested the behavioral relevance of PFC KORs for dysphoria in rats. Infusion of a KOR antagonist into the PFC was sufficient to prevent KOR agonist-induced CPA, clearly identifying the limbic cortex as a necessary substrate for this behavioral effect.

Collectively, results from these various methodological approaches also suggest that NAc-projecting DA neurons express KOR in pre-synaptic terminals, but not in soma and dendrites (33), while PFC-projecting DA neurons express KOR in both compartments (13, 54, 55) (**Figure 1**). At the molecular level, it is currently unknown how DA neurons may control KOR trafficking to distinct cellular compartments as a function of their projection targets. We speculate that the type of electrophysiological feedback (excitatory from the cortex, inhibitory from striatal medium spiny neurons) provided to the VTA by each region may be implicated. Alternatively, cell-autonomous processes might be involved, with distinct transcriptomic profiles in NAc- and PFC-projecting DA neurons leading to distinct KOR post-translational modifications and trafficking. To experimentally address the latter hypothesis, technological advances now allow researchers to distinguish transcriptomes from neuronal populations sharing a common cell-body location but with distinct projections (56). Alternatively, retrograde tracing may be coupled with knockin reporter mice expressing opioid receptors in fusion with fluorescent proteins [such mice are currently available for mu and delta, but not kappa, opioid receptors (57, 58)].

Table 1 | Kappa opioid receptor function in reward regulation.

Species/model	KOR-targeting compound	Behavioral paradigm	Route of administration (dose)	Timing of administration	Outcome of behavioral paradigm	Reference
Rats	U69593	CPP + CPA	s.c. (0.16 mg/kg)	Prior to drug conditioning	KOR-induced aversion	(27)
Rats	U50488H	CPA	Intra-NAc (10 μg) Intra-VTA (0.33, 1 μg) Intra-PFC (1, 3.3 μg) Intra-LH (3.3 μg)	Prior to drug conditioning	KOR-induced aversion	(49)
Rats	U50488H	Social play	i.p. (1, 3 mg/kg)	1 h prior to testing	Reduced social play	(74)
Rats	U50488H	Cocaine SA	i.p. (1.2, 2.5 mg/kg)	5 min prior to testing	Dose-dependent decrease in SA	(60)
Mice	U50488H	Morphine SA	i.p. (5, 10 mg/kg)	15 min prior to testing		
Rhesus monkeys	EKC U50488H	Cocaine SA	i.v. (0.01, 0.032 mg/kg/h) i.v. (0.1 mg/kg/h)	10 consecutive days (23 h/day)	Dose-dependent decrease in SA Reduced SA	(65)
Rats	U50488H	EtOH oral SA (24 h access)	i.p. (10 mg/kg)	6 h prior to drinking measures	Reduced EtOH drinking	(62)
Rhesus monkeys	Enadoline Mr2033 Bremazocine	Cocaine SA	i.v. (0.001, 0.0032 mg/kg/h) i.v. (0.0032, 0.01 mg/kg/h) i.v. (0.0032 mg/kg/h)	10 consecutive days (23 h/day)	Dose-dependent decrease in SA Reduced SA	(66)
Rats	U69593	Cocaine SA	s.c. (0.32 mg/kg)	15 min prior to testing	Reduced SA	(68)
Rats	U69593	Cocaine-reinforced cocaine SA	s.c. (0.32 mg/kg)	15 min prior to testing	Reduced maintenance of SA	(67)
Rats	U50488H	EtOH SA (2 h access)	i.p. (2.5, 5, 10 mg/kg)	15 prior to ethanol access	Dose-dependent decrease in EtOH intake	(63)
Rats	U69593	ICSS	i.p. (0.25, 0.5 mg/kg)	45 min prior to testing	Reduced ICSS threshold	(78)
Mice	Salvinorin A	CPA	i.p. (1, 3.2 mg/kg)	Prior to drug conditioning	KOR-induced aversion	(85)
Mice	nor-BNI	WIN 55.212-2 SA	s.c. (5 mg/kg)	4 hr prior to first session	Enhanced maintenance of self-administration	(70)
Pdyn KO mice	Ø	WIN 55.212-2 SA	Ø	Ø		
Pdyn KO mice	Ø	Nicotine SA	Ø	Ø	Enhanced acquisition of nicotine SA	(69)
Mice	U50488H	EtOH CPP	i.p. (1, 3 mg/kg)	10 min prior to drug conditioning	Reduced EtOH CPP	(64)
Rats	Salvinorin A	ICSS	i.p. (2 mg/kg)	45 min prior to testing	Increased ICSS threshold with lowered breakpoint	(83)
Pdyn KO mice	Ø	EtOH CPP	Ø	Ø	Increased EtOH CPP	(72)
	Ø	EtOH SA (24 hr access)	Ø	Ø	Increased EtOH drinking	
DAT KOR-cKO mice	U69593	CPA	s.c. (0.32 mg/kg)	Prior to drug conditioning	No KOR-induced aversion	(12)
Rats	U50488	ICSS	i.p. (5, 10 mg/kg)	45 prior to testing	Higher dose-dependent increase in ICSS threshold in female than male rats	(87)
Mice	U50488	CPA	i.p. (2.5, 10 mg/kg)	Prior to each drug conditioning session	Dose-dependent difference in aversion between females and males	(76)

This table lists papers discussed in Part 1 of the main text. CPA, conditioned place aversion; CPP, conditioned place preference; SA, self-administration; ICSS, intracranial self-stimulation; EtOH, ethanol; LH, lateral hypothalamus; NAc, nucleus accumbens; PFC, prefrontal cortex; VTA, ventral tegmental area; nor-BNI, norbinaltorphimine; EKC, ethylketocyclazocine.

Table 2 | Kappa opioid receptor function in mood regulation.

Species/ model	KOR-targeting compound	Behavioral paradigm	Route of administration (dose)	Timing of administration	Outcome of behavioral paradigm	Reference
Rats	nor-BNI	LH	Local into the NAc (1 μg/side)	72 h prior to testing	Reduced failure number and latency to escape	(88)
Rats	nor-BNI GNTI	FS	i.c.v. (20 μg) i.p. (0.3–3 mg/kg)	72 h prior to testing 23 h prior to testing	Reduced immobility and increased swimming Reduced immobility	(42)
Rats	ANTI					
Pdyn KO mice	Ø	FS-induced analgesia	Ø	Ø	Loss of FS-induced analgesia	(90)
Mice	nor-BNI		i.p. (10 mg/kg)	60 min prior to FSS		
Rats	JDTic nor-BNI	FS	s.c. (0.3–3 mg/kg) s.c. (1, 10 mg/kg)	23 h prior to testing	Reduced immobility with increased swimming	(41)
Pdyn KO mice	Ø	SD-induced analgesia		Ø	Loss of SD-induced analgesia	(92)
Mice	nor-BNI		i.p. (10 mg/kg, daily)	1 h prior to first SD trial		
Rats	nor-BNI	FS	i.c.v. (20 μg/kg)	24 h prior to testing	Reduced immobility with increased swimming	(99)
Pdyn KO mice	Ø	FS-paired odorant aversion & Footshock CPA	Ø	24 h prior to testing	No aversion from FS-paired odor or footshock	(37)
Mice	nor-BNI		i.p. (10 mg/kg)			
Mice	nor-BNI	SD-induced analgesia	Local into the DRN (2.5 μg/side)	5–7 days prior to testing	Loss of SD-induced analgesia	(38)
Mice	Buprenorphine	FS Novelty-induced hypophagia	i.p. (0.25 mg/kg)	24 h prior to testing	Reduced immobility Decrease in latency to approach	(45)

This table lists papers discussed in Part 2 of the main text. CPA, conditioned place aversion; LH, learned helplessness; FS, forced swim; SD, social defeat; NAc, nucleus accumbens; DRN, dorsal raphe nucleus; nor-BNI, norbinaltorphimine; GNTI, 5′-guanidinonaltrindole; ANTI, 5-acetamidinoethylnaltrindole.

Activity of the dynorphin/KOR pathway on DA neurotransmission and in CPA has obvious implications for addiction-related behaviors, as observed for a variety of drugs of abuse in self-administration paradigms [see in Ref. (59) for an exhaustive review]. KOR agonists dose-dependently decrease morphine self-administration in rats and mice (60, 61). Similar inhibitory effects of KOR activation were found for ethanol (62–64), cocaine (61, 65–68), nicotine (69), and cannabis (70), and these were associated with reduced drug-induced DA release (for example, cocaine, see in Ref. (71); ethanol, see in Ref. (72)). Globally, these results provide robust evidence for an inhibitory effect of KOR on the rewarding effects of drugs of abuse, and recent findings suggest that natural rewards, such as social interactions, may also be affected. In prairie voles, a monogamous rodent species, maintenance of mating pair bonds relies on the expression of aggressive behaviors toward novel conspecifics. Interestingly, this form of "social aversion" has been shown to be mediated by KOR signaling within the NAc (73). In rodents, social play represents a highly studied, naturally occurring behavior that recruits DA neurons and triggers potent reinforcement. Systemic activation of KOR decreased social play in both rats (74, 75) and mice (76). These findings are relevant to our understanding of depression in human as anhedonia, or the altered perception of rewarding properties of everyday-life stimuli (including social interactions), is a hallmark of this condition. Therefore, while KOR-dependent modulation of DA and reward was initially conceptualized and explored in addiction paradigms, it is now becoming clear that it also has strong implications for mood disorders (17, 18). CPA reflects the interaction of several neurobiological mechanisms, corresponding to three psychological constructs: learning, motivation, and hedonia. Intra-cranial self-stimulation (ICSS) is another paradigm assessing these three aspects: in this operant conditioning, animals learn to self-administer brief electrical pulses into specific brain regions (usually the medial forebrain bundle (77)). Systemic activation of KOR was found to induce an anhedonic-like state in ICSS, as indicated by increased stimulation threshold (78). In the latter work, the stimulation electrode was placed in the lateral hypothalamus, strengthening previous evidence for KOR-dependent regulation of hedonic state occurring outside the NAc (49). Further, Potter et al studied the kinetics of KOR agonist-induced ICSS modulation following acute and repeated injections (79). The KOR agonist Salvinorin-A increased the stimulation threshold, and this acute effect persisted with daily injections over an 8-day period. Interestingly, repeated injections also triggered delayed and opposite effects, as evidenced by decreased ICSS stimulation threshold 24 h post-injection, suggesting that opponent processes (80, 81) had developed.

The neuronal pathway potentially linking hypothalamic KOR activity with DA transmission and reward has been poorly studied. Recent elegant data using electron microscopy, electrophysiology, ICSS, and cocaine self-administration (47, 48), suggest an antagonistic interplay between orexin and dynorphin peptidergic systems. The hypocretin/orexin system is composed of neurons originating in the lateral hypothalamus and projecting to several mesolimbic structures (82). Importantly, orexin and dynorphin were found to act as co-transmitters in neurons of the hypothalamus (47): the two peptides co-localize in synaptic vesicles,

Table 3 | Kappa opioid receptor function at the interface of reward and mood regulation.

Species/ model	KOR-targeting compound	Behavioral paradigm	Route of administration (dose)	Timing of administration	Outcome of behavioral paradigm	Reference
Mice Pdyn KO mice	nor-BNI	FS-induced cocaine CPP	i.p. (10 mg/kg) Ø	1 h prior to testing Ø	Loss off FS-induced potentiated preference	(90)
Rats	JDTic	Footshock-induced/cocaine-primed reinstatement of cocaine SA	i.p. (10, 30 mg/kg)	24 h prior to testing	Loss of footshock-induced reinstatement	(41)
Mice	U50488H	Cocaine CPP	i.p. (5 mg/kg)	360, 60, 30, 15 min prior to testing	Time-dependent potentiation or suppression of preference	(105)
KOR KO mice Mice Pdyn KO m*ce*	Ø nor-BNI Ø	FS-induced cocaine CPP SD-induced cocaine CPP	Ø i.p. (10 mg/kg) Ø	Ø 1 h prior to testing Ø	Loss off FS-induced potentiated preference Loss off SD-induced potentiated preference	(92)
Mice Pdyn KO mice KOR KO mice	nor-BNI Ø	Footshock-/FS-induced/cocaine-primed reinstatement of cocaine CPP	i.p. (10 mg/kg) Ø	1 h prior to testing Ø	Loss off footshock- and FS-induced reinstatement	(104)
Mice	nor-BNI	SD-induced reinstatement of cocaine CPP	Local into the DRN (2.5 μg/side)	5–7 days prior to testing	Loss of SD-induced reinstatement	(38)
Mice	Zyklophin	FS-induced/cocaine-primed reinstatement of cocaine CPP	s.c. (3 mg/kg, daily)	60 min prior to FSS exposure	Loss off FS-induced reinstatement	(103)
Mice Pdyn KO mice	nor-BNI Ø	FS-induced reinstatement of EtOH SA	i.p. (10 mg/kg) Ø	60 min prior to FSS exposure Ø	Loss of FS-induced self-administration	(106)
Rats	nor-BNI U50488	EPM after 24 h of withdrawal from EtOH EPM	i.p. (20 mg/kg) i.p. (10 mg/kg)	24 h prior to testing 10 min prior to testing	No reduced open arm exploration Reduced open arm exploration	(125)
Rats	nor-BNI	FS-induced reinstatement of cocaine SA	Local into the VTA (2.5 μg/side)	24 h prior to FSS exposure	Loss of FS-induced reinstatement of self-administration	(112)
Rats	nor-BNI U50488	EPM after 6 weeks of withdrawal from EtOH	i.p. (20 mg/kg) i.p. (10 mg/kg)	24 h prior to testing	No reduced open arm exploration Reduced open arm exploration	(135)
Mice	nor-BNI	U50488-induced reinstatement of cocaine CPP	Local into the LC (2.5 μg/side)	5–7 days prior to testing	Reduced KOR-induced reinstatement	(39)
Mice	U50488	FS-induced reinstatement of cocaine CPP	i.p. (5 mg/kg)	30 min prior to testing	Enhanced FS-induced reinstatement	(162)
Rats	nor-BNI	Yohimbine-induced reinstatement of heroin SA	i.p. (20 mg/kg)	48 h prior to testing	Reduced yohimbine-induced reinstatement	(109)
Rats	nor-BNI	FS-induced reinstatement of cocaine SA	i.p. (10 mg/kg)	22 h prior to FSS exposure	Loss of FS-induced reinstatement of self-administration	(113)
KOR KO mice	Ø	Social behaviors after 4 weeks of withdrawal from heroin	Ø	Ø	Loss of social avoidance	(139)
Mice Rats	LY2456302	FS EtOH SA	*per os* (10 mg/kg) *per os* (3, 10 mg/kg, daily)	60 min prior to testing 60 min prior to testing	Reduced immobility Dose-dependent decrease in EtOH consumption	(144)

This table lists papers discussed in Part 3 of the main text. FS, forced swim; CPP, conditioned place preference; SA, self-administration; SD, social defeat, EPM, elevated plus maze; EtOH, ethanol; DRN, dorsal raphe nucleus; VTA, ventral tegmental area; LC, locus coeruleus; nor-BNI, norbinaltorphimine.

and are co-released upon electrical hypothalamic stimulation. The authors further showed that orexin and dynorphin act within the VTA to stimulate and inhibit, respectively, the excitability of DA neurons, thereby bi-directionally modulating reward (in ICSS experiments), and self-administration of cocaine (and potentially other drugs of abuse). In the VTA, most cells (65%) were found to be common targets for both orexin and dynorphin. Based on previous evidence, future experiments may test the hypothesis that VTA DA neurons expressing both KOR and orexin receptors project preferentially to the PFC rather than the NAc.

Overall, data on KOR function in the regulation of reward highlights the importance of assessing the full spectrum of peptides and neurotransmitters expressed along the mesolimbic pathway and associated neuronal circuits. Determining how this network dynamically evolves under chronic pathologic conditions will be an exciting endeavor.

KAPPA OPIOID RECEPTOR: A STRESS SYSTEM IMPLICATED IN DEPRESSION PATHOPHYSIOLOGY

In parallel to these studies on reward, recent data have demonstrated that the KOR also controls emotional responses, in particular during stressful experiences. Pharmacological studies in rodents indicate that the dynorphin/KOR system regulates mood-related behaviors. In rats, systemic administration of KOR agonists and antagonists showed pro- and antidepressant-like effects, respectively, in the forced swim (FS) and learned helplessness (LH) tests [see in Ref. (10, 41–45) for a review]. Consistent with CPA studies, systemic KOR activation decreased DA release in ventral (44, 83), dorsal (84, 85), and striatal regions, while local NAc injection of a KOR agonist mimicked the prodepressant-like effect of systemic treatment (86). These data further confirm that KOR-dependent modulation of DA is implicated in both mood- and addiction-related behaviors (28). Interestingly, KOR-dependent prodepressant-like effects may be modulated by gender (87), an important aspect considering that the prevalence of depression is higher in women. Using ICSS, the authors found that the KOR agonist-induced increase in ICSS stimulation threshold was higher in male than female rats. This effect was independent from circulating levels of gonadal hormones, and was not accounted for by sex differences in pharmacokinetics of the agonist. Rather, sex differences in KOR agonist-induced neuronal activation, as revealed by c-fos staining, were found in the BNST and PVN, but not in the NAc or amygdala. Therefore, in addition to the mesolimbic pathway, sex-specific KOR-dependent regulation of hedonic tone may also occur at the level of the BNST and PVN, two structures controlling stress-responses and emotions.

Adding to pharmacological studies targeting KOR, there is also evidence that dynorphins provide an endogenous tonic regulation of mood-related traits (43, 88). In the NAc, medium spiny neurons expressing the DA D1-receptor are known to synthesize and release dynorphins under the control of the cAMP response element binding protein (CREB). Accordingly, prodynorphin levels were decreased in the NAc of transgenic mice overexpressing a dominant negative form of CREB. This effect was associated with decreased behavioral despair in the LH paradigm. Consistently, a recent study reported that *Pdyn* knock-down (by viral expression in the NAc of an anti-*Pdyn* short hairpin RNA) decreased depressive-like behavior in the FS test (46).

Beyond baseline emotional responses, data indicate that activity of the dynorphin/KOR system is potentiated by stress. Acute, but not chronic, restraint stress was shown to sensitize KOR-dependent CPA (89). Also, repeated exposure to FS stress produced a prodepressant-like effect that was blocked by the KOR antagonist nor-BNI, and was absent in *Pdyn* KO mice (90). Dynorphins were further demonstrated to modulate repeated stress-dependent aversive conditioning (37). Mice trained to associate a given odor with FS stress robustly avoided that odor. This avoidance behavior was not observed in *Pdyn* KO mice, and was blocked in WT mice by pre-treatment with a KOR antagonist. Similarly, a context repeatedly paired with footshocks was aversive in WT mice; but again, this effect was absent in *Pdyn* KO mice, and prevented by KOR antagonist pre-treatment. Importantly, the authors showed that corticotropin releasing factor (CRF) release in the central nervous system is likely the primary event responsible for stress-induced recruitment of the dynorphin/KOR system. Results indicated that systemic injection of CRF triggered KOR phosphorylation, as revealed using a phospho-KOR antibody. Further, stress-induced CPA (mimicked by systemic or intracerebroventricular injection of CRF or the CRF_2-receptor agonist Urocortin III) was absent in *Pdyn* KO mice, and blocked by nor-BNI pre-treatment. Following stress exposure, KOR activation, and phosphorylation was identified in several brain structures, including the basolateral amygdala, hippocampus, dorsal raphe, VTA, and NAc. Altogether, these data show that dysphoric aspects of stress behaviorally manifest when CRF stimulates dynorphin release, yielding KOR activation (37).

Stress is a complex physiological process that has a primarily adaptive value, but that can trigger pathological events during prolonged and excessive stressful experiences. Recently, interactions between stress and the KOR have been investigated using more sophisticated and ethologically relevant models of depression. In nature, confrontation among conspecific animals potentially generates significant consequences in terms of control over resources, access to mates, and social positions. For example, the resident–intruder social defeat paradigm (91) is a naturalistic model characterized by potent aggressive interactions that are unpredictable and inescapable, thereby inducing several anhedonia-like symptoms such as diminished sexual pursuit and decreased sucrose preference (52). McLaughlin and colleagues were the first to reveal the role of dynorphins and KOR in transducing the effects of social stress (92). Mice exposed to repeated social defeats over 3 days showed a characteristic defeated postural response, as well as an increased nociceptive threshold, or stress-induced analgesia (SIA, observed in a tail withdrawal latency assay). Both aspects were prevented in mice pre-treated with a KOR antagonist, or lacking the *Pdyn* gene. Another important feature of the social defeat model is that its effects show high inter-individual variability, both in rats and among inbred mice, such that animals can be typically separated into susceptible and resilient groups (93). Along this line, according to Bérubé et al. (94), expression levels of dynorphins in the NAc differ among susceptible and resilient Sprague–Dawley rats. Increased dynorphin mRNA levels (measured by qPCR) were found in the ventral striatum of susceptible rats (NAc shell, coherent with previous mice data), while surprisingly increased levels

were observed in the dorsal striatum of resilient individuals, suggesting that the regulation of DA and mood by dynorphin and KOR may be more complex than anticipated. Additional studies will be necessary to further substantiate this hypothesis. In contrast, another study reported no change in dynorphin levels in VTA or NAc of socially defeated Long–Evans rats (95). Discordance between these two studies might be explained by the different strains used, or the absence of a distinction between resilient and susceptible Long–Evans rats in the latter study. Of note, Nocjar et al. (95) found decreased dynorphin-A, as well as decreased orexins A and B, in the hypothalamus of defeated rats. Therefore, combined regulation of VTA DA neurons activity by these two antagonistic peptides might mediate defeat-induced KOR-dependent social aversion, and be impaired following social defeat.

We previously discussed (Part 1) how the KOR may display differential cellular localization across the two populations of VTA DA neurons projecting to the NAc or to the PFC. A recent report suggests that this anatomical dissociation may have relevance for the understanding of the effects of chronic social defeat. Chaudhury and colleagues (96) showed that the selective inhibition of VTA DA neurons projecting either to the NAc or to the PFC, respectively, promoted resilience or susceptibility to repeated social defeat. Due to its selective cellular localization, it is tempting to speculate that the KOR may mediate prodepressant-like symptoms induced by the inhibition of the VTA-PFC DA pathway.

In addition to DAergic signaling, new findings suggest that 5-HT transmission may also be modulated by KOR in stress- and social defeat-based models of depression. Electrophysiology experiments (97, 98) initially demonstrated that the KOR regulates 5-HT neurons at the level of the dorsal raphe nucleus (DRN), a main 5-HT brain nucleus. Importantly, rescue experiments showed that the selective re-expression of KOR in the DRN of KOR KO mice is sufficient to restore the CPA induced by infusion of a KOR agonist in the NAc (38). Together with previous findings, these results indicate that KOR in the PFC, and KOR expressed by neurons present in the DRN, which target the NAc (that are likely to be 5-HT neurons), are necessary and sufficient, respectively, for the expression of KOR agonist-induced aversion. At the molecular level, acute social defeat was shown to trigger phosphorylation of KOR and the p38α kinase in the DRN (14). Recruitment of p38α in 5-HT neurons is essential, as defeat-induced social avoidance was abolished in cKO mice in which p38α is specifically deleted from serotonin transporter (SERT)-expressing neurons (p38α-cKO^{SERT}). Phosphorylated p38α in turn promotes SERT translocation to the plasma membrane, thereby increasing 5-HT reuptake and likely mediating social avoidance. Electrophysiological recordings in brain slices (40) also showed that KOR activation dampens excitability of DRN 5-HT neurons through two mechanisms: the pre-synaptic inhibition of glutamatergic inputs, and the post-synaptic stimulation of G-protein-gated inwardly rectifying potassium channels (GIRKs). Repeated exposure to FS stress impairs post-synaptic, but not pre-synaptic, effects of KOR activation. Importantly, stress-induced inhibition of KOR-mediated GIRK currents was abolished in p38α-cKO^{SERT} mice. Finally, recent evidence suggests that KOR regulation of DA and 5-HT neurons may converge at the level of the NAc to produce dysphoric and depressive-like effects. Repeated FS stress selectively increased cell-surface expression of SERT in the ventral striatum, but not in other regions examined (dorsal striatum, hippocampus, PFC, amygdala, or DRN). This effect of stress on SERT was prevented by pharmacological blockade of KOR signaling in the NAc, but not in the DRN (16). Altogether, stressful experiences appear to recruit a CRF-dynorhin-KOR-p38α-GIRK signaling cascade within DRN 5-HT neurons, as well as KOR activation in the NAc. These molecular adaptations in turn lead to up-regulation of SERT function in the NAc, and ultimately affect DA function to produce behavioral symptoms. Whether similar DRN signaling is also involved in more prolonged mood-related deficits, in particular in the context of chronic exposure to drugs of abuse (Part 3), has yet to be determined.

In addition to 5-HT and DA circuits, other possible sites of KOR-dependent mood regulation notably include hippocampal neurogenesis and noradrenergic (NA) transmission. One report found that in rats, the antidepressant-like effect of the KOR antagonist nor-BNI (99) associated in the hippocampus, as well as in other structures (e.g., frontal cortex, amygdala, hippocampus, and endopiriform cortex), with increased mRNA levels of BDNF, a neurotrophic factor controlling synaptic plasticity and neurogenesis. Further studies are required to better understand the relevance of this KOR/BDNF interaction.

KAPPA OPIOID RECEPTOR AT THE INTERFACE OF DEPRESSION AND ADDICTION

We have summarized the role of KOR in the regulation of reward processes (Part 1), and in the modulation of stress-responses and affective states (Part 2). Based on these data, several groups have recently explored how the KOR may mediate the interplay between addiction and depression. The relationship between these two disorders is likely bi-directional: addicts show a strong lifetime risk for anxiety or depressive disorders, while, conversely, depressed patients frequently abuse drugs to self-medicate their depressive symptoms. Both aspects are currently being addressed in animal models.

STRESS SENSITIVITY, RELAPSE, AND THE EMERGENCE OF DEPRESSIVE SYMPTOMS IN ADDICTED INDIVIDUALS

Stress-induced relapse during the course of addiction

Rodent models of CPP and drug self-administration have been extensively used to investigate various triggers for relapse, or the re-initiation of drug-seeking behaviors. Following a period of repeated conditionings, or stable drug self-administration, animals are repeatedly re-exposed to CPP or operant chambers in the absence of drug, so that drug-seeking and instrumental responding are no longer reinforced and progressively disappear, a process referred to as extinction. Importantly, after extinction has been achieved, relapse can be triggered through re-exposure (i.e., "priming") to the drug of abuse (drug-induced reinstatement), or through exposure to an acute stressor (stress-induced reinstatement). Classically, stressful experiences represent major lifetime risk factors for the emergence of depressive (100) and addictive (101) disorders. In addition, drugs of abuse potentiate the neurobiological and behavioral effects of a variety of stressors, which in turn may potentiate the effects of drugs of abuse in a vicious circle (see below the stress-induced reinstatement of

CPP) (102). Therefore, addiction and stress interact tightly, and the underlying neurobiological mechanisms represent factors contributing to the comorbidity between addiction and stress-related psychopathology.

Based on available evidence implicating KOR in stress effects (Part 2), researchers went on to probe the role of this receptor in stress-induced reinstatement. Overall, results demonstrate that KOR signaling critically mediates stress-induced reinstatement for a variety of drugs of abuse. In rats, pre-treatment with a KOR antagonist (either JDTic or nor-BNI) significantly decreased stress-induced (footshock), but not cocaine-induced, reinstatement of cocaine self-administration (41). In mice, similar findings were obtained for both stress- and drug-induced reinstatement of cocaine-seeking in a CPP assay [using a new systemically active KOR peptidergic antagonist with short duration of action (103)]. Further, exposure to acute or repeated stress reinstated cocaine CPP in WT, but not in KOR or *Pdyn* KO mice, nor following pharmacological KOR blockade (104). Stress and the KOR also interact at the level of cocaine-context associative conditionings: stress is classically known to potentiate cocaine CPP, and this effect is mimicked by systemic KOR activation (105). Therefore, the KOR mediates stress/cocaine interactions during initial drug exposure, as well as following extinction.

Consistent with cocaine data, genetic and pharmacological approaches showed that stress-induced reinstatement of ethanol consumption similarly relies on dynorphin and KOR in both CPP and self-administration paradigms (106). These results support the notion that the KOR is a pro-addictive agent during stress exposure, in contrast with its inhibitory action on acute reinforcing properties of drugs of abuse (see Part 1). As will be discussed below, clarifying this apparent paradox will require systematically determining which KOR populations are recruited in the entire brain following stress events (and following release of central CRH and systemic corticosteroids), and how this stress-induced signaling differs from KOR activation (by endogenous dynorphins or systemic pharmacological agents) in naïve, unstressed animals.

At the neuro-anatomical level, findings across several drugs of abuse and stressful modalities suggest that stress-induced reinstatement of drug-seeking relies on multiple interactions between the KOR and monoaminergic systems, as well as several forebrain limbic structures. In the DRN, results are in line with previously mentioned data on KOR-dependent CPA. Social stress-induced reinstatement of cocaine CPP was abolished after the conditional deletion of p38α in 5-HT neurons, as shown using p38α-cKO^{SERT} mice (14). In the context of nicotine addiction, FS stress-induced activation of dynorphin/KOR signaling was shown to potentiate nicotine CPP (20), an effect that could be prevented by infusion of nor-BNI in the amygdala. In the latter brain structure, recent studies have started unraveling, which neurons express the KOR (see below). Additional studies will be necessary to assess where KOR-positive amygdala neurons send projections, and whether dysregulation of nicotinic receptors, the direct nicotine targets, occurs in this or another brain region following stress exposure.

Functional interactions between NA transmission and the dynorphin/KOR system also contribute to stress-induced reinstatement of drug-seeking. Anatomical studies initially showed that the KOR is expressed in multiple cellular compartments within the LC, the main NA brain nucleus. Light and electron microscopy showed that KOR prominently co-localizes with the vesicular glutamate transporter and CRF (107), as well as with preprodynorphin (108), in axon terminals of the LC. The KOR is also expressed by LC NA neurons, as KOR immunoreactivity was found in TH-positive somatodendritic processes (108). Electrophysiological recordings indicated that KOR activation in the LC stably attenuates the neuronal activation achieved by recruiting excitatory, or CRF-positive, inputs. In contrast, KOR activation had no effect on spontaneous LC neurons activity (107), suggesting that KOR agonists predominantly recruit pre-synaptic KORs under basal conditions. At the behavioral level, KOR/NA interactions were recently investigated in the context of heroin self-administration (109). Systemic Yohimbine injection was used to precipitate relapse, based on the property of this compound to activate the HPA axis and NA neurons (acting as an antagonist at α_2 NA inhibitory autoreceptors). Results showed that Yohimbine produced a significant reinstatement in control rats, but not in rats pre-treated with the KOR antagonist nor-BNI. Because this study used systemic administration of Yohimbine and nor-BNI, it is difficult to conclude whether the observed effects resulted from KOR blockade in the LC, or in another brain region, following Yohimbine-induced activation of the stress system (potentially leading to widespread CRF and dynorphin release). Another recent report dissected these mechanisms with better anatomical resolution, taking advantage of the simpler behavioral model of KOR agonist-induced reinstatement of cocaine CPP (39). Blockade of the KOR selectively in the LC partly prevented KOR-induced reinstatement. Consistently, rescuing the KOR in the LC of KOR KO mice partially restored KOR-dependent CPP reinstatement. Like other monoaminergic circuits (110, 111), physiological activity of NA neurons relies on multiple receptor subtypes, including inhibitory α_2-autoreceptors and post-synaptic β_1- and β_2-heteroreceptors. Selective pharmacological agents were used to show that the inhibition of NA neurons (α_2-receptor agonist), or the blockade of NA action at post-synaptic β_1-receptors (β_1-antagonist), both potentiated KOR-induced reinstatement. These results suggest a model whereby stress- and KOR-mediated inhibition of NA neurons contributes to relapse, and are in line with previous data showing that LC KOR activation locally decreases neuronal activity. Interestingly, both cocaine-induced reinstatement of cocaine CPP, as well as KOR-induced CPA, were unaffected by manipulations of NA signaling, suggesting that the KOR/NA interplay selectively mediates stress-related aspects of drug-seeking. In the previously mentioned study, Yohimbine precipitated relapse while it is considered an activator of both the HPA axis and NA neurons. Reconciling both studies, one might speculate that these initial stimulatory effects of Yohimbine may be followed and ultimately counteracted by CRF- and KOR-induced inhibition of LC NA neurons, leading to relapse. Finally, these data raise several questions for future studies: how are CRF-receptors and KOR interacting in the LC? Which molecular signaling pathways are recruited in LC neurons following KOR activation, and are they similar to those described in the DRN? Which forebrain structures are impacted upon LC KOR activation?

Very recently, synaptic plasticity has emerged as another level of analysis to better understand KOR-mediated reinstatement of

drug-seeking. Based on previous evidence that: (i) stress impairs long-term potentiation (LTP, a form of long-lasting enhancement in synaptic transmission between two neurons) in the VTA, and that (ii) KOR regulates the mesolimbic pathway (Part 2), a recent report explored KOR modulation of LTP in the VTA as a function of stress (112). Results showed that systemic pharmacological blockade of KOR prevented stress-induced inhibition of LTP at GABAergic synapses (LTP_{GABA}), but not stress-induced potentiation of excitatory synapses, within the VTA. KOR activation in the VTA was sufficient to mimic the effect of stress, and to block LTP_{GABA} in DA neurons. Importantly, intra-VTA nor-BNI infusion, *prior* to FS stress, was shown to prevent stress-induced reinstatement of cocaine self-administration. The same group of investigators further characterized the kinetics of the stress/KOR interplay (113) by looking at KOR and the glucocorticoid receptor (GR), which is activated during stressful experiences and the systemic endogenous release of corticosteroids. Following FS stress, the GR was transiently recruited (during 1 day), whereas at least 4 days of tonic activation of the KOR was necessary to mediate long-lasting effects of stress on LTP_{GABA} in DA neurons. Consistently, blocking KOR signaling *after* FS stress prevented reinstatement of cocaine self-administration. Globally, these two studies strongly suggest that GR- and KOR-dependent blockade of LTP_{GABA} in DA neurons crucially mediates stress-induced reinstatement of drug-seeking. Based on these data, it appears likely that in models of stress response and addiction, distinct plasticity processes might also occur across multiple brain regions following KOR activation.

Overall, the dynorphin/KOR system is critically implicated in relapse across a variety of animal paradigms and drugs of abuse, through complex interactions with 5-HT, DA, and NA signaling. Under baseline conditions, acute activation of the KOR inhibits the reinforcing properties of drugs of abuse (Part 1). In contrast, rodent data suggest that in humans, recruitment of the KOR during stressful life experiences may mediate reinstatement of drug-seeking in addicted individuals trying to achieve abstinence from the drug, and may therefore contribute to the maintenance of addiction.

Emergence of depressive symptoms in addicted individuals

Enhanced stress-reactivity during prolonged exposure to, and abstinence from, drugs of abuse contributes to the emergence of depressive symptoms, which may then evolve into chronic conditions independently from the addictive disorder.

Chronic exposure to drugs of abuse has been shown to potentiate endogenous signaling through the KOR. Repeated exposure to cocaine increased dynorphins concentrations in the striatum and substantia nigra in rats (114). Similarly, prolonged heroin self-administration led to increased *Pdyn* expression in the NAc shell and the central nucleus of the amygdala, with no effect on *Penk,* the gene encoding the enkephalin opioid peptides acting preferably at MOR and DOR (115). Chronic alcohol has also been associated with increased dynorphin expression and release in the NAc (116, 117) and the amygdala (118). As already mentioned, increased dynorphin release in the NAc likely occurs through a cAMP–CREB signaling pathway (119). Accordingly, drugs of abuse increase DA release in the NAc, leading to enhanced and prolonged activation of the DA D1-receptor, a receptor that couples to stimulatory G_s-proteins. This in turn increases intra-cellular cAMP formation, and increases CREB binding to its genomic response elements, leading to increased expression of the *Pdyn* gene. These findings have been substantiated in humans in frontal cortical regions which, similar to the NAc, receive dense DA innervation. In a study examining post-mortem tissues from 14 alcoholics versus 14 healthy controls, increased *Pdyn* mRNA and dynorphin peptides A and B were observed in the dorsolateral PFC, as well as increased KOR mRNA in the orbito-frontal cortex, whereas, no change was found for other opioid peptides and receptors (120) in these regions.

Because of its robust prodepressant-like activity (Part 2), increased expression of the dynorphin/KOR system following prolonged exposure to drugs of abuse has been implicated in the aversive symptoms of acute withdrawal, as well as in the emergence of depressive symptoms during long withdrawal phases or abstinence. Negative affect drives drug consumption (the "self-medication" hypothesis), thereby reinforcing drug-seeking and contributing to addiction severity. In addition, drug-induced emotional disruption may also possibly lead, in vulnerable individuals, to depressive disorders evolving independently from the initial substance abuse. In rodent models, acute withdrawal from chronic ethanol exposure is associated with negative emotional states [see for examples in Ref. (121–124), including behavioral traits usually described as anxiety- (125) or depression-related (126)]. It is likely that both of these dimensions of emotional responses interact (127–129), and that withdrawal-induced anxiety-like behaviors may potentiate depressive symptomatology. In rats, ethanol-dependence can be established by chronic and passive exposure to an ethanol liquid diet (125) or to ethanol vapors (130). In Wistar rats (125), dependence has been shown to manifest as enhanced anxiety-like behavior (as assessed in the elevated plus maze test) during acute withdrawal, and this effect was blocked by systemic treatment with the KOR antagonist nor-BNI. Kissler et al. (130) also observed that acute withdrawal from ethanol-dependence associates with increased alcohol operant self-administration, and an increase in 22-kHz ultrasonic vocalizations, which represents "*an ethologically valid behavior that easily discriminates negative affective states*" (131). These behavioral changes associated with increased Dynorphin-A immunoreactivity in the capsular region of the central amygdala (CeA) and increased agonist-stimulated G-protein coupling of KOR [as measured using the classical [^{35}S]-GTPγS method (132)]. Blockade of KOR in the CeA was shown to prevent escalated ethanol self-administration in dependent rats. The effect of this local manipulation on ultrasonic vocalizations was not assessed; however, it is likely that CeA KOR signaling may contribute to negative affect following chronic ethanol exposure. At the circuitry level, localization of KOR in the amygdala and its physiological relevance has only begun to be appreciated, and recent results indicate that the receptor mainly locates on pre-synaptic terminals of GABAergic neurons (133). Consistently, administration of a KOR agonist and an antagonist onto slice preparations of amygdala rat tissue, respectively decreased and increased GABAergic transmission [miniature IPSCs, (21)]. Surprisingly, these two compounds were found to have inverse effects following daily sessions of cocaine self-administration, and

respectively induced increased and decreased GABAergic activity. These effects were observed only in rats that escalated cocaine consumption during long (6 h) sessions of self-administration, but not in rats showing stable cocaine consumption during short (1 h) sessions. Therefore, while chronic exposure to drugs of abuse potentiate dynorphin/KOR signaling, it is also possible that loss-of-control over drug-taking may specifically modify the net impact of KOR activation on specific neuronal circuits (as exemplified here in the CeA), possibly due to changes in cell types expressing the KOR, or in the cellular localization of KOR. At the behavioral level, CeA micro-infusion of nor-BNI attenuated the heightened anxiety-like behavior (in the defensive burying paradigm) that was observed during withdrawal from chronic, experimentally delivered, cocaine injections. While this effect of KOR blockade should also be tested following voluntary cocaine consumption, these results clearly suggest that amygdala KORs control emotional responses during cocaine withdrawal.

During the repeated cycles of intoxication and withdrawal that characterize addiction, some environmental cues progressively associate with negative affective states, and may then produce aversive effects independently of any drug exposure [even including withdrawal-like symptoms (134)]. Along this line, Berger et al. (19) showed that air-puff induced 22-kHz ultrasonic vocalizations are potentiated during withdrawal from ethanol-dependence (induced by a 2-week forced exposure to ethanol vapors), and this effect was dose-dependently blocked by systemic KOR antagonism. In another set of experiments, the authors associated a neutral odor (almond scent), with the aversive properties of systemic KOR activation. Interestingly, re-exposure to this conditioned odor was shown to potentiate ethanol operant self-administration in non-dependent rats, and this effect was blocked by KOR systemic blockade. Likewise, in humans, re-exposure to contextual cues that have been repeatedly paired with withdrawal-induced negative affect may produce a KOR-dependent dysphoric state and potentiate drug-seeking, thereby contributing to the maintenance of addiction and the emergence of depressive symptoms.

Emotional consequences of drugs of abuse extend well beyond the acute withdrawal phase, defined as the period during which the drug is cleared from the body. A recent study examined the long-term KOR-dependent changes associated with protracted withdrawal from ethanol (135). Rats were fed a liquid alcohol diet for 25–30 days, using oral self-administration in a two-bottle choice paradigm. Six weeks following ethanol removal, anxiety-like behaviors (measured immediately following a 20-min restraint stress, in the elevated plus maze) were potentiated in ethanol-abstinent rats. This effect was blocked by nor-BNI pre-treatment 24 h before testing, suggesting that increased stress-reactivity of the dynorphin/KOR system may persist for very long periods following initial ethanol exposure. Our group recently expanded this growing body of evidence to opiate abuse, and implicated KOR in emotional deficits during long-term drug abstinence in mice. We first showed that morphine abstinence progressively leads to the emergence of increased behavioral despair (in the tail suspension test) and social withdrawal (136, 137). Both deficits were detected 4 weeks, but not 1 week, following chronic experimentally delivered high morphine doses. Chronic *per os* treatment with the antidepressant Fluoxetine (a selective serotonin reuptake inhibitor) during the 4-week abstinence period reversed morphine-induced deficits. Further, 5-HT metabolism (136) and 5-HT1A-receptor function (138) were dysregulated during morphine abstinence, in particular in the DRN, suggesting an important contribution of 5-HT mechanisms. Strengthening this model, we characterized a slightly different kinetic pattern using heroin (139): at 4 weeks of abstinence, only social withdrawal was detected in heroin-pretreated mice; at 7 weeks of abstinence, this initial symptom was accompanied by increased behavioral despair (in the FS test). Importantly, we showed that this robust decrease in social interactions (observed across both morphine and heroin abstinence) relies on the activation of both MOR and KOR (139): this phenotype was absent: (i) in cKO mice, in which the MOR was specifically deleted in the DRN prior to heroin treatment; and (ii) in constitutive KOR KO mice. Considering previous data on a 5-HT and DA interplay at the level of the NAc in models of KOR-dependent CPA and cocaine CPP, an interesting possibility is that similar monoamine interactions may contribute to emotional disruption during opiate abstinence, potentially through similar molecular cascades.

An important task for future research will be to explore emotional-like responses in the context of more sophisticated models of addictive-like behaviors. In a phylogenic and translational perspective, and using self-administration paradigms, several groups have been able to transpose DSM-IV addiction criteria into reproducible, drug-induced behavioral abnormalities, including the emergence of compulsive drug-seeking and drug-taking despite adverse consequences (140, 141). We speculate that such aberrant patterns of drug intake may also lead to stronger and more prolonged emotional deficits in rodents, and may represent better models of the emotional comorbidity associated with addiction. Such approaches also have the potential to reveal, in a dimensional approach, the behavioral traits that not only predict the transition to compulsive drug use (such as high impulsivity), but also the risk of emotional comorbidity.

Collectively, the rapidly expanding KOR literature has stimulated great interest in the development of KOR antagonists as pharmacotherapies for depression and anxiety disorders, as well as to improve stress regulation and reduce dysphoria in the context of addiction. Although some KOR ligands have not demonstrated optimal pharmacological properties, others have been shown to be viable drug candidates (142). In summary, KOR antagonists may (i) block stress-induced potentiation of drug consumption, (ii) prevent stress-induced relapse during abstinence periods, and (iii) limit negative emotional states during both acute withdrawal and more prolonged abstinence periods. Although long-term follow-ups and well-controlled studies are methodologically challenging in drug addicts, these results are coherent with a clinical report in depressed opiate abusers of the beneficial effects of buprenorphine, a dual MOR agonist/KOR antagonist (compared to methadone, a pure MOR agonist) (23); another study, however, failed to detect a difference between these two compounds (143). Intensive research in KOR pharmacology has already produced a plethora of short- [Zyklophin (103), LY-2456302 (144, 145)] and long-acting (nor-BNI, GNTI, JDTic) antagonists (146). Future studies will have to carefully analyze their respective signaling properties depending

on the structural conformation they achieve with the KOR, i.e., the promising field of biased agonism [see for example in Ref. (147–150)]. Additional specificities may emerge when comparing KOR signaling across rodent and human species (151), or as a function of genetic polymorphisms (152–154). Also, the recent possibility of studying human KOR *in vivo*, using PET-Scans with the radiotracer 11C-LY2795050 (155), is promising. In the long-term, pharmacogenomic approaches have the potential to predict individualized treatment modalities targeting the KOR, and may therefore become the key to efficient clinical prescriptions.

WHEN DEPRESSION PRECEDES ADDICTION

To address the neurobiological mechanisms of comorbidity between depression and addiction, another complementary approach in animal models is to study how depressive-like states may potentiate behavioral effects and patterns of consumption of drugs of abuse (36, 156). Compared to the inverse causal relationship implicated in comorbidity, the later aspect has been poorly studied, and very few studies have explored the potential role of the KOR.

In this framework, available rodent evidence is inconsistent, and chronic stress-based models of depression have been associated with either increased effects of drugs of abuse (a sensitization of reward pathways that would be consistent with the human comorbidity) or decreased effects. Krishnan et al. (93) showed that mice that are susceptible to chronic social defeat effects, who develop long-lasting depressive-like features, including decreased sucrose preference and social avoidance, also show a significant CPP at cocaine doses that are not reinforcing in undefeated mice, or in defeated but resilient mice.

Chronic mild stress is another model of depression, which is based on the unpredictable exposure of rodents to multiple mild stressors, typically over 4–8 weeks. This model is extensively used because of its face, construct, and predictive validities (157–159). The most common behavioral output in chronic mild stress (CMS) experiments is a decreased preference (over water) for a sucrose solution, or anhedonia. This anhedonic phenotype also seems to extend to the reinforcing properties of drugs of abuse, as decreased CPP for amphetamine (160) and morphine (161) has been reported following CMS in rats. Surprisingly, there is no available study, to our knowledge, on CMS effects in KOR KO mice: is KOR expression potentiated in stressed WT mice? In which brain regions? Would KOR KO mice be protected against the effects of chronic stress? Addressing this gap in the literature, Al-Hasani et al. recently explored the effects on reinstatement of CPP of three stressful modalities: CMS, a "sub-chronic social defeat" (a shorter 5-day form of social defeat), and a single acute FS stress (162). Results showed that, as previously described, acute stress potentiates KOR-mediated reinstatement of cocaine CPP. In contrast, both CMS and sub-chronic social defeat were found to attenuate KOR agonist-dependent reinstatement of cocaine and nicotine CPP. As expected, drug-induced reinstatement of cocaine or nicotine CPP was unaffected by CMS, adding to previous evidence on the specific implication of KOR in stress-induced relapse. These counterintuitive results suggest that, at least in rodent models, CMS may have protective or adaptive effects against drug relapse, a notion that fits poorly with epidemiological and clinical findings in humans.

Overall, we speculate that anhedonia-like behaviors following either CMS or prolonged social defeat may decrease the acute reinforcing properties of drugs of abuse (as assessed using place preference paradigms of drug conditioning, extinction, and relapse), possibly implicating a KOR-dependent mechanism. At the same time, stress-induced anhedonia may also potentiate the emergence of compulsive drug-taking during chronic voluntary consumption of drugs of abuse, hence favoring the entry into addiction. To explore this possibility, future studies will ideally combine two sets of advanced behavioral paradigms: CMS or chronic social defeat first, followed by extended operant drug self-administration. The plethora of cKO mice now available should prove useful in better understanding the role of KOR in these combined preclinical approaches of comorbidity.

FUTURE DIRECTIONS AND CONCLUSION

A major challenge in the future will be to unravel dynamic adaptations of the endogenous dynorphin/KOR system as mood and reward disruption emerge and evolve. This issue is of significant clinical relevance considering the chronicity of these two conditions. In particular, available evidence indicates that the KOR exerts multiple controls over the main monoamines in rodents. Interestingly, addiction research suggests that repeated exposure to drugs of abuse disrupts mutual inhibitory feedback mechanisms between monoaminergic nuclei, which may mediate long-term behavioral dysfunction (163, 164). Whether such mechanisms also impair KOR-dependent mood regulation is an intriguing hypothesis in the context of comorbidity.

Accumulating evidence in the KOR field has recently prompted clinicians to undertake brain imaging studies and clinical trials (22). Very recently, the first PET-Scan study using a radioactive KOR antagonist was able to demonstrate significant and widespread disruption of KOR *in vivo* availability in subjects suffering from fear and dysphoric symptoms following severe trauma exposure (165). While results are nicely consistent with animal data on KOR and the mesolimbic pathway, they also suggest that other brain regions, currently poorly explored in preclinical settings, may be equally important (e.g., thalamus and insular cortex). Additional studies will be required to further assess KOR availability in well-characterized cohorts of depressed, addicted, and comorbid subjects. Finally, from a pharmacological point of view, the rapidly evolving field of biased agonism (or ligand-directed signaling) raises great hopes for KOR-targeting therapeutics (149). A major goal in the field of G-protein-coupled receptors is the identification of distinct signaling pathways that may operate to control specific behavioral responses. In the near future, such approaches will likely aid in the development of antidepressants acting as KOR antagonists and devoid of potentially associated adverse effects (e.g., hyperalgesia).

In conclusion, we have summarized in the present review the large body of evidence supporting the role of KOR in regulating reward and mood. We have also described how this receptor is ideally placed to mediate strong interactions between two frequent and severe psychiatric disorders, addiction, and depression.

Altogether, preclinical research on the KOR exemplifies how transversal studies across multiple animal models have the potential to identify brain mechanisms that contribute to transdiagnostic pathophysiological processes, and therefore represent key therapeutic targets for the management of comorbidity, one of the most prominent global issues in mental health.

ACKNOWLEDGMENTS

The authors thank Dr. Laura Fiori for critical reading of the manuscript. This work was supported by the Agence Nationale de la Recherche (ANR-Abstinence).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 September 2014; accepted: 13 November 2014; published online: 08 December 2014.
*Citation: Lalanne L, Ayranci G, Kieffer BL and Lutz P-E (2014) The kappa opioid receptor: from addiction to depression, and back. Front. Psychiatry **5**:170. doi: 10.3389/fpsyt.2014.00170*
This article was submitted to Molecular Psychiatry, a section of the journal Frontiers in Psychiatry.
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Psychopharmacology (2015) 232:1957–1971
DOI 10.1007/s00213-014-3826-5

ORIGINAL INVESTIGATION

Dissociation of heroin-induced emotional dysfunction from psychomotor activation and physical dependence among inbred mouse strains

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Received: 2 July 2014 / Accepted: 20 November 2014 / Published online: 9 December 2014
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Abstract
Rationale Opiate addiction is a brain disorder emerging through repeated intoxication and withdrawal episodes. Epidemiological studies also indicate that chronic exposure to opiates may lead in susceptible individuals to the emergence of depressive symptoms, strongly contributing to the severity and chronicity of addiction. We recently established a mouse model of heroin abstinence, characterized by the development of depressive-like behaviors following chronic heroin exposure.
Objectives While genetic factors regulating immediate behavioral responses to opiates have been largely investigated, little is known about their contribution to long-term emotional regulation during abstinence. Here, we compared locomotor stimulation and physical dependence induced by heroin exposure, as well as emotional dysfunction following abstinence, across mice strains with distinct genetic backgrounds.
Methods Mice from three inbred strains (C57BL/6J, Balb/cByJ, and 129S2/SvPas) were exposed to an escalating chronic heroin regimen (10–50 mg/kg). Independent cohorts were used to assess drug-induced locomotor activity during chronic treatment, naloxone-precipitated withdrawal at the end of chronic treatment, and emotional-like responses after a 4-week abstinence period.
Results Distinct behavioral profiles were observed across strains during heroin treatment, with no physical dependence and low locomotor stimulation in 129S2/SvPas. In addition, different behavioral impairments developed during abstinence across the three strains, with increased despair-like behavior in 129S2/SvPas and Balb/cByJ, and low sociability in 129S2/SvPas and C57BL/6J.
Conclusions Our results indicate that depressive-like behaviors emerge during heroin abstinence, whatever the severity of immediate behavioral responses to the drug. In addition, inbred mouse strains will allow studying several aspects of mood-related deficits associated with addiction.

Keywords Addiction · Heroin · Abstinence · Depression · Sociability · Physical dependence · Locomotion

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Introduction

Opiates such as heroin remain widely abused drugs (Tarabar and Nelson 2003) with a strong inherent potential for addiction, a complex process developed by contribution of both environmental (Ducci et al. 2009) and genetic (Goldman et al. 2005; Xu et al. 2004) factors. Addiction to opiates is a chronic brain disorder defined mainly as a vicious cycle in which drug intoxication is followed by the emergence, when the drug is no longer available, of a withdrawal state characterized by negative emotions and somatic symptoms. Both intoxication and withdrawal states feed into drug craving, leading to drug-seeking behavior and compulsive drug use (Koob and Le Moal 2008; Lutz and Kieffer 2013). The major challenge to escape from this vicious cycle is to maintain a protracted drug-free or abstinent state. Somatic symptoms of withdrawal diminish in a short period of time, but the aversive emotional state can persist for several months (Jaffe 1990; Martin and Jasinski 1969). Epidemiological retrospective studies clearly document a strong link between opiate addiction and major depressive disorders (Kessler et al. 2005). In particular, mood disorders frequently arise following a history of chronic opiate exposure, in the specific context of abstinence (Nunes et al. 2004; Peles et al. 2007).

Animal models have been instrumental in our understanding of the interactions between environmental and genetic factors that together contribute to the emergence of psychiatric disorders, including substance use disorders (Koob 2008), as well as anxiety or depression (Cryan and Holmes 2005). In particular, these models have been used to explore the emotional consequences of protracted opiate withdrawal. Despair-like behaviors were detected during spontaneous opiate withdrawal, from 6 days (Anraku et al. 2001) up to 2–3 weeks in the rat (Grasing and Ghosh 1998) and after 9 days in the mouse (Hodgson et al. 2009). Building on these initial studies, we established recently a model of opiate abstinence in which depressive-like behaviors emerge during extended withdrawal periods. Following exposure to escalating doses of morphine (Goeldner et al. 2011; Lutz et al. 2011) or heroin (Lutz et al. 2014), abstinent mice progressively developed, over 4 to 7 weeks, increased despair-like behavior and low sociability.

A powerful strategy to probe genetic factors of psychiatric disorders is to study inbred mouse strains, which largely differ across a wide range of behavioral and pharmacological responses (Cryan and Holmes 2005; Vannoni et al. 2014). In particular, inbred mouse strains strongly differ in opiate-induced psychomotor activation (Schlussman et al. 2008; Szumlinski et al. 2005) and physical dependence (Kest et al. 2002; Klein et al. 2008b). In the present study, we aimed to explore the contribution of genetic background to behavioral consequences of heroin exposure, during both chronic treatment and abstinence from the drug. We assessed locomotor stimulation and physical dependence induced by chronic heroin treatment, as well as long-term emotional-like consequences of abstinence, across three inbred strains: C57BL/6J mice (B6, as in our previous work (Lutz et al. 2014)), Balb/cByJ mice (Balb), and 129S2/SvPas mice (SvPas). Our results indicate that increased behavioral despair or low sociability develops during abstinence in a strain-dependent manner, even in the SvPas strain that exhibits low levels of physical dependence and locomotor sensitivity to heroin.

Methods

Subjects Eight-week-old male B6, Balb, and SvPas (Charles River Laboratories, France) mice were habituated to housing during 2 weeks (4/cage; 12H light/dark cycle; food and water ad libitum). Experimental procedures followed ethical guidelines (IGBMC ComEth saisine 2012-0126) and have been conducted at the ICS (http://www.ics-mci.fr/service_neurobiology_behaviour_tests.html).

Drugs Heroin (Francopia, France) and naloxone hydrochlorides (Sigma Aldrich, France) were prepared in saline solution (0.9 % NaCl) and injected at a volume of 10 ml/kg.

Heroin regimen Mice received intraperitoneal (IP) twice daily (8 AM–6 PM) injections with escalating doses of heroin (10 to 50 mg/kg for 5 days, followed by a single 50 mg/kg injection on day 6), or saline solution as control. To avoid potentially reciprocal social influences between saline- and heroin-treated mice (Cole et al. 2012; Hofford et al. 2011), each housing cage contained only saline-treated or only heroin-treated mice. Independent groups of mice from each strain were used to measure drug-induced locomotion during the heroin treatment (Experiment 1), physical dependence at the end of heroin treatment (Experiment 2), and emotional-like responses after 4 weeks of abstinence, in animals that experienced spontaneous withdrawal from heroin (i.e., in the absence of naloxone injection, Experiment 3). Mice were weighed during heroin treatment and abstinence (Fig. 1 and Table 1). Note that data for B6 in Experiments 2 and 3 have been partly reported in a recent study (Lutz et al. 2014) and are shown here for comparison.

Locomotor activity during heroin regimen (Experiment 1) After PM heroin injections on days 1, 3, and 5, mice were placed individually in an actimeter chamber (Imetronic, France) equipped with two infrared beams. Locomotor activity was measured as the number of crossovers (successive beam breaks) during an overnight session (food and water being provided ad libitum). Mice remained in their home cages after AM injections, as well as after PM injections on days 2 and 4. In both actimeter chambers and housing cages, light was switched on and off at 7 AM and 7 PM.

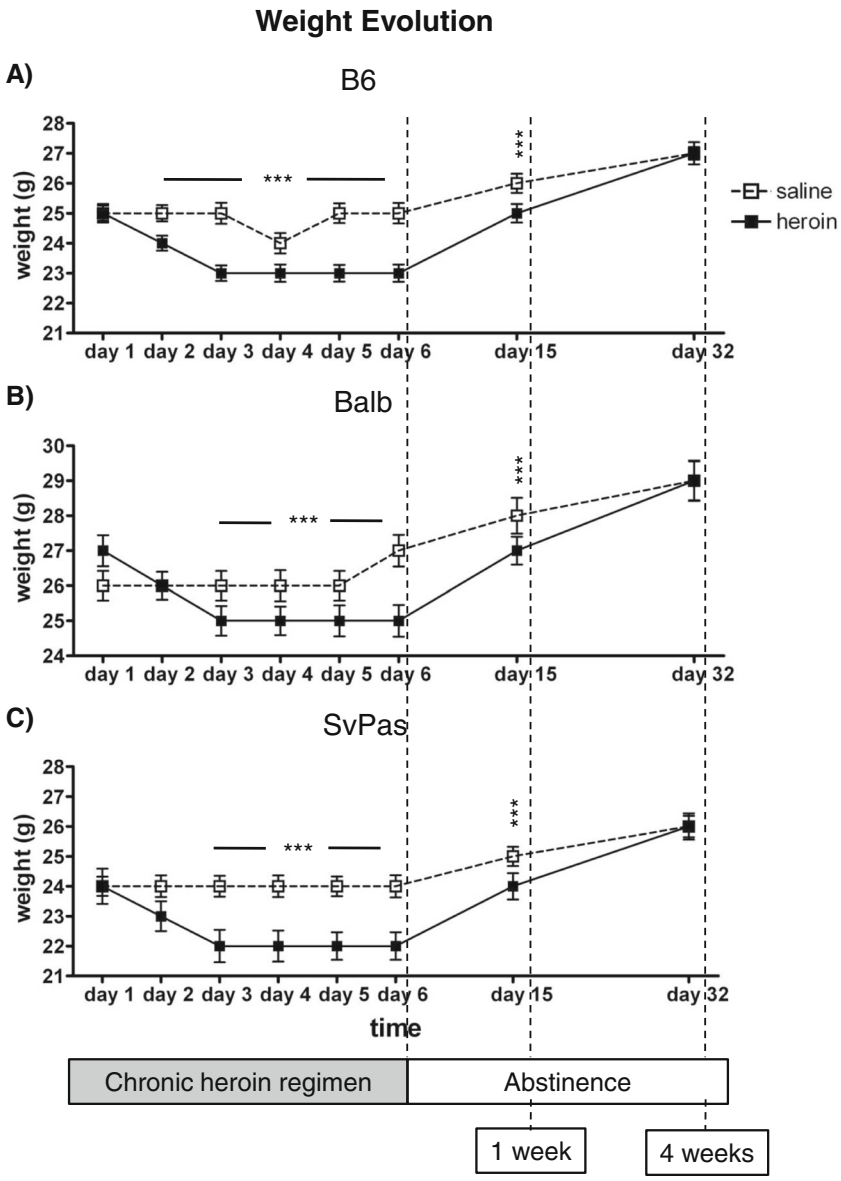

Fig. 1 Weight evolution during chronic heroin treatment and 4-week abstinence in three inbred mouse strains. As an index of general opiate effects, B6 (n=24 mice/group, data from (Lutz et al. 2014), Balb and SvPas (n=12 mice/group/strain) were weighed daily during chronic treatment, and after 1 week and 4 weeks. This figure depicts results from Experiment 3. Raw data for experiments 2 and 3, as well as statistical results from all three experiments are summarized in Table 1. Overall, chronic heroin treatment produced significant body weight loss across the three strains studied, with rapid recovery of normal weight during the first few weeks of abstinence. Accordingly, heroin-treated mice of each strain (**a**–**c**) showed significantly decreased body weights compared with their saline-treated counterparts during chronic treatment and after 1 week of abstinence, but not after 4 weeks of abstinence. Data are mean±SEM. ***p<0.001, ANOVA main effect of treatment

Naloxone-precipitated withdrawal (Experiment 2) Physical dependence to heroin was assessed by a subcutaneous (SC) injection of 1 mg/kg naloxone, 1 h after the last heroin or saline IP injection on day 6 (Matthes et al. 1996). Mice were placed in a Plexiglas box (30×15×15 cm) and upon naloxone administration; withdrawal signs were scored for 20 min, and a global withdrawal score was calculated for each mouse as described previously (Le Merrer et al. 2011).

Emotional consequences of heroin abstinence (Experiment 3) Our previous studies showed that, following morphine (Goeldner et al. 2011) or heroin (Lutz et al. 2014) treatment, emotional deficits progressively strengthen with time and are stronger after 4 than after 1 week of abstinence. Therefore, in the present work, following heroin treatment, mice experienced spontaneous withdrawal (i.e., in the absence of naloxone injection) and were left drug-free during 4 weeks in their home cages. Behavioral testing was then performed every other day (except for open-field (OF) and social interactions (SI) performed in two consecutive days) in the following order: light/dark (LD, Crawley and Goodwin 1980), OF (Crawley 1985), SI (File and Hyde 1978), tail suspension (TS, Steru et al. 1985), forced swim (FS, Porsolt et al. 1977), Y-maze (YM, Sarter et al. 1988), and sucrose preference (SP, Pothion et al. 2004; Willner et al. 1987), as previously described (Lutz et al. 2014).

Sucrose Preference. All sessions were conducted overnight. On the first session, mice were habituated to individual housing with access to two bottles containing water. On the following two sessions, one bottle was randomly exchanged with a bottle containing 0.8% sucrose solution. For SvPas mice, two additional

Table 1 Weight analysis during chronic heroin treatment and abstinence in three inbred mouse strains. B6, Balb, and SvPas mice were exposed to heroin during 5 (Experiment 1) or 6 (Experiments 2 and 3) days (see "Methods" and Fig. 1)

		C57BL/6J		Balb/cJ		129Sv/Pas	
		Saline	Heroin	Saline	Heroin	Saline	Heroin
Day 1	Exp. 1	24±0.66	23±0.39	28±0.61	26±0.85	26±1.67	24±0.81
	Exp. 2	24±0.30	25±0.46	28±0.67	28±0.33	25±0.35	26±0.40
Day 2	Exp. 1	24±0.68	22±0.53	28±0.66	25±0.85	24±1.59	22±0.74
	Exp. 2	24±0.32	24±0.43	28±0.65	27±0.28	25±0.31	26±0.33
Day 3	Exp. 1	24±0.63	22±0.24	28±0.56	25±0.80	25±1.63	22±0.59
	Exp. 2	24±0.32	23±0.41	28±0.83	26±0.28	26±0.32	25±0.38
Day 4	Exp. 1	25±0.67	21±0.26	28±0.49	24±0.91	24±1.69	21±0.73
	Exp. 2	24±0.28	23±0.38	29±0.86	26±0.23	25±0.36	25±0.33
Day 5	Exp. 1	24±0.69	21±0.25	28±0.51	24±0.88	25±1.71	22±0.71
	Exp. 2	24±0.31	23±0.38	29±0.75	25±0.24	26±0.40	25±0.35
Day 6	Exp. 2	24±0.32	22±0.34	28±0.81	25±0.17	26±0.41	25±0.29
	Three-way ANOVA (F; p values)						
		Time	Treatment		Genotype	Interaction	
	Exp. 1	$F(5.170)=37.8$; $p<0.001$	$F(1, 34)=15.3$, $p<0.001$		$F(2.34)=7.7$; $p=0.002$	$F(2.34)=0.2$; $p=0.85$	
	Exp. 2	$F(5, 240)=104.3$; $p<0.001$	$F(1.48)=11.8$; $p=0.001$		$F(2, 48)=35.1$; $p<0.001$	$F(2, 48)=3.1$; $p=0.06$	
	Exp. 3 (Fig. 1)	$F(7, 616)=146.9$; $p<0.001$	$F(1, 88)=11.1$, $p=0.001$		$F(2, 88)=27.3$; $p<0.001$	$F(2, 88)=0.6$; $p=0.54$	

As an index of general opiate effect, mice were weighed daily during chronic heroin treatment, and after 1 week and 4 weeks of abstinence (see also Fig. 1). Consistently in all three experiments, we found main effects of time, strain, and treatment, with no interaction between the two latter factors. *post-hoc* analyses for Experiments 1 and 2 revealed that heroin treatment reduced body weight across three strains ($p<0.001$) and that Balb mice showed overall higher body weight compared with B6 and SvPas ($p<0.001$). B6 data in experiments 2 and 3, but not Experiment 1, have been previously reported (Lutz et al. 2014) and are shown here for comparison. Data are mean±SEM

sessions with a 5.5 % sucrose concentration were performed to reach a significant preference over water. Sucrose bottles were counterbalanced and alternated in position over successive sessions. Mice were placed back in their home cages during daytime. Bottles were weighed before and after each session, and SP was defined as: SP=[(sucrose solution intake)/(total fluid intake)]×100.

Statistical analysis Statistical analysis was performed using two-way analysis of variance (ANOVA) with strain and treatment as independent variables. For locomotor activity and weight analyses, we used a three-way mixed ANOVA, with treatment and strain as between-subject factors and dose or time as within-subject factors. When significant interactions were detected between main variables (including for *post-hoc* inter-strain comparisons), *post-hoc* analyses were performed using Fisher's PLSD test. Statistical significance was defined as $p<0.05$.

Results

Experiment 1. We investigated locomotion on alternate days of the chronic heroin treatment (at 10-, 30-, and 50-mg/kg heroin doses). Analysis of crossover counts (Fig. 2a) revealed significant effects of treatment [$F(1, 34)=209.65$; $p<0.001$], strain [$F(2, 34)=41.29$; $p<0.001$], and dose [$F(2, 68)=42.09$; $p<0.001$]. Heroin-treated mice of each strain showed increased crossover counts compared with their saline-treated counterparts, and this increase was in proportion to dose (treatment×dose interaction [$F(2, 68)=42.09$; $p<0.001$]). There was an interaction [$F(2, 34)=34.76$; $p<0.001$] between treatment and strain, suggesting that heroin-induced increase in locomotion is strain-dependent. *post-hoc* analysis showed that crossover counts did not differ among saline-treated groups ($p=0.91$ for Balb/B6, $p=0.56$ for Balb/SvPas, $p=0.47$ for B6/SvPas comparisons) while, in heroin-treated mice, crossover count was the highest in Balb and the lowest in SvPas ($p<0.001$). *post-hoc* comparisons also revealed significant differences across the three heroin doses ($p=0.04$ for 10 vs. 30 mg/kg; $p<0.001$ for 10 vs. 50 mg/kg; $p=0.047$ for 30 vs. 50 mg/kg) in heroin-treated B6. In heroin-treated Balb, analysis showed a significant difference between 10 and 30 mg/kg ($p<0.001$), and between 10 and 50 mg/kg ($p<0.001$) heroin doses without a significant difference between 30 and 50 mg/kg ($p=0.09$). In contrast, there was no significant difference between heroin doses in SvPas ($p=0.60$ for 10 vs. 30 mg/kg; $p=0.15$ for 10 vs. 50 mg/kg; $p=0.34$ for 30 vs. 50 mg/kg). Therefore, our escalating heroin regimen did not produce increasing hyperlocomotor effects in SvPas, in contrast with Balb and B6.

We then analyzed the kinetic of heroin-induced locomotion in each strain (Fig. 2b, c, d). Only the first 5 h of actimeter sessions are represented, since heroin effect was no longer present after this time point in either strain. Analysis of cross-over counts for each individual strain revealed significant effects of treatment [B6, $F(3, 24)=16.12$; Balb, $F(3, 20)=45.06$; SvPas, $F(3, 24)=5.35$; $p<0.001$] and time [B6, $F(9, 216)=39.94$; Balb, $F(9, 180)=87.39$; SvPas, $F(9, 216)=21.11$; $p<0.001$] with significant interactions [B6, $F(27, 216)=6.88$; Balb, $F(27, 180)=17.78$; SvPas, $F(9, 216)=2.76$; $p<0.001$]. *post-hoc* analysis indicated that, in B6, heroin-induced increase in locomotion was significant for 90 min at 10 mg/kg, for 120 min at 30 mg/kg, and for 270 min at 50 mg/kg heroin doses. In Balb, this effect was significant for 150 min at 10-mg/kg, for 240 min at 30-mg/kg, and for 300 min at 50-mg/kg heroin doses. In SvPas, this effect was significant for 90 min at 10-mg/kg, for 120 min at 30-mg/kg, and for 210 min at 50-mg/kg heroin doses, indicating that, as expected, higher doses achieved longer locomotor effects across the three strains.

Experiment 2 To investigate physical dependence to heroin, withdrawal was precipitated using naloxone (see Table 2 for statistical analysis of main effects). Analysis of jumps (Fig. 3a) revealed a main effect of strain with no effect of treatment and no interaction between treatment and strain. *post-hoc* strain comparisons showed that Balb presented higher number of jumps compared with SvPas ($p=0.02$) but not B6 ($p=0.13$). There was no difference in the number of jumps between B6 and SvPas ($p=0.22$).

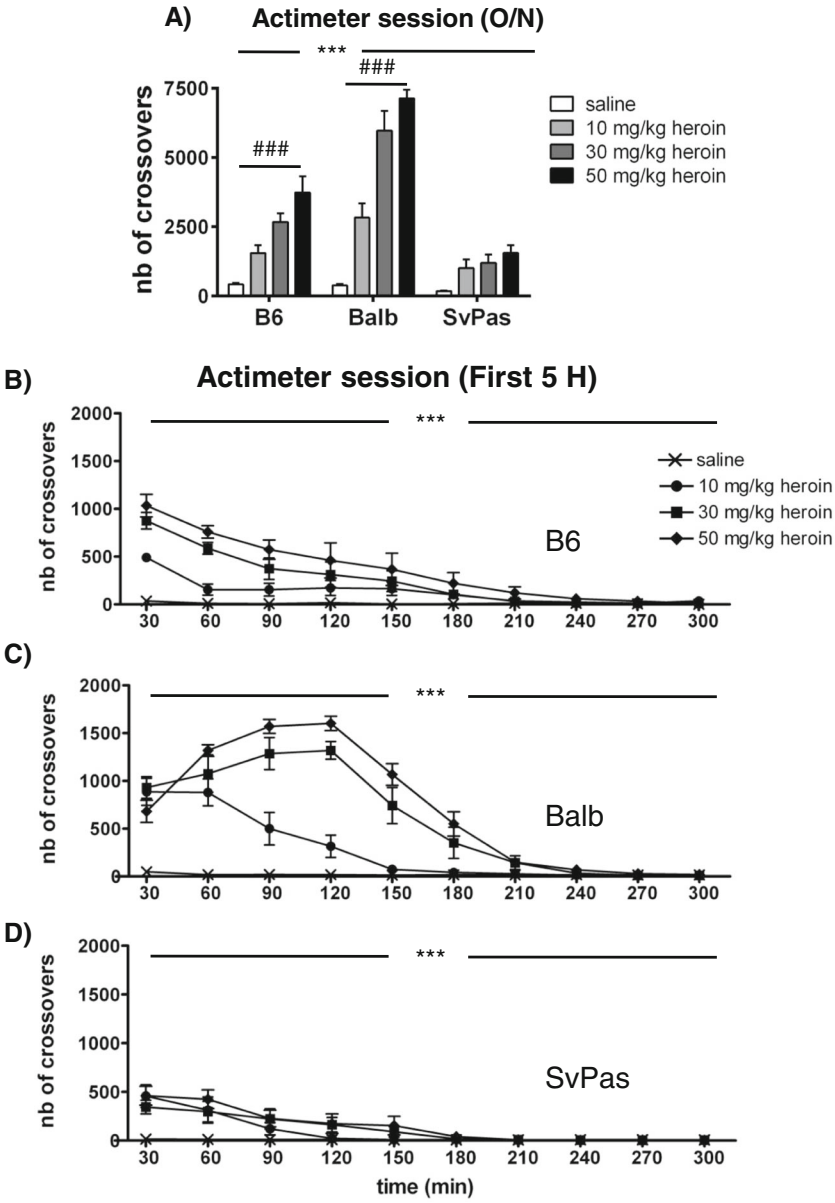

Fig. 2 Heroin-induced locomotor stimulation in three inbred mouse strains. B6, Balb, and SvPas mice received chronic injections with increasing doses of heroin (10, 20, 30, 40, and 50 mg/kg, IP, twice daily) or saline (n=6–7 mice/group/strain) during 5 days, and their locomotor responses were measured every other day during overnight actimeter sessions. Heroin dose-dependently increased the total number of crossovers (**a**) across the three strains. Analysis of first 5 h of locomotor responses (**b**–**d**) to heroin showed the dose-dependent locomotor stimulation in mice from each strain. Data are mean±SEM. ***$p<0.001$, ANOVA, main effect of heroin treatment. #$p<0.05$, ###$p<0.001$, *post-hoc* comparisons of heroin dose

Table 2 Signs of naloxone-precipitated withdrawal from chronic heroin treatment in three mouse strains

	C57BL/6J		Balb/cJ		129Sv/Pas	
	Saline	Heroin	Saline	Heroin	Saline	Heroin
Wet dog shakes	0.9±0.4	5.9±1.1$^{\#\#\#}$	0.1±0.1	5.3±1.2$^{\#\#\#}$	0±0	0±0
Scratching	1.8±1	1.6±1	0±0	0±0	0±0	0±0
Sniffing	0±0	4.6±1.1$^{\#\#\#}$	0±0	2.6±0.8$^{\#\#}$	0±0	0±0
Body tremor	0.1±0.1	1±0.4$^{\#\#}$	0.7±0.4	2.1±0.3$^{\#\#\#}$	0±0	0±0
Ptosis	0±0	1.4±0.5$^{\#\#\#}$	0±0	0.7±0.3$^{\#}$	0±0	4±0$^{\#\#\#}$
Teeth chattering	0±0	3.8±0.2$^{\#\#\#}$	0±0	0±0	0±0	0.4±0.3$^{\#}$
Piloerection	0.3±0.1	3.9±0.1$^{\#\#\#}$	0.4±0.2	1.3±0.4$^{\#}$	1±0.7	3.9±0.1$^{\#\#\#}$
	Two-way ANOVA (F; p values)					
	Treatment		Genotype		Interaction	
Global withdrawal score	$F(1, 48)=259.9$; $p<0.001$		$F(2, 48)=65.5$; $p<0.001$		$F(2, 48)=42.6$; $p<0.001$	
Wet dog shakes	$F(1, 48)=35.3$; $p<0{,}001$		$F(2, 48)=12.9$; $p<0{,}001$		$F(2, 48)=8.1$; $p<0{,}001$	
Paw tremors	$F(1, 48)=203.1$; $p<0.001$		$F(2, 48)=72.6$; $p<0.001$		$F(2, 48)=44.3$; $p<0.001$	
Scratching	$F(1, 48)=0.5$; $p=0.48$		$F(2, 48)=1.2$; $p=0.31$		$F(2, 48)=0.4$; $p=0.71$	
Sniffing	$F(1, 48)=32.9$; $p<0.001$		$F(2, 48)=10.3$; $p<0.001$		$F(2, 48)=10.3$; $p<0.001$	
Jumping	$F(1, 48)=1.5$; $p=0.22$		$F(2, 48)=3.3$; $p=0.047$		$F(2, 48)=2.9$; $p=0.061$	
Body tremor	$F(1, 48)=12.9$; $p<0.001$		$F(2, 48)=14.7$; $p<0.001$		$F(2, 48)=3.3$; $p=0.045$	
Ptosis	$F(1, 48)=128.5$; $p<0.001$		$F(2, 48)=27.5$; $p<0.001$		$F(2, 48)=27.5$; $p<0.001$	
Teeth chattering	$F(1, 48)=201.9$; $p<0.001$		$F(2, 48)=149.8$; $p<0.001$		$F(2, 48)=149.8$; $p<0.001$	
Piloerection	$F(1, 48)=105.3$; $p<0.001$		$F(2, 48)=15.9$; $p<0.001$		$F(2, 48)=12.5$; $p<0.001$	

B6, Balb, and SvPas (n=7–8 mice/group/strain) were exposed to escalating heroin treatment or saline during 6 days. One hour after the last injection on day 6, mice were injected subcutaneously with 1 mg/kg naloxone, and several withdrawal signs were observed and summed up to a global score (see also Fig. 3). B6 data have been previously reported (Lutz et al. 2014) and are shown here for comparison. Data are mean±SEM

#$p<0.05$, ##$p<0.01$, ###$p<0.001$, *post-hoc* comparisons for the effect of heroin treatment in each strain

Contrasting with our previous study using B6 mice (Lutz et al. 2014), precipitated withdrawal across three strains did not lead to a global increase in the number of jumps. Therefore, studying genetic factors that influence physical dependence to opiates requires either (1) the use of much higher naloxone doses (e.g., 50 mg/kg,

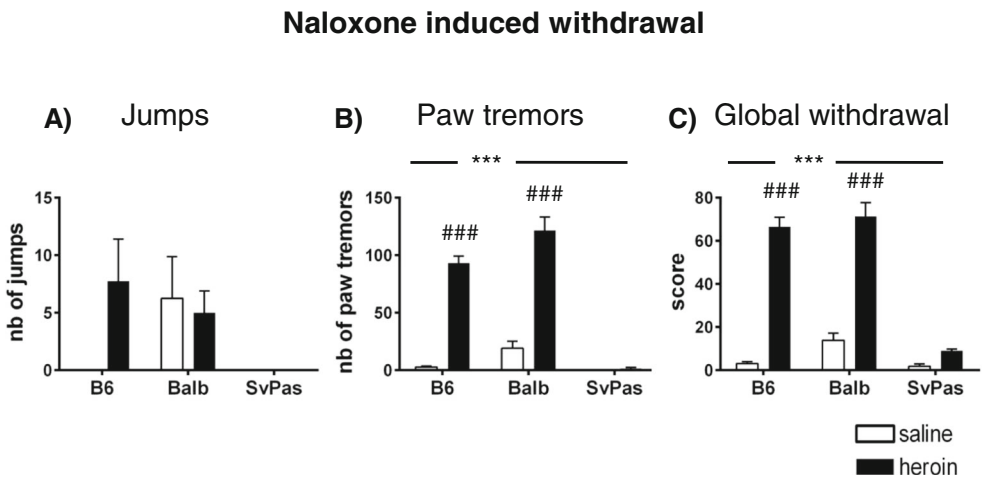

Fig. 3 Naloxone-precipitated heroin withdrawal in three inbred mouse strains. B6 (data from (Lutz et al. 2014), Balb, and SvPas were exposed to chronic heroin treatment or saline (n=7–8 mice/group/strain) during 6 days (10, 20, 30, 40, and 50 mg/kg, IP, twice daily with single IP on day 6). One hour after the last injection on day 6, mice were injected subcutaneously with 1 mg/kg naloxone, and several withdrawal signs were observed and combined into a global score (see "Methods"). Naloxone administration induced jumps (**a**) in heroin-treated B6, and both saline- and heroin-treated Balb. No jumps were observed in SvPas. Naloxone administration increased the number of paw tremors (**b**) in heroin-treated B6 and Balb, but not in heroin-treated SvPas. **c** Heroin treatment induced physical dependence in B6 and Balb but not in SvPas. Significant effects of strain are depicted in the text. Data are mean±SEM. ***$p<0.001$, ANOVA, main effect of heroin treatment; ###$p<0.001$, *post-hoc* comparisons for the effect of treatment in each strain

(Klein et al. 2008b)) , when only jumps are quantified or (2) the observation of several withdrawal signs, as shown below.

We also analyzed the number of paw tremors (Fig. 3b) and found a main effect of strain. *post-hoc* strain comparisons showed that Balb and SvPas exhibited the highest and the lowest numbers of paw tremors, respectively ($p<0.001$). We also found an effect of treatment with an interaction between treatment and strain. *post-hoc* analysis indicated that heroin-treated B6 and Balb showed significantly higher number of paw tremors than their saline-treated counterparts ($p<0.001$); with heroin-treated Balb showing higher number of paw tremors than heroin-treated B6 ($p=0.001$). The number of paw tremors in heroin-treated SvPas was similar to their saline-treated counterparts ($p=0.86$) and was strikingly lower than in heroin-treated mice of the other two strains ($p<0.001$ for both comparisons). When comparing saline-treated groups, a small significant increase of paw tremor was found in Balb ($p=0.022$ for Balb/B6, $p=0.025$ for Balb/SvPas), suggesting that this strain shows higher baseline (i.e., in the absence of heroin) sensitivity to naloxone.

Analysis of global withdrawal scores (GWS, Fig. 3c) revealed main effects of strain and treatment, with an interaction, suggesting that, as expected, the severity of heroin physical dependence is strain-dependent. *post-hoc* analysis showed that GWS of heroin-treated B6 and Balb were significantly higher than their saline-treated counterparts ($p<0.001$) but were similar ($p=0.30$) among each other. In contrast, heroin-treated SvPas showed GWS that was not different from their saline-treated counterparts ($p=0.16$), and that was strikingly lower ($p<0.001$) compared with heroin-treated B6 and Balb. Similar to paw tremor results, *post-hoc* analysis of saline-treated groups revealed higher baseline sensitivity to naloxone in Balb.

Experiment 3 Statistical analyses of main effects are summarized in Table 3.

LD. Following 4 weeks of abstinence, we analyzed the behavior of saline- and heroin-treated mice. Analysis of the number of entries into compartments (Fig. 4a) showed no significant effect of either strain or heroin treatment, and no interaction. Analysis of the percentage of time spent in the lit compartment (Fig. 4b) revealed a main effect of strain but no effect of treatment and no interaction. *post-hoc* strain comparisons indicated that SvPas showed the highest level of anxiety

Table 3 Results from statistical analyses of heroin treatment, mouse strain, and interaction effects in behavioral paradigms performed during experiment 3 (Figs. 4, 5, and 6)

	Two-way ANOVA (F; p values)				
			Treatment	Genotype	Interaction
LD	Entries		$F(2, 65)=0.88$; $p=0.42$	$F(2, 65)=0.05$; $p=0.82$	$F(2, 65)=1.19$; $p=0.31$
	% Time in lit compartment		$F(1, 65)=0.44$; $p=0.51$	$F(2, 65)=26.58$; $p<0.001$	$F(2,65)=1.01$; $p=0.37$
OF	Distance travelled		$F(1, 89)=0.34$; $p=0.56$	$F(2, 89)=149.88$; $p<0.001$	$F(2, 89)=0.47$; $p=0.63$
	% Time in center		$F(1, 89)=2.02$; $p=0.16$	$F(2, 89)=39.98$; $p<0.001$	$F(2, 89)=1.90$; $p=0.16$
SI	Sniffing	Duration	$F(1, 41)=21.6$; $p<0.001$	$F(2, 41)=44.8$; $p<0.001$	$F(2, 41)=6.3$; $p=0.004$
		Counts	$F(1, 41)=47.8$; $p<0.001$	$F(2, 41)=11.1$; $p<0.001$	$F(2, 41)=5.1$; $p=0.01$
	Following	Duration	$F(1, 41)=2.4$; $p=0.13$	$F(2, 41)=7.4$; $p=0.002$	$F(2, 41)=2.2$; $p=0.13$
		Counts	$F(1, 41)=3.6$; $p=0.06$	$F(2, 41)=4.6$; $p=0.02$	$F(2, 41)=1.2$; $p=0.30$
	Paw contact	Duration	$F(1, 41)=3.0$; $p=0.09$	$F(2, 41)=9.3$; $p<0.001$	$F(2, 41)=4.0$; $p=0.03$
		Counts	$F(1, 41)=2.2$; $p=0.14$	$F(2, 41)=8.4$; $p=0.001$	$F(2, 41)=6.2$; $p=0.004$
	Social behaviors	Duration	$F(1, 41)=16.2$; $p<0.001$	$F(2, 41)=37.82$; $p<0.001$	$F(2, 41)=7.48$; $p=0.002$
		Counts	$F(1, 41)=35.4$; $p<0.001$	$F(2, 41)=15.6$; $p<0.001$	$F(2, 41)=6.8$; $p=0.003$
	Self-grooming	Duration	$F(1, 41)=5.2$; $p<0.001$	$F(2, 41)=68.65$; $p<0.001$	$F(2, 41)=26.43$; $p<0.001$
		Counts	$F(1, 41)=2.1$; $p=0.15$	$F(2, 41)=51.5$; $p<0.001$	$F(2, 41)=5.7$; $p=0.006$
TS	Immobility (s)		$F(1, 89)=0.54$; $p=0.46$	$F(2, 89)=3.42$; $p=0.04$	$F(2, 89)=2.62$; $p=0.08$
	Latency to first immobilization		$F(1, 89)=5.10$; $p=0.023$	$F(2, 89)=5.01$; $p=0.008$	$F(2, 89)=4.26$; $p=0.017$
FS	Immobility (s)		$F(1, 89)=19.82$; $p<0.001$	$F(2, 89)=37.15$; $p<0.001$	$F(2, 89)=4.80$; $p=0.01$
	Latency to first immobilization		$F(1, 89)=20.91$; $p<0.001$	$F(2, 89)=18.57$; $p<0.001$	$F(2, 89)=6.42$; $p=0.003$
YM	% Spontaneous alternation		$F(1, 79)=1.85$; $p=0.18$	$F(2, 79)=14.30$; $p<0.001$	$F(2, 79)=2.88$; $p=0.06$
SP	% Sucrose preference		$F(1, 84)=0.93$; $p=0.34$	$F(2, 84)=39.54$; $p<0.001$	$F(2, 84)=0.95$; $p=0.39$

B6 ($n=24$ mice/group), Balb and SvPas ($n=12$ mice/group/strain) were treated with increasing doses of heroin or saline during 6 days, followed by 4 weeks of spontaneous withdrawal (i.e., without any naloxone injection at the end of the chronic heroin treatment). B6 data have been previously reported (except for LD, see Lutz et al. 2014) and are shown here for comparison

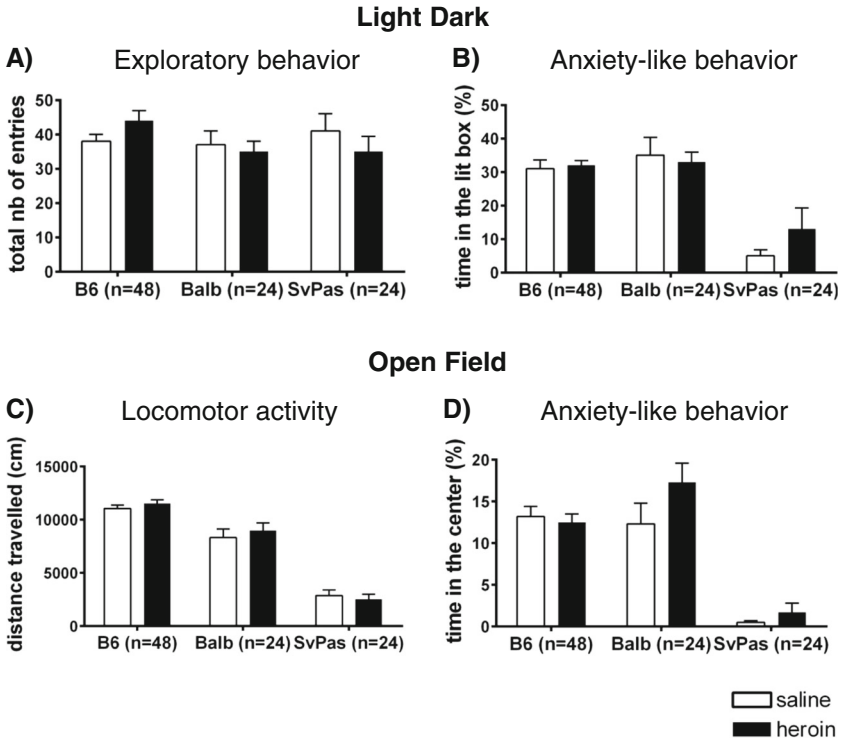

Fig. 4 Anxiety-like behaviors during heroin abstinence in three inbred mouse strains. B6 (n=24 mice/group, data from (Lutz et al. 2014), Balb and SvPas (n=12 mice/group/strain) were treated with increasing doses of heroin or saline during 6 days (10, 20, 30, 40, and 50 mg/kg, IP, twice daily with single IP on day 6). After 4 weeks of abstinence (i.e., no naloxone injection at the end of the chronic heroin treatment), we analyzed anxiety-like (LD and OF) and social behaviors. Heroin abstinence had no effect on total number of entries to each compartment (**a**) or on the time spent in the lit compartment (**b**) in the light/dark box. Similarly, heroin abstinence did not affect the distance traveled (**c**) or the time spent in the center zone (**d**) of the OF. Significant effects of strain are depicted in the text. Data are mean±SEM

against either B6 or Balb (p<0.001) with no difference between B6 and Balb (p=0.57).

OF. Analysis of locomotor activity (Fig. 4c, distance traveled) indicated a significant effect of strain, with no effect of heroin treatment and no interaction. *post-hoc* strain comparisons showed that locomotor activity was the lowest in SvPas and the highest in B6 (p<0.001). We also analyzed the percentage of time spent in the center of the OF (Fig. 4d) and found a main effect of strain with no effect of treatment or interaction. Post hoc strain comparisons indicated that the anxiety-like behavior was the highest in SvPas (p<0.001) and similar between B6 and Balb (p=0.19).

SI. We assessed sociability by measuring the duration and counts of distinct and total social behaviors, as well as self-grooming (Fig. 5). Heroin abstinence had potent effects on several parameters, as summarized in Table 3. *post-hoc* analyses found decreased duration and counts of sniffing (Fig. 5a and b) in B6 (p<0.001) and SvPas (p=0.03 for duration, p<0.001 for counts), but not in Balb mice (p=0.67 for duration, p=0.21 for counts). Heroin abstinence reduced both duration and counts of the following behavior (Fig.5c and d) in B6 (p=0.003 for duration, p=0.006 for counts) but not in either Balb or SvPas. Similarly, both the duration and counts of the paw contact behavior (Fig. 5e and f) were lower in B6 only (p<0.001), with no effect in Balb or SvPas strains.

Analysis of the duration of these three social behaviors pooled together (Fig. 5g) showed that strain had a strong effect on social interaction time, with B6 showing higher duration than Balb and SvPas (p<0.001), and with no difference between Balb and SvPas (p=0.61). Analysis also revealed a main effect of treatment and an interaction. *post-hoc* comparisons showed that heroin abstinence reduced the duration of social behaviors in B6 (p<0.001) but not in Balb (p=0.79) or SvPas (p=0.15). We also counted the number of social behaviors (Fig. 5h) and found a significant effect of strain. *post-hoc* strain comparisons showed that the number of social behaviors was higher in B6 compared with either Balb (p=0.004) or SvPas (p<0.001). We also found a significant effect of treatment and an interaction. *post-hoc* comparisons revealed that heroin abstinence decreased the number of social behaviors in both B6 (p<0.001) and SvPas (p=0.004) but not in Balb (p=0.48).

Analysis of the duration of self-grooming (Fig. 5i) found an effect of strain, with B6 showing the highest (p<0.001) and Balb showing the shortest (p=0.029, Balb/SvPas) duration. We also found an effect of treatment and an interaction. Post hoc comparisons indicated that heroin abstinence increased the duration of self-grooming in B6 (p<0.001) but not in SvPas (p=0.09) or Balb (p=0.52). Analysis of the number of self-grooming episodes (Fig. 5j) revealed an effect of strain. *post-hoc* strain comparisons showed that self-grooming counts were higher in B6 compared with either Balb or SvPas (p<0.001), with no difference between the two latter strains (p=0.09). Our results also showed a treatment×strain interaction. *post-hoc* comparisons revealed that heroin abstinence increased self-grooming counts in B6 (p<0.001) but not in either Balb (p=0.94) or in SvPas (p=0.47).

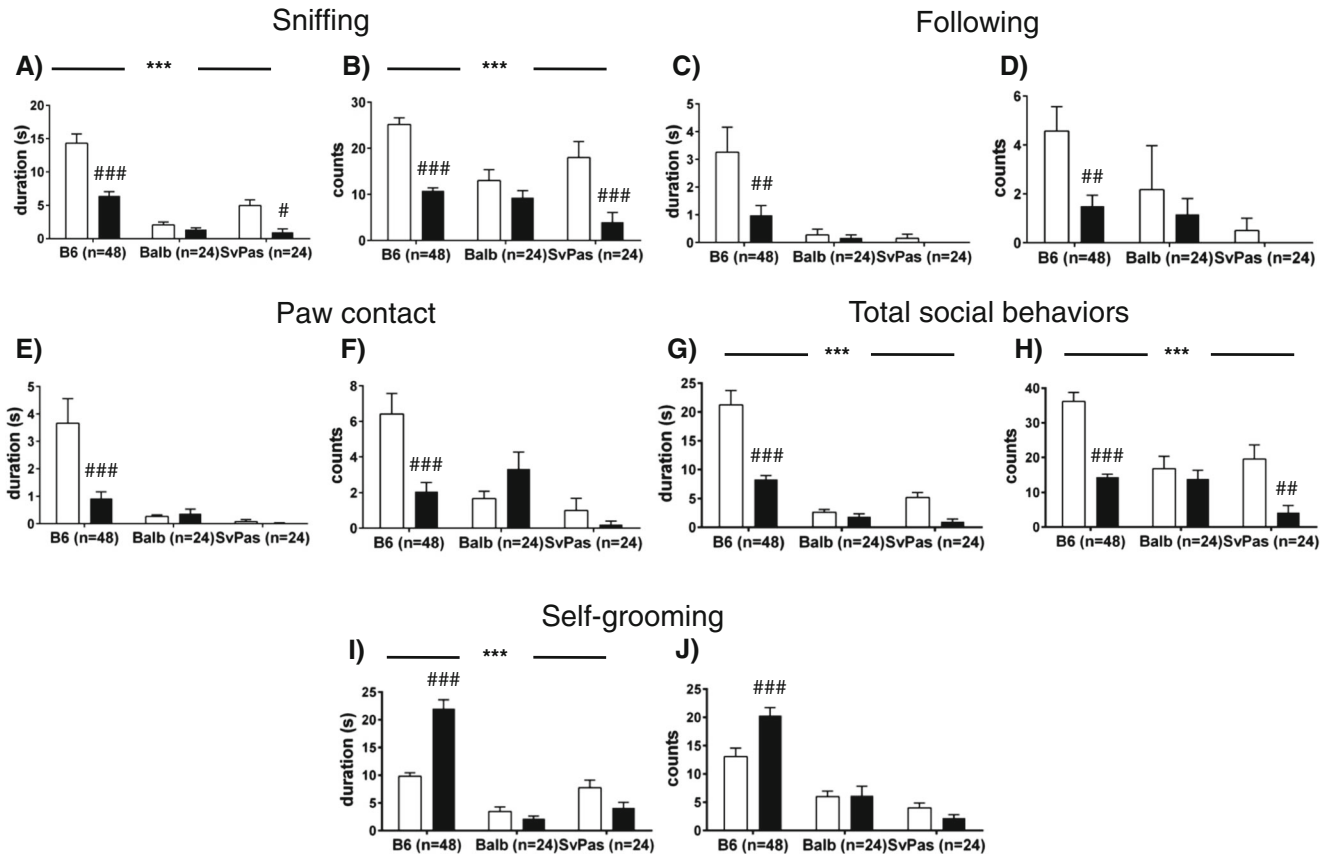

Fig. 5 Social behaviors during heroin abstinence in three inbred mouse strains. B6 (n=24 mice/group, data from (Lutz et al. 2014)), Balb (n=12 mice/group/strain) and SvPas (n=10 mice/group/strain) were treated with increasing doses of heroin or saline during 6 days (10, 20, 30, 40, and 50 mg/kg, IP, twice daily with single IP on day 6; same animal cohorts as in Fig. 4). After 4 weeks of abstinence (i.e., no naloxone injection at the end of the chronic heroin treatment), we measured the duration and counts of three types of social behaviors, as well as self-grooming. Statistical results are summarized in Table 3. Heroin abstinence decreased both the duration (**a**) and counts (**b**) of sniffing in B6 and SvPas, but not in Balb mice. Heroin abstinence also reduced both the duration and counts of the following (**c**+**d**) and paw contact (**e**+**f**) parameters in B6 mice, while these effects were absent in the two other strains. When considering all three parameters together, heroin abstinence reduced the total duration (**g**) of social behaviors only in B6, while it reduced the counts (**h**) of social behavior in both B6 and SvPas. Heroin abstinence increased both the total duration (**i**) and counts (**j**) of self-grooming in B6 mice, with no effects in Balb or SvPas. Significant effects of strain are depicted in the text. Data are mean± SEM. *** p<0.001, ANOVA, main effect of heroin abstinence; #p<0.05, ## p<0.01, ###p<0.001, *post-hoc* comparisons for the effect of heroin abstinence in each strain

TS. Analysis of immobility (Fig. 6a) found a main effect of strain with no effect of treatment and no interaction. *post-hoc* comparisons showed that immobility duration was higher in B6 compared to Balb (p=0.01) but not to SvPas (p=0.21), with no difference between the two latter strains (p=0.26).

Analysis of latency to first immobility (Fig. 6b) showed a significant effect of strain. *post-hoc* strain comparisons showed that SvPas had reduced latency compared with B6 (p=0.037) and Balb (p=0.003) with no difference between the two latter strains (p=0.14). We also found an effect of treatment and an interaction. *post-hoc* comparisons indicated that heroin abstinence decreased the latency to first immobilization in both Balb (p=0.038) and SvPas (p=0.026) but not in B6 (p=0.31).

FS. Despair-like behaviors were further evaluated in the FS. Analysis of the duration of immobility (Fig. 6c) revealed an effect of strain. *post-hoc* strain comparisons indicated that B6 showed the longest duration (p<0.001), with SvPas showing longer duration of immobility (p=0.001) than Balb. We also found a significant effect of treatment with an interaction. Post hoc comparisons revealed that heroin abstinence increased immobility in both Balb (p=0.033) and in SvPas (p<0.001) but not in B6 (p=0.44). Analysis of the last 4 min of immobility provided similar results (data not shown).

We then measured the latency to first immobilization (Fig. 6d) and found a significant effect of strain. *post-hoc* strain comparisons showed that SvPas had decreased latency compared to both B6 and Balb (p<0.001) with no difference between the two latter strains (p=0.93). Importantly, we also found a significant effect of treatment with an interaction. Post hoc comparisons showed that heroin abstinence reduced latency in both SvPas (p<0.001) and Balb (p=0.014) but not in B6 (p=0.74).

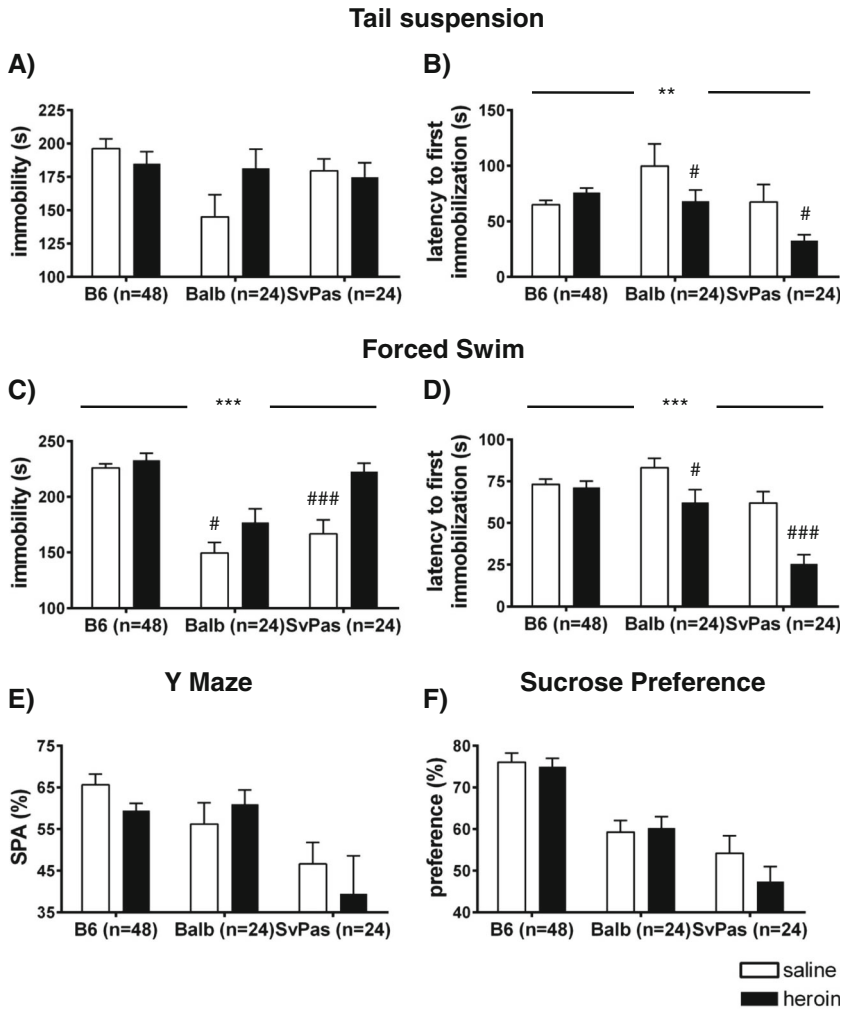

Fig. 6 Despair-like behaviors, working memory, and SP during heroin abstinence in three inbred mouse strains. B6 (n=24 mice/group, data from (Lutz et al. 2014)), Balb (n=12 mice/group/strain) and SvPas (n=11 mice/group/strain) were treated with increasing doses of heroin or saline during 6 days (10, 20, 30, 40, and 50 mg/kg, IP, twice daily with single IP on day 6; same animal cohorts as in Fig. 4). After 4 weeks of abstinence (i.e., no naloxone injection at the end of the chronic heroin treatment), we analyzed despair-like behaviors (TS and FS), working memory (YM), and SP. In TS, heroin abstinence had no effect on the duration of immobility (**a**), but decreased the latency to first immobilization in Balb and SvPas (**b**). In FS, heroin abstinence increased the duration of immobility (**c**) and reduced latency to first immobilization (**d**) in Balb and SvPas but not in B6. Finally, heroin abstinence did not affect working memory (**e**) or hedonic tone (**f**) (as revealed using spontaneous alternation and preference for a sucrose solution, respectively) across the three strains. Significant effects of strain are depicted in the text. Data are mean±SEM. **p<0.01, ***p<0.001, ANOVA, main effect of heroin abstinence; #p<0.05, ###p<0.001, *post-hoc* comparisons for the effect of heroin abstinence in each strain

YM. Analysis of the percent of spontaneous alternation (SPA, Fig. 6e) found an effect of strain only. *post-hoc* comparisons indicated that SvPas showed lower percent of SPA than B6 (p<0.001) and Balb (p=0.001), with no difference (p=0.06) between the two latter strains.

SP Finally, analysis of the percent of sucrose preference (Fig. 6f) revealed a main effect of strain with no effect of treatment or interaction. *post-hoc* strain comparisons indicated that B6 had a higher SP% than either Balb or SvPas (p<0.001), with Balb having higher preference than SvPas (p=0.005). Mice of the latter strain showed no preference for 0.8 % sucrose (t-test against a theoretical mean of 50 %, p=0.34) but did show preference for 5.5 % sucrose (Pothion et al. 2004) (p<0.001, data not shown).

Discussion

Opiates trigger potent reinforcing effects and physical dependence, and rodent studies have demonstrated that both aspects heavily rely on genetic factors (Klein et al. 2008a; Schlussman et al. 2008). Long-term opiate abuse has also been consistently associated with an increased lifetime risk for anxiety and depressive disorders (Grant et al. 2004; Grella et al. 2009).

Whether the latter aspect is also determined by genetic predispositions remains comparatively poorly understood. Here, using our mouse model of abstinence, we explored heroin-induced emotional disruption in three inbred strains. Results indicate strain-specific levels of physical dependence and locomotor activation during chronic heroin exposure. Furthermore, increased behavioral despair and low sociability emerged in a strain-dependent manner following prolonged heroin abstinence.

Inbred mouse strains exhibit baseline behavioral differences. Mouse strain differences have been reported for a variety of behaviors, and results from the present study are largely consistent with previous findings. First, we observed in two paradigms (LD and OF) higher levels of anxiety-like behaviors in SvPas compared with B6 or Balb, similar to previous data (Mandillo et al. 2008; Tang and Sanford 2005). Furthermore, we found no difference between Balb and B6. When comparing the two latter strains, previous studies reported variable results, with slightly higher anxiety-like behavior in Balb (Mandillo et al. 2008), or no difference (Belzung et al. 2000; Lalonde and Strazielle 2008). Taken together, results from these studies and ours clearly indicate that SvPas present high baseline levels of anxiety-like behavior, while the detection of subtle differences between B6 and Balb mice depends on experimental conditions. Second, our results indicate that direct physical interactions in freely moving animals are lower in Balb and SvPas compared with B6, in line with previous studies that quantified exploration of encaged congeners (Moy et al. 2007; Nadler et al. 2004). Finally, we found a modest increase in despair-like behavior in B6 that was most clearly detected in the FS. This effect was not previously reported (Dulawa et al. 2004; Liu and Gershenfeld 2001; Norcross et al. 2008), suggesting that strain differences in this paradigm depend on experimental procedures.

Locomotor responses and physical dependence to heroin show strain differences. We then explored heroin-induced behaviors during chronic treatment. Injections with increasing heroin doses induced a severe body weight loss across the three strains. Similar effects were previously reported for several mu opioid receptor-targeting opiates and are classically attributed to both locomotor activation and complex effects on feeding behaviors (Papaleo et al. 2007; Rouibi and Contarino 2012; Ziauddeen et al. 2013). Another major facet of systemic opiate effects in mice is the well-known, dose-dependent locomotor stimulation characterized by high horizontal activity and muscular rigidity (Fadda et al. 2005). Here, heroin-induced hyperlocomotion was strikingly strain-dependent, with strongest and weakest effects in Balb and SvPas, respectively. In previous studies, similar levels of hyperlocomotion were measured between B6 and SvPas following acute morphine IP injections with doses ranging from 4 to 32 mg/kg (Belknap et al. 1998). Also, hyperlocomotion induced by heroin (1.25 to 20 mg/kg, IP) was comparable across B6 and 129P3/J, another 129 substrain (Schlussman et al. 2008). There is some evidence, however, for a decreased sensitivity to locomotor effects of opiates in 129-related strains compared to B6. Accordingly, lower morphine-induced locomotion (3 mg/kg, SC) was found in 129X1/SvJ compared with B6 (Murphy et al. 2001). Furthermore, four injections with a very low heroin dose (0.1 mg/kg, IP) were sufficient to induce locomotor sensitization in B6 but not in 129X1/SvJ (Szumlinski et al. 2005). Within this line, the present study found a clear and strong difference, with dose-dependent effects in B6 but not SvPas, which were detected using high heroin doses (10 to 50 mg/kg). Therefore, the modest difference between B6 and 129-related strains is likely to be better detected at very high opiate doses. Regarding Balb, the only available study, to our knowledge, used low morphine doses and failed to detect a significant difference with B6 (Belknap et al. 1998): Again, the high-dose regimen used here may be more sensitive. While these variations may stem from initial differences in basal locomotion across strains (Bailey et al. 2010), this is unlikely to be the case in the present study, as we did not observe any difference between saline-treated groups. Alternatively, at such high doses, heroin-induced rigidity may be more (SvPas) or less (B6 and Balb) severe across strains, thereby differentially competing with locomotor activation. Overall, strain differences in heroin-induced locomotion also rely on pharmacokinetic factors: While comprehensive studies have been performed in B6 (Andersen et al. 2009; Boix et al. 2013; Koek et al. 2012), other strains have been largely unexplored. Pharmacodynamic factors may also be at play, including differences in expression and function of the many neurotransmitter systems mediating these effects of opiates: mu opioid receptor (Bailey et al. 2010), dopamine (Schlussman et al. 2011), serotonin (Fadda et al. 2005), and GABA (Schlussman et al. 2013), among others. Further studies will be necessary to demonstrate the contribution of such strain differences in the context of opiate-induced locomotion.

We also examined naloxone-precipitated withdrawal. First, following repeated handling and saline injections (i.e., in the absence of any heroin exposure), systemic administration of the general opioid antagonist naloxone produced discrete signs of withdrawal in Balb but not in B6 or SvPas. Very few studies have compared endogenous opioid activity and behavioral effects of naloxone across mice strains. Results suggest strong differences in baseline mu opioid receptor expression (Schlussman et al. 2011) and function (Bailey et al. 2010) across B6, 129P3/J, and DBA/2J mice, as well as mild behavioral effects of naloxone in B6 (Gorris and van Abeelen 1981; Schlussman et al. 2013). While, to our knowledge, no similar data are available for Balb and SvPas strains,

our results suggest that repeated, stressful manipulations might more potently activate the endogenous opioid system in Balb than in B6 and SvPas. Second, we found that physical dependence to heroin is strikingly lower in SvPas compared with the other two strains, consistent with previous reports that primarily focused on jumps as the main manifestation (Kest et al. 2002; Klein et al. 2008b; Schlussman et al. 2011). Importantly, even when combining several signs into a global score, we could not detect a significant withdrawal syndrome in SvPas (Fig. 3c). We speculate that this striking insensitivity might be explained by low expression of the mu opioid receptor along noradrenergic pathways critically mediating opiate physical dependence (Delfs et al. 2000; Gowing et al. 2014).

Heroin abstinence leads to low sociability and despair-like responses in a strain-dependent manner The present study failed to detect any effect of heroin abstinence on anxiety-like behaviors in any of the three strains examined, in two paradigms (LD and OF). Similarly, our previous studies reported no effect of opiate abstinence, either (1) 1, 4, or 7 weeks after exposing adult B6 to escalating morphine or heroin regimens (Goeldner et al. 2011; Lutz et al, 2014) or (2) 4 weeks after exposing adolescent B6, B6N, or Balb to morphine (Lutz et al. 2013). Data from other groups provided inconsistent results and reported either decreased or increased anxiety-like behaviors, using elevated plus maze (Buckman et al. 2009; Grasing et al. 1996) or conditioned defensive burying paradigm (Harris and Aston-Jones 2001). Overall, the effects of repeated opiate exposure on anxiety-like behaviors appear complex and highly dependent upon the species, opiate doses, duration of withdrawal, and behavioral outputs under study. Hence, rodent models have been poorly successful so far in modeling comorbidity between opiate addiction and anxiety disorders.

We also studied social behaviors 4 weeks after heroin exposure. We found that heroin abstinence reduced sociability in both B6 and SvPas. This effect appears extremely robust in our reference B6 strain because: (1) Consistent results have been observed for morphine abstinence by our group (Goeldner et al. 2011) and others (Jia et al. 2013; Zanos et al. 2014), as well as for heroin abstinence in six independent cohorts that we generated over the last 3 years (see Lutz et al. (2014) and Lalanne et al. (in preparation)); (2) this effect persists until 7 weeks of heroin abstinence (Lutz et al. 2014); and (3) this effect is observed across three types of social contact (Fig. 5). In SvPas, decreased social drive only manifested with significant effects on the number and duration of sniffing (the most frequent social behavior in this strain), suggesting that heroin abstinence has a milder impact on sociability compared with B6. In Balb, the duration and number of global and specific social behaviors were unchanged during abstinence: This absence of effect might be explained, at least in part, by the very low baseline levels of social behaviors observed in saline-treated mice pairs from this strain. This explanation, however, is unlikely to be true in SvPas mice: While saline-treated pairs of Balb and SvPas mice showed similar numbers of sniffing and global behaviors, the effect of heroin was still detectable in SvPas. Therefore, results suggest that Balb strain may be insensitive to detrimental heroin effects on sociability. During social encounters, we also measured the duration and number of self-grooming behaviors, which are enhanced in heroin-pretreated B6 pairs. Because abstinent B6 mice show a strongly decreased drive to interact with unfamiliar congeners, we interpret this result as suggestive of a displacement behavior that may allow limiting the SI. In contrast, heroin pretreatment had no effect on grooming in SvPas and Balb, further strengthening the notion that, in these strains, neuronal substrates of sociability are less sensitive to negative effects of heroin abstinence. We recently developed, on a mixed B6-SvPas 50/50 % genetic background, a conditional knockout strategy of the mu opioid receptor, the main molecular target of heroin (Lutz et al. 2014). Results showed that poor sociability following a 4-week abstinence period rely on the recruitment of mu opioid receptors located in the dorsal raphe nucleus (DRN), a major source of serotonergic innervation in the central nervous system. Whether expression and function of the mu opioid receptor in the DRN differ among inbred strains, and contribute to differential heroin effects on sociability, is an interesting possibility. Another mechanism potentially contributing to social avoidance in heroin abstinent mice is the dysregulation of brain oxytocinergic system. A recent study showed that 7 days of spontaneous withdrawal from chronic morphine treatment led to impaired sociability and decreased hypothalamic oxytocin peptide levels. Interestingly, treatment with an oxytocin analogue attenuated the development of these deficits (Zanos et al. 2014). Whether inbred strains differ in the expression and function of the oxytocinergic system remains to be explored.

Finally, we analyzed depression-related traits. Our results reveal that heroin abstinence increased despair-like behaviors in both Balb and SvPas but not in B6. In Balb and SvPas, this effect was consistent across two paradigms: TS (latency to first immobilization) and FS (both duration of immobility and latency to first immobilization). Considering that these two tests show different sensitivities to experimental conditions and environmental factors (Miller et al. 2010), and engage different neurochemical substrates (Renard et al. 2003), our data suggest that heroin abstinence robustly affects mood regulation in Balb and SvPas. Therefore, these two strains and B6 should all prove useful to study specific molecular substrates of the distinct mood-related deficits associated with opiate addiction (i.e., despair in Balb and SvPas; low sociability in B6). Importantly, despite the very low levels of hyperlocomotion and physical dependence observed during heroin treatment in SvPas, both low sociability and despair-

like behaviors were observed in this strain following abstinence. These results argue against the long-standing notion that the severity of the acute withdrawal state, encountered repeatedly during cycles of drug intoxication and abstinence, is directly related to the negative emotional consequences of addiction. Rather, the strong dissociation observed here in SvPas suggests that depressive-like features in opiate abstinence may depend on different adaptive processes in the brain than physical dependence. Of note here is that nicotine and cocaine, for example, are potent drugs of abuse that also strongly associate with comorbid depressive symptoms while producing very low or no physical dependence.

Finally, we assessed hedonic processes in heroin-abstinent mice using SP (Fig. 5f). Although previous rat data suggest that morphine abstinence reduces the motivational properties of natural reinforcers (Zhang et al. 2007), our results suggest that heroin abstinence does not affect the hedonic aspect of sucrose consumption, even in Balb and SvPas mice that showed evidence of despair-like behaviors. It is nevertheless possible that, in the latter strains, longer abstinence durations or repeated cycles of drug exposure and abstinence may ultimately lead to anhedonia.

In conclusion, we show that prolonged abstinence from chronic heroin exposure strain-dependently triggers delayed social avoidance and behavioral despair, even when immediate heroin-induced locomotor activation and physical dependence were minimal. Altogether, our results provide a valid model for studying the highly prevalent comorbidity between opiate addiction and depression. As this model is based on passive drug exposure and only partially recapitulates the human condition, future studies should explore emotional long-term consequences of heroin abstinence in rodent models involving voluntary drug intake (Ahmed and Koob 1998), as well as following the emergence of compulsive drug use (Deroche-Gamonet et al. 2004).

Acknowledgments We thank the Mouse Clinical Institute (Illkirch France) for help with behavioral experiments and protocols. We also thank Claire Gaveriaux-Ruff and Abdel Mouttalib-Ouagazzal for constant scientific exchanges. This work was supported by Ministère de l'Enseignement Supérieur et de la Recherche (GA), Fondation pour la Recherche Médicale (PEL), Fondation Fyssen (PEL), Fondation Bettencourt-Schueller (PEL), Canadian Institutes for Health Research (PEL), Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, and Agence Nationale de la Recherche (ANR-Abstinence).

Financial disclosure The authors have no conflict of interest to declare.

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Addiction Biology

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ORIGINAL ARTICLE

doi:10.1111/adb.12392

Kappa opioid receptor antagonism and chronic antidepressant treatment have beneficial activities on social interactions and grooming deficits during heroin abstinence

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ABSTRACT

Addiction is a chronic brain disorder that progressively invades all aspects of personal life. Accordingly, addiction to opiates severely impairs interpersonal relationships, and the resulting social isolation strongly contributes to the severity and chronicity of the disease. Uncovering new therapeutic strategies that address this aspect of addiction is therefore of great clinical relevance. We recently established a mouse model of heroin addiction in which, following chronic heroin exposure, 'abstinent' mice progressively develop a strong and long-lasting social avoidance phenotype. Here, we explored and compared the efficacy of two pharmacological interventions in this mouse model. Because clinical studies indicate some efficacy of antidepressants on emotional dysfunction associated with addiction, we first used a chronic 4-week treatment with the serotonergic antidepressant fluoxetine, as a reference. In addition, considering prodepressant effects recently associated with kappa opioid receptor signaling, we also investigated the kappa opioid receptor antagonist norbinaltorphimine (norBNI). Finally, we assessed whether fluoxetine and norBNI could reverse abstinence-induced social avoidance after it has established. Altogether, our results show that two interspaced norBNI administrations are sufficient both to prevent and to reverse social impairment in heroin abstinent animals. Therefore, kappa opioid receptor antagonism may represent a useful approach to alleviate social dysfunction in addicted individuals.

Keywords abstinence, addiction, heroin, kappa opioid receptor, serotonin, sociability.

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INTRODUCTION

Addiction is a chronic brain disorder with devastating consequences for individuals and their social life (Volkow, Baler & Goldstein 2011; Everitt 2014). Natural history of the disease has been classically conceptualized as a vicious cycle. Drug intoxications initially produce positive subjective effects but are followed by aversive signs of withdrawal when pharmacological drug effects unfold. In turn, withdrawal feeds into a 'preoccupation' stage, where drug craving irresistibly drives drug-seeking behaviors and precipitates relapse (Koob & Volkow 2010). Escaping this vicious cycle and maintaining abstinence is a lifelong challenge for addicted individuals.

Several causes contribute to interrupt drug abstinence. External factors, such as stressful life experiences and drug-associated environments (Koob & Volkow 2010), are well-studied determinants for relapse. Alteration of emotional homeostasis during the course of the disease represents yet another key factor, which is less understood. Abstinence, notably from opiate abuse, is characterized by a symptomatology encompassing

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anxiety and depressive disorders, as well as social isolation (Grella *et al.* 2009). Emotional and social dysfunction in addicted subjects is a major concern that associates with a more severe and longer clinical course, as well as higher relapse rates during abstinence periods (Bakken, Landheim & Vaglum 2007). Several rodent paradigms have been used to study the various facets of emotional deficits associating with opiate abuse. In summary, chronic opiate exposure was found to potentiate stress vulnerability (Blatchford *et al.* 2005), defensive behaviors (Harris & Aston-Jones 1993, 2001) and depressive-like behaviors (Grasing & Ghosh 1998; Anraku *et al.* 2001; Hodgson *et al.* 2009; Jia *et al.* 2013). Recently, we established a novel mouse model of drug abstinence focused on social behaviors. We showed that abstinence from both morphine and heroin, two prototypical opiates, progressively leads over the course of 4 weeks to the emergence of a long-lasting, low sociability phenotype (corresponding to both decreased interactions and increased grooming when encountering a new congener), which also associates with depressive-like features (Goeldner *et al.* 2011; Lutz & Kieffer 2013; Lutz *et al.* 2014).

Rigorous clinical studies have been conducted to evaluate classical antidepressants in the context of emotional comorbidities of addiction (Nunes & Levin 2006). These studies reported mixed results, some positive but some negative, emphasizing the need to investigate other therapeutic options. Over the last few years, animal studies have identified the kappa opioid receptor (KOR) as a promising target for innovative antidepressant strategies (Bruchas, Land & Chavkin 2010; Knoll & Carlezon 2010; Lalanne *et al.* 2014), prompting clinicians to assess the potential of targeting this particular opioid receptor to treat emotional symptoms in drug abuse. Buprenorphine, for example, is a complex opiate drug that activates the mu opioid receptor (MOR), the main molecular mediator of reinforcing properties of opiates, but is also an antagonist at the KOR. Considering the treatment of addiction, buprenorphine was used primarily as a substitution to illegal opiates because of its MOR activity; interestingly, some authors reported beneficial effects on emotional distress (Gerra *et al.* 2004) possibly because of KOR blockade. Recent studies have also investigated combined administration of buprenorphine and MOR antagonists, in order to achieve KOR blockade while limiting the risk of abuse associated with MOR activation. Results have revealed significant improvement of depressive symptoms (Ehrich *et al.* 2014; Almatroudi *et al.* 2015), further reinforcing the possibility that KOR antagonism may have intrinsic antidepressant potential.

Because MOR and KOR exert complex and distinct controls over emotions and sociability, animal studies and clinical trials that target both receptor types may be difficult to interpret. Also, systemic KOR-targeting therapies have not been investigated in animal models of social comorbidities of opiate addiction. In the present study, we hypothesized that KOR signaling may play a role in the emergence of low sociability in opiate addicts and evaluated the efficacy of a pure KOR antagonist in a validated mouse model of social deficits in heroin abstinence. We examined the ability of norBNI to prevent low sociability in heroin abstinent mice and to reverse this phenotype after it has been established, using the antidepressant fluoxetine (FLX) as a comparison. Our results indicate that these two pharmacological interventions had protective effects during heroin abstinence, in prevention and reversion experiments, against both low social interactions and increased grooming.

METHODS

Animals

Male C57BL/6JCrl mice (Charles Rivers Laboratories, St.-Germain-sur-l'Arbresle, France) were habituated to housing conditions during 2 weeks and were 10-week old at the beginning of chronic heroin exposure. Animals were housed four/cage and maintained under standard laboratory conditions (12-hour light–dark cycle with lights on at 7 AM; food and water available *ad libitum*). All experimental procedures were performed according to standard ethical guidelines (European Union Council Directive of 22 September 2010, directive 2010/63/UE and IGBMC-ICS ethical comity, Com'Eth).

Heroin treatment

Heroin (Francopia, Gentilly, France) was administered intraperitoneally (i.p.) twice daily (8 AM and 6 PM) with escalating doses (10, 20, 30, 40, 50 mg/kg for 5 days, followed by a single 50 mg/kg injection on day 6) or saline solution as a control. We previously showed that this regimen induces a strong physical dependence, one of the hallmarks of opiate addiction in human (Lutz *et al.* 2014). Compared with our previous studies on morphine abstinence (20–100 mg/kg morphine doses) and based on systematic analyses of opiate-induced physical dependence across inbred mouse strains (Kest *et al.* 2002; Klein *et al.* 2008), we selected heroin doses divided by a factor of two (10–50 mg/kg). Although the signs of withdrawal qualitatively differ (refer to Lutz *et al.* 2014), the global scores for acute naloxone-precipitated withdrawal are comparable across these two morphine and heroin regimens. To avoid reciprocal social influences between saline- and heroin-treated mice (Cole *et al.* 2012), housing cages contained only saline-treated or only opiate-treated mice. Following the 6-day heroin regimen, animals experienced spontaneous withdrawal in their home cages and

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were maintained drug free (i.e. 'abstinent') for 4 or 8 weeks in prevention or reversion experiments, respectively.

Fluoxetine treatment

The 10 mg/kg FLX dose was chosen based on our previous studies (Goeldner *et al.* 2011; Lutz *et al.* 2014). Briefly, the amount of FLX (Sigma-Aldrich, Lyon, France) supplemented to regular chow was based upon initial body weight of animals and daily average food intake: A 30 g mouse consuming 4 g/day (dry weight) of chow supplemented with 0.3 mg FLX received an approximate 10 mg/kg/24 hour dose. The 10 mg/kg was chosen as, in pilot experiments (refer to supplementary online material in Goeldner *et al.* 2011), it did not alter food intake during the 3 weeks of treatment and was ineffective on despair-related behaviors in naïve mice. In contrast, a higher (30 mg/kg) FLX dose was rejected as it severely reduced food intake and produced signs of serotonergic overdosage in some animals.

Norbinaltorphimine treatment

Norbinaltorphimine (norBNI) (Tocris Bioscience, Lille, France) is a long-acting antagonist with a greater than 100-fold selectivity for KOR over other opioid receptors (Metcalf & Coop 2005). Two injections of norBNI were administered i.p. over a 4-week period (Melief *et al.* 2011) and at a 10 mg/kg dose (McLaughlin *et al.* 2006; Bruchas *et al.* 2007a) with saline solution as a control. Housing cages contained only saline-treated or only norBNI-treated mice.

(*trans*)-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl] benzene-acetamide treatment

(*trans*)-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cycloxhexyl] benzene-acetamide (U50,488H; Sigma Aldrich, Lyon, France) was administered subcutaneously (s.c.), as previously described (Simonin *et al.* 1998), at a 20 mg/kg dose.

Behavioral testing

Following repeated heroin injections, mice were maintained drug free in their home cages. Social behaviors were investigated 4 or 8 weeks later in a familiar environment (open-field arena), as previously described (Goeldner *et al.* 2011; Lutz *et al.* 2014).

Social interactions

Pairs of unfamiliar mice, from different home cages but of similar weight, were placed simultaneously for 10 minutes in the open-field arena, indirectly lit at 50 lux. Prior habituation to the arena and dim lighting both favor social interactions in poorly anxiogenic conditions (Goeldner *et al.* 2011). While in the present study, social interactions were measured using pairs of mice from the same treatment condition, our previous work (Lutz *et al.* 2014) suggests that similar effects of abstinence are observed when heroin-treated mice interact with naïve, opiate-free animals. Using an ethological keyboard, we measured the number of occurrences and the total duration of social interaction behaviors (sniffing, following and pawing contact), as well as of the individual grooming behavior.

Tail immersion

The 52°C water is a commonly used nociceptive stimulus for opioid analgesia testing (Vaught & Takemori 1979; Mogil, Wilson & Wan 2001). Latency to withdraw the tail was measured by stopwatch. A cutoff time of 15 seconds was used to prevent heat-related tissue damage. For each mouse, pain-reflex latency was first measured at baseline. Then, U50,488H was administered with saline solution as a control, followed 30 minutes later (peak effect of the drug, refer to Bruchas *et al.* 2007b; Munro *et al.* 2012) by another measure of the pain reflex latency.

Statistical analyses

All data is expressed as mean ± SEM. Statistical analyses were performed using one- and two-way analyses of variance (ANOVA) with between-subjects (heroin abstinence, norBNI injections and FLX pellets) and within-subjects (U50,488H-induced analgesia) factors, in accordance with the experimental design. In case of significant interaction following ANOVA, multiple comparisons between groups were performed using Fischer's *post hoc*. Statistical significance was defined as $P < 0.05$.

RESULTS

Low sociability during heroin abstinence can be both prevented and reversed by chronic antidepressant treatment with FLX

We showed previously that prolonged abstinence from chronic heroin exposure leads to the emergence of social withdrawal, a stable phenotype that can be detected at least from 4 to 7 weeks following the end of heroin treatment (Lutz *et al.* 2014). Here, in a first set of experiments ($n = 128$ mice, refer to timeline in Fig. 1a), we examined the ability of chronic antidepressant treatment to prevent (FLX administered during weeks 2 to 5) or reverse (FLX administered during weeks 5 to 9) heroin abstinence-induced social deficit. For each prevention or reversion experiment ($n = 64$ mice), two animal cohorts ($n = 32$ mice/cohort; $n = 16$ mice/saline or heroin group in each cohort) were processed independently, and results were

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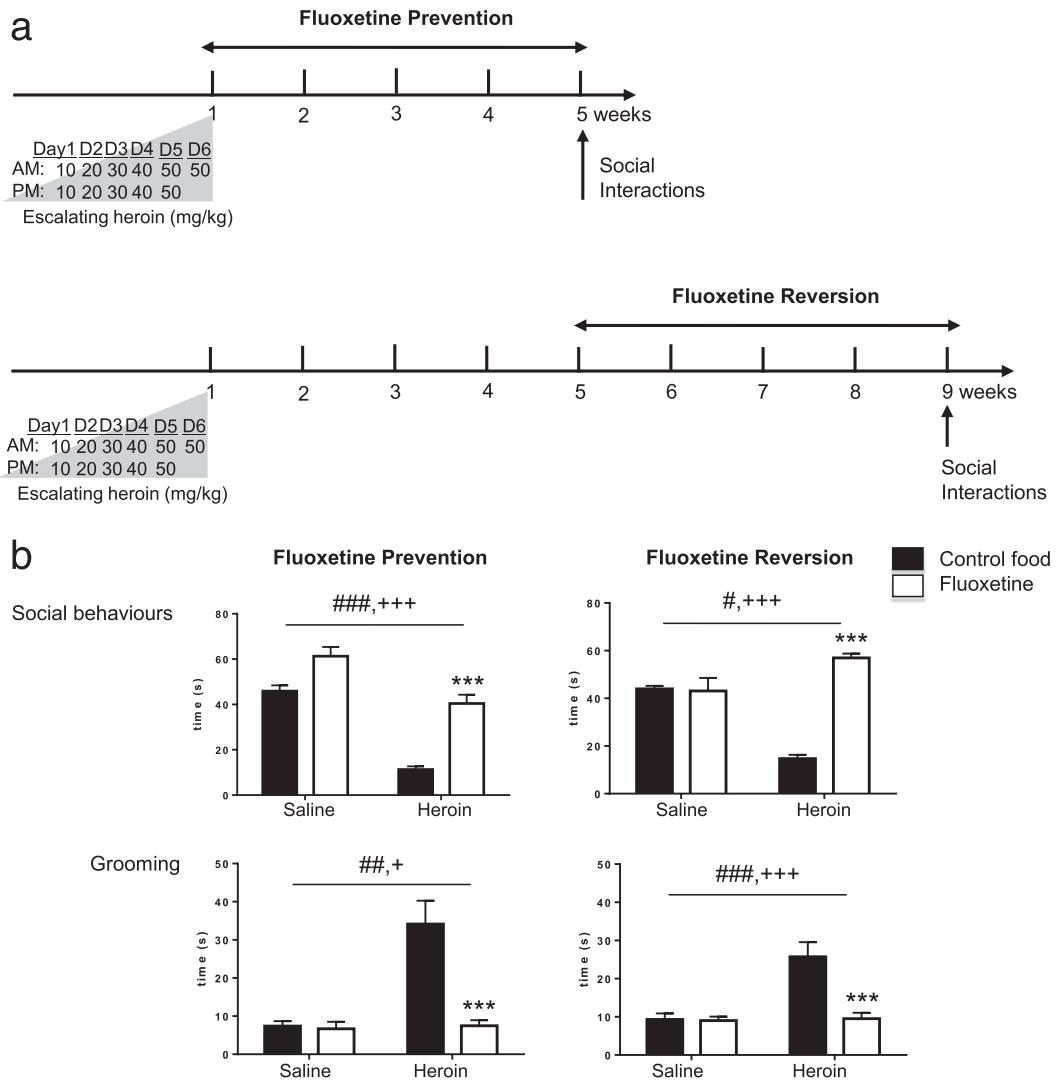

Figure 1 Chronic FLX treatment can both prevent and reverse low sociability in heroin abstinent mice. (a) Timeline for 'Fluoxetine Prevention' (upper panel) and 'Fluoxetine Reversion' (lower panel) experiments. Following heroin treatment, mice were maintained drug-free to experience spontaneous withdrawal for 4 weeks in prevention or 8 weeks in reversion experiments, followed by behavioral testing (Social Interactions). Mice were fed FLX-supplemented pellets (10 mg/kg/24 hours) during weeks 2 to 5 or weeks 5 to 8, in prevention or reversion experiments, respectively. (b) Social Interactions in 'Fluoxetine Prevention' (left panel) and 'Fluoxetine Reversion' (right panel) experiments. Consistent with our previous findings, heroin abstinence reduced social behaviors and potentiated grooming. Both of these deficits were prevented (left panel) and reversed (right panel) by 4 weeks of per os FLX treatment. Data represented as mean ± SEM. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, ANOVA, main effect of heroin. +$P < 0.05$, +++$P < 0.001$, ANOVA main effect of FLX. ***$P < 0.001$, *post hoc* FLX effect in heroin-FLX food mice as compared with heroin-control food mice

pooled. FLX-supplemented pellets were replaced by normal pellets 48 hours before behavioral testing to avoid acute effects of the drug. Results of the prevention experiments were consistent with our previous findings (Fig. 1b, left panels) (Lutz *et al.* 2014) and showed that heroin abstinence decreases social behaviors while potentiating self-grooming. We note also that no aggressive behaviors could be detected in heroin abstinent mice under our experimental conditions. Statistical analyses (two-way ANOVA) showed that social exploration time (sum of the durations of sniffing, pawing contact and following between pairs of mice of same treatment group) was affected by heroin pre-exposure [$F(1,21) = 61.7$, $P < 0.0001$] as well as by FLX treatment [$F(1,21) = 30.6$, $P < 0.0001$], with no significant interaction [$F(1,21) = 2.11$, $P > 0.05$]. Post hoc analyses showed a decrease in social interactions in heroin-control food mice, as compared with both saline-control food ($P < 0.0001$), and heroin-FLX ($P < 0.0001$), mice. Heroin abstinence increased grooming [$F(1,21) = 13.2$, $P < 0.005$], a behavior that may represent an attempt to limit or avoid social contact (refer to our previous studies for a discussion). This effect was prevented by FLX [$F(1,21) = 8.4$, $P < 0.05$], with a significant interaction [$F(1,21) = 7.4$, $P < 0.05$]. Post hoc analysis showed that

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heroin-pretreated mice fed regular chow (heroin-control food) spent more time grooming than saline controls (saline-control food, $P < 0.001$) or than heroin abstinent mice fed FLX pellets ($P < 0.001$). Therefore, a chronic antidepressant treatment targeting the serotonergic (5-HT) system is able to promote social interactions and limit aberrant grooming during long-term withdrawal, consistent with our previous observation of dynamic adaptations within 5-HT circuits during this time period (Goeldner *et al.* 2011; Lutz *et al.* 2011). We next wondered whether chronic FLX treatment might be beneficial in abstinent mice when administered at a later stage, after neurochemical adaptations have developed and low sociability has established (Fig. 1b, right panels). In these reversion experiments, two-way ANOVA analysis of social behaviors duration found significant detrimental and beneficial effects of heroin [$F(1,25) = 6.7$, $P < 0.05$] and FLX [$F(1,25) = 48.5$, $P < 0.0001$], respectively, with a significant interaction [$F(1,25) = 52.4$, $P < 0.0001$]. Post hoc analyses showed a decrease in social interactions in heroin-control food mice, as compared with both saline-control food ($P < 0.0001$), and heroin-FLX ($P < 0.0001$), mice. Similar to results from prevention experiments, we also found that increased grooming induced by heroin pre-exposure [$F(1,25) = 17.4$, $P < 0.0005$] is reversed by FLX [$F(1,25) = 16.6$, $P < 0.0005$], with a significant interaction [$F(1,25) = 14.1$, $P < 0.005$]. Post hoc analyses further confirmed that heroin-control food mice exhibit significantly longer grooming behaviors compared with both saline-control food ($P < 0.0001$), and heroin-FLX ($P < 0.0001$), mice. Altogether, the data show that a chronic FLX treatment is able to both prevent and reverse social withdrawal, which has developed during heroin abstinence.

Two norBNI injections are sufficient to significantly block KOR signaling during a 4-week period

The long-lasting KOR antagonist activity of norBNI was described originally by several groups of investigators to last *in vivo* around 14 to 21 days (in both rodents, Bruchas *et al.* 2007b; Munro *et al.* 2012; Patkar *et al.* 2013, and rhesus monkeys, Butelman *et al.* 1993). While extremely long compared with most opiates, the reported duration of norBNI effects is shorter than the 4-week abstinence period in our model. We therefore determined the number of injections necessary to block KOR signaling during the entire 4-week abstinence period.

To this aim, we tested the analgesic effect of the KOR agonist U50,488H 4 weeks after a single i.p. norBNI injection (refer to timeline in Fig. 2a and results in Fig. 2b, left bottom panel). As expected, two-way ANOVA showed a significant analgesic effect of U50,488H, with an increase in the tail withdrawal latency [$F(1,10) = 11.87$, $P < 0.01$]. We noted, however, that norBNI had no statistically significant effect [$F(1,10) = 1.99$, $P = 0.18$], with no interaction between U50,488H and norBNI [$F(1,10) = 1.9$, $P = 0.19$], indicating that the later compound no longer significantly antagonized the KOR 4 weeks after a single injection. We then tested KOR-dependent antinociception following two norBNI injections separated by a 2-week interval (refer to timeline in Fig. 2a and results in Fig. 2b, right bottom panel). Results again confirmed the effect of U50,488H [$F(1,9) = 11.74$, $P < 0.01$]. Importantly, we also found a significant effect of norBNI [$F(1,9) = 7.3$, $P < 0.05$] with a tendency for an interaction [$F(1,9) = 4.23$, $P = 0.06$]. Post hoc analyses confirmed a significant analgesic effect of U50,488H in saline-treated controls ($P < 0.005$) but not in norBNI-treated mice ($P = 0.37$). Accordingly, we decided to use a two-injection paradigm for later experiments in heroin abstinence mice.

In this experimental series, we also examined whether long-term blockade of endogenous KOR signaling might impact on the expression of social behaviors in naive mice. Social interactions were tested prior analgesic testing to limit stress effects of the pain assay. Results showed that in the single norBNI injection paradigm (Fig. 2b, left upper panel), norBNI had no significant effect on either social interactions ($P = 0.32$) or self-grooming ($P = 0.82$). Similarly, two injections of norBNI (Fig. 2b, right upper panel) had no effect on social ($P = 0.11$) or grooming ($P = 0.10$) behaviors. We conclude that, under basal conditions, sociability levels are not controlled by endogenous KOR signaling in adult mice, a finding that is consistent with our previous results in KOR knockout animals (Lutz *et al.* 2014).

Low sociability during heroin abstinence is both prevented and reversed with two systemic injections with the KOR antagonist norBNI

We next examined whether the two-injection norBNI regimen, which is sufficient to block KOR signaling over a 4-week period, prevents the emergence of low sociability in heroin abstinent animals. Similar to our experiments using FLX ($n = 128$ mice, refer to timeline in Fig. 3a), we examined whether chronic blockade of the KOR would prevent or reverse abstinence-induced deficits.

In the prevention experiment ($n = 64$ mice), the two norBNI injections were performed 24 hours and 2 weeks following the last heroin injection. Results revealed a significant effect of heroin abstinence [$F(1.28) = 18.4$, $P < 0.001$] and a significant interaction between abstinence and norBNI [$F(1,28) = 16.1$, $P < 0.001$], on the total duration of social behaviors (Fig. 3b, left upper panel). NorBNI had no main effect [$F(1,28) = 2.8$, $P = 0.11$]. Post hoc analyses confirmed that, in the absence of any norBNI exposure, social behaviors were decreased in heroin-pretreated compared with saline-

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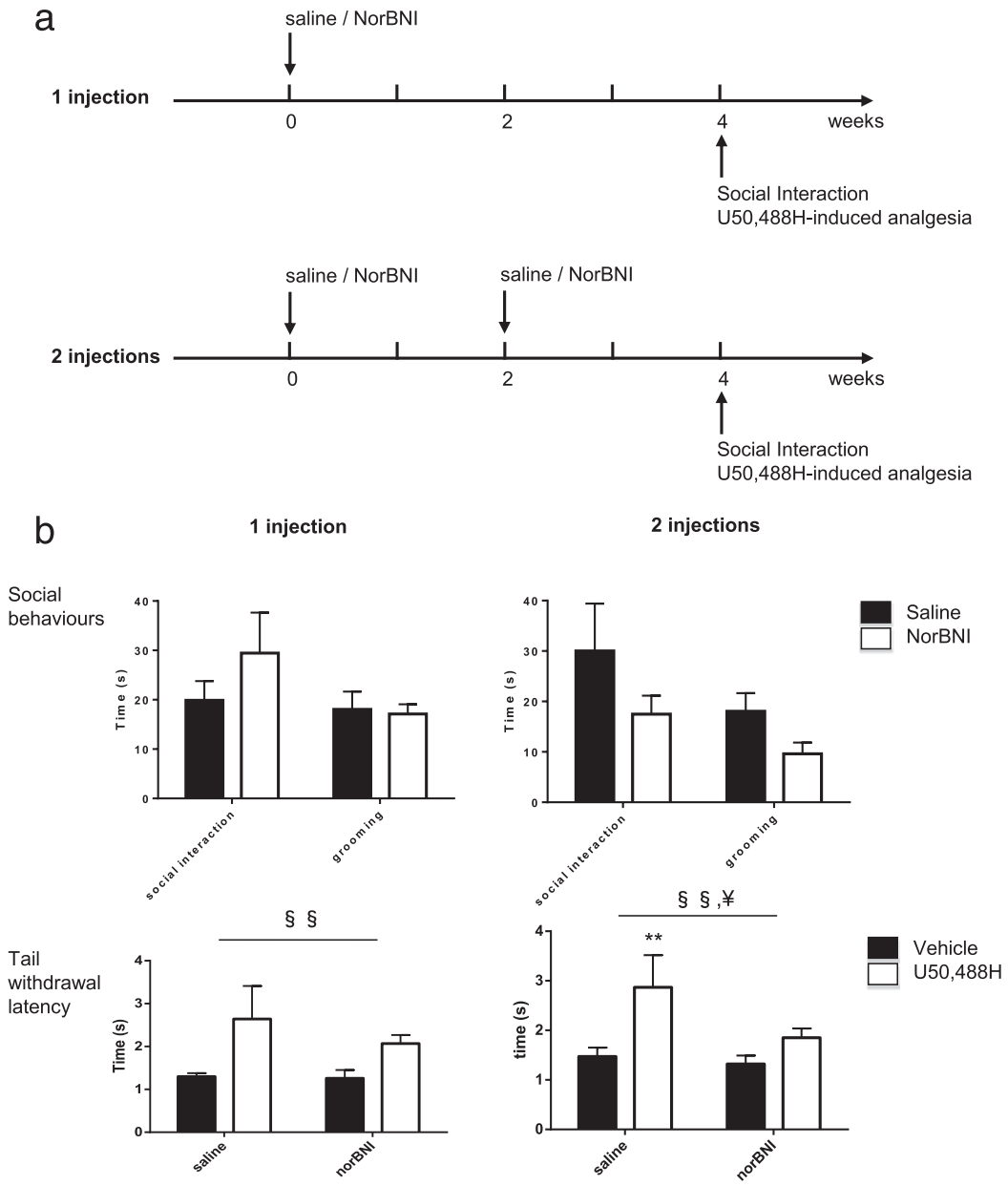

Figure 2 Two norBNI injections are sufficient to achieve chronic blockade of the kappa opioid receptor over 4 weeks. (a) Experimental timeline to assess chronic KOR blockade by one (upper panel) or two (lower panel) norBNI injections. Mice received one or two injections of norBNI (10 mg/kg, i.p.) over a 4-week time period. Four weeks after the first norBNI injection, mice underwent social interaction testing followed by U50,488H-induced analgesia (20 mg/kg, i.p.). (b) Social interactions (upper panels) and tail immersion (lower panels) in mice after one (left panels) or two (right panels) norBNI injections. Neither one nor two norBNI injections had significant effect on social behaviors and grooming. While the analgesic effect of U50,488H was still detectable 4 weeks after a single norBNI injection, two norBNI injections were able to prevent U50,488H-induced analgesia, in the 52°C tail immersion assay. Data represented as mean ± SEM. §§$P < 0.01$, ANOVA, main effect of U50,488H. ¥$P < 0.01$, ANOVA, main effect of norBNI. **$P < 0.01$, *post hoc* comparisons for the effect of U50,488H

pretreated abstinent animals ($P < 0.0001$). Importantly, two norBNI injections were sufficient to restore sociability to normal values in heroin-pretreated groups ($P < 0.001$), a striking effect that matches the efficacy of chronic FLX. As for grooming (Fig. 3b, left bottom panel), we found significant effects of heroin [$F(1,28) = 13.3$, $P < 0.005$] and norBNI [$F(1,28) = 6.5$, $P < 0.05$], with an interaction [$F(1.28) = 5.54$, $P < 0.05$]. Post hoc analyses revealed that, in the absence of norBNI exposure, heroin pretreatment significantly increased grooming behaviors ($P < 0.0005$). This effect was completely reversed by KOR blockade, as shown when comparing norBNI- and saline-treated mice in the two heroin abstinent groups ($P < 0.005$). Altogether, these results clearly demonstrate

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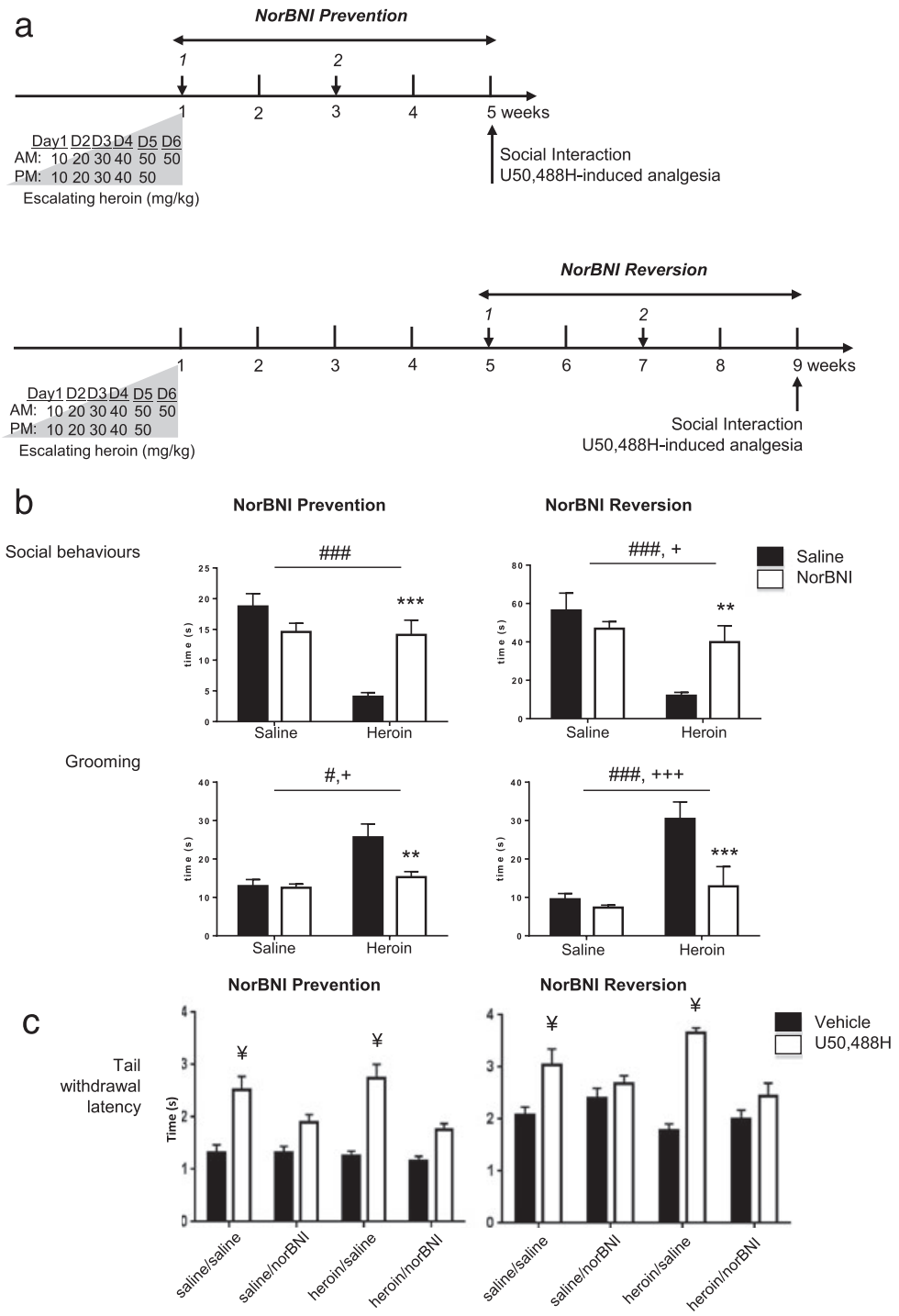

Figure 3 Chronic kappa opioid receptor norBNI antagonism prevents and reverses low sociability in heroin abstinent mice. (a) Timeline for 'norBNI Prevention' (upper panel) and 'norBNI Reversion' (lower panel) experiments. Following heroin treatment, mice experienced spontaneous withdrawal for 4 or 8 weeks, in prevention or reversion experiments, respectively. Two norBNI i.p. injections were used to achieve KOR blockade over a 4-week period. In 'norBNI Prevention' experiments, norBNI was administered 24 hours and 2 weeks after the last heroin injection. In 'norBNI Reversion' experiments, injections were 4 and 6 weeks after heroin treatment. (b) Social Interactions in 'norBNI Prevention' (left panels) and 'norBNI Reversion' (right panels) experiments. In the absence of norBNI, heroin abstinence decreased social behaviors and increased grooming. Two norBNI injections were sufficient to prevent and reverse these deficits. (c) Tail immersion in 'norBNI Prevention' (left panel) and 'norBNI Reversion' (right panel) experiments. To confirm chronic KOR blockade, mice were tested for U50,488H-induced analgesia (20 mg/kg, i.p.) in the 52°C tail immersion test, after social interaction testing. norBNI significantly blocked U50,488H-induced analgesia in both heroin abstinent and control mice, in prevention as well as in reversion experiments. Data represented as mean ± SEM. #$P < 0.05$, ###$P < 0.001$, ANOVA, main effect of heroin; +$P < 0.05$, +++$P < 0.001$, ANOVA, main effect of norBNI; **$P < 0.01$, ***$P < 0.001$, *post hoc* norBNI effect in heroin-norBNI mice as compared with heroin-control mice; ¥$P < 0.005$, post hoc comparisons for the effect of U50,488H

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that two norBNI injections are sufficient to completely block heroin abstinence effects on sociability, as well as on self-centered, grooming behaviors.

Next, we explored the efficacy of norBNI in reversing the effects of heroin. In these reversion experiments ($n = 64$ mice), the two norBNI injections were performed 4 and 6 weeks following the last heroin injection, when impaired sociability is already prominent (Lutz *et al.* 2014). Results from the two-way ANOVA analysis showed that heroin abstinence [$F(1,17) = 21.1$, $P < 0.0005$] and norBNI [$F(1,17) = 5.5$, $P < 0.05$] had opposed and significant effects on social behaviors (Fig. 3b, right upper panel), with a strong interaction between the two factors [$F(1,17) = 13.1$, $P < 0.005$]. Post hoc group comparisons confirmed that, in the absence of norBNI administration, sociability was strongly impaired following a 8-week abstinence period ($P < 0.0001$). In addition, comparing the two heroin-pretreated groups showed that this deficit was potently reversed by norBNI ($P < 0.0001$). We also analyzed the duration of grooming (Fig. 3b, right bottom panel) and found effects of both heroin [$F(1,17) = 20.9$, $P < 0.0005$] and norBNI [$F(1,17) = 29.7$, $P < 0.0001$], as well as a significant interaction [$F(1,17) = 21.6$, $P < 0.001$]. Post hoc analyses further demonstrated that heroin abstinent mice show increased grooming compared with saline controls ($P < 0.0001$), an effect that was not observed following norBNI injections ($P < 0.0001$). Altogether, our results indicate that norBNI can be administered in long-term abstinent mice to reverse heroin-induced social withdrawal.

Compared with our preliminary analyses in naive mice (refer to Fig. 2), here norBNI was administered to animals experiencing significantly higher levels of stress (with repeated i.p. injections), which may potentially modify (and likely potentiate, Bruchas *et al.* 2010; Knoll & Carlezon 2010; Lalanne *et al.* 2014) endogenous KOR activity. To confirm that the two-injection norBNI regimen was still antagonizing KOR activity at the time of behavioral testing, U50,488H-induced antinociception was measured in both prevention and reversion experiments, following social interaction testing. Results showed that in prevention experiments (Fig. 3c, left panel), heroin pre-exposure had no effect [$F(1,56) = 3.1$, $P = 0.09$], suggesting that chronic MOR activation does not interfere with later KOR-mediated analgesia. In contrast, we found that norBNI [$F(1,56) = 25.1$, $P < 0.0001$] and U50,488H [$F(2,56) = 109.0$, $P < 0.0001$] had strong effects, with a significant interaction between these two treatments [$F(1,56) = 25.6$, $P < 0.0001$]. Post hoc group comparisons further indicated that U50,488H significantly increased tail withdrawal latency in saline-saline and heroin-saline groups ($P < 0.0005$) but not in saline-norBNI or heroin-norBNI groups ($P > 0.05$). In the reversion experiments (Fig. 3c, right panel), U50,488H had a significant effect [$F(1,46) = 159.7$, $P < 0.0001$], with a tendency for an effect of norBNI [$F(1,46) = 3.5$, $P = 0.065$], but not of heroin [$F(1,46) = 2.8$, $P = 0.10$]. We finally observed a strong interaction between U50,488H and norBNI [$F(2,46) = 42.9$, $P < 0.0001$], and post hoc comparisons found that U50,488H had significant effects in saline-saline ($P < 0.005$) and heroin-saline ($P < 0.0005$) groups but not in saline-norBNI or heroin-norBNI groups ($P > 0.05$). Overall, these results indicate that norBNI effectively blocked the KOR throughout abstinence.

DISCUSSION

Interactions with congeners represent major determinants of emotional well-being and resilience in both animal species and human. Disruption of social relationships is a hallmark of addiction (whatever the drug of abuse considered) and has been recognized as one of the diagnostic criteria most strongly associated with disease severity (Hasin *et al.* 2013). From a therapeutic perspective, medications that could help in restoring proper social functioning in addicted individuals are of great interest. Recently, we established an animal model of opiate abstinence in which impaired sociability (decreased interactions and increased grooming during a new social encounter) stands as a robust and long-lasting phenotype. While this model rely on experimentally delivered opiate injections and does not recapitulate important core dimensions of addiction (such as loss of control over drug seeking), our data indicate that it has both face (strong physical dependence, low sociability and negative affective state during withdrawal) and predictive (beneficial effects of antidepressant medication) validities for the human condition (American Psychiatric Association 1994; Hyman, Malenka & Nestler 2006; George, Koob & Vendruscolo 2014). Here, building on this model, we further demonstrate that abstinence-induced social dysfunction is reversible and can be normalized by the 5-HT antidepressant FLX after it has established. Importantly, results from the present study further indicate that, in the same mouse model, two systemic injections with the KOR antagonist norBNI are sufficient to completely restore social behaviors to normal levels. Therefore, we propose that chronic blockade of the KOR may represent a powerful strategy for the management of social withdrawal in addicted individuals, alone or in combination with classical antidepressants.

Previous studies from our group (Goeldner *et al.* 2011) and others (Fadda *et al.* 2005; Tao & Auerbach 2005; Ferreira & Menescal-de-Oliveira 2012) indicate that chronic opiate exposure strongly activates the 5-HT system (an essential mediator of social behaviors), as revealed by increased 5-HT release and turnover across several brain regions, and also potently activates the hypothalamus–

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pituitary–adrenal stress axis. Our previous study showed that, with the exception of 5-HT levels in the dorsal raphe nucleus (DRN), all these effects attenuated with time while, in contrast, emotional deficits gradually developed along abstinence (Goeldner *et al.* 2011; Lutz *et al.* 2011). In particular, social avoidance strengthened to become significant after 4 weeks of drug withdrawal and persisted during at least three more weeks in the case of heroin (Lutz *et al.* 2011). Importantly, social withdrawal could not be attributed to changes in motor activity, anxiety-like behavior or hedonic tone (Lutz *et al.* 2014; Ayranci *et al.* 2015) and occurred before the emergence of despair-like behavior at 7 weeks of abstinence (Lutz *et al.* 2014), suggesting that deficient sociability precedes mood disruption during ongoing heroin abstinence. Further, we found that low sociability in abstinent mice was prevented when a chronic FLX treatment, known to potentiate 5-HT signaling, was initiated immediately after the last heroin injection. In the present study, we reproduced this finding and also examined whether delayed FLX administration, that is, after the 4-week abstinence period, would also be beneficial and reduce social behavior perturbations. Our results clearly indicate a complete reversion of the aberrant phenotype, with similar efficacy compared with prevention experiments, indicating that FLX has the capacity to restore normal functioning even after social avoidance has fully developed. Neurochemical and circuit mechanisms of FLX effects are unknown at this stage. For both prevention and reversion experiments, FLX could produce a true reversion of neuroadaptations that have developed either upon chronic opiate exposure (prevention experiment) or during abstinence (reversion experiment). Alternatively, FLX may trigger new adaptations that counteract the former modifications to restore normal behaviors. Future studies will be required to determine whether FLX-mediated restoration of social behaviors solely rely on the modulation of serotonergic neurons or recruit other neurotransmitters such as glutamatergic or noradrenergic signaling (the latter being tightly coupled with 5-HT neurons and known to be regulated by the KOR; Al-Hasani *et al.* 2013).

Because the KOR has prodepressant properties and has been recently suggested to inhibit social behaviors (Lutz & Kieffer 2013; Bilkei-Gorzo *et al.* 2014; Robles *et al.* 2014), we hypothesized that KOR signaling may play a role in the emergence of low sociability during opiate abstinence. We recently obtained some evidence supporting this hypothesis, as knockout (KO) mice for the KOR developed a strikingly lower social withdrawal upon heroin abstinence, compared with controls (Lutz *et al.* 2014). In the later experiment, however, we noted that the intensity of physical dependence to heroin was also attenuated in the KOR mutant line, as shown in precipitated withdrawal experiments. Considering that physical withdrawal represents an aversive experience, it is possible that the milder withdrawal syndrome experienced by KOR KO mice during heroin exposure may contribute, at least partly, to the absence of social avoidance following abstinence in these mutants. In the present study, we used a pharmacological strategy to gain temporal control over KOR blockade. NorBNI was administered 24 hours after the end of intermittent heroin injections, thereby leaving unchanged the severity of physical withdrawal episodes that occurred after each heroin challenge. After establishing that two interspaced norBNI injections are sufficient to block KOR over 4-week duration (Fig. 2), we found that this two-injection regimen completely prevents the emergence of social impairment in abstinent mice. Therefore, we conclude that this phenotype does not result from the recruitment of KOR activities during repeated cycles of heroin intoxication and withdrawal but rather originates from KOR signaling during the abstinence period. Finally, we note that norBNI had no impact on sociability in control animals, indicating that this treatment specifically influenced long-term neuroadaptations that result from chronic heroin exposure and incubate during abstinence.

Similar to our reasoning with 5-HT signaling and FLX, we hypothesized that blockade of KOR activity could be beneficial both to prevent the development of social withdrawal during abstinence and also to reverse low sociability that has been established in abstinent animals. The KOR antagonist treatment was delayed and initiated after 4 weeks of abstinence, when the social phenotype is already constituted. Results indicate that norBNI injections fully reverse the heroin-induced phenotype with similar efficacy compared with the preventive norBNI intervention. These findings suggest that systemic norBNI administration may prove useful even in addicted subjects who already show signs of severe social isolation.

Results from these norBNI experiments strikingly mirror those obtained using FLX. A robust body of literature has recently demonstrated that KOR-dependent signaling pathways within DRN 5-HT neurons (such as the p38α kinase) mediate aversive responses in models of acute stress and stress-induced reinstatement of cocaine or nicotine drug seeking (Bruchas *et al.* 2007a; Land *et al.* 2009; Bruchas *et al.* 2011). Data also indicate that the nucleus accumbens (NAc) may be an important endpoint of KOR-dependent regulation of 5-HT neurons, which would ultimately affect dopaminergic signaling to produce behavioral effects (Bruchas *et al.* 2011; Schindler *et al.* 2012). Within this line, Zan *et al.* very recently provided interesting evidence in the specific context of opiate abstinence (Zan *et al.* 2015). Following chronic morphine exposure in mice, the authors showed that KOR blockade in the NAc could prevent abstinence-induced depressive-like behaviors and anhedonia (in the forced swim and sucrose preference tests, respectively). Together with our

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own results, the later findings therefore suggest that the NAc is critically located at the interplay of 5-HT and dopaminergic systems to control emotional homeostasis during opiate abstinence.

Several rodent studies have explored the effects of chronic MOR activation on the expression and function of the KOR and its endogenous ligands, the dynorphins A and B peptides. Results suggest a complex pattern of responses, including different and even opposite changes in dynorphin levels depending on (i) the brain region, with an increase in the striatum and a decrease in the hippocampus and pituitary gland (Nylander, Vlaskovska & Terenius 1995a, 1995b); (ii) the timepoint, with an increase in the NAc after 4, but not 2 weeks, of morphine withdrawal in mouse (Zan *et al*. 2015); and also (iii) genetic factors, with increased and decreased dynorphins in the VTA of Fischer and Lewis rats, respectively (Nylander *et al*. 1995a). A complete description of both dynorphin/KOR and 5-HT systems in the present heroin abstinence model, including FLX and norBNI treatment effects, will be instrumental to understand interactions between the two systems and design further molecular manipulations at targeted brain sites, including NAc as well as DRN or other brain sites involved in the social brain.

The present study is not without limitations, as heroin was delivered by the experimenter, during a relatively short period and at doses that likely do not produce acute reinforcing effects (Schlussman *et al*. 2008). Additional studies will be necessary to explore whether similar social dysfunction emerges following distinct drug exposure regimens (e.g. during longer periods, Williams *et al*. 2012, at doses that classically produce conditioned place preference, Schlussman *et al*. 2008, or in self-administration paradigms, Picetti *et al*. 2012) and its sensitivity to antidepressant or KOR antagonist medications. Beyond negative affective consequences of drug exposure, these additional paradigms have the potential to recapitulate other aspects of human addiction (such as loss of control over drug seeking).

In conclusion, previous studies focusing on rodent models of cocaine, nicotine, ethanol and opiate addictions consistently indicated that KOR antagonists may help prevent relapse episodes driven by stressful experiences or even drug-associated contexts (refer to Lalanne *et al*. 2014 for a recent review). Our study further expands the utility of KOR targeting compounds and suggests that they may also promote higher social functioning in addicted individuals. Importantly, two norBNI administrations only were sufficient to match the efficacy of a prolonged FLX administration. Therefore, the well-established long-acting properties of available KOR antagonists may help to improve compliance in opiate-dependent patients, who typically prove difficult to retain over long-term follow-ups. Two clinical trials recently reported mixed results on the tolerability of two opiates with antagonist activity at the KOR (JDTic, Buda *et al*. 2015, buprenorphine, Karp *et al*. 2014), with evidence for a potential cardiac toxicity (Buda *et al*. 2015). Future studies will be necessary to characterize the risk/benefit balance of these compounds when used as antidepressants in clinical populations, notably in the context of opiate addiction.

Acknowledgements

We thank Pr André Dufour for his help with statistical analyses.

ROLE OF FUNDING SOURCES

We thank the Mouse Clinical Institute (Illkirch France) for help with behavioral experiments and protocols. This work was supported by Fondation Fyssen (PEL), Fondation Bettencourt-Schueller (PEL), Canadian Institutes of Health Research (PEL), American Foundation of Suicide Prevention (PEL), Ministère de l'Enseignement Supérieur et de la Recherche (GA and PEL), Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Agence Nationale de la Recherche (ANR-12-BSV4-0028 ABSTINENCE, PEL and BLK), Frame program Investissements d'Avenir (ANR-10-IDEX-0002-02 and ANR-10-LABX-0030-INRT ; LL, GA, DF, CGR, KB, BLK), and the National Institutes of Health (NIH-NIDA #005010).

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European Neuropsychopharmacology (2015) 25, 100–112

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REVIEW

The cannabinoid system and visual processing: A review on experimental findings and clinical presumptions

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Received 2 June 2014; received in revised form 19 September 2014; accepted 4 November 2014

KEYWORDS
Cannabis;
Endocannabinoid system;
Visual information processing;
Neurotransmission;
Visual dysfunctions;
Driving impairments

Abstract
Cannabis is one of the most prevalent drugs used worldwide. Regular cannabis use is associated with impairments in highly integrative cognitive functions such as memory, attention and executive functions. To date, the cerebral mechanisms of these deficits are still poorly understood. Studying the processing of visual information may offer an innovative and relevant approach to evaluate the cerebral impact of exogenous cannabinoids on the human brain. Furthermore, this knowledge is required to understand the impact of cannabis intake in everyday life, and especially in car drivers. Here we review the role of the endocannabinoids in the functioning of the visual system and the potential involvement of cannabis use in visual dysfunctions. This review describes the presence of the endocannabinoids in the critical stages of visual information processing, and their role in the modulation of visual neurotransmission

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http://dx.doi.org/10.1016/j.euroneuro.2014.11.002
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and visual synaptic plasticity, thereby enabling them to alter the transmission of the visual signal. We also review several induced visual changes, together with experimental dysfunctions reported in cannabis users. In the discussion, we consider these results in relation to the existing literature. We argue for more involvement of public health research in the study of visual function in cannabis users, especially because cannabis use is implicated in driving impairments.
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1. Introduction

Cannabis use is very widespread in industrialized countries. In the United States, the lifetime prevalence of cannabis use is estimated at 42%, and the prevalence in the year preceding the interview at 21.5% (Degenhardt et al., 2008). In the European Union, some 75.5 million people aged 15–64 years old report having already smoked cannabis at least once in their lives, and 23 million Europeans used cannabis in the year preceding interview (European Monitoring Centre for Drugs and Drug Addiction, and Publications Office of the European Union, 2010). Regular cannabis use may have long-term health consequences. Part of these effects is comparable to that of tobacco, especially when smoked without a filter, such as bronchopulmonary cancers (Mehra et al., 2006), chronic respiratory diseases (Tetrault et al., 2007), arteritis (Sauvanier et al., 2002) and reproductive disorders (Holt et al., 2005; Vescovi et al., 1992). However, other outcomes are more directly linked to cannabis itself, such as psychiatric and cognitive effects. For example, higher risk of developing schizophrenia is correlated with cannabis use (Casadio et al., 2011; Moore et al., 2007). In a recent cohort study, 20 years of regular cannabis use was found to result in a significant intelligence quotient (IQ) decrease and major impairment in executive functions and processing speed (Meier et al., 2012). More specifically, the best-documented cognitive impairments due to regular cannabis use involve executive functions (Bolla et al., 2002; Pattij et al., 2008; Verdejo-García et al., 2006), memory (Bolla et al., 2002; Solowij and Battisti, 2008), and attention (Solowij et al., 1995). Studying the impact of cannabis use on these cognitive functions is particularly relevant from a clinical point of view. However, the precise neural mechanisms underlying impairments due to cannabis use, in particular those in cognitive functions critical for car driving, are still being debated. Consequently, modeling the effects of exogenous cannabinoids on the human brain on this basis is problematic because these high-level cognitive functions are tightly integrated and involve many brain areas (Aggleton, 2014; Funahashi and Andreau, 2013; Somers and Sheremata, 2013).

Low-level vision may therefore be a good candidate for conceptualizing the neural impact of regular cannabis use for several reasons. Despite the evolution of mammals, the visual function displays good preservation of functional and anatomical characteristics across species (Yoon et al., 2013), thus allowing the translation of findings between animals and humans. A broad range of techniques – electrophysiological, imaging and pharmacological – can be used alone or coupled to each other to study anatomical details and physiological or molecular mechanisms of the visual system (Disney et al., 2007; Katzner et al., 2011). For example, pharmacological modulation of gabaergic and glutamatergic receptors by the administration of agonists or antagonists may be useful techniques. In the human being, studying the earliest stages of visual processing, for example at the retina level, has the advantage of being less sensitive to attentional variation during experimental measurements, thus eliminating the bias of nonspecific generalized attentional deficit, which is difficult to control (Knight and Silverstein, 2001). Besides this fundamental question, and considering that the visual function seems to be altered in cannabis users, it is now crucial to obtain more insight on how cannabis use may change visual perception, especially given the pervasive role of vision in human everyday life. For example, cannabis users drive, and so we need to know whether or not regular intake of cannabis affects the visual ability of drivers.

This review looks at the involvement of the endocannabinoid system in visual processing, and whether exogenous cannabinoids may affect visual function. We review human and animal studies that have examined the distribution of cannabinoid receptors and ligands, together with those that have investigated the involvement of the cannabinoid system in neurotransmission and synaptic plasticity of visual information processing. We also review studies that have shown visual changes and experimental visual dysfunctions following both acute and chronic cannabis use. These results are discussed on the basis of the existing data in the literature. Since cannabis constitutes the most prevalent illicit drug implicated in road fatalities, we also argue for more involvement of public health research in the study of visual function among cannabis users (Hartman and Huestis, 2013).

2. Experimental procedures

A search for relevant articles was conducted in the Pubmed and Google Scholar databases using the following keywords (“CANNABIS” OR “CANNABINOID” OR “MARIJUANA” OR “THC”) AND (“VISION” OR “VISUAL PROCESSING” OR “VISUAL SYSTEM” OR “VISUAL CORTEX” OR “RETINAL PROCESSING” OR “RETINA” OR “THALAMUS”). All results up to February 28, 2014 were examined for the selection process. Relevant publications were chosen through an individual independent selection of titles by three authors (TS, VL, RS). The articles selected had to be written in English and be related to the topic of the review. Additionally, a manual search was performed on the bibliography of each selected article.

3. Cannabis and the endocannabinoid system

3.1. Main psychoactive components

The psychotropic effects of *Cannabis sativa* L. are mediated by two major compounds: Δ9-tetrahydrocannabinol (THC) (Gaoni and Mechoulam, 1964; Mechoulam et al., 1967) and cannabidiol (CBD) (Mechoulam and Shvo, 1963). The concentration of THC contained in cannabis consumed has been constantly increasing since the 1960s, raising the ratio of THC to CBD (Potter et al., 2008). THC may increase the risk of psychotic disorders in a specific high-risk population (Bossong and Niesink, 2010), whereas recent findings suggest that CBD may have antipsychotic properties (Schubart et al., 2014).

3.2. Receptors

Cannabinoid compounds are mainly active on CB1 and CB2 receptors (Devane et al., 1988; Herkenham et al., 1990; Howlett et al., 1986; Matsuda et al., 1990; Munro et al., 1993). CB1 receptors are predominantly expressed in the mammalian central nervous system (CNS), with a higher density in basal ganglia, hippocampus, cerebellum and cerebral cortices, correlated with the motor and cognitive regions of the brain (Glass et al., 1997; Herkenham et al., 1991, 1990; Hohmann and Herkenham, 1999; Mailleux et al., 1992; Moldrich and Wenger, 2000; Tsou et al., 1998; Westlake et al., 1994). CB2 receptors are mainly detected in the peripheral and immune tissues (Berdyshev, 2000; Munro et al., 1993; Sugiura and Waku, 2000; Wilson and Nicoll, 2001), but it appears that CB2 receptors also have a functional presence in the CNS at a lower level than CB1 receptors (Ashton et al., 2006; Gong et al., 2006; Lu et al., 2000; Onaivi et al., 2006, 2008; Ross et al., 2001; Van Sickle, 2005).

3.3. Main endocannabinoids

In mammals, two main compounds act on endocannabinoid receptors: *N*-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) (Devane et al., 1992; Mechoulam and Hanus, 2000). Anandamide is involved post-synaptically through CB1 and CB2 receptors as a retrograde messenger to modulate the release of neurotransmitters (Egertová and Elphick, 2000; Glass and Northup, 1999), and expresses more affinity for CB1 than CB2 receptors (Felder et al., 1995). 2-AG acts pre-synaptically and has more affinity for CB1 receptors (Hanus et al., 2001; Marzo et al., 2004; Sugiura and Waku, 2000). Recent findings have shown that 2-AG is more readily detected in the brain than anandamide, and that it exerts less affinity for CB1 receptors than anandamide (Chevaleyre et al., 2006). In goldfish, anandamide and 2-AG appear to be expressed in all brain areas, with a higher level of anandamide in the hypothalamus and a higher level of 2-AG in the telencephalon (Valenti et al., 2005). In rats and humans, anandamide has been found in the cortex, hippocampus, striatum and cerebellum (Felder et al., 1995), correlated with higher levels of CB1 receptors, and in the thalamus, correlated with low levels of CB1 receptors (Egertová and Elphick, 2000; Felder et al., 1996).

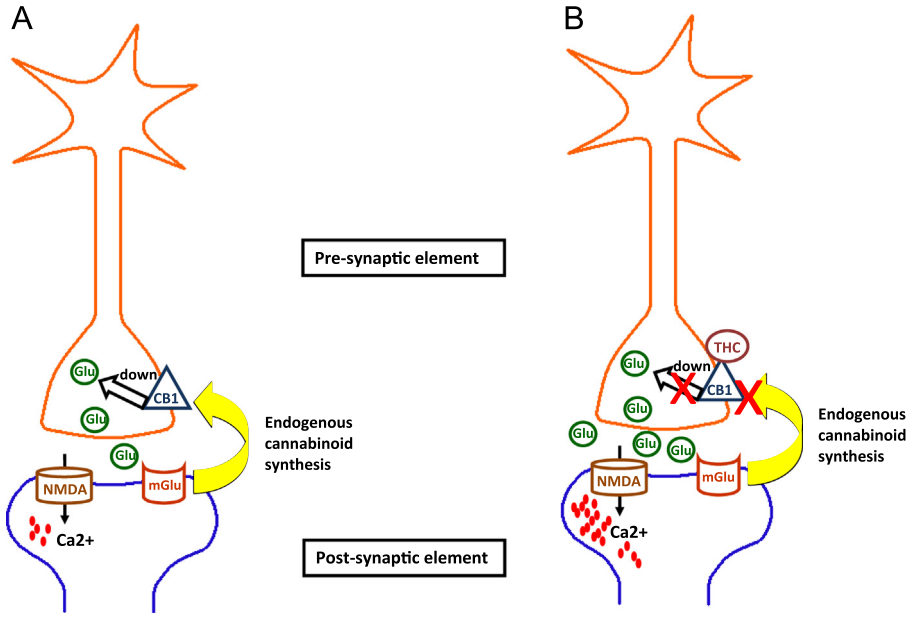

Figure 1 Role of the cannabinoid system in the regulation of neurotransmission in a glutamatergic synapse (A) and dysregulation induced by exogenous cannabinoids (B). (A) The synaptic release of glutamate (Glu) results in a post-synaptic influx of calcium (Ca^{2+}) through NMDA receptors (NMDA). The glutamate receptor (mGlu) is then stimulated, engaging a post-synaptic synthesis of endocannabinoids. Consequently, they regulate, through cannabinoid receptors CB1 (CB1), the synthesis and release of glutamate, thus preventing an increase in post-synaptic influx of Ca^{2+}. (B) The blockade of CB1 receptors by exogenous cannabinoids such as THC inhibits the pre-synaptic regulation of glutamate release, leading to an excess of post-synaptic influx of Ca^{2+}. This therefore induces apoptosis of the cell.

3.4. Synaptic process

CB1 receptors are detected at the level of pre-synaptic localization in the brain neurons (Mechoulam and Parker, 2013). They play a post-synaptic regulatory role by modulating the release of neurotransmitters such as gamma-aminobutyric acid (GABA) and glutamate (Mechoulam and Parker, 2013; Straiker and Mackie, 2006; Wilson and Nicoll, 2001). The stimulation of a glutamatergic neuron results in a synaptic release of glutamate, which induces a post-synaptic influx of calcium through NMDA receptors (Bossong and Niesink, 2010). The post-synaptic increase in the Ca^{2+} concentration activates the process of synaptic strengthening (Bossong and Niesink, 2010), and by stimulating the post-synaptic metabotropic glutamate receptor mGlu engages a post-synaptic synthesis of endocannabinoids. Through CB1 pre-synaptic receptors, endocannabinoids achieve a regulatory role on pre-synaptic glutamate release, which prevents an excessive post-synaptic release of calcium (Bossong and Niesink, 2010; Yazulla, 2008). Exogenous cannabinoids, such as THC, prevent the pre-synaptic regulation of glutamate release induced by endocannabinoids by blocking the cannabinoid CB1 receptor, and may lead to an excess of post-synaptic calcium influx, thus accelerating the pruning of the post-synaptic part of the synapse and the apoptosis of the cell (Bossong and Niesink, 2010; Yazulla, 2008). The role of the cannabinoid system in the synaptic process of a glutamatergic neuron is presented in Figure 1.

4. The endocannabinoid system and vision

Visual perception is a complex mechanism that embraces the cognitive processes for the retrieval of information from environmental light in order to construct a meaningful representation of the environment. Briefly, visual processing begins in the retina with the absorption of light by the photopigment of the photoreceptors, thus initiating the conversion of light into neural activity (Hoon et al., 2014). The retina also contains amacrine and horizontal cells acting as interneurons, and which join together bipolar and photoreceptor cells respectively, and Müller cells, which have a glial function (Hoon et al., 2014). The visual information is relayed to the brain by the optic nerve formed by the axons of the ganglion cells (Hoon et al., 2014). The first main relay in the brain's visual pathways is in the thalamus, where the lateral geniculate nucleus performs differential processing of contrast, spatial distribution and temporal sequence (Tootell et al., 1988). The information is then transmitted to the occipital cortex, especially in the primary and secondary visual cortex, which provides knowledge about forms, movements or colors (Livingstone and Hubel, 1988). From there, the ventral pathway goes to the frontal lobe through the temporal lobe, while the dorsal pathway goes to the frontal lobe through the parietal lobe. These two pathways perform differential processing, allowing the identification and localization of the information, and help to form a coherent, complex representation of the environment (Livingstone and Hubel, 1988). All the areas briefly presented here are densely interconnected, allowing retroactive complex interactions (Nowak et al., 1997). Analysis of the literature on cannabis and vision yields information on three main stages in this visual processing that are of interest because of their critical role and their accessibility for measurement: retina, thalamus and visual cortex.

4.1. Retinal localization

Animal studies have shown that cannabinoid CB1 and CB2 receptors are expressed in the retina of several species. CB1 and CB2 receptors are detected in the retina of goldfish (Cottone et al., 2013; Straiker et al., 1999a; Yazulla et al., 2000), rodents (Buckley et al., 1998; Cecyre et al., 2013; Lalonde et al., 2006; López et al., 2011; Lu et al., 2000; Porcella et al., 1998; Straiker et al., 1999a; Zabouri et al., 2011) and non-human primates (NHP) (Bouskila et al., 2012, 2013; Straiker et al., 1999a). CB1 receptors are found in the retina of the chick and the tiger salamander (Straiker et al., 1999a).

In addition, CB1 and CB2 receptors are expressed in the human retina. CB1 receptors are detected in the human retina in the inner plexiform layer (IPL), outer plexiform layer (OPL), two synaptic layers of the retina, inner nuclear layer, ganglion cell layers, outer segments of photoreceptor cells and retinal pigmentary epithelium (RPE) cells (Porcella et al., 2000; Straiker et al., 1999b; Wei et al., 2009). CB2 receptors are expressed in the human RPE cells (Wei et al., 2009). Recent findings suggest that the deletion of CB1 receptors in the human RPE may protect RPE cells from oxidative damage, the key mechanism of age-related macular degeneration (AMD) (Wei et al., 2013). This suggests a possible modulation of information transmission by the cannabinoid system at the retina level.

2-AG and anandamide, two major ligands of CB1 and CB2 receptors, have also been detected in the retina. In animal species, 2-AG and anandamide are expressed in rat and bovine retina (Bisogno et al., 1999; Straiker et al., 1999a), while anandamide is found in goldfish, porcine and bovine retina (Bisogno et al., 1999; Glaser et al., 2005; Matsuda et al., 1997). In humans, 2-AG is expressed at a high level in the retina (Chen et al., 2005; Matias et al., 2006), whereas anandamide is detected at a lower level in the retina (Chen et al., 2005; Matias et al., 2006; Stamer et al., 2001). Interestingly, a recent study showed that their concentrations might be changed in diabetic retinopathy (DR) and AMD (Matias et al., 2006), also showing a possible involvement of cannabinoid ligands in the retinal pathologic processes.

The retina constitutes the best documented level of visual information processing concerning the regulatory role of the cannabinoid system. A number of animal studies have highlighted the involvement of the cannabinoid system in the synaptic process, thus allowing the modulation of the neurotransmission at the retina level. First, the cannabinoid system is involved in the regulation of several inward and outward ionic channels. For instance, cannabinoid agonists induce a dose-dependent reversible modulation of calcium, potassium and chloride currents in bipolar, rod, cone and ganglion cells (Fan and Yazulla, 2003, 2004, 2005, 2007; Lalonde et al., 2006; Opere et al., 2006; Straiker et al., 1999a; Straiker and Sullivan, 2003; Yazulla et al., 2000; Zhang et al., 2013). Additionally, THC may inhibit the

monoamine oxidase activity in the retina, thus regulating the level of neurotransmitters (Gawienowski et al., 1982). Also, cannabinoid agonists alter the spontaneous post-synaptic currents within retinal ganglion cells (Middleton and Protti, 2011). Finally, by acting on ionic currents and electrical potentials, the cannabinoid system may modulate the release of several neurotransmitters such as dopamine, noradrenaline, GABA, and glutamate (Middleton and Protti, 2011; Opere et al., 2006; Schlicker et al., 1996; Straiker and Sullivan, 2003; Weber and Schlicker, 2001). By their likely involvement in the retinal neurotransmission, cannabinoid agonists affect phototransduction, leading to alterations in the retinal sensitivity, which was studied using whole-cell patch-clamp recordings performed from ON-bipolar cells and cones (Fan and Yazulla, 2005; Struik et al., 2006; Yazulla et al., 2000). Interestingly, such involvement of the cannabinoid system in the modulation of the retinal response has been confirmed by ERG measurements. The retinal function may be assessed by several ERGs using different stimulations. Flash ERG measurements allow the assessment of the functional properties of the photoreceptors and bipolar–Müller cell complex following a flash stimulation. Pattern ERG evaluates the macular and ganglion cell functions in response to a reversible black and white checkerboard, and multifocal ERG explores the spatial characteristics of central retinal cone function in response to multiple hexagons distributed over a screen (Holder et al., 2010). Using fERG under both scotopic and photopic conditions, mice lacking CB1 or CB2 receptors presented modifications of a-wave and b-wave amplitudes (Cecyre et al., 2013). These waves respectively represent an electronegative component generated by the photoreceptors and an electropositive component originating from bipolar cells and Müller cells.

Besides, Chaves et al. (2008) have shown the involvement of the cannabinoid system in plasticity mechanisms occurring between the retina and the thalamus. Using immunohistochemistry, immunoblotting, and real-time PCR techniques, they have demonstrated that retinal ablation increases the level of CB1 protein in the controlateral optic tectum in the adult chick brain. These findings suggest that the cannabinoid system participates in the neuroprotection of the visual function and plays a major role in development, through synaptic plasticity, of the visual system. Such functions may therefore be disrupted by exogenous cannabinoids.

Based on these findings, the cannabinoid system may modulate the human visual function at the retina level by its involvement in the retinal transmission of the visual signal (Laprevote et al., 2015). Since alterations at the central level in dopamine and serotonine neurotransmission may affect the ERG measurements and thus alter the retinal response, the retinal function may constitute a possible biological marker of brain neurochemistry (Lavoie et al., 2014).

4.2. Thalamus localization

CB1 receptors are heterogeneously distributed in the thalamus in rats (Herkenham et al., 1990, 1991; Moldrich and Wenger, 2000; Tsou et al., 1998), mice (Yoneda et al., 2013), NHP (Herkenham et al., 1990; Yoneda et al., 2013), and humans (Glass et al., 1997; Herkenham et al., 1990). Interestingly, CB1 receptors are detected in the lateral geniculate nucleus (LGN), the colliculus superior and the suprachiasmatic nucleus in the rat and mouse (Herkenham et al., 1991; Yoneda et al., 2013), and in the LGN and the colliculus superior in humans (Glass et al., 1997). Both the LGN and the colliculus superior have neural connections with the primary visual brain area, and relay visual information from the retina to the primary visual cortex (Sherman and Guillery, 2002).

Dasilva et al. (2012) have shown that the administration of cannabinoid agonists induces an excitatory effect on a part of cells and an inhibitory effect on another part of the cells in the LGN of adult rats, thus revealing two cell populations. These effects were mediated by the activation of CB1 receptors, and were blocked by a cannabinoid antagonist. As a consequence, both spontaneous and visual activities of the cells were altered. According to these findings, cannabinoid agonists, through CB1 receptors, may modulate, at a cellular level, the visual neurotransmission from the thalamus to the visual cortex. This demonstrates the potential ability of cannabis to disrupt the processing of visual information at the thalamic level.

4.3. Cortical localization

The visual cortex forms a later stage in visual information processing. CB1 and CB2 receptors are detected in the primary (V1) and secondary (V2) visual cortex of rats and mice (Gong et al., 2006; Herkenham et al., 1991; Onaivi et al., 2006; Tsou et al., 1998; Yoneda et al., 2013). In NHP, CB1 receptors have also been found in V1 and V2 (Ong and Mackie, 1999). Interestingly, CB1 receptors are more strongly expressed in V2 (layers I, II, IV, V, VI) than in V1 (layers I, II, III, IVA, IVB, IVC, V, VI) in humans (Glass et al., 1997).

Like in the retina and the LGN, the cannabinoid system is able to modulate the neural transmission in the adult visual cortex. Interestingly, Ohiorhenuan et al. (2014) have recorded the electroencephalogram (EEG), local field potentials (LFP) and single-unit activity of V1 and V2 in adult NHP. A decrease in EEG power, LFP power and LFP coherence was induced by the administration of a cannabinoid agonist. Additionally, at the level of individual neurons they found an increase in the latency and duration of the neuronal response. Taken together, these findings show the involvement of the cannabinoid system in the modulation of the neuronal activity of the visual cortex at the level of individual neurons as well as at the level of neuronal networks.

Besides, the developmental role of the cannabinoid system during early postnatal development in visual cortical plasticity has been recently established in animal studies. This knowledge warrants attention because it may inform us on the potential effects of cannabis use during pregnancy. In mice aged from postnatal day 13 (P13) to P27, the administration of a cannabinoid agonist significantly reduced both the amplitude and the frequency of inhibitory post-synaptic currents (IPSC) in layer IV of V1 (Garkun and Maffei, 2014). Using the ocular dominance plasticity, a model of cortical plasticity Liu et al. (2008) found that during

Table 1 Summary of cannabinoid functions in critical stages of visual processing.

Visual structures	Functions of cannabinoids	Species	References
Retina	Ionic channels	Tiger salamander	Straiker et al. (1999a), Straiker and Sullivan (2003)
		Goldfish	Fan and Yazulla (2003, 2004, 2005, 2007), Yazulla et al. (2000)
		Rat	Lalonde et al. (2006), Zhang et al. (2013)
		Bovine	Opere et al. (2006)
	Enzymatic activity	Bovine	Gawienowski et al. (1982)
	Release of neurotransmitters	Tiger salamander	Straiker and Sullivan (2003)
		Guinea-pig	Schlicker et al. (1996), Weber and Schlicker (2001)
		Mouse	Middleton and Protti (2011)
		Bovine	Opere et al. (2006)
	Synaptic plasticity	Chick	Chaves et al. (2008)
	Retinal sensitivity	Goldfish	Fan and Yazulla (2005), Struik et al. (2006), Yazulla et al. (2000)
	Functioning of photoreceptors and bipolar-Müller cells	Mouse	Cecyre et al. (2013)
	Oxidative damage	Human	Wei et al. (2013)
Thalamus	Neurotransmission	Rat	Dasilva et al. (2012)
Cortex	Neurotransmission	Non-human primate	Ohiorhenuan et al. (2014)
	Synaptic plasticity	Mouse	Garkun and Maffei (2014), Liu et al. (2008), Huang et al. (2008), Jiang et al. (2010a, 2010b)

postnatal development, the blockade of cannabinoid receptors prevented the ocular dominance shift in layer II/III, but not in layer IV. As observed in long-term depression (LTD), cannabinoid ligands are involved in the heterosynaptic LTD (hetero-LTD) of excitatory synapses, which is predominant from P7 to P14 (Crozier et al., 2007; Huang et al., 2008). Finally, the cannabinoid system plays a major role in the maturation of GABAergic transmission between eye opening and puberty (Jiang et al., 2010a, 2010b).

The endocannabinoid system is detected through the critical stages of visual information processing. As this system is involved in neural transmission and synaptic plasticity in the visual cortex, exogenous cannabinoids may lead to impairment of the visual function. The main functions of cannabinoids in these crucial stages of visual processing are summarized in Table 1.

5. Cannabis use and human vision

5.1. Case reports and case series

Several publications have related that acute use of cannabis results in improved visual function and contributes to visual side-effects. The most surprising report concerns Jamaican fishermen, whose night vision improved shortly after smoking *C. sativa*, and who were able to navigate safely through coral reefs in night conditions (West, 1991). This observation was recently confirmed by showing that the administration of dronabinol, a synthetic THC, resulted in a dose-related increase in scotopic sensitivity, and that inhaling a mixture of *C. sativa* and tobacco resulted in an enhanced dark adaptation and scotopic sensitivity (Russo et al., 2004). In both situations, vision was improved. Furthermore, Consroe et al. (1997) report that patients with multiple sclerosis used cannabis to reduce some of their symptoms including visual symptoms such as double vision or vision dimness. On the other hand, a study conducted nearly four decades ago, which examined the analgesic effect of THC, found that single oral dose administration of THC induced visual side-effects such as blurred vision (Noyes et al., 1975). Interestingly, all these effects are acute consequences of cannabis use.

Besides these acute visual changes, several case series have described residual visual effects of regular cannabis use. A case report describes the occurrence of flickering black spots in a man after cessation of five years of daily cannabis use, and persisting for 8 months after drug abstinence (Laffi and Safran, 1993). Similarly, a case series reported several visual disturbances (visual distortions, illusions of movement, color vision disturbances, flashbacks) in eight regular cannabis smokers, which persisted after cessation of use (Lerner et al., 2011). These findings showing the involvement of cannabinoid use in visual abnormalities are isolated, and only one study, to our knowledge, has studied the impact of chronic cannabis use on a large number of visual parameters compared with a non-user group (Dawson et al., 1977). In this large study, only small differences were measured concerning Snellen acuity, intra-ocular pressure, pupil response and color vision. Unfortunately, contrast sensitivity was not evaluated in cannabis users in this study.

5.2. Experimental studies

Despite these clinical presumptions, few studies have evaluated the earliest stages of visual processing in cannabis users. Even so, experimental tests of the visual function have shown several impairments. For example, regular cannabis users have increased foveal glare recovery time after smoking a cigarette containing 8 or 15 mg of THC (Adams et al., 1978). This might demonstrate a possible direct acute action of THC on retinal processing. Ehrenreich et al. (1999) have shown that the use of cannabis between ages 12 and 16 years was associated with impairments in visual scanning in adulthood. Similarly, chronic cannabis users with early age drug consumption have shown longer response times and a higher number of fixations on a square stimulus than controls in a visual scanning task (Huestegge et al., 2002), and both are residual effects of cannabis use. In a study assessing the residual impact of regular cannabis use on visuomotor integration, the authors showed that cannabis affected the strength of the binding between task-relevant stimulus and response features (Colzato and Hommel, 2008). This suggests a critical role of dopaminergic transmission, in particular through CB1 receptors, in visuomotor integration, which may be modulated by cannabinoids. Binocular depth inversion (BDI) is an illusion of visual perception informative of impaired visual processing. It is sensitive and regularly used in psychiatric and addictive disorders such as psychotic states and alcohol withdrawal (Emrich, 1989; Schneider et al., 1998, 2002). Both acute and chronic cannabis use may induce a reduction of BDI, showing acute and residual effects of cannabis, respectively (Emrich et al., 1991; Leweke et al., 1999; Semple et al., 2003).

A few studies using cerebral imaging have investigated the consequences of cannabis use on brain areas involved in visual processing. A radial visual checkerboard in alterning block viewed during functional magnetic resonance imaging (fMRI) was used to assess the acute impact of 10 mg of THC and 600 mg of CBD compared with placebo on visual processing in healthy volunteers (Winton-Brown et al., 2011). THC decreased activation in the secondary visual cortex, which was activated in the placebo condition, and increased activation in the primary visual cortex in the right hemisphere, compared with placebo. Also, CBD increased activation in the right occipital lobe compared with placebo. Interestingly, during visual processing, THC and CBD have similar effects on brain response in several regions and opposite effects in others. Additionally, in MRI, an interesting study has reported as a residual consequence of cannabis use a significant reduction in global and bilateral thalamic volume in cannabis users compared with a non-user group in a population at high familial risk of developing schizophrenia (Welch et al., 2011). Since the thalamus relays the visual information between the retinal and cortical pathways, and has neural connections with the retina and the primary visual cortex, the loss of thalamic volume due to cannabis use may have potential consequences on the processing of visual information.

Several studies performing visually evoked potential measurements in cannabis users have shown inconsistent results, in particular for the P300 component. This is a positive wave that represents the amount of attentional resources involved in stimulus characterization (Polich, 2007). For example, cannabis users showed a reduction in the amplitude of the visual P300 compared with non-users when all subjects were psychiatric inpatients. However, when users and non-users were screened to be healthy participants, no difference was seen (Patrick et al., 1995). In this situation, psychiatric disorders and drugs could disturb the results. In addition, in an older study that evaluated the consequences of the inhalation of THC on many cardiovascular, respiratory and neurological parameters, no difference was shown on visually evoked potentials (Low et al., 1973). More recently, visually event-related potentials recorded 2 h after acute intakes of different THC doses during a visual selective attention task showed a dose effect on several parameters (Böcker et al., 2010). A significant acute dose effect on the amplitude of P300 and SFD 80, which is related to perception of high versus low spatial frequency gratings, was observed. This suggests a non-selective decrease in attentional or processing resources. Evaluation of steady-state visually evoked potential (SSVEP), which represents a type of EEG response of visual occipital brain areas to periodic visual stimulation, has shown residual effects of cannabis, such as impairments in SSVEP and transient N160 component in cannabis users (Skosnik et al., 2006). The transient N160 component is a negative component representing a main stage of processing in the recognition and extraction of a visual pattern (Simon et al., 2007). Interestingly, female cannabis users and cannabis users with early age drug consumption showed decreased power values at 18 Hz in SSVEP. Additionally, the transient N160 component appears to be reduced in cannabis users. Based on these findings, cannabis use may affect both early and later cortical stages of visual information processing.

These impairments in visual processing are correlated with previous clinical observations. In both situations, cannabis use is implicated in clinical and electrophysiological visual dysfunctions. As cannabis use is implicated in visual dysfunctions, the impact of regular cannabis use on visual information processing warrants evaluation for its consequences on public health.

6. Discussion

This review examines the broad involvement of the cannabinoid system in visual processing, and the potential consequences of regular cannabis use. The cannabinoid system is closely involved in the visual function for several reasons. Endocannabinoids are expressed through critical stages of the visual system, such as in the retina, the LGN and the visual cortex. The modulation of the cannabinoid system by exogenous agonists or antagonists may disrupt the neural transmission in the retina, the thalamus and the visual cortex, thereby modifying the processing of the visual information. In the longer term, the endocannabinoid system may play a dynamic role in the development and function of the visual system through synaptic plasticity.

Besides the potential role of the cannabinoid system on the early processing of visual information, there is still a lack of knowledge about the potential effect of regular cannabis use on the human visual function. Nonetheless, acute and regular cannabis uses are correlated with alterations of human vision. Interestingly, case reports and case

series have shown that cannabis use can lead to visual disturbances, reduction of visual symptoms and improvement of the visual function. Furthermore, experimental studies have reported several dysfunctions at the retina and cortical level in visual information processing of cannabis users. Based on these findings, we need more studies, for example clinical prospective studies in cannabis users versus healthy controls, that evaluate visual information processing in cannabis users in order to find out whether cannabis use disturbs visual function. Such information is critical and constitutes a major public health challenge, since cannabis is one of the most prevalent drugs detected in drivers' blood or oral fluid, and is implicated in motor vehicle accidents (MVAs) (Hartman and Huestis, 2013). The association between cannabis and driving impairments has been clearly established in the United States and the European Union (Hartman and Huestis, 2013; Raes and Verstraete, 2006). For example, driving under the influence of cannabis is correlated with increased crash risk and decreased driving ability (Hartman and Huestis, 2013). Car drivers classified as positive for cannabis therefore represent an expanding target audience for prevention campaigns in public healthcare provision. A better knowledge of the impact of cannabis on cognitive functions critical for driving is therefore crucial, especially for the decrease in mortality and morbidity on roads and for the development of appropriate legislation. Further investigation to evaluate the visual function in regular cannabis users and to assess the impact on regular cannabis use on driving is needed.

On this basis, studying the visual function in regular cannabis users represents a relevant and original approach allowing the evaluation of the impact of exogenous cannabinoids on an accessible, well-studied function (Palczewski, 2011). Currently, mounting evidence suggests that visual processing represents an additional interesting measure to explore neural dysfunctions associated with mental disorders (Javitt, 2009; Yoon et al., 2013). The visual system forms one of the early information processing entities, and has now been largely studied (Palczewski, 2011). Much technological progress has been recently made in the study of structural and functional characteristics of the visual system (Disney et al., 2007; Katzner et al., 2011; Palczewski, 2011), such as the development of non-invasive methods yielding valuable information on the central level of neurotransmitter, for example magnetic resonance spectroscopy (MRS). Additionally, there are standardized measures to examine different stages of visual processing. Concerning the retina, standard clinical electroretinography allows the evaluation of the retinal function in dark and light adaptation to differentiate the rod and cone responses (Marmor et al., 2009). Scotopic ERG evaluates the rod response and the response of the ON-bipolar cells, while photopic ERG evaluates cone response and simultaneous ON and OFF pathways (Holder et al., 2010). The contrast sensitivity function assesses spatial specificity, and temporal discrimination of the visual system is evaluated by both visual backward masking (Giersch and Herzog, 2004) and simultaneity threshold measurements (Brown et al., 2003). These behavioral measurements can be extended by electrophysiological ones such as event-related potentials (ERP) to evaluate temporal dynamics of early visual processing. Cerebral imaging coupled with the previous behavioral paradigms then allows the evaluation of the cerebral areas involved in visual information processing.

There are many possible reasons for the difficulties met in developing clinical studies assessing the impact of cannabis consumption (Carlin et al., 1972; Weil et al., 1969). Firstly, because cannabis is an illicit drug in most countries, cannabis users may be suspicious and reluctant to participate in a clinical study. They may think that the main goal of studying cannabis is to prove its toxic effects and thus plead against its legalization. Secondly, it is important to control composition and route of administration to limit some variations in measurements. Cannabinoid agonists may be administrated by oral, intra-venous or inhaled routes, and this may change the dose ingested. Similarly, the frequency and extent of use may vary with the type of drug (Huestis, 2007). Furthermore, the evolution of the concentration of THC and CBD contained in the cannabis consumed complicates the understanding of dose-related effects of each compound. Thirdly, the acute administration of cannabis induces impairments in cognitive functions (Crean et al., 2011; Lundqvist, 2005; Solowij and Battisti, 2008), making it difficult to perform any kind of measurement, and especially to interpret the results. Fourthly, cannabis may be associated with other psychoactive drugs, complicating the extraction of the specific effect of cannabis use (González Pérez et al., 1995). A number of studies that have evaluated the cognitive functions in cannabis users have already overcome these various difficulties. Consequently, it is critical to obtain data regarding the impact of cannabis intake on the visual function in regular users.

Several methodological considerations have to be borne in mind for future research on cannabis. One is to accurately evaluate, with a consistent measurement tool, the dose of cannabinoids consumed to seek a dose–effect relationship. Also, cannabis may induce acute, residual or withdrawal effects that can be evaluated and so reported to provide a clear picture of the consequences of cannabis use. In studies assessing the residual effects of cannabis, measurements must be performed some time after the last cannabis use to prevent acute cognitive deficits such as attentional and awareness disturbance (Knight and Silverstein, 2001). The use of licit or illicit drugs associated with cannabis use makes screening for these drugs necessary, as they are liable to disrupt the functioning of several biological systems, for example thiamine deficiency in alcohol use, thus altering the cannabinoid function. In future studies, urine analyses should therefore be systematically performed to rule out these confounding factors. Some substances such as tobacco or alcohol may also be evaluated by questionnaires. Finally, since cannabis is widely used with tobacco, for example smoked as a mixture (Agrawal et al., 2012), a control group of tobacco users may be needed to differentiate the effects of each drug.

Developing new models and biomarkers assessing the neurotoxicity of substance consumption and allowing the early detection of these disorders now represents a new challenge in the neurosciences. For example in schizophrenia, the development of tasks that allow the evaluation of visual perception has been added to the Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) battery (Carter et al., 2011). Thus further work is needed to develop valid, reproducible,

reliable and easy-to-use markers of neural activity in cannabis users, thereby facilitating the screening of these disorders and the understanding of neural mechanisms underlying related deficits. Visual function may provide some of these markers.

7. Conclusion

Although visual abnormalities have been observed in cannabis users, few studies have yet evaluated the impact of cannabis use on visual information processing. Recent findings in animal and human studies have shown the distribution of endocannabinoids throughout the visual system and their involvement in critical mechanisms such as visual synaptic plasticity and visual neurotransmission. Such knowledge may enhance our understanding of the biological mechanisms underlying cannabis-related deficits, and so prompt more studies in cannabis users. Additionally, technological advances such as the development of precise, non-invasive measurement, together with standardized protocols, currently allow the rigorous assessment of visual function and ensure reproducible results. Consequently, further investigation of the visual function at the retinal and cortical levels after both acute and chronic cannabis use is needed to provide a clearer picture of visual dysfunctions due to cannabis use. We see that studying the effects of cannabis use is far from straightforward. This review outlines some methodological considerations that may help future research on this public health issue.

Role of funding source

This work was funded by a French National Research Agency Grant (ANR-12-SAMA-0016-01) and by the French "Mission Interministérielle de Lutte contre la Drogue et la Toxicomanie".

Contributors

All the authors contributed to write the manuscript, concurred with the submission and have approved the final manuscript.

Conflict of interest

All the authors declare that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

This work was supported by the French National Research Agency and the French "Mission Interministérielle de Lutte contre la Drogue et la Toxicomanie".

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