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Oscillations dans la bande de fréquence gamma dans des modèles de rongeurs pour la schizophrénie

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Gamma Frequency Oscillations in Rodent Models for Schizophrenia

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"I don't know anything, but I do know that everything is interesting if you go into it deeply enough"

— *Richard P. Feynman*

ENGLISH ABSTRACT

Background

Schizophrenia is a devastating psychiatric illness most prominently characterised by symptoms such as hallucinations, disordered thought and delusions. However, patients also exhibit a variety of sensory and cognitive deficits including alterations in visual processing, sensory gating, working memory and attention. The pathophysiological mechanisms behind these cognitive impairments are still unknown, but they are thought to result from abnormal activity both within and between cortical areas, and in how brain networks code, communicate and combine information (Andreasen et al., 1999; Friston, 1999; Phillips and Silverstein, 2003).

Neural oscillations, in particular those in the gamma frequency band (γ, 30–80 Hz), are purported to support the coordination of activity in diverse brain regions and to combine the inputs of disparate neural networks (Engel et al., 1997; Singer, 1999; Buzsaki and Draguhn, 2004). This type of functional integration is essential for cognitive processes, such as attention, memory and perception, and the associated γ oscillations are altered in neurobiological disorders, including schizophrenia (Tallon-Baudry and Bertrand, 1999; Fries et al., 2001; Melloni et al., 2007). Patients with schizophrenia have increased ongoing cortical γ activity during psychotic episodes and hallucinations (Spencer et al., 2004; Ffytche, 2008) and extensive disruptions (both increases and decreases) in sensory-evoked and task-induced γ oscillations (see Gandal et al., 2012 and Uhlhaas and Singer, 2010 for reviews). These findings lead to the hypothesis that excessive ongoing γ activity represents an aberrant network 'noise', disrupting information processing and communication in neural circuits. This viewpoint provides a theoretical framework for understanding the information processing deficits in schizophrenia.

Gamma oscillations are generated by recurrent inhibition through the action of networks of parvalbumin (PV) containing GABAergic interneurons, a neuronal subtype that is reduced in number in patients with schizophrenia (Lewis et al., 2005; Glausier et al., 2014). Non-competitive N-Methyl-D-aspartate receptor (NMDAr) antagonists, such as ketamine and MK-801 (well-known psychotomimetic substances), quickly and transiently increase the power of ongoing γ oscillations in rodents (Pinault, 2008) and in humans (Hong et al., 2010). The hypothesis of NMDAr hypofunction is currently widely investigated at both clinical and experimental levels (Krystal et al., 1994; Kocsis et al., 2013) and provides a biological basis for how abnormal γ oscillations may arise in schizophrenia. The work presented in this thesis consists of a series of studies into the effects of pharmacological and genetic manipulations on ongoing and sensory-evoked γ oscillations in rodent models for schizophrenia.

Aims

The goal of this thesis is to increase our understanding of the pathophysiological properties of γ oscillations in rodent models for schizophrenia and to explore how this electrophysiological marker may be used to evaluate novel compounds with antipsychotic properties. Three separate studies were conducted using behavioural, electrophysiological, pharmacological and genetic tools.

Aim 1 – Effects of chronic administration of antipsychotics on ketamine-induced behavioural and electrophysiological abnormalities

Current antipsychotic medications alleviate the symptoms in less than half of patients with schizophrenia, with limited efficacy in the negative and cognitive symptom domains (Leucht et al., 2008). For this reason the development of novel

antipsychotic medications is a necessary goal to improve treatment of this complex disease. Translational biomarkers of antipsychotic efficacy would be useful tools for the development of potential novel medications. A single systemic injection of a subanesthetic dose of ketamine or MK-801 in rodents induces a dose-dependent increase in both the power of ongoing γ oscillations and in locomotor activity (Pinault, 2008; Hakami et al., 2009). Hyperlocomotion in rodents is commonly employed as a model for psychosis (Van den Buuse et al., 2005). Clinical studies have demonstrated that abnormalities in γ activity in schizophrenia patients are associated with psychotic symptomatology (Baldeweg et al., 1998; Gordon et al., 2001; Spencer, 2009). These findings suggest that NMDAr antagonist-induced increases in the power of γ oscillations may represent a useful electrophysiological biomarker of an acute psychotic-like state (Lee et al., 2003). Furthermore, previous work from our laboratory (Jones et al., 2012) demonstrated that single injections of typical and atypical antipsychotics modulate the power of γ oscillations and reduce ketamine-induced behavioural effects. This study aimed to investigate the efficacy of conventional pharmacological treatments and a novel metabotropic glutamate receptor (mGluR) agonist (LY379368) in a chronic dosing paradigm that reflects clinical treatment, in an attempt to evaluate the translational properties of the model.

Aim 2 – Gamma frequency activity and NMDA receptors in neuregulin 1 mutant mice: implications for schizophrenia.

Neuregulin 1 (Nrg1), a protein coded by a leading candidate 'risk' gene for schizophrenia (Stefansson et al., 2002), Nrg1 mRNA and protein expression are altered in schizophrenia (Hashimoto et al., 2004; Law et al., 2006). Although the pathophysiological mechanisms by which altered Nrg1 expression or function results in vulnerability to develop schizophrenia is not known, several lines of evidence link Nrg1 and NMDAr function (Geddes et al., 2011). Nrg1 signalling is

important for the development of PV interneuron circuits, the interneuron subtype that contributes to the generation of γ oscillations. Furthermore, ablation of ErbB4 (the Nrg1 receptor) on PV interneurons leads to a range of abnormal synaptic and circuit level activities, including altered γ frequency activity (Wen et al., 2010; del Pino et al., 2013). Mice genetically engineered to be heterozygous for the transmembrane domain of the Nrg1 protein (Nrg1 TM HET mice) possess a behavioural phenotype relevant to schizophrenia, exhibiting hyperlocomotion, increased anxiety, impairments in working memory and social interaction deficits (Stefansson et al., 2002; Karl et al., 2007; Duffy et al., 2010; Chesworth et al., 2012). This study examined ongoing γ activity and expression of NMDAr in mice containing a heterozygous mutation in the transmembrane domain of Nrg1 with the aim to characterise electrophysiological singularities of this genetic model.

Aim 3 – Contribution of the corticothalamic pathway in the ketamine-induced reduction of the γ signal-to-noise ratio

There is a growing body of literature indicating involvement of dysfunctional thalamic networks in the pathophysiology of schizophrenia, specifically in the observed disturbances in function-related γ frequency oscillations (Cronenwett and Csernansky, 2010; Pinault, 2011; Woodward et al., 2012). A postulated mechanism of these impairments is reduced NMDAr activation at glutamatergic synapses on GABAergic interneurons (Carlen et al., 2011). We previously demonstrated that a single subanesthetic dose of ketamine transiently increases the power of ongoing γ oscillations and decreases sensory-evoked γ oscillations in the rat somatosensory thalamocortical (TC) network (Kulikova et al., 2012), reducing the γ signal-to-noise ratio; (defined as the power of the sensory-evoked γ response above the power of the baseline γ noise). Both the glutamatergic TC neurons and the GABAergic thalamic reticular nucleus (TRN) neurons are innervated by glutamatergic corticothalamic (CT) axons originating from layer VI. The goal of the

present study was to assess the contribution of the CT pathway in the ongoing and sensory-evoked γ oscillations in the somatosensory thalamus.

Methodology

Aim 1 – The effects of chronic antipsychotic administration on the electrophysiological and behavioural effects of acute NMDAr antagonism was assessed by implanting adult male Wistar rats with subcutaneous osmotic pumps and electrocorticogram (ECoG) recording electrodes. Animals received treatment with subcutaneous (s.c.) haloperidol (0.25 mg/kg), clozapine (5 mg/kg), LY379268 (0.3 mg/kg) or vehicle (10% acetic acid) for 28 days during which they then underwent weekly recordings of electrophysiological activity. On day 26 ketamine (5 mg/kg, s.c.) was administered and concurrent ECoG and locomotor activity were recorded. Behavioural data was objectively quantified using Ethovision tracking software and ECoG data were analysed using a combination of fast-Fourier transforms (FFT) and wavelet analysis. Results were compared with those generated previously using acute treatment with antipsychotic medications.

Aim 2 – To characterise the electrophysiological and behavioural effects of a mutation in the transmembrane domain of the Neuregulin 1 gene, female Nrg1 TM HET mice and wild-type controls were implanted with ECoG recording electrodes and underwent a series of electrophysiological recordings and behavioural tests. Ongoing electrophysiological activity was recorded during periods of quiet wakefulness in animal's home cages, as well as during a ketamine (10 mg/kg, s.c.) challenge in an open-field behavioural chamber. Locomotor activity was assessed in the open field under saline and ketamine conditions. Sensory-triggered γ activity was assessed using an auditory-evoked paradigm in acoustic behavioural chambers again under both saline and ketamine conditions. Electrophysiological data were

analysed using a combination of FFT and wavelet decomposition techniques. mRNA expression of ErbB4 and NMDAr subunits, as well as NR2B phosphorylation levels were measured in the prelimbic cortex using qPCR and Western blotting.

Aim 3 – To investigate the contributions of the CT pathway to thalamic ongoing and sensory-evoked γ activities we performed a series of in-vivo electrophysiological studies in lightly anaesthetised adult Wistar rats. Local-field potential and multi-unit activities were simultaneously recorded at the three key sites of the somatosensory TC system. We recorded the activities of 1) the medial part of the ventral posterior nucleus (VPm) – the principal target of trigeminal nerve input to the thalamus. 2) The thalamic reticular nucleus (TRN) – a GABAergic structure with γ pacemaker properties that exclusively innervates the dorsal thalamus and receives innervation from both the VPm and the Layer VI CT pathway (Pinault, 2004). 3) Layer VI of the corresponding somatosensory cortex. Electrical stimulation of the vibrissae was applied to evoke somatosensory activity and electrophysiological measures were recorded under saline and ketamine (2.5 mg/kg, s.c.) conditions. Further experiments were conducted with tetrodotoxin (TTX, a sodium channel blocker) applied directly to the cortical surface to block cortical feedback. Data generated were analysed with time-frequency decomposition using wavelet techniques, this allowed for the qualitative and quantitative assessment of ongoing and sensory-evoked γ activities.

Results

Aim 1 – Significant and sustained decreases in the power of ongoing γ oscillations was measured after chronic administration of haloperidol (64% decrease) or clozapine (43% decrease), but not with LY379268 (2% increase). Chronic administration of all three drugs attenuated the ketamine-induced γ and locomotor

hyperactivities. When compared to the acute effects of antipsychotic administration, the chronic delivery of both haloperidol and clozapine was able to significantly reduce the transient abnormal electrophysiological effects that follow a single sub-anaesthetic dose of ketamine.

Aim 2 – Nrg1 TM HET mutant mice displayed an increase in the power of ongoing cortical γ compared to wild-type mice (p<0.0001), reduced auditory-evoked γ activity (p<0.0001) as well as a dampened γ power response to ketamine. Nrg1 mice exhibited increased baseline hyperlocomotion compared to wild type animals, but both genotypes showed a similar locomotor response to ketamine. ErbB4 and NMDAr subunit mRNA expression was unaffected by Nrg1 genotype, but NR2B phosphorylation levels were significantly lower in Nrg1 mutant mice. Nrg1 mice displayed an altered γ frequency oscillation phenotype that is relevant to the pathophysiology of schizophrenia. The reduced phosphorylation of the NMDAr NR2B subunit presents a potential molecular mechanism in the generation of these abnormal γ rhythms.

Aim 3 – A single injection of ketamine quickly and transiently increased the power of ongoing γ oscillations and simultaneously decreased both the amplitude and the γ power of the sensory-evoked responses in both the thalamus and the cortex. The sensory-evoked response recorded in the VPm and TRN was characterised principally by both short-latency (4.3±0.1 ms) and long-latency (10.8±0.1 ms) negative components. The late response was hypothesised to be the result of CT feedback. Local application of TTX in the related cortex abolished the amplitude of the late response and decreased the amount of the associated γ power. The amplitude of the late response was highly correlated (r2=0.9, p<0.001) with the power of the sensory-evoked γ oscillations, indicating that the CT pathway significantly contributed in the sensory-evoked γ in the thalamus.

Conclusions

Gamma oscillations have been demonstrated to play an important role in a variety of cognitive processes. There is increasing evidence that the cognitive abnormalities and symptoms seen in schizophrenia are related to dysfunctional networks, which exhibit abnormal γ oscillations; a potential hallmark of the pathophysiology of the disease. The three separate studies, which form the basis of the present thesis, used electrophysiological singularities (γ hyperactivity) recorded in rodent models for schizophrenia and psychotic states to increase our understanding of the pathophysiology of schizophrenia. The outcome of this thesis work could lead to the development of more robust and effective translational models for psychiatric disorders.

Aim 1 – The study of chronic antipsychotic treatment both verifies and expands our previous work demonstrating that antipsychotic medications affect γ oscillations, this result validates a potential biomarker of antipsychotic efficacy and further increases the relevance of γ oscillations to the schizophrenia disease state. It also develops a model that appears to be responsive to chronic, but not acute, antipsychotic treatment. The increased efficacy of haloperidol and clozapine under chronic treatment conditions recapitulates the clinical situation, which strengthens the validity to this model.

Aim 2 – The presence of distinct γ frequency electrophysiological abnormalities in Nrg1 mutant animals is the first evidence of an abnormal γ oscillatory as a potential endophenotype in the Nrg1 transmembrane domain mutant mouse, providing a neurophysiological correlate of the behavioural abnormalities observed in this model. It provides further evidence of the γ signal-to-noise ratio decreases in schizophrenia models and it is also an important extension of our γ oscillation model into a genetic foundation.

Aim 3 – Investigating the contribution of the CT pathway in the ongoing and somatosensory-evoked γ oscillations demonstrated that the cortex plays a great role in the generation of thalamic γ oscillations during information processing and that ketamine reduces the CT-mediated sensory γ signal-to-noise ratio.

Thus, the specific results from each study extend certain aspects of our understanding of the pathophysiology of schizophrenia and offer potential new methods for the development of therapeutic concepts. In particular, we show that, by using various tools (behavioural, electrophysiological, pharmacological and genetic), we can study, in rodent models (pharmacological or genetic), both the state and the functionality of neural circuits through the dynamics of spontaneous and sensory-evoked γ oscillations and use them to test molecules having antipsychotic properties.

RÉSUMÉ FRANÇAIS

Contexte

La schizophrénie est une maladie psychiatrique invalidante caractérisée principalement par des symptômes tels que des hallucinations, des troubles de la pensée et des idées délirantes. Les patients présentent également une variété de déficits sensoriels et cognitifs incluant des altérations dans le traitement des informations sensorielles, dans la mémoire de travail et l'attention. Les mécanismes physiopathologiques à l'origine de ces troubles cognitifs demeurent inconnus, mais de nombreux travaux laissent à penser qu'ils résulteraient d'une activité anormale au sein de diverses aires corticales et leurs interactions fonctionnelles ainsi que dans la manière dont les circuits du cerveau codent, communiquent et transmettent les informations (Andreasen et coll, 1999; Friston, 1999; Phillips et Silverstein, 2003).

Les oscillations neuronales, en particulier dans la bande de fréquence gamma (γ, 30-80 Hz) sont reconnues pour jouer un rôle important dans la coordination (synchronisation spatio-temporelle) des activités de diverses régions du cerveau (Engel et coll., 1997; Singer, 1999; Buzsaki and Draguhn, 2004). Ce type d'intégration fonctionnelle est essentiel dans les processus complexes comme la cognition, l'attention, la mémoire et la perception, tous étant associés à des oscillations γ, lesquelles sont perturbées dans de nombreux désordres neurobiologiques complexes comme la schizophrénie (Tallon-Baudry and Bertrand, 1999; Fries et coll., 2001; Melloni et coll., 2007). Le cortex de patients atteints de schizophrénie développe une augmentation des oscillations γ durant des épisodes psychotiques et des hallucinations (Baldeweg et coll., 1998; Spencer et coll., 2004; Ffytche, 2008) ainsi que d'importantes perturbations (augmentation comme diminution) dans les oscillations γ évoquées par les voies sensorielles (Gandal et coll., 2012; Uhlhaas and Singer, 2010). Ces résultats nous conduisent à formuler

l'hypothèse selon laquelle l'activité γ spontanée (ou activité basale) représente, au sein des réseaux neuronaux, un « bruit » persistant et gênant à leur bon fonctionnement, ainsi perturbant le traitement de l'information et de la communication dans les circuits en question. Ce point de vue offre un cadre théorique pour la compréhension des déficits du traitement de l'information dans la schizophrénie.

Les oscillations γ sont engendrées essentiellement par une inhibition récurrente assurée par l'action collective d'interneurones GABAergiques contenant de la parvalbumine (PV), un sous-type neuronal qui est réduit en nombre chez les patients atteints de schizophrénie (Lewis et coll., 2005). Les antagonistes non-compétitifs des récepteurs N-méthyl D-aspartate (rNMDA), comme la kétamine et le MK-801 (des substances psychotomimétiques connues), augmentent rapidement et transitoirement la puissance des oscillations γ chez l'homme (Hong et coll., 2010) et le rongeur (Pinault, 2008; Hakami et coll, 2009). L'hypothèse d'un hypofonctionnement des rNMDA est actuellement largement étudiée au niveau clinique et expérimental (Krystal et coll., 1994; Kocsis et coll., 2013) pour comprendre les mécanismes responsables des anomalies fonctionnelles dans les oscillations γ observées dans la schizophrénie. Cette thèse se compose d'une série d'études sur les effets de manipulations pharmacologiques et génétiques sur les oscillations γ spontanées et évoquées, par l'activation des voies sensorielles, dans des modèles murins pour la schizophrénie.

Objectifs

L'objectif de cette thèse est de comprendre les propriétés physiopathologiques des oscillations γ au sein de modèles murins pour la schizophrénie et de chercher comment utiliser ce marqueur électrophysiologique pour tester des molécules avec

des propriétés (potentiellement) antipsychotiques. Trois études distinctes ont été réalisées avec des outils comportementaux, électrophysiologiques, pharmacologiques et génétiques.

Objectif 1 – Effet d'une administration chronique d'antipsychotiques sur les effets comportementaux et électrophysiologiques induits par la kétamine

Les traitements antipsychotiques actuels apaisent les symptômes de moins de la moitié des patients atteints de schizophrénie avec une efficacité limitée pour les symptômes négatifs et cognitifs (Leucht et coll., 2008). C'est pourquoi, le développement de nouveaux médicaments antipsychotiques est un objectif essentiel pour traiter efficacement cette maladie complexe. Des biomarqueurs translationnels de l'efficacité antipsychotique représenteraient un outil important dans le développement de ces nouveaux médicaments. Les antagonistes non-compétitifs des rNMDA induisent, chez le rongeur, une augmentation de la puissance des oscillations γ spontanées et une augmentation de l'activité locomotrice (Hakami et coll., 2009), un modèle couramment utilisé pour les états psychotiques aigus (Van den Buuse et coll., 2005). Les résultats cliniques montrent que des anomalies de l'activité γ chez des patients atteints de schizophrénie est liée à la symptomatologie psychotique (Baldeweg et coll, 1998; Gordon et coll, 2001; Spencer, 2009). Ces résultats suggèrent que les augmentations induites par les antagonistes des rNMDA dans la puissance γ représentent un bio-marqueur électrophysiologique d'un état rappelant un état psychotique aigu (Lee et coll., 2003). De plus, des travaux antérieurs de notre laboratoire (Jones et coll., 2012) démontrèrent que les antipsychotiques typiques et atypiques modulent profondément la puissance des oscillations γ et réduisent les effets comportementaux induits par la kétamine. Cette étude visait à évaluer l'efficacité des traitements pharmacologiques classiques ainsi que celle d'un nouvel agoniste des récepteurs métabotropiques au glutamate

(mGluR) (LY379368) avec des doses similaires à celles utilisées en clinique, et ce, afin d'évaluer les propriétés translationnelles de ce modèle.

Objectif 2 – Activité γ et rNMDA chez des souris mutantes (Neuregulin 1) : implications pour la schizophrénie.

La Neuregulin 1 (Nrg1), une protéine codée par un gène « risque » candidat pour la schizophrénie (Stefansson et coll, 2002), l'ARNm Nrg1 et l'expression de protéines sont modifiés dans la schizophrénie (Hashimoto et coll., 2004; Law et coll., 2006). Bien que les mécanismes physiopathologiques qui lient les modifications d'expression Nrg1 à la vulnérabilité à la schizophrénie ne soient pas connus, plusieurs indices lient le gène Nrg1 au fonctionnement des rNMDA (Geddes et coll., 2011). La Signalisation Nrg1 est importante pour le développement de circuits des interneurones PV+, et ces interneurones à décharge rapide contribuent à la genèse d'oscillations γ et l'ablation de ErbB4 (récepteur Nrg1) sur les interneurones PV+ conduit à une série d'activités synaptiques anormales, comprenant une modification de l'activité γ (Wen et coll., 2010; del Pino et coll., 2013). Les souris génétiquement modifiées pour être hétérozygote pour le domaine transmembranaire de la protéine Nrg1 (souris Nrg1 TM HET) possèdent un phénotype comportemental pertinent pour la schizophrénie, présentant une hyperlocomotion, une augmentation de l'anxiété, des troubles de la mémoire de travail et des déficits d'interaction sociale (Stefansson et coll., 2002; Karl et coll., 2007; Duffy et coll., 2010; Chesworth et coll., 2012). Notre étude portait sur les oscillations γ et l'expression des rNMDA chez la souris contenant une mutation hétérozygote dans le domaine transmembranaire de Nrg1 dans le but de caractériser des singularités électrophysiologiques chez ce modèle génétique.

Objectif 3 – Contribution de la voie corticothalamique dans la réduction induite par la kétamine du rapport signal γ-bruit γ

Un nombre croissant d'arguments cliniques et expérimentaux soutiennent l'hypothèse selon laquelle les circuits neuronaux impliquant le thalamus sont dysfonctionnels dans la physiopathologie de la schizophrénie, plus précisément dans les perturbations des oscillations γ fonctionnelles observées (Cronenwett and Csernansky, 2010; Pinault, 2011; Woodward et coll., 2012). Ces déficits seraient sous-tendus par un mécanisme impliquant un hypofonctionnement des rNMDA au niveau des synapses glutamatergiques sur des interneurones GABAergiques (Carlen et coll., 2011). Nous avons déjà démontré dans le système thalamocortical (TC) du rat que l'administration systémique d'une dose subanesthésique de kétamine augmente transitoirement la puissance des oscillations γ spontanées et diminue les oscillations γ évoquées par l'activation des voies sensorielles (Kulikova et coll., 2012), ainsi réduisant le rapport puissance du signal γ (de nature sensoriel)/puissance du bruit γ (oscillations basales). Les neurones TC glutamatergiques et du noyau réticulaire thalamique (TRN) GABAergique sont massivement innervés par les axones corticothalamiques (CT), glutamatergiques, issus de la couche VI. L'objectif de cette étude était d'évaluer la contribution de la voie CT dans les oscillations γ spontanées et évoquées dans le thalamus somatosensoriel.

Méthodologie

Objectif 1 – Les effets d'une administration chronique d'antipsychotiques sur les effets aigus, électrophysiologiques et comportementaux, de la kétamine furent testés à la suite de l'implantation, chez le rat Wistar male adulte, avec une pompe osmotique sous-cutanée (s.c.) et des électrodes pour effectuer un

électrocorticogramme (ECoG). Ainsi les animaux recevaient une perfusion sous-cutanée d'un traitement contenant de l'halopéridol (0,25 mg/kg), de la clozapine (5 mg/kg), du LY379268 (un agoniste aux récepteurs mGluR2/3, 0,3 mg/kg) ou un véhicule (acide acétique à 10%) durant 28 jours au cours desquels des enregistrements ECoG hebdomadaires furent effectués. Au jour 26, une dose subanesthésique (5 mg/kg, s.c.) de kétamine fut injectée par voie sous-cutanée puis les activités, locomotrice et ECoG, furent simultanément enregistrées. Les données électrophysiologiques furent analysés en combinant des transformées de Fourier rapides (FFT) et une analyse par ondelettes. Les résultats furent comparés à ceux précédemment obtenus en utilisant un traitement aigu avec les mêmes substances dotées de propriétés antipsychotiques.

Objectif 2 – Afin de caractériser les effets électrophysiologiques et comportementaux d'une mutation dans le domaine transmembranaire du gène Nrg1, des souris Nrg1 TM HET femelles furent implantées avec des électrodes d'enregistrement corticales ECoG et furent soumises à une série d'enregistrements électrophysiologiques et des tests comportementaux. L'activité électrophysiologique spontanée était enregistrée au cours des périodes de veille calme dans leur cage, ainsi que lors d'une administration de kétamine (10 mg/kg, s.c.). L'activité locomotrice fut évaluée dans une enceinte appropriée sous condition saline ou kétamine. L'activité γ sensorielle évoquée fut évaluée à l'aide d'un paradigme de stimulation auditive dans des enceintes comportementales acoustiques, et ce, sous la condition saline ou kétamine. Les données électrophysiologiques furent analysées en utilisant une combinaison de FFT avec des techniques de décomposition en ondelettes. L'expression de l'ARNm d'ErbB4 et des sous-unités rNMDA ainsi que les niveaux de phosphorylation de NR2B furent mesurés dans le cortex prélimbique.

Objectif 3 – Afin d'étudier la contribution de la voie CT dans les activités thalamiques γ, spontanées et évoquées, nous effectuâmes une série d'études électrophysiologiques in vivo sur des rats adultes Wistar sous anesthésie légère. Plus précisément, des potentiels de champ extracellulaires et des activités multi-unitaires étaient enregistrés simultanément aux trois sites clés du système TC somatosensoriel. Ainsi étaient enregistrées les activités 1) de la partie médiane du noyau ventral postérieur (VPM) - la principale entrée du nerf trijumeau du thalamus ; 2) du noyau réticulaire thalamique (TRN), une structure GABAergique avec des propriétés de type pacemaker dans la bande de fréquence γ, qui innerve exclusivement le thalamus dorsal et qui est innervé par les neurones TC du VPM et par les neurones CT issus de la couche VI du cortex somatosensoriel ; et 3) des neurones de cette couche VI (Pinault, 2004). La voie somatosensorielle était activée par une stimulation électrique des vibrisses, et les enregistrements simultanés étaient effectués en condition saline ou kétamine (2,5 mg/kg, s.c.). D'autres expériences furent réalisées avec de la tétrodotoxine (un bloquant des canaux sodiques) appliquée directement sur la surface corticale afin de bloquer les activités de la voie CT. Les données obtenues furent traitées par une analyse temps-fréquence en utilisant la technique des ondelettes, ainsi permettant d'appréhender qualitativement et quantitativement les activités γ spontanées et évoquées.

Résultats

Objectif 1 – Une baisse significative et durable de la puissance des oscillations γ spontanées fut observée à la suite d'une administration chronique d'halopéridol (diminution de 64 %) ou de clozapine (diminution de 43 %), mais pas avec le LY379268 (augmentation de 2%). Les effets aigus de l'injection de kétamine (augmentations significatives de la puissance des oscillations γ spontanées et de

l'activité locomotrice) furent considérablement atténués durant l'administration chronique de chacun des trois médicaments comparativement à la condition contrôle (véhicule). Par rapport aux effets d'une administration aiguë d'antipsychotiques, l'administration chronique d'halopéridol ou de clozapine réduisit de manière significative les effets électrophysiologiques d'une injection de kétamine.

Objectif 2 – Le cortex des souris mutantes Nrg1 TM HET montrait une augmentation de la puissance des oscillations γ corticales spontanées par rapport aux souris de type sauvage ($p < 0.0001$), une réduction de la l'activité γ évoquée (à la suite de l'activation des voies auditives ; $p < 0.0001$) ainsi qu'une diminution de la puissance de l'hyperactivité γ spontanée transitoire induite par la kétamine. Les Souris Nrg1 présentaient aussi une augmentation de leur activité locomotrice basale par rapport aux souris sauvages, mais pas de réponse locomotrice singulière mesurable après une injection de kétamine à une dose subanesthésique. L'expression d'ARNm ErbB4 et des rNMDA n'étaient pas affectée par le génotype Nrg1, mais les niveaux de phosphorylation NR2B étaient significativement plus faibles chez les souris mutantes Nrg1. Ces souris présentaient une hyperactivité γ spontanée anormalement élevée, un endo-phénotype présentant une certaine pertinence relative à la physiopathologie de la schizophrénie. Une réduction de la phosphorylation de la sous-unité NR2B des rNMDA est un mécanisme moléculaire candidat potentiel dans la genèse de ces rythmes γ anormaux.

Objectif 3 – Un injection unique de kétamine (à une dose subanesthésique) augmentait, rapidement et transitoirement, la puissance des oscillations γ spontanées et simultanément diminuait l'amplitude des réponses sensorielles évoquées au sein du thalamus et du cortex ainsi que la puissance des ondes γ correspondantes. Les réponses évoquées dans le VPM et le TRN étaient composées de 2 ondes négatives à courte (4.3 ± 0.1 ms) et longue (10.8 ± 0.1 ms) latences.

Cette onde tardive étaient interprétée comme étant la manifestation d'un rétrocontrôle CT. L'application locale de TTX sur le cortex atténua l'amplitude de la réponse tardive et diminua la puissance des oscillations γ associées. L'amplitude de la réponse tardive était hautement corrélée ($R2 = 0.9$, $p < 0.001$) avec la puissance des oscillations γ évoquées, indiquant que la voie CT contribuait de manière significative aux oscillations γ de nature sensorielle enregistrées au sein du thalamus.

Conclusions

Les oscillations γ jouent un rôle important dans divers processus cognitifs. Il y a de plus en plus d'éléments indiquant que les désordres cognitifs et les symptômes observés dans la schizophrénie sont liés à un dysfonctionnement des circuits neuronaux se manifestant par des anomalies dans les oscillations γ, un marqueur potentiel de la physiopathologie de la maladie. Les trois études distinctes de ce travail de thèse utilisèrent des singularités électrophysiologiques (hyperactivité γ) enregistrées dans des modèles murins pour la schizophrénie et pour des états psychotiques. Notre travail devrait conduire au développement de modèles translationnels plus efficaces et plus robustes de désordres psychiatriques complexes et donc nous aider à élargir notre connaissance de la physiopathologie de la schizophrénie.

Objectif 1 - L'étude d'un traitement chronique par une molécule de type antipsychotique parachève nos travaux précédents sur les effets aigus de médicaments antipsychotiques sur les oscillations γ, validant ainsi ces oscillations comme un biomarqueur potentiel pour étudier l'efficacité de substances dotées de propriétés antipsychotiques potentielles. Ce travail donne du poids sur la relation étroite entre les oscillations γ et la schizophrénie. Il développe aussi un modèle qui

semble être plus sensible à un traitement chronique qu'à un traitement aigu par un antipsychotique. L'efficacité d'un traitement chronique avec l'halopéridol ou la clozapine reproduit la situation clinique, ce qui ajoute du poids à la validité de ce modèle.

Objectif 2 – Les oscillations γ anormalement excessives dans le cortex des souris mutantes Nrg1 sont interprétées comme un endo-phénotype potentiel, un corrélat neurophysiologique des anomalies comportementales observées dans ce modèle génétique. Il fournit un argument supplémentaire en faveur de l'hypothèse selon laquelle il y a une diminution du rapport signal-bruit durant le traitement de l'information dans la schizophrénie.

Objectif 3 – En étudiant la contribution de la voie CT dans les oscillations γ spontanées et évoquées (par les voies somatosensorielles), nous démontrons que la voie CT joue un rôle majeur dans la genèse d'oscillations γ thalamiques durant le traitement de l'information et que la kétamine réduit le rapport signal γ-bruit γ dans la transmission CT.

Ainsi, les résultats spécifiques à chaque étude alimentent certains aspects de notre connaissance de la physiopathologie de la schizophrénie et offre une voie nouvelle pour le développement de concepts thérapeutiques. Plus particulièrement, nous montrons qu'en utilisant divers outils (comportementaux, électrophysiologiques, pharmacologiques et génétiques) nous pouvons étudier, sur des modèles murins (pharmacologiques ou génétiques), l'état et la fonctionnalité de circuits neuronaux au travers de la dynamique des oscillations γ spontanées et évoquées, par exemple par l'activation de voies sensorielles, et les utiliser pour tester des molécules ayant des propriétés antipsychotiques.

DECLARATION

This is to certify that

    i.   The thesis comprises only my original work towards the PhD except where indicated in the Preface,

    ii.   due acknowledgement has been made in the text to all other material used,

    iii.   the thesis is fewer than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Signature _____   Date _____

PREFACE

The following sections of this thesis were contributed to by colleagues and collaborators:

Résumé Français *Front Matter*

Mr. Audran Chamarty and Associate Professor Didier Pinault of the Faculté de Médecine, Université de Strasbourg, France assisted in the translation of the English Abstract into French

Acute antipsychotic experiments *Chapter 2*

Ms. Maya Reddy of the Department of Medicine, University of Melbourne, Australia conducted experiments investigating acute administration of antipsychotics prior to my PhD. This data was reanalysed and used in addition to the original experiments performed in Chapter 2.

Tissue collection and qPCR assessment of NMDA receptor expression
Chapter 3

Dr. Leonora Long of the Schizophrenia Research Institute, Sydney, Australia performed the tissue collection, qPCR assessment of NMDA receptor expression in neuregulin 1 mutant mice, and generated Figure 3.6

Western blot assessment of NMDA receptor phosphorylation *Chapter 3*

 Dr. Shijie Liu of the Department of Medicine, University of Melbourne, Australia performed Western Blots of phosphorylated NMDA receptor protein and generated Figure 3.7.

In-vivo electrophysiology studies of the somatosensory pathway *Chapter 4*

Experiments in this chapter were performed under the supervision of Associate Professor Didier Pinault of the Faculté de Médecine, Université de Strasbourg, France.

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ABBREVIATIONS

AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ACSF	Artificial cerebral spinal fluid
ASSR	Auditory steady-state response (ASSR)
CT	Corticothalamic
ECoG	Electrocorticogram
EPS	Extrapyramidal side effects
ERP	Event-related potential
EPSC	Exitatory post-synaptic current
FFT	Fast Fourier transform
GABA	Gamma-Aminobutyric Acidx
GAD$_{67}$	Glutamic acid decarboxylase 67 kDA
HFO	High frequency oscillation
i.m.	Intra-muscular
i.p.	Intra-peritoneal
i.v.	Intra-venous
LFP	Local field potential
MRI	Magnetic Resonance Imaging
mRNA	Messenger ribonucleic acid
mGluR	Metabotropic glutamate receptor
MMN	Mismatch negativity
NAA	N-acetylaspartic acid
NAAG	N-acetylaspartylglutamate
NMDA	N-Methyl-D-aspartate
Nrg1	Neuregulin 1
PV	Parvalbumin
PCP	Phencyclidine
PET	Positron emission tomography
PSD95	Post synaptic density protein 95
PPI	Prepulse inhibition
s.c.	Sub-cutaneous
TTX	Tetrodotoxin
TRN	Thalamic reticular nucleus
TC	Thalamocortical
TM HET	Transmembrane heterozygous
VL	Ventral lateral nucleus
VPm	Ventral posteromedial nucleus

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CHAPTER 1

LITERATURE REVIEW

Schizophrenia is a debilitating mental disorder characterised by a breakdown in normal thought processes, blunted emotional responses and varied cognitive deficits. The pathological processes that lead to the development of schizophrenia are still unknown despite ongoing efforts to elucidate these processes over the past 100 years. There is a growing conception in modern neuroscience that the cognitive functions of the brain are the result of coordinated patterns of activity across distributed neuronal circuits. This view enables a powerful theoretical explanation of schizophrenia, that it is the result of dysconnection amongst functional neuronal networks. Gamma oscillations (γ, 30-80 Hz) are a key mechanism that drives the synchronisation of neural activity and disruptions in gamma oscillations represent a potential mechanistic explanation for the dysconnectivity hypothesis. The cellular and molecular mechanisms that govern the generation of gamma frequency neural oscillations intersect with the substantial body of evidence regarding the behavioural, molecular and physiological pathologies of schizophrenia. The following literature review will detail the symptoms and neuropathology of schizophrenia and describe how the hypothesis of dysconnection in the functional neural networks that govern cognitive processes is a cogent one. A description of the neurochemical hypothesis of NMDAr hypofunction and how it converges with the dysfunction hypothesis and γ oscillations will be made. Finally the use of animal models and evidence of NMDAr induced γ oscillatory dysfunction will be detailed.

1.1

Schizophrenia

Schizophrenia is a chronic, severe, and debilitating mental disorder that is characterised by disordered thought, hallucinations, delusions and diverse cognitive deficits (Harrison, 1999). First identified and named in the early 1900's (Bleuler, 1950; Kraeplin, 1919), schizophrenia has long been a confounding disorder; difficult to treat, devastating to those that suffer from it and resisting attempts by scientists to understand it. To this date we still have limited understanding of the fundamental causes of schizophrenia and a reciprocally poor ability to treat those that have it. Approximately 1 percent of the population worldwide develops schizophrenia during their lifetime, and treatment and care of sufferers is estimated to occupy 2-3 % of national healthcare budgets around the world (Knapp et al., 2004). Schizophrenia is also a major cause of disability, being ranked by the World Health Organisation as the 8th leading cause of disability worldwide (Rössler et al., 2005). Despite the prevalence, the high costs, and degree of suffering caused by schizophrenia treatment, options are still limited in their effectiveness, with approximately 29% of patients diagnosed with schizophrenia recovering to a level described as 'good' or better (Jalensky, 2000). As a result of these concerning statistics more research is needed into the underlying causes of schizophrenia and its varied symptomatology.

1.1.1 Symptoms

Schizophrenia is a heterogenous disease, with a large variety of symptoms. The varied nature of schizophrenia symptomatology can be seen between different patients and even within individuals across the course of their disease. The most prominent symptoms are the sometimes bizarre hallucinations and delusions and these capture the imagination of both the public and health professionals and

researchers; however, there is a much larger and more varied symptomatology than these. The symptoms of schizophrenia are broken down into three key domains – positive, negative and cognitive symptoms (van Os and Kapur, 2009). Positive symptoms include the psychotic symptoms and represent traits and actions that patients with schizophrenia possess that aren't seen in healthy individuals. Negative symptoms describe a range of disruptions to normal behaviours and actions that are missing or absent in patients. Lastly, cognitive symptoms are a range of deficits in normal cognition that have been observed in patients with schizophrenia. The negative and cognitive symptoms can be hard to distinguish as being central to the disease, yet they can have some of the most profound impacts on a patients well-being and recovery over time and are the hardest to treat (Elvevåg and Goldberg, 2000).

Positive Symptoms

Hallucinations – The most common form is auditory hallucinations, typically manifesting as one or more voices that are often quite derogatory and violent. World Health Organisation studies suggest that internationally, more than 70 percent of people with schizophrenia have auditory hallucinations, and that percentage may be higher in industrialised societies (Girolamo, 1996).

Delusions – Defined as "A false belief based on incorrect inference about external reality that is firmly sustained despite what almost everyone else believes and despite what constitutes incontrovertible and obvious proof or evidence to the contrary" (World Health Organisation, 1992).

Thought Disorder – Unusual or dysfunctional ways of thinking, sometimes manifesting as garbled and nonsensical speech.

Motor Symptoms – Can manifest in two opposite extremes; patients may present with agitated and highly repetitive motions or conversely, almost complete catatonia (a lack of movement).

Negative Symptoms

Affective flattening – A reduction in the ability to express and display emotion. This can include reductions in facial expressions and gestures as well as speech. Can also affect the ability to understand and interpret emotion in others.

Avolition – The loss of will or drive, patients can lack the ability to act on plans even when it causes them great difficulties.

Anhedonia – A loss of the ability to enjoy or gain or pleasure from almost any activity. Anhedonia is one of the least treatable and most persistent negative symptoms.

Alogia – is a reduction in verbal communication, literally a lack of speech seen in some patients. In some ways it can be considered "negative thought disorder" (Sadock et al., 2014). This can include an increased latency to response, short verbal responses, and a complete lack of spontaneous speech.

Cognitive Symptoms

Specific cognitive deficits have been identified in patients with schizophrenia in addition to the positive and negative symptoms listed above, they include reductions in: Working memory, attention, verbal learning and memory, visual learning and memory, reasoning and problem solving, speed of processing and social cognition (Green et al., 2000; 2004; Keefe and Harvey, 2012).

1.1.2 Diagnosis

There is currently no objective test to definitively recognise schizophrenia, and diagnosis is made by mental health professionals in accordance with the Diagnostic and Statistical Manual of Mental Disorders-Fourth edition (DSM-IV) released by the American Psychiatric Association or the International Classification of Diseases (ICD-10) administered by the World Health Organisation. There are slight differences between the two systems, but overall there is a high degree of agreement between them.

The major differences are in criteria concerning the length of symptomatic presentation required to make a diagnosis. The DSM-IV-requires a duration of 6 months (with at least 1 month of active symptoms) as opposed to only 1 month in the ICD-10. The primary diagnostic criteria is the presence of one 'first-rank' positive symptom such as bizarre delusions of control or auditory hallucinations of voices commenting on behaviour or two concurrent symptoms covering other hallucinations, thought disorder, movement disorders or negative symptoms.

The most recent version of the DSM, the fifth edition (DSM-5) was released in 2013 and made some minor changes to the existing diagnosis. The special status of bizarre delusions and verbal auditory hallucinations was reduced so that a single symptom is no longer sufficient for a diagnosis of schizophrenia. Also the specific subtypes of schizophrenia (i.e., paranoid, disorganised, catatonic) were removed, as they had been shown to have limited stability, low reliability, and poor validity (Tandon et al., 2013). It is excepted the further revision to the ICD 11 will make similar changes (Braff et al., 2013).

1.1.3 Epidemiology

Schizophrenia is typically reported to have an onset in late adolescence with appearance in childhood or adulthood occurring rarely (Sadock et al., 2014). The lifetime prevalence of schizophrenia has been shown as ranging from $1.4 - 4.6$ per 1000 people worldwide (Jablensky, 2000) and is commonly reported as affecting 1% of the population, with recent estimates of prevalence across Europe supporting this figure (Wittchen et al., 2011). It is also most-commonly reported to have a slightly higher prevalence in males than females. A recent meta-analysis, a systematic review incorporating 43 individual studies, covering over 100,000 reported cases of schizophrenia largely supports this characterisation, with some caveats. Men were found to have a 1.15 fold (95% CI $1.00 - 1.31$) greater risk than women to be diagnosed with schizophrenia. Men showed a peak in the incidence of schizophrenia at ages 20-29, with a median rate of $4.15/10,000$ person-years followed by a rapid drop off in incidence throughout later life (van der Werf et al., 2012). Women had a much lower peak incidence at this age, $1.71/10,000$ person-years but show an elevated risk throughout the ages 30-39.

It has been estimated that the treatment of schizophrenia utilises from $2 - 3\%$ of the worldwide health budget (Knapp et al., 2004). Estimates in Australia place the direct financial costs of the disease at \$1.85 billion in 2001, nearly \$50,000 per person for each of more than 37,000 Australians with the illness (SANE, 2002). A 2012 estimate places the cost at €93.9 billion across the European Union (Olesen et al., 2011). In addition to the financial costs there is a burden of the disease in terms of pain, suffering and loss of life. Schizophrenia causes significant disability in sufferers, being ranked by the World Health Organisation as the 8th leading cause of disability worldwide (Rössler et al., 2005). The estimated cost to healthy life in Australia was the loss of 22,616 years to schizophrenia in one year alone (SANE, 2002). Moreover, the burden of disease

was greater for schizophrenia than for ovarian cancer, rheumatoid arthritis or HIV/AIDs, and of similar magnitude to leukaemia and melanoma. Although schizophrenia is a heterogeneous disorder with a wide variety of symptoms and severity, overall patients have much poorer outcomes compared to other psychiatric illnesses (Jobe and Harrow, 2005). For example one study showed that up to 47% of patients first presenting with schizophrenia may achieve symptom remission within five years, the overall rate of recovery during early years of the illness is shown to be less than 14% within two years or longer (Robinson, 2004).

Patients with schizophrenia also exhibit much higher rates of mortality than unaffected populations. A systematic review covering 25 nations showed that patients with schizophrenia have a risk of mortality 2.5 times that of the general population (Saha et al., 2007). Patients also show a correspondingly reduced life expectancy of $12 - 15$ years lower than their unaffected cohorts. Devastatingly, suicide is incredibly prevalent amongst patients with schizophrenia with some studies reporting as many as 10% of all patients committing suicide (Caldwell and Gottesman, 1990). More recent studies report a lifetime prevalence of suicide at 4.9% amongst schizophrenic patients, considerably high compared to 0.01% in the general population (Palmer et al., 2005). Schizophrenia is a devastating disease, causing extreme burdens both financially and emotionally to society and the families of people suffering from the disease.

1.1.4 Importance of Cognitive Symptoms

The dramatic and sometimes bizarre nature of the positive symptoms of schizophrenia has led to their dominance in the conception of schizophrenia, both in the public at large and amongst health professionals and researchers. The focus on positive symptoms has important ramifications for our understanding and the treatment of the disease, leaving the negative and cognitive symptoms of

schizophrenia to remain under-recognised and often under-appreciated as a major cause of suffering and disability in patients.

The earliest descriptions of schizophrenia gave a much greater prominence to the negative symptoms. The first clinicians to formally describe schizophrenia (Kraepelin and Bleuler) both made mention of the emotional dullness or deterioration present in patients. Bleuler claimed it "stands at the forefront of the clinical picture" (Bleuler, 1950). Later attempts to formalise and make more robust and reliable diagnostic systems have placed a greater emphasis on the psychotic and positive symptoms. These efforts, such as Kurt Schnieder's description of 'first-rank symptoms' (Schneider, 1959) included various forms of hallucinations, bizarre delusions and experiencing thoughts and feelings as the actions of outside influences. The presence of one of these symptoms, in the absence of clear intoxication or other brain injury or illness, was (and often still is) taken as sufficient for making a diagnosis of schizophrenia (Sadock et al., 2014).

This increased focus on the importance of the positive symptoms of schizophrenia rose to prominence at around the same time as the discovery of the applicability and usefulness of antipsychotic medications. This had an unfortunate effect as both the diagnosis and the treatment of schizophrenia became mainly concentrated with the positive and psychotic symptoms (Turner, 2007). Furthermore, psychotic symptoms are uniquely responsive to antipsychotic medications, which have limited (if any) effects on the negative and cognitive symptoms of schizophrenia (Goldberg et al., 2007; Szoke et al., 2008). Effective treatment soon became focused on maximising antipsychotic dosages in order to minimise positive symptoms.

Because of this focus on the positive symptoms, the negative and cognitive symptoms have been largely unrecognised and under-appreciated as a major factor in the poor outcomes seen with schizophrenia. Thankfully in the last several decades

an increased appreciation of the importance of the negative and cognitive symptom domains has arisen. It has even been claimed that negative symptoms are the most important symptoms in schizophrenia, because the severity of negative symptoms predicts long-term disability better than the severity of positive symptoms (Leeson et al., 2009). Negative and cognitive symptoms may also be the most significant predictor of disease outcome; the severity of negative symptoms and cognitive symptoms has a large influence on future work performance and social relationship (Milev et al., 2005). Negative symptoms are also more stable across time than positive symptoms (Arndt et al., 1995).

The importance of the cognitive symptom domain has also risen with new attempts to improve diagnostic and treatment paradigms in schizophrenia. Cognitive impairment associated with schizophrenia is now viewed as a potential psychopharmacological target for treatment (Hyman and Fenton, 2003; Miyamoto et al 2012). Although cognition is not a formal part of the current diagnostic criteria for schizophrenia, both the DSM-IV and ICD-10 make several references to cognitive abnormalities in their descriptions of schizophrenia. There is also increasing evidence that neurocognitive abnormalities are present before manifestation of psychotic symptoms (the 'prodromal' stage) (Simon et al., 2007). This gives rise to the possibility that cognitive screening may be able to detect schizophrenia prior to the disease onset, opening up a range of treatment possibilities not previously available. It may also be feasible to include some cognitive testing parameters in more robust and objective diagnostic tools. Diagnostic and scientific experts have increasingly expressed the idea that neurocognitive impairment is a core feature of the illness and not simply the result of the symptoms or the current treatments of schizophrenia (Elvevåg and Goldberg, 2000; Keefe and Harvey, 2012).

The greatest evidence for the importance of cognitive deficits to the disease is the prevalence and magnitude of the deficits seen. In several cognitive fields, multiple studies have demonstrated impairments in patients with schizophrenia is as large as two standard deviations lower than the healthy controls (Harvey and Keefe, 2001; Heinrichs and Zakzanis, 1998; Keefe et al., 2006). Up to 98% of patients with schizophrenia have lower cognitive function than is predicted by their parents' education level (Keefe et al., 2005). Also, twin studies performed with monozygotic twins discordant for schizophrenia show almost all twins diagnosed with schizophrenia have lower cognitive functioning than their unaffected sibling (Goldberg et al., 1990).

Cognitive impairments and negative symptoms cause profound hardship and disability in the lives of patients with schizophrenia. Treatments options that specifically target these symptom domains are also extremely limited to non-existent at this time. Finally cognitive symptoms seem to exist prior to the onset of the more dramatic positive and negative symptoms, potentially giving insight into the pathophysiological causes underlying the disease as well as providing a potential pathway to the development of new diagnostic and treatment options.

1.1.5 Aetiology

There is currently no single accepted biological mechanism that leads to the development of schizophrenia, nor is there an established specific aetiology; however, considerable evidence from a variety of genetic twin and familial studies identifies a clear genetic role in the disease, as well as a significant interaction between environmental factors and a genetic predisposition (Sullivan et al., 2003). There are also multiple anatomical, structural and neurochemical differences observed in patients with schizophrenia that can give us insight into both the

underlying causes of schizophrenia and the mechanisms by which the myriad symptoms of schizophrenia are manifested.

1.1.5.1 Environment

The lifetime prevalence of schizophrenia is fairly consistent across countries, ethnicities and cultures (Bromet and Fennig, 1999). There are however, some notable exceptions that support the notion that external environmental factors can impact on the pathogenesis of schizophrenia. Twin studies suggest a small but significant portion of schizophrenia is due to shared environmental influences that are likely prenatal in origin (Sullivan et al., 2003). In support of this the majority of environmental factors identified as contributing towards schizophrenia in epidemiological studies are prenatal stressors. These factors include extremely stressful events occurring to the mother during the pregnancy, such as the emotional impact of losing close relatives during the first trimester (Khashan et al., 2008) or medical complications during pregnancy such as maternal bleeding (Cannon et al., 2002). There is also evidence to suggest that women with diabetes (Van Lieshout and Voruganti, 2008) and older than 35 (Wohl and Gorwood, 2007) at the time of conception can increase the risk of offspring developing schizophrenia. Maternal infection during pregnancy, specifically influenza or rubella, has also been suggested to increase the risk of developing schizophrenia (Brown and Susser, 2002), as has maternal exposure to famine (Susser et al., 1996).

Complications during delivery that potentially cause fetal hypoxia have also been shown to increase the risk of schizophrenia (Cannon et al., 2002). People with schizophrenia also have more minor physical anomalies than healthy controls, which may reflect increased prenatal insults (McDonald and Murray, 2000). Children born in winter and spring also display an increased risk of developing schizophrenia (Bradbury and Miller, 1985), as do children born at higher latitudes (Kinney et al., 2009). This has led to the hypothesis that perinatal vitamin D deficiency is a risk

factor for schizophrenia (McGrath, 1999; McGrath et al., 2003). There is also increased risks seen from living in an urban environment (Krabbendam and Van Os, 2005) and using cannabis during adolescence (Arseneault et al., 2002). The majority of epidemiological evidence concerning environmental factors in the development of schizophrenia points towards pre and perinatal insults that may affect development. This leads to a hypothesis that schizophrenia can be affected by developmental challenges, particularly those that can impinge upon the healthy development and maturation of the nervous system (McGrath et al., 2003).

1.1.5.2 Genetics

Schizophrenia has a well-established relationship to genetic factors; twin and familial studies have demonstrated an increased risk of schizophrenia in the relatives of sufferers (Tsuang, 2000). The risk of developing schizophrenia increases with the degree of shared genetics; in monozygotic twins the risk of developing schizophrenia approaches 50% when one sibling has the disease, is 6%–17% in first degree relatives (e.g., parents, siblings or children) and 2%–6% in second degree relatives (uncles/aunts) (Gottesman, 1991). In spite of the clear heritability of the disease, the consensus view in the field is that "schizophrenia is best viewed as a complex trait resulting from both genetic and environmental etiological influences" (Sullivan, 2005). With the rise of modern genotyping technology and the bioinformatic capabilities to analyse the enormous amounts of data such techniques generate, the genetics of schizophrenia has been put under great scrutiny. Genome-wide association studies initially had poor success, with nearly no positive associations observed and those with significant results were inconsistent and rarely replicated (Sullivan, 2005; Girard et al., 2012). More recent studies with larger sample sizes have successfully identified a range of genetic loci associated with

schizophrenia (Ripke et al., 2012; Shi et al., 2012; Yue et al,. 2012) , a result which emphasises the heterogeneous nature of schizophrenia (Girard et al,. 2012).

Genetic association studies have had considerably more success, with a range of genes identified, many of which have been replicated and show a clear neurobiological connection to the disease. A recent large meta-analysis identified a total of 24 polymorphisms across 16 genes that conferred significant risk for schizophrenia (Allen et al., 2008). The majority of the reliably and repeatedly identified schizophrenia risk genes influence the nervous system. For example *DISC1* encodes for a protein named disrupted in schizophrenia 1, which was first identified in a genome wide association study (Millar et al., 2000). This protein has been shown to play a role in neuronal migration, neurite outgrowth, neuronal maturation, synaptic transmission, and plasticity (Ross et al., 2006).

Genetic alterations in proteins related to neurotransmitters, their metabolism or their receptors are commonplace (Lewis and Moghaddam, 2006). For example *GAD1* encodes the enzyme glutamic acid decarboxylase (GAD) which is responsible for catalysing the production of gamma-aminobutyric acid (GABA) from glutamic acid (Addington et al., 2005). GABA deficits are one of the most replicated post-mortem histological findings in schizophrenia (Lewis et al., 2005) as well as playing an important role in synchronised neural activity (Gonzalez-Burgos and Lewis, 2012; Lewis et al., 2012). Several genes identified as risk factors for schizophrenia can influence the function of glutamatergic signalling pathways (Harrison and Owen, 2003). These include *DAAO* (Chumakov et al., 2002), which encodes an enzyme that metabolises D-serine, an endogenous modulator of NMDArs, *GRM3*, which encodes the mGlu3 subtype of metabotropic glutamate receptors (Egan et al., 2004) and *neuregulin 1* (Stefansson et al., 2002) which can influence the expression of NMDA receptors through activation of Erb4 receptors. The large genetic heterogeneity associated with schizophrenia has also been attributed to an

increased prevalence of *de novo* mutations (Xu et al., 2008) amongst patients with the disease. It has recently been reported that *de novo* mutations in patients with schizophrenia are over-represented in proteins associated with NMDArs (Fromer et al., 2014).

These various lines of genetic evidence provide support for a conception of schizophrenia as resulting from dysfunctional communication and information transfer in the nervous system. Neurotransmitters are the chemical messengers of the nervous system and genetic alterations in these systems alter the brain's ability to transfer information and coordinate neuronal activity.

1.2

Treatment of Schizophrenia

The treatment of schizophrenia is primarily based on pharmacological intervention, through the administration of antipsychotic medications. Psychotherapy and social outreach programs are also utilised, chiefly to improve patient's social integration and create beneficial patterns of behaviour. Schizophrenia is often a highly isolating condition and non-pharmacological interventions are often centred on improving quality of life issues (Bengtsson-Tops and Hansson, 1999; Browne et al., 1996). However, therapeutic interventions are almost always secondary to psychopharmacolgy in schizophrenia care, with antipsychotic medications the mainstay of treatment. As such, the following review will focus on pharmacotherapy in schizophrenia.

Antipsychotics are broadly divided into two classes: the 'typical' antipsychotics – the first generation of drugs specifically designed for the treatment of psychotic symptoms, primarily defined as being high-affinity dopamine D_2 receptor antagonists; and the 'atypical' or second generation antipsychotics –

developed later and generally having a lower specificity for D_2 receptors while also targeting a wide variety of other neurotransmitter targets, including serotonin (5-HT), noradrenaline and muscarinic receptors (Miyamoto et al., 2004).

Although antipsychotic medications are currently the most effective treatment available they do not achieve success in treating all patients, with approximately 29% of patients diagnosed with schizophrenia ever recovering to a level described as 'good' or better (Jablensky, 2000). Furthermore, many patients with severe schizophrenia show no response to either typical or atypical antipsychotics (Buckley et al., 2001; Mouaffak et al., 2006). Up to a third of all these treatment resistant individuals will only show a poor or partial response to pharmacotherapy (Suzuki et al., 2012). Therefore it is important to develop new antipsychotic medications that have improved efficacy and side effect profiles.

1.2.1 Typical Antipsychotics

Prior to the development of pharmacological interventions, primary treatment for psychotic conditions was the confinement of patients. The first true antipsychotic drug discovered was chlorpromazine. First synthesised in 1950, chlorpromazine's sedative properties led it to being trialled in psychiatric patients with noted success. Chlorpromazine is still often used as a benchmark of treatment efficacy when evaluating different medications (Adams et al., 2014). With the success of chlorpromazine a variety of similar drugs were developed and tested for efficacy; over 51 typical antipsychotics were approved for use and 12 are still used to some extent today. Haloperidol is probably the most widespread typical antipsychotic in use today, used in the treatment of acute psychosis and to manage aggressive and delusional symptoms in a wide variety of disorders (Settle and Ayd, 1983). The typical antipsychotics produce a wide-variety of side effects, most prominently symptoms resembling Parkinson's disease. These include a variety of movement

disorders including dystonia and akathisia, collectively referred to as extrapyramidal side effects (EPS). The pervasiveness and significant adverse consequences of short-term and long-term motor side effects associated with classic antipsychotic drugs led to a search for antipsychotic drugs that would be as efficacious but without the risk of EPS.

1.2.2 Atypical Antipsychotics

Clozapine was the first 'atypical' or second-generation antipsychotic drug discovered and remains the classical example of its class. It was found to be an effective antipsychotic but does not lead to the development of EPS in patients. Unfortunately clozapine can in rare cases lead to a fatal blood condition known as agranulocytosis (Kane, 1988), which has limited it's widespread use. Other second-generation medications were introduced including risperidone, olanzapine, quetiapine and ziprasidone in an effort to provide the therapeutic benefits of clozapine without the associated risk of haematological disorders. The atypical antipsychotics are generally shown to have similar effects to the typical antipsychotics in reducing the positive symptoms of schizophrenia, with some limited evidence showing an increased efficacy (Davis et al., 2003; Geddes et al., 2000; Leucht et al., 1999). A lower incidence of EPS is the major advantage of the atypical antipsychotics compared with most typical medications (Leucht et al., 2009). The exception is clozapine, which has shown considerable efficacy in treatment refractory patients (Chakos et al., 2001; Tuunainen et al., 2002), that is patients who do not respond to any other treatments. The potentially lethal side-effects of clozapine limits it's use to the most treatment resistant cases.

The atypical drugs do show some improvements in the reduction of negative symptoms, although this has been theorised to be a result of the beneficial side-effect profiles (Rosenheck et al., 2003) or an ability to ameliorate secondary causes

of negative symptoms, such as depression and anxiety (Tollefson and Sanger, 1997). A subset of the atypical medications (including clozapine) are sometimes found to show modest improvements in treating the cognitive symptoms of the disease (Thornton et al., 2006; Woodward et al., 2005), when compared to the typical medications. Despite the lack of EPS these second generation drugs still have problematic side-effects; primarily they alter glucose and lipid metabolism (Henderson et al., 2005; Koro et al., 2002) and have a propensity to induce weight gain (Allison et al., 1999).

1.2.3 Pharmacology

The typical antipsychotics are all high affinity antagonists at dopamine D_2 receptors and this is thought to be the primary site of action for their antipsychotic efficacy, as well as the pathway that leads to the development of EPS (Nasrallah and Tandon, 2000). There is a strong correlation between the dosage necessary to see a therapeutic effect in these drugs and their affinity for the D_2 receptor (Creese et al., 1976; Seeman, 1987; Seeman et al., 1976). This is further supported by numerous in-vivo imaging studies (Remington and Kapur, 1999). These imaging studies reveal that the therapeutic effects of antipsychotics do not typically appear until dopamine D_2 receptor occupancy reaches 65-70% (Farde et al., 1992; Kapur et al., 1996; 2000b; Nordström et al., 1993). EPS side effects have been shown to be associated with D_2 occupancy over 80% (Farde et al., 1992). It is important to note here that the relatively slow onset of antipsychotic effects, typically estimated at 1-2 weeks (Gelder et al., 2000; Nasrallah and Tandon, 2000) is not consistent with the rapid D2 receptor blockade induced by these drugs and this is still an area of considerable research (Agid et al., 2003; 2006; Seeman, 2011).

The second-generation atypical antipsychotics received their name from the lack of EPS appearing at effective doses. There remains some debate about what

exactly constitutes 'atypicality' but a general consensus includes a lower affinity for D_2 receptors and a high affinity for 5-HT$_{2A}$ receptors. This leads to the hypothesis that the ratio of affinity for 5-HT$_{2A}$ versus D_2 receptors was the crucial target for 'aytpical' antipsychotics (Meltzer et al., 1989). Many atypical antipsychotics still produce significant (> 70%) occupancy of D_2 receptors at therapeutic doses, although clozapine and quetiapine have lower affinities (Kapur et al., 2000a; 1999; Nordström et al., 1995). These confounding results highlight that dopamine antagonism is important, but indicate antipsychotic efficacy is not dependent on reaching some threshold of D_2 occupancy.

Some of the most widely prescribed and effective atypical antipsychotics: Clozapine, risperidone, olanzapine and ziprasidone, reach 5-HT$_{2A}$ receptor occupany levels of greater than 80% (Farde et al., 1992; Kapur et al., 1998; 1999; Miyamoto et al., 2004). It is thought that this 5-HT$_{2A}$ antagonism modulates dopamine transmission, ameliorating the EPS effects of dopamine blockade. Beyond this it is unclear what benefits 5-HT$_{2A}$ antagonism has (Lieberman et al., 1998), although potential benefits against depression and anxiety have been reported, as well as increased efficacy against negative symptoms (Leucht et al., 2009). Clozapine also has partial agonist effects at 5-HT$_{1A}$ receptors and this effect has been hypothesised to improve negative symptoms and cognitive impairment by enhancing dopamine release in the PFC (Miyamoto et al., 2004).

Typical antipsychotic agents also have varying degrees of activity at serotonin, muscarine, noradrenaline and histamine receptors (Nasrallah and Tandon, 2000). It is generally thought that these non-dopaminergic targets do not reflect specific targets in the treatment of psychotic symptoms. These targets do result in a variety of adverse effects, with predictable side-effects arising from the specific receptor profile. For example blockade of histamine H_1 receptors is related to weight gain (Kroeze et al., 2003), while blocking the α1 adrenergic receptor

contributes to hypotension and sedative effects (Nasrallah, 2007). There are some slight exceptions, such as muscarinic M_1 receptor agonism, an effect that is unique to clozapine, and is thought to underlie it's superior efficacy (Davies et al., 2004). However, there is no conclusive evidence that any pharmacological action other than D_2 receptor blockade plays a significant role in antipsychotic efficacy (Miyamoto et al., 2012).

Given the relevance of NMDAr and the glutamate system to schizophrenia (see Section 1.4 for review) it is interesting to note that some antipsychotic drugs can modulate NMDAr function (Lidsky et al., 1997). Clozapine and haloperidol have been shown to effect the phosphorylation levels and extracellular availability of NMDAr's through intracellular mechanisms (Leveque et al., 2000). Clozapine itself has a very low affinity for the NMDA receptor (Shim et al., 1999) and has been shown to have subunit specific inhibitory effects (Levine et al., 2003). It has also been proposed that antipsychotic drugs can enhance NMDAr function by increasing circulating dopamine in the prefrontal cortex (Chen and Yang, 2002). In a wide range of preclinical animal studies, pre-treatment with antipsychotics, particularly the atypical drugs can attenuate the behavioural and physiological effects of NMDAr antagonists (Bakshi and Geyer, 1995; Corbett et al., 1995; Duncan et al., 1998; Jones et al., 2012).

Thus there is reasonable evidence that certain antipsychotic drugs, in certain situations are able to exert complex alterations to glutamate function and NMDAr activities (Bressan et al., 2005). Unfortunately the mechanisms through which antispsychotic treatment is able to attenuate NMDAr antagonists psychotomimetic effects is poorly understood. It is possible longterm treatment with antipsychotics is modulating the number, density or phosphorylation states of NMDAr. However, to date numerous animal studies have produced inconsistent result; showing increases, decreases and no changes in various brain region and for various glutamate

receptors. (Giardino et al., 1997; McCoy et al., 1998; Ossowska et al., 1999; Spurney et al., 1999).

Developing new antipsychotic treatments that specifically target NMDAs is a difficult proposition, due to the ubiquity of NMDAr's through the nervous system. Selectively targeting NMDAr's to only have beneficial antipsychotic effects would be difficult if not impossible. One potential alternative target that has become of interest in recent years is the group II metabotropic glutamate receptors, ($mGluR_{2/3}$). These are G protein coupled receptors that are thought to modulate glutamatergic release, making them viable targets for schizophrenia treatment.

Targeting mGluR's presents a more selective target to modulate glutamatergic and NMDAr function and have received a considerable amount of attention as potential targets for therapeutic intervention in schizophrenia (Vinson and Conn, 2012). $mGluR_2$ in particular are a promising target; these receptors are found on presynaptic neurons outside of the synaptic density. They are thought to play a role in monitoring excessive glutamate release and when activated work to inhibit the further release of glutamate (Scanziani et al., 1997). This role for mGluR's would ameliorate one of the downstream effects of NMDAr antagonists, which is excessive glutamate release (Moghaddam et al., 1997). A selective $mGluR_{2/3}$ agonist LY404039 has shown promising efficacy in this field and a successful phase II clinical trial was completed (Patil et al., 2007). LY404039 was as effective as a conventional atypical drug, olanzapine and even showed improved efficacy against negative symptoms and has a beneficial side effect profile (Adams et al., 2013). This is an important development as this is the first report of a drug with no D_2 receptor antagonism having efficacy in treating the symptoms of schizophrenia and will help guide future research into the underlying causes and pathophysiology of the disease.

1.3

The Dysconnection Hypothesis

In the last several decades, stimulated by the rise of modern non-invasive imaging techniques, a new hypothesis has arisen that explains the complex symptomatology of schizophrenia as the result of pathological interactions between brain circuits – the dysconnectivity hypothesis. The idea that schizophrenia is not caused by a specific and localised neurological defect, but is instead the result of abnormal and disrupted communication between brain regions has its roots in some of the earliest descriptions of schizophrenia. As far back as 1906 the prominent neuroanatomist Carl Wernicke proposed that psychosis is the result of an anatomical defect in the fibre projections of the brain (Wernicke, 1906). Even the name schizophrenia itself, coined by Blueler in the early 20th century (Bleuler, 1950), derives from the Greek for 'split mind', evoking the concept of disconnection. Technological limitations meant that until recently the only method available to study this hypothesis was through the use of post-mortem brain tissue from patients, and the concept languished for decades.

With the advent of modern non-invasive imaging techniques in the 1980's and beyond we have gained the ability to study both the structure and function of intact, living brains using technologies such as positron emission tomography (PET), magnetic resonance imaging (MRI) and electroencephalography (EEG). Remarkably this concept from the earliest years of the twentieth century has been given substantial empirical support in recent years. The concept that schizophrenia is the result of abnormal activity and connectivity distributed throughout the brain has undergone a renaissance in recent years (Friston, 1999; 2002; Stephan et al., 2009; Uhlhaas, 2013). The dysconnectivity hypothesis was formalised by (Friston, 2002) stating that:

"schizophrenia can be understood in cognitive terms, and in terms of pathophysiology, as a failure of functional integration within the brain. Functional integration refers to the interactions of functionally specialised systems (i.e., populations of neurons, cortical areas and sub-areas), that are required for adaptive sensorimotor integration, perceptual synthesis and cognition. Functional integration is mediated by the influence that the dynamics or activity of one neuronal system exerts over another and therefore rests on the connections among them."

The body of evidence supporting this work has grown to include both structural and functional abnormalities, highlighting not just anatomical differences but also differences in the patterns of neural activity in patients with schizophrenia (Uhlhaas, 2013). The advent of modern techniques to gain insight into neuronal functioning such as EEG and functional magnetic resonance imaging (fMRI), has given insight not just into pathological conditions such as schizophrenia, epilepsy and Parkinson's but also into the dynamic nature of healthy cognitive functioning.

The concept that complex interaction between various brain regions serves as a neurological substrate for higher cognitive functioning has grown to dominate cognitive neuroscience (Bullmore and Sporns, 2009). The formation and governance of these networks of interacting neuronal circuits is achieved through the coupling together of neurons functionally relevant to the task, while inhibiting those that are not (Fries, 2005). In this understanding the synchronised and coordinated activity of disparate neuronal regions and circuits is essential for cognitive function and even consciousness (Uhlhaas et al., 2009). The dramatic and heterogeneous symptoms of schizophrenia are posited to arise as a result of abnormal, 'dysfunctional' activity between brain regions, a pathology of neural dynamics (Uhlhaas, 2013).

An important distinction to make with regard to the dysconnection hypothesis is that it does not imply a simple separation or breakage in the neural

connectivity (be that structural or functional) in schizophrenia. The prefix 'dys' means bad or ill, and is specifically chosen to emphasise that the connectivity observed in schizophrenia is abnormal and differs from what is seen in healthy individuals, but does not imply the directionality of these changes. Reductions in connectivity certainly are observed and constitute a part of the constellation of changes that make up schizophrenia, but equally important are increases in connectivity that can lead to aberrant neural function. For example excessive ongoing or baseline γ activity has been associated with hallucinations and the positive symptoms of schizophrenia (Baldeweg et al., 1998). Similarly it has been reported that patients with schizophrenia have a hyperactive default-mode network and that they are less able to task switch and modulate this activity down in response to cognitive demands (Whitfield-Gabrieli et al., 2009). The dysconnection hypothesis incorporates all aspects of aberrant functional connectivity.

1.3.1 Imaging Evidence

The modern origins of the dysconnectivity hypothesis are in the rise of noninvasive neuroimaging techniques in the 1980s and 1990s, including PET and fMRI. As these technologies were developed and then applied to studying the ongoing activity in the living brains of patients with schizophrenia they started to reveal patterns of abnormal and irregular activity distributed across the entire brain. These abnormal patterns of activity eventually gave rise to the dysconnection hypothesis; the impairment of the ability to coordinate action and communicate information across the brain is the fundamental cause schizophrenia (Friston, 2002; Stephan et al., 2009). The evidence for this comes from a variety of different studies and methodologies, which show a remarkable agreement with the central hypothesis that there is disruption in the coordinated patterns of activity in schizophrenia.

For example, an early resting-state PET study found that "schizophrenic subjects showed derangements in the pattern of interactions among brain areas". This was quickly followed by other PET studies showing dysfunctional networks of brain activation involving the prefrontal cortex and limbic system (Weinberger et al., 1992) and the temporal lobe (Friston et al., 1992). Following these initial studies, further evidence supporting the dysconnectivity hypothesis has arisen from a number of sources. Studies utilising PET and then eventually fMRI have found significant abnormalities in the functional connectivity between the temporal and frontal regions of the brains of people suffering from schizophrenia (Friston et al., 1996; Lawrie et al., 2002; Meyer-Lindenberg et al., 2005).

Most recently the concept of the default-mode network has arisen in fMRI studies. This describes the network of brain regions that are active when an individual is at rest, i.e. not engaged in any task specific activity. The default-mode network is characterised by coherent neuronal activity across the network, which is inhibited when task specific activity is initiated (Buckner et al., 2008). The default-mode network has been shown to display altered and dysfunctional patterns of activity in patients with schizophrenia (Broyd et al., 2009; Garrity et al., 2007; Zhou et al., 2007). There is also a comprehensive body of research that has established a pattern of abnormal γ frequency activity in patients with schizophrenia, resulting from both sensory and cognitive task related paradigms. γ frequency activity is hypothesised to serve as the major mechanism for forming and coordinating transient networks of neural processes, so these deficits hold a particular relevance to the dysconnectivity hypothesis, and are reviewed in detail in Section 1.5.2.

1.3.2 Structural Changes

Changes in the anatomical structure of the brain are a widely replicated finding in schizophrenia (Harrison, 1999) and there is considerable anatomical evidence to support the dysconnection hypothesis. The majority of studies on structural changes in schizophrenia report increases in ventricle size and decreases in brain volume, including differences in the volume of the grey and white matter of the brain as well as more specific differences in brain structures such as the hippocampus (Wong and Van Tol, 2003), amygdala (Lawrie and Abukmeil, 1998; Nelson et al., 1998), prefrontal cortex, thalamus (Konick and Friedman, 2001), anterior cingulate (Baiano et al., 2007) and corpus callosum (Woodruff et al., 1995).

Changes in both the corpus callosum and thalamus have implications for communication amongst and between different brain regions. Both of these regions are highly connected to various cortical regions and play specific roles in the transfer and synchronisation of information across the brain (Berlucchi, 1999). Thalamo-cortical (TC) circuits are important generators of γ oscillations (Ribary et al., 1991; Whittington et al., 2000), rhythmic fluctuations in the electrical activity of the brain that are proposed to coordinate the communication of groups of neurons (discussed in detail in Section 1.5). TC circuits are proposed to synchronise various regions of the brain serving to integrate perceptual information (Llinas et al., 1998). Deficits in the structure and function of these areas would have a significant impact on the synchronised activity of the brain, resulting in the functional dysconnections which are hypothesised to underlie the symptomatology of schizophrenia (Friston, 1999).

Related to these findings of disruptions in anatomically highly connected areas of the brain are studies of white matter tracts with modern imaging techniques such as diffusion tensor imaging. These studies utilise an MRI scanner but instead of producing images of brain structures, they are sensitive to the directional flow of

water. This enables diffusion tensor studies to determine the size, strength and integrity of white matter tracts in the brain (Basser and Pierpaoli, 2011). The consensus result is that patients with schizophrenia have reduced fractional anisotropy (FA), a measure that reflects fibre density, axonal diameter, and myelination in white matter (Kanaan et al., 2005). Several studies found reductions in FA across the whole brain, (Agartz et al., 2001; Ardekani et al., 2003; Hubl et al., 2004), and four studies have found specific reductions in the corpus callosum (Agartz et al., 2001; Ardekani et al., 2003; Foong et al., 2000; Hubl et al., 2004).

1.3.3 Molecular Abnormalities

Postmortem studies of neural tissue from patients with schizophrenia have consistently provided evidence of deficits in the GABAergic system in patients with schizophrenia (Lewis et al., 2005). GABA-expressing interneurons are essential for the generation of γ frequency oscillations, which connects this molecular change seen in patients with the function dysconnection hypothesised to result in the symptoms of schizophrenia (Gonzalez-Burgos and Lewis, 2012; Uhlhaas, 2013). One of the earliest studies that examined neurochemical deficits found a reduction of GABA concentrations in the thalamus and nucleus accumbens in patients as compared to age-matched controls (Perry et al., 1979). A similar study found reductions in both GABA and the GABA transporter GAT-1 in the prefrontal cortex (Ohnuma et al., 1999).

Another related finding is that the 67kD isoform of glutamic acid decarboxylase (GAD_{67}), the enzyme that catalyses the reaction to synthesise GABA, is also altered in patients with schizophrenia (Lewis et al., 2005). The density of neurons that express GAT-1 and GAD_{67} mRNA was found to be decreased throughout the cortex (Akbarian et al., 1995; Guidotti et al., 2000; Hashimoto et al., 2003; Volk et al., 2000; 2001). The reduction in GAD_{67} expression is in fact

the most robust and widely replicated finding in post-mortem histopathological studies (Akbarian and Huang, 2006). *GAD1*, the gene that codes for the enzyme GAD_{67}, is a schizophrenia risk gene (Addington et al., 2005). Deficits in *GAD1* mRNA expression have also been reported from post-mortem tissue studies of schizophrenia patients (Akbarian and Huang, 2006; Hashimoto et al., 2008; Huang and Akbarian, 2007; Straub et al., 2007).

GABA-expressing neurons represent an enormous diversity of cell types, which differ in morphology, connectivity, neurochemistry and physiology (Buzsaki et al., 2004; Maccaferri and Lacaille, 2003; Markram et al., 2004; Monyer and Markram, 2004; Whittington and Traub, 2003). Of the several subtypes of GABA-expressing cells, fast-spiking cells that express that calcium binding protein parvalbumin (PV+ cells) hold special significance for the generation of γ frequency oscillations (Bartos et al., 2007; Cunningham et al., 2006; Hajos et al., 2004; Klausberger et al., 2003). There is increasing evidence that PV+ cells are specifically disrupted in patients with schizophrenia (Lewis et al., 2005), which provides the cellular and molecular basis for the disruption of γ frequency oscillations.

(Lewis et al., 2005) demonstrated that PV+ interneurons show a specific reduction in the synthesis of GABA in patients with schizophrenia. The same group demonstrated that the number of cells containing PV mRNA that express a detectable level of GAD_{67} mRNA is decreased by as much as 45% in the prefrontal cortex in schizophrenia patients (Hashimoto et al., 2003). It was also revealed that the expression of PV itself in these cells was significantly reduced, as compared to the levels of other calcium binding proteins in a separate population of GABAergic cells (Hashimoto et al., 2003). There are other results that indicate a dysfunction in PV+ subtypes of GABAergic neurons; the density of axon terminals of chandelier cells (one of two classes of PV+ interneuron) have been found to be decreased by

up to 40% in patients with schizophrenia (Pierri et al., 1999; Woo et al., 1998), indicating reduced inhibitory tone in patients.

Post-mortem studies have also demonstrated abnormalities in the glutamate system that interacts with GABAergic deficits in schizophrenia. The density of GABAergic neurons that express the NR2A subunit of the NMDAr has been shown to be decreased in the anterior cingulate cortex of patients with schizophrenia (Woo et al., 2004). A similar result has been found in prefrontal cortex, with reductions in the number of neurons that express both GAD_{67} and NR2A in schizophrenia patients (Woo et al., 2008). Overall there is conclusive evidence for a specific and prevalent deficit in GABA related enzymes, particularly in the PV+ interneurons. These neurons are essential for the generation and maintenance of cortical rhythms that are involved in coordinating neuronal communication and information processing. The deficits commonly found in these cells provide evidence of cellular and molecular alterations that can potentially lead to abnormal γ oscillations and in turn the functional dysconnection of neural circuitry responsible for the cognitive deficits and perceptual symptoms seen in schizophrenia.

1.3.4 Electrophysiological Abnormalities

There are a wide variety of perceptual responses that can be measured in humans using scalp recorded electroencephalogram (EEG). These responses generate stereotypical neurological and electrical activity that can be measured and analysed to assess information processing and perceptual function. Many of these techniques reveal specific abnormalities in patients with schizophrenia further supporting the hypothesis that a widespread dysfunction in neuronal communication is prevalent in the disease. Note that a detailed review of γ frequency activity is covered in Section 1.5.3 and so this section focuses on other electrophysiological measures.

P50 auditory evoked potentials – The P50 wave is defined as the amplitude of positive-going wave that can be recorded in EEG and occurs approximately 50 ms after an auditory stimulus. In healthy subjects the P50 amplitude is suppressed when there is another audio stimuli 500 ms prior. P50 suppression represents a form of sensory gating, a process whereby the brain modulates its response to stimuli and theoretically prevents overstimulation by filtering incoming information (Freedman, 1996). Several studies have demonstrated that P50 suppression is impaired in patients with schizophrenia (Clementz et al., 1997; 1998; Freedman et al., 2001). As reported earlier there is a genetic connection to this paradigm and schizophrenia; animal studies have demonstrated that blockade of α7 containing nicotinic acetylcholine receptors leads to a loss of inhibition following auditory stimulation (Stevens et al., 1998) and the gene that codes for α7 is a risk gene for schizophrenia (Leonard and Freedman, 2006).

P300 event-related potential – like the P50, the P300 is defined as the amplitude of the positive-going cortical EEG potential that occurs roughly 300 ms following a target stimulus in an auditory 'oddball' paradigm. In the oddball paradigm the subject is presented a series of tones in which a small percentage (typically 5 - 10 %) vary in some way from the others, such as pitch or length. Subjects must respond to these 'oddball' stimuli. The P300 is thought to represent the interaction of multiple neuronal circuits including the frontal cortex, hippocampus and thalamus (Kiehl et al., 2001). Again patients with schizophrenia are regularly reported to show reduced amplitudes of P300 potentials (Ford et al., 1994; Turetsky et al., 1998).

Mismatch negativity (MMN) – is a negative-going amplitude visible in scalp recorded EEG, that is generated in response to an unexpected or 'deviant' stimuli. In this paradigm subjects listen to a long series of repetitive tones and a very infrequent 'deviant' tone is occasionally played. The MMN response is thought to

reflect an automatic memory comparison processes that is designed to perceive change (Näätänen et al., 2005). The MMN is a relatively low level perceptual process and can be elicited regardless of whether the subject is attending to the stimuli. Patients with schizophrenia show reduced MMN as compared to healthy controls (Alain et al., 1998; Umbricht and Krljes, 2005). Interestingly the administration of an NMDAr antagonist has also been shown to reduce the MMN amplitude (Umbricht et al., 2000).

Sleep Spindles – reductions in the amount of sleep spindle activity have been reported in patients with schizophrenia (Ferrarelli et al., 2007; 2010; Wamsley et al., 2012). Spindles are a key feature of stage 2 non-rapid eye movement sleep; generated by TC circuits spindles are visible in scalp recorded EEG as bursts of 12–15Hz activity (De Gennaro and Ferrara, 2003). Spindles have been correlated with sleep dependent memory processes (Fogel and Smith, 2011) and spindle abnormalities in patients have been linked to memory deficits seen in the disease (Manoach et al., 2004; 2010).

To summarise there are consistent and robust deficits seen in a number of electrophysiological markers of brain function in patients with schizophrenia. These differences reflect altered and dysfunctional information processing and perceptual processes and give further support to the hypothesis that a pathological dysconnection in functional neural networks is the underlying cause of the symptoms of schizophrenia.

1.3.5 Cause of Dysconnection

The leading hypothesis for the cause of the dysconnectivity is that it results from a defect in the functional coupling of neurons due to impairments in synaptic plasticity (Friston, 1999). Specifically, abnormal regulation of NMDAr-dependent

synaptic plasticity has been proposed to results in the dysconnection seen in schizophrenia (Stephan et al., 2006). NMDAr activity controls plasticity at glutamatergic synapses by altering the function and or number of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Stephan et al., 2009). This is achieved by NMDAr activation dependent phosphorylation of AMPA receptor subunits and altering the trafficking of AMPA receptors from the post synaptic density (Bagal et al., 2005; Montgomery and Madison, 2004; Passafaro et al., 2001).

Alterations to synaptic plasticity can have a far reaching impact on the functional and structural connectivity of a neural network; for instance, the strength of functional coupling between neurons is a product of the strength of input they receive at excitatory glutamatergic synapses (Zhang and Poo, 2001). Furthermore the strength of this functional coupling determines the survival of connections and neurons in the process of developmental pruning (Hua and Smith, 2004). Deficiencies in the ability to regulate synaptic plasticity can have far reaching effects that could explain the involvement of environmental and gene interactions in schizophrenia (Sullivan et al., 2003) as well as the integral involvement of developmental processes (Uhlhaas, 2013).

Neural oscillations are a fundamental mechanism that enable coordinated brain activity during cognitive functioning (Fries, 2005). Oscillatory activity can establish precise temporal correlations between distributed neuronal responses. Oscillations in the γ frequency range in particular have been linked to the synchronisation of cortical networks and a range of perceptual and cognitive processes (Herrmann et al., 2010). Disruption to the neural oscillations that serve to precisely coordinate disparate neuronal circuits presents another hypothesis for the functional dysconnectivity seen in schizophrenia (Stephan et al., 2009; Uhlhaas, 2013). These two hypotheses are not mutually exclusive, in fact they are intimately

related; excitatory glutamatergic input onto PV+ interneurons is the proposed mechanism through which gamma oscillations and synchronised neuronal activity is generated (Gonzalez-Burgos and Lewis, 2008).

Disruption to NMDAr activity resulting in dysfunctional synaptic plasticity is a putative mechanism that may underlie γ oscillation abnormalities in schizophrenia. NMDAr hypofunction is already a prominent hypothesis for the origins of schizophrenia and there is an extensive body of clinical research investigating γ oscillations in patients. The next two sections of this literature review will summarise current knowledge regarding these potential mechanisms of schizophrenia and establish the rationale for studying the fundamental nature of these phenomena, in an effort to better understand the pathophysiology of schizophrenia.

The dysconnection hypothesis brings together the various disparate threads of molecular, cellular and functional abnormalities observed in schizophrenia and provides a robust and comprehensive account of the disease. The dysconnection hypothesis is ideally suited for translational research into the mechanisms that underlie the cognitive deficits and functional dysconnectivity in schizophrenia, as it provides clear molecular and physiological targets that can be investigated in animal and cellular translational models (Uhlhaas, 2013). In particular electrophysiolgical recordings can be made in animal models and readily compared to EEG data acquired in the clinic, with standardised analysis techniques allowing comprehensive comparisons. The dysconnection hypothesis provides a comprehensive explanation for the symptoms, pathology and cause of schizophrenia. It allows for robust preclinical exploration of these factors and provides a framework for incorporating the findings of works such as that performed in this thesis.

1.4

NMDA Receptor Hypofunction

The NMDAr hypofunction hypothesis for schizophrenia was first proposed in the late 1980's based on observations that non-competitive NMDAr antagonists such as phencyclidine (PCP) and ketamine could elicit symptoms reminiscent of schizophrenia in healthy subjects (Krystal et al., 1994). These findings gave rise to the concept that the symptoms of schizophrenia are the result of reduced or abnormal NMDAr functioning (Olney, 1995). This idea has since undergone significant revision and advancement, with new findings from the fields of genetics and molecular biology adding further support to the hypothesis. Theoretical advances in the conceptualisation of schizophrenia have also increased the relevance of the NMDAr hypofunction model. Pre and postsynaptic NMDArs govern synaptic plasticity (Hua and Smith, 2004), the molecular process responsible for learning and memory and that determines the functional coupling between neurons. This makes NMDArs uniquely positioned in the nervous system to disrupt learning, memory and perception (Bliss and Collingridge, 1993), the key cognitive domains disrupted in patients with schizophrenia. Modulating synaptic strengths due to experience also affects the functional connectivity between neurons. Deficits in the ability to modulate the effective coupling between neuronal circuits results in deficits in connectivity across the brain (Stephan et al., 2009). Furthermore, NMDArs are essential in the generation and coordination of neural oscillations (Nakazawa et al., 2012). What began as a simple concept based on the psychotomimetic activity of NMDAr antagonists has evolved into a comprehensive hypothesis of neuronal dysconnection in schizophrenia.

1.4.1 Glutamate and NMDA Receptors

Glutamate is the most abundant excitatory neurotransmitter in the central nervous

system – many excitatory neurons utilise glutamate and it is estimated that half of all of neurons in the brain release it (Meldrum, 2000; Purves et al., 2011). Glutamate receptors can be split into two discrete categories, the ionotropic and metabotropic glutamate receptors. Ionotropic receptors are defined as receptors that when activated (through shifting membrane potential or ligand binding) allow the flow of ions through a central pore in the receptor. There are three known families of ionotropic glutamate receptors: AMPA, kainate and NMDArs. They received their names from the glutamate analogues used to identify them as distinct receptors with separate properties. The metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors that modify neuronal functioning through intracellular signalling cascades and are thought to play a role in learning and memory through adjusting synaptic excitability and glutamate release.

The NMDAr is a heterotetramer – made of four discrete subunits of distinct types, NR1, NR2 or NR3 (also referred to as GluN1, 2 and 3). Two NR1 subunits are essential to form a functioning receptor with the remaining two composed of either NR2 or NR3 subunits. The NR3 subunits were the last to be identified and show the smallest homology to the other units, they remain less studied and poorly understood and there is limited evidence for much endogenous expression of NR1/NR3 receptors (Low and Wee, 2010). NR1 subunits contain a glycine-binding domain, while NR2 have a glutamate binding domain giving NMDArs the unique property of requiring the binding of two separate agonists to activate. They also have another unique property; they are "doubly gated" requiring a simultaneous change in membrane voltage and the binding of a ligand in order to open. This is caused by the presence of a Mg^{++} ion in the channel pore, meaning that even when the ligand gated channel opens, unless there is a corresponding depolarisation to expel the Mg^{++} ion, there may not be any inward current flow. These unique ligand and voltage gating characteristics of NMDArs allows them to play a distinctive role in the nervous system, ideally suited to integrating information from multiple

pathways (Kantrowitz and Javitt, 2010), acting as a 'coincidence detector' (Seeburg et al., 1995), filtering sub-threshold perceptual information (Butler et al., 2008) and detecting aberrant events (Javitt, 2000); these properties are all essential for learning and perception.

Once open, NMDArs are permeable to calcium, unlike most other ligand gated ion channels. Unlike the other ions (Na^+, K^+ or Cl^-), Ca^{++} doesn't just effect membrane potential, it is metabolically active as well. Ca^+ is in fact, essential for the processes of long term potentiation and depression (Artola et al., 1990). These processes are the primary physiological events thought to underlie learning and new memory formation in cortex and hippocampus, consistent with the well described memory deficits associated with schizophrenia (Javitt, 2009). Lastly, the kinetics of NMDArs are also unique. NMDArs mediate relatively slow excitatory postsynaptic potentials. While other ionotropic glutamate receptors have depolarisations lasting 10's of milliseconds, NMDArs depolarise the cell for up to 100's of milliseconds. These slow channel kinetics makes them more sensitive to bursts of released glutamate as opposed to single inputs, which is consistent with a role in gating and information integration (Magleby, 2004). In addition, the complexity of unblocking kinetics makes NMDArs highly sensitive to timing, facilitating to spike-timing-dependent synaptic plasticity (Kampa et al., 2004). NMDArs are uniquely positioned in the nervous system to play a role in learning, perception, and memory. These processes are all disrupted in schizophrenia, which means that any theory that includes disruption to NMDAr or glutamate function is positioned theoretically to explain a vast range of the cognitive, and perceptual symptoms of schizophrenia.
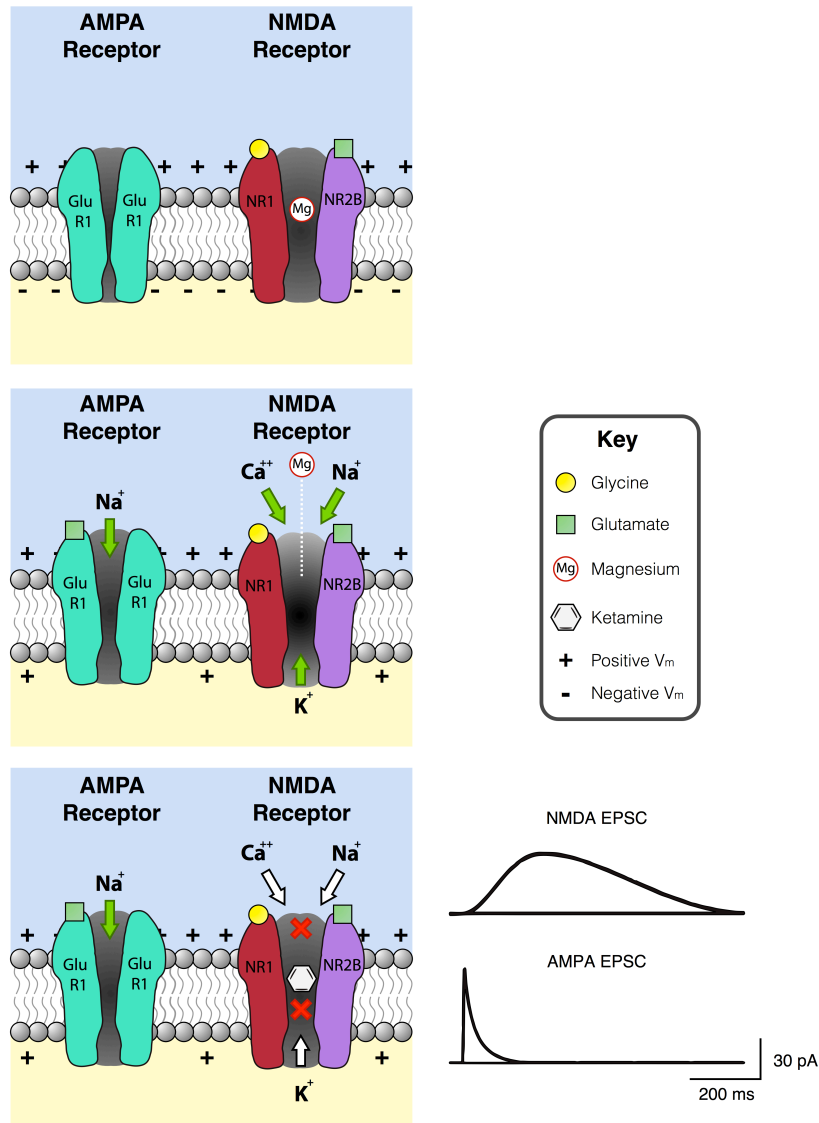
Figure 1.1 – A schematic Representation of NMDA Receptor Voltage Activation

The NMDAr has several unique properties that underlie its specific role in the nervous system and have relevance to pathophysiology of schizophrenia. The NMDAr is doubly gated, requiring both the binding of ligands as well as membrane depolarisation to occur before it opens to ion flow. In this schematic the different potential configurations of the NMDAr are shown. In the top panel glycine and glutamate have bound to the NMDAr and the pore is open, however the membrane potential is still highly negative and a Mg^{++} ion is present, blocking the central pore of the receptor. In the middle panel, nearby AMPA receptors have also been activated by glutamate, these channels open and the influx of Na^+ ions has depolarised the membrane. This allows the Mg^{++} ion to exit the pore and Na^+ and Ca^{++} ions to enter. In the bottom panel the AMPA receptor is active, the membrane is depolarised but the non-competitive NMDAr antagonist ketamine is blocking the flow of ions through the NMDAr pore. Ketamine binds to the PCP site inside the NMDAr pore; as a result it does not 'compete' with glutamate binding and requires the channel to be open before it can inhibit activity. To the bottom right are idealised representations of NMDA and AMPA receptor excitatory post-synaptic currents (EPSCs). The NMDA receptor has much slower kinetics, resulting in excitatory currents that last up to 10 times longer than non-NMDAr glutamate receptors.

1.4.2 NMDA Receptor Antagonists in Humans

The NMDAr hypofunction hypothesis of schizophrenia arose in the late 1980's when the neurochemistry underlying the psychotomimetic actions of phencyclidine (PCP) and ketamine was revealed to be dependent on their ability to block neurotransmission at NMDArs (Javitt and Zukin, 1991). PCP was the first known NMDAr antagonist and had been reported to provoke a broad range of schizophrenia-like symptoms since as early as 1959 (Luby et al., 1959). Similar reports abounded of recreational users of PCP presenting to hospitals and psychiatric care and being initially diagnosed with schizophrenia in the midst of acute psychotic episodes (Fauman et al., 1976).

PCP was eventually removed from clinical use due to its potency and the propensity for abuse. Ketamine, another non-competitive NMDAr antagonist has similar psychotomimetic effects, but a lower NMDAr affinity (Anis et al., 1983) and a much shorter half-life (Idvall et al., 1979). Ketamine has a strong record for safety and remains in common use clinically as an anaesthetic and analgesic in healthy adults and children (Green and CotÃ, 2009; Green et al., 1998). It is also currently undergoing pre-clinical trials in the treatment of depression where it shows promising results (Berman et al., 2000; Ibrahim et al., 2011; Zarate et al., 2006). When utilised in the clinical setting as a model for schizophrenia NMDAr antagonists are typically administered intravenously and the resulting plasma concentrations are monitored and adjusted to achieve a steady, specific dosage.

Ketamine in particular is rapidly metabolised and its effects can change dramatically over relatively small changes in plasma concentration (Krystal et al., 1994). The onset of symptoms is rapid, occurring in as short as 5 minutes (Moghaddam and Krystal, 2012). The psychotic symptoms typically presented include disorganised thought, paranoia, confusion and visual and auditory hallucinations. There is also an amotivational presentation, with blunted affect and

withdrawal observed (Cohen et al., 1962; Goff and Coyle, 2001). Ketamine treatment also causes transient cognitive deficits reminiscent of schizophrenia, while sparing overall global function (Malhotra et al., 1997; Newcomer et al., 1999).

The use of NMDAr antagonists as pharmacological models of schizophrenia quickly gained interest as research revealed that NMDAr blockade triggered not just the positive symptoms of schizophrenia, but also the negative and cognitive symptoms in otherwise healthy subjects (Krystal et al., 1994; 1999; Malhotra et al., 1997; Newcomer et al., 1999; Oye et al., 1992; Vollenweider et al., 1997). This was a marked difference to existing pharmacological models of psychosis or schizophrenia, which typically utilised dopaminergic stimulants like amphetamine or serotonergic hallucinogens such as lysergic acid diethylamide (LSD) or mescaline. These alternative pharmacological models can reproduce the positive symptoms, such as disordered thought, motor agitation and hallucinations but produced little of the negative symptoms such as apathy and withdrawal (Gouzoulis-Mayfrank et al., 2005; Krystal et al., 2005). Not only does ketamine/PCP produce a broader and more comprehensive pharmacological model of schizophrenia's symptoms, it was found that the phenomenological nature of ketamine-induced thought disorder in healthy subjects closely resembled the nature of thought disorder in schizophrenia patients (Adler et al., 1998; 1999).

There have also been studies demonstrating that in patients with schizophrenia an acute treatment with ketamine will transiently induce a psychotic episode, exacerbating positive symptoms (Lahti et al., 1995; 2001; Malhotra et al., 1997). Schizophrenia patients reported that the nature of the ketamine-induced positive symptoms in these studies were similar to when they occur naturally. Specifically over 70% of patients reported hallucinations and delusions that reminded them of their 'typical' positive symptoms during a spontaneous exacerbation (Lahti et al., 2001). In addition to the positive and negative symptoms

of schizophrenia, NMDAr antagonists have also been shown to elicit specific cognitive deficits that are typical of schizophrenia. Particular alterations in executive function that are common and quite specific in schizophrenia have been reported, including deficits in attention, working memory, abstract reasoning, planning and judgement (Hetem et al., 2000; Krystal et al., 1994; 1999; Malhotra et al., 1997; Newcomer et al., 1999).

As well as the neuropsychological effects that acute NMDAr blockade can induce, there are numerous physiological abnormalities that are transiently induced in healthy subjects that are also commonly observed in patients with schizophrenia. These include deficits in electrophysiological measures such as MMN (Umbricht et al., 2000) and ERPs (Hong et al., 2010). There were also alterations to physiological characteristics such as smooth eye tracking (Avila, 2002; Radant et al., 1998), prepulse inhibition (PPI) and cortical blood flow (measured with fMRI) (Abel et al., 2003).

Ketamine also causes significant dissociative symptoms – feelings of detachment from and withdrawal from reality – in a large proportion of healthy subjects administered the drug. Dissociative states are not generally thought of as symptoms of schizophrenia, although they have been suggested to be a part of the schizophrenia prodrome, the early symptoms preceding or marking the onset of the disease (Møller and Husby, 2000). Interestingly the nature of hallucinations induced by ketamine and PCP administration is also more relevant to the early onset of schizophrenia than the chronic disease. In the early course of schizophrenia both auditory and visual hallucinations and disturbances are common, whereas auditory hallucinations dominate later (McGhie and Chapman, 1961). In light of this and the phenomenological similarity between ketamine administration and acute psychotic episodes, the acute ketamine challenge may be best thought of as a model of acute onset schizophrenia, or a psychotic episode rather than later, more chronic, phases

(Kantrowitz and Javitt, 2010). In summary there is considerable neuropsychological and physiological evidence that ketamine and other NMDAr antagonists provide a useful and comprehensive pharmacological model of schizophrenia's symptoms, providing insight into potential mechanisms and pathways.

1.4.3 Molecular and Genetic Evidence

As well as the neuropsychological and physiological evidence that acute NMDAr antagonism elicits symptoms reminiscent of schizophrenia in healthy subjects, there is considerable molecular evidence obtained from patients with schizophrenia. One of the very first proposals that abnormalities in the glutamatergic system were related to schizophrenia was based on molecular evidence gathered from patients. Low levels of glutamate were observed in the cerebral spinal fluid of patients with schizophrenia (Kim et al., 1980). This finding received limited traction at the time, as it was reproduced by some (Bjerkenstedt et al., 1985; Macciardi et al., 1990), but not all studies that further investigated the issue (Gattaz et al., 1982; Perry, 1982; Tsai et al., 1998). Further investigations into glutamate and related molecules in patients with schizophrenia have yielded more promising results. Such as an *in vivo* single photon emission tomography study that revealed reduced NMDAr binding in the hippocampus of untreated patients with schizophrenia, an effect that was reversed in patients taking antipsychotic medications (Pilowsky et al., 2005).

Post-mortem studies of brain tissue found that patients with schizophrenia had lower levels of glutamate in the prefrontal cortex and hippocampus, and lower levels of aspartate (another ligand for NMDArs) than healthy controls (Tsai and Coyle, 1995). The same study made the novel finding that N-acetylaspartylglutamate (NAAG) levels were higher in the hippocampus of patients. NAAG is a neuropeptide (a small protein-like molecule that can signal neurons) and

consists of N-acetylaspartic acid (NAA) bound to a glutamate molecule. NAAG has been demonstrated to be an NMDAr antagonist (Puttfarcken et al., 1993), although this finding is presently a matter of considerable debate (Begeron and Coyle, 2012). Less controversially NAAG acts as an agonist at mGluR$_3$s (Wroblewska et al., 1998), that in turn inhibit glutamate release (Sánchez-Prieto et al., 1996). The precise regulation of NAAG, NAA and glutamate are complex (Tsai et al., 1995) but these results further indicate abnormal glutamate transmission and NMDAr hypofunction; whether these glutamate-related molecules are altered as a byproduct or a primary cause is not yet established.

Numerous other studies of post-mortem tissue from patients with schizophrenia have examined glutamate receptor binding, transcription, subunit protein and mRNA expression across numerous different brain areas (Geddes et al., 2011; Snyder and Gao, 2013). These results broadly support the NMDAr hypofunction hypothesis; showing reductions in NMDAr subunit proteins in the temporal lobe (Beneyto et al., 2007) prefrontal cortex (Beneyto and Meador-Woodruff, 2008), thalamus (Ibrahim et al., 2000) and hippocampus (McCullumsmith et al., 2007). Other commonly reported changes include alterations in the level of NMDAr affiliated structural proteins such as post synaptic density protein 95 (PSD95) (Beneyto and Meador-Woodruff, 2008; Dracheva et al., 2001) and synapse associated protein 102 (Beneyto and Meador-Woodruff, 2008; Dracheva et al., 2001; Kristiansen et al., 2006). Binding studies utilising autoradiographay have produced more mixed results; increases in kainate receptors in the prefrontal cortex, decreases of both AMPA and kainate in the hippocampus, and inconsistent results for NMDArs across the brain (Snyder and Gao, 2013).

One final molecular finding of particular interest to the theoretical underpinnings of the NMDAr hypofunction hypothesis of schizophrenia is the NMDAr subunit switch. Numerous studies have indicated that during adolescence

there is a NMDAr subunit switch i.e. NR2B subunits are replaced with NR2A or NR3A to NR3B (Monyer et al., 1994; Sheng et al., 1994; Snyder et al., 2013; Wang and Gao, 2009; Wang et al., 2008b). This change in NMDAr subunits marks a transition to adult neural processing (Dumas, 2005) and creates a situation where NMDArs are particularly susceptible to environmental and genetic risk factors (Spear, 2000). The fact that this coincides with the typical age of onset of schizophrenia is of particular interest and further adds to the evidence of the centrality of NMDAr hypofunction to schizophrenia.

There are numerous genes that have been linked to or shown some involvement to schizophrenia, a large proportion of which are related to NMDAr or glutamate function (Harrison and Owen, 2003). For instance, a recent large meta-analysis of genome wide association studies for schizophrenia 'risk' genes (Allen et al., 2008) showed that polymorphisms in GRIN2B, the gene that encodes the NR2B subunit of the NMDAr, is a significant risk factor for schizophrenia. GRM3, which encodes the mGluR$_3$ subtype of metabotropic glutamate receptors has also been identified as a risk gene (Egan et al., 2004) .

A series of papers published across 2002 and 2003 revealed a total of seven separate risk genes for schizophrenia, practically all of which converged on the glutamate system (Harrison and Owen, 2003). One of the first and best replicated genes identified as a risk for schizophrenia, *Nrg1* (Munafo et al., 2006; Stefansson et al., 2002; Williams et al., 2003), has considerable interactions with NMDArs and the glutamate pathway. The receptor for Nrg1, ErbB4 (which has itself been shown to have a genetic link to schizophrenia (Norton et al., 2006)) shares a common anchoring region on the PSD-95 structural protein with NMDAr (Garcia et al., 2000). It has also been shown that Nrg1 activity in the prefrontal cortex increases the association between ErbB4 and the NR1 subunit, and can lead to a reduction in

NR2A phosphorylation and decreases in NMDAr activity through internalisation of the receptor. (Gu et al., 2005; Hahn et al., 2006).

Other genes also have direct links to NMDAr function: *DAAO* and *G72* are interrelated and affect NMDArs – DAAO metabolises D-serine, an endogenous modulator of the NMDArs (Mothet et al., 2000) and G72 is thought to initiate the activity of DAAO (Chumakov et al., 2002). Dysbindin is localised to presynaptic terminals, and may participate in the formation and maintenance of synapses, and in signal transduction (Benson et al., 2001; Straub et al., 2002). *COMT* acts directly on dopamine neurotransmission, (Gogos et al., 1998) and almost certainly could alter glutamate transmission through the various links between dopamine and glutamate transmitter systems (Grace, 1991).

1.4.4 NMDA Receptor Hypofunction and Other Neurotransmitters

The first prominent theory about the neurochemistry underlying schizophrenia was the dopamine hypothesis, which stated that the symptoms of schizophrenia arose from a hyper-dopaminergic state in the frontal cortex of patients (Seeman, 1987). This view arose due to dopamine antagonist properties of the antipsychotic drugs developed in the 1950s and become widely accepted, as the antipsychotic drugs were quite successful at treating the positive symptoms of schizophrenia (Seeman and Lee, 1975). As the initial success of pharmacological treatment waned and the unmet need of negative and cognitive symptoms grew in importance, it was realised the dopamine hypothesis was not sufficient to fully explain all aspects of schizophrenia (Thaker and Carpenter, 2001) and as discussed throughout this section, there is substantial evidence that a dysfunction of NMDArs fulfils that role. However, any alternate theory still needs to account for the same evidence answered by the dopamine hypothesis, which is still a prominent account in the field. The major evidence supporting dopamine involvement in schizophrenia pathophysiology is that dopamine D_2 receptor antagonist drugs are effective in

treating the positive symptoms of schizophrenia and a substantial body of evidence showing altered dopamine levels in patients (Howes and Kapur, 2009).

As stated earlier glutamate is the most abundant neurotransmitter in the brain, and NMDArs are broadly distributed throughout the nervous system (Meldrum, 2000). This enables glutamatergic transmission to regulate the function of other neurons that have also been implicated in the pathophysiology of schizophrenia. For example, the bursting firing pattern of dopaminergic neurons (the stereotypical response pattern of these neurons), requires NMDAr activation to occur (Johnson et al., 1992). Studies with NMDAr antagonists have also produced evidence linking the two neurochemical hypotheses. Ketamine treatment can induce increased dopamine release in the striatum (Smith et al., 1998), simultaneously with an increase in positive symptoms (Breier et al., 1998). These studies demonstrate that an NMDAr blockade can lead to a hyper-dopaminergic state. Further evidence associating NMDAr hypofunction with excessive dopamine activity are studies of amphetamine-triggered dopamine release. Patients with schizophrenia show a higher level of dopamine release following amphetamine administration than healthy controls (Abi-Dargham et al., 1998; Laruelle et al., 1996). Similar effects are seen in healthy subjects pretreated with a dose of ketamine (Kegeles et al., 2000; 2002), indicating altered NMDAr function may lead to hyper-dopaminergic states.

Glutamatergic systems are also linked to GABAergic interneurons, another neurotransmitter system that has been strongly implicated in the pathophysiology of schizophrenia. See Section 1.3.3 for a summary of GABAergic pathology in schizophrenia, but to briefly summarise, considerable histological evidence shows reductions in PV and GAD_{67} expression in patients with schizophrenia (Lewis et al., 2005). PV+ GABAergic interneurons are essential for the generation of synchronised activity amongst neuronal ensembles, in particular γ frequency

oscillations (Bartos et al., 2007; Mann and Paulsen, 2007). There is increasing evidence that deficits and synchrony and neural dynamics is a major pathophysiological aspect of schizophrenia (Reviewed in section 1.5.3) and that PV+ GABAergic interneuron dysfunction is the mechanism through which these alterations occur.

Several lines of evidence indicate that NMDArs specifically contribute to GABAergic dysfunction. GABAergic interneurons are disproportionally more sensitive to NMDAr antagonists than pyramidal neurons (Greene et al., 2000; Grunze et al., 1996; Olney, 1995). Secondly, repeated administration of NMDAr antagonists causes depletion in levels of GAD_{67} and PV in cortical areas (Behrens et al., 2007; Cochran et al., 2003; Keilhoff et al., 2004; Rujescu et al., 2006), causing effects similar to that seen in histological examination of patients post-mortem tissue. Recent studies utilising cell-specific techniques to ablate NMDArs on PV+ interneurons in mice revealed these animals display a range of behavioural and cognitive deficits reminiscent of schizophrenia, and a concurrent increase in the power of ongoing γ oscillations (Carlen et al., 2012; Korotkova et al., 2010).

The mechanisms by which NMDAr may affect PV+ interneurons are still unclear, but one hypothesis posits that the calcium permeability of the NMDAr channel plays a role. PV is a calcium binding protein and its physiological role is as a calcium buffer; it quickly sequesters free Ca^{++} in the intracellular space and reduces available Ca^{++} concentrations. Reduced PV expression may be a compensatory reduction that reflects reduced Ca^{++} entry into PV-containing interneurons and reduced GAD_{67} may reflect reduced excitatory NMDAr drive onto PV+ interneurons. The associations between NMDAr hpyofucntion and PV+ interneuron excitatory drive provide a link to disruptions in γ oscillations and synchronisation. NMDAr function is essential for the proper maintenance and generation of γ frequency activity, leading to a conception of schizophrenia as the

product of deficits in NMDAr function, leading to abnormal γ frequency activity which results in a dysconnection in functional neural networks and a breakdown in neural communication.

1.5

Neural Oscillations

Oscillatory activity is an intrinsic characteristic of neuronal tissue, observed in the very first human EEG recordings by Hans Berger in the 1920's (Berger, 1929). Berger reported the existence of the alpha wave – an 8-12 Hz rhythm that dominated his recordings when subjects closed their eyes. There has since been an enormous interest in the nature and function of these rhythms: what role did they play, and how were they generated?

As these oscillatory patterns were observed to change in ways related to the activity of the subject being recorded, they gave rise to the hope that we could gain insight into the activity of the living, functioning and intact brain through the study of these oscillations. There is a growing view in cognitive neuroscience that the higher order cognitive processes result from the complex interplay of highly structured and organised brain activity (Engel et al., 2001), and that by studying the neural dynamics of oscillatory activity we can gain insight into them (Herrmann et al., 2010). This approach has borne fruit in recent years as the functional role of brain oscillations has become more fully understood; oscillations have been demonstrated to play a role in governing the complex flow of information and activity in the human brain (Buzsaki and Watson, 2012; Fries, 2009).

When viewing raw EEG recorded from a human scalp it can give the impression of being a rapidly changing seemingly random, chaotic process. However, this is the result of the EEG signal captured from the scalp consisting of

the summation of multiple different frequency bands overlaid on top of each other (Buzsaki, 2006). These frequency bands were labelled for the order they were discovered in, and in increasing order are delta (δ, 1-4 Hz), theta (θ, 4-8 Hz), alpha (α, 8-12 Hz), beta (β, 13-29 Hz), gamma (γ, 30-80 Hz), (Figure 1.2). In recent years advances in recording equipment and analysis technologies has allowed the study of even higher frequency bands including 'high gamma' (80-140 Hz) (Canolty et al., 2006) and hippocampal ripples (140-220 Hz) (Buzsaki and da Silva, 2012). These different frequency bands are posited to be generated through different neuronal and network mechanisms and be related to different activities. For example, α oscillations are associated with a relaxed, unoccupied brain; they decrease with attention and focus (Klimesch, 1999). In contrast θ band activity is associated with memory tasks and the strength of θ oscillations has been shown to increase with memory load and when engaged in memory related tasks (Kahana et al., 2001; Nunez, 2000).

The power of the EEG has the property that as the frequency increases the power of the related oscillations decreases at a proportional rate i.e. $1/f$ (Freeman et al., 2000); the faster frequencies have considerably lower power than the lower frequencies. The precise origin of this property is still up for debate (Bédard and Destexhe, 2009; Logothetis et al., 2007), but has given rise to several concepts. Lower frequency oscillations are generally understood to involve larger volumes of neural tissue, incorporating larger numbers of neurons and propagating over larger distances (Stein and Sarnthein, 2000). This is due to both the $1/f$ property and the fact that their slower oscillatory speeds allow synchronisation at larger distances. The axonal conductance times over long distances can prevent faster oscillations over large distances. The faster oscillations are thought to be more constrained in space, therefore generated locally (Csicsvari et al., 2003; Steriade, 2001). These

multiple time and distance domains are proposed to allow the brain to operate at multiple temporal and spatial scales (Buzsaki et al., 2004).
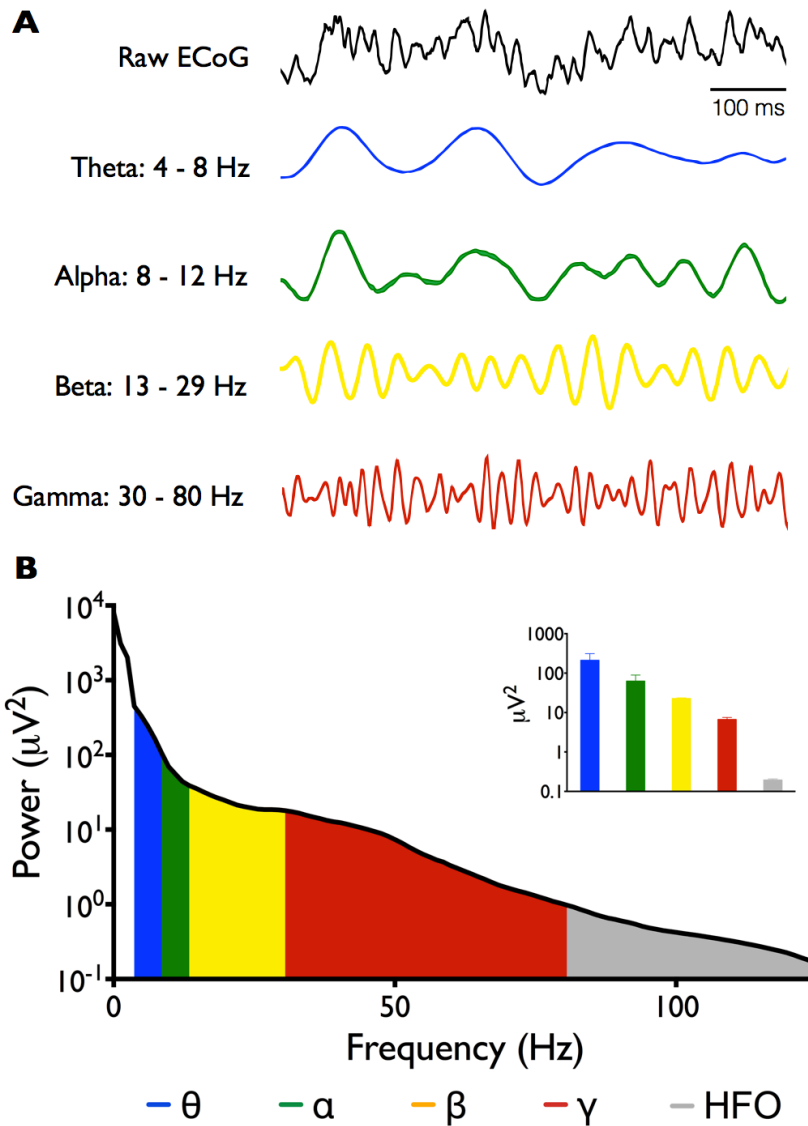
Figure 1.2 – Representative ECoG Trace, Frequency Bands and Power Spectrum

The top panel represents a raw ECoG recording taken from the cortical surface of a Wistar rat. Shown underneath are representations of the same recording period, filtered to display specific frequency bands. The bottom panel shows a stereotypical power spectrum. This graph uses a log scale on the Y-axis and the distinct frequency bands are highlighted in different colours.

Neural oscillations spontaneously emerge in brain regions as the result of several intrinsic cellular and circuit properties (Destexhe and Sejnowski, 2003; Somers and Kopell, 1993; Whittington and Traub, 2003). These properties range from the type, location and number of specific ion channels in cell membranes (Llinas, 1988), the presence or absence of electrical gap junctions (Draguhn et al., 1998; Gibson et al., 1999), through to the reciprocal excitation and inhibition between networks of large numbers of neurons (Whittington and Traub, 2003). The specific mechanisms governing γ oscillations are detailed in Section 1.5.4.

Studying the function and purpose of neural oscillations is a rapidly expanding field of neuroscience that has come to overlap multiple fields from physiology, psychology, computational neuroscience and cognitive neuroscience. A general concept has arisen in which synchrony between neurons, neural ensembles and circuits is an essential process for combining distributed information processing in the brain (Buzsaki, 2006; Buzsaki et al., 2004; Fries, 2009; Uhlhaas et al., 2009). Oscillations represent a 'low energy' mechanism for entraining synchronisation in neural networks (Buzsaki and Draguhn, 2004). Spontaneously generated oscillations are rhythmic changes in the membrane potential and hence the propensity to fire of individual neurons. This equates to rhythmic changes in both the likelihood of firing and the sensitivity to synaptic inputs of the oscillating cells (Fries, 2005). This allows the period of oscillations to govern the communication between neurons and within neuronal ensembles. Communication and the transfer of information will be enabled and aided in neurons that are firing in phase with surrounding oscillations and inhibited or blocked in those out of phase. The exact timing of spikes controls how well information is transmitted from one brain region to another (Softky and Koch, 1993) and synchronous inputs from upstream neurons to a target neuron can summate more effectively and lead to an action potential. This synchronisation of neuronal firing and oscillations has been proposed to enable wide ranging computational processes in the brain, at the simplest conception the rhythmic

activity in neural networks influences communication and the passage of information between neurons (Fries, 2009).

The study of oscillations can be used to give insight into neurological diseases; oscillatory patterns of activity and abnormal traits reveal the mechanisms at play in pathological conditions, and can be used to develop treatments and diagnostic tools (Herrmann and Demiralp, 2005; Traub and Whittington, 2010; Uhlhaas and Singer, 2010). For example both Parkinson's disease and epilepsy can be thought of as diseases of oscillations (Traub and Whittington, 2010). Epilepsy represents hyper-synchronous activity, where a seizure is brought on by aberrant oscillatory activity spreading and dominating the brain leading to a loss of consciousness (Fisher et al., 2005). Parkinson's disease represents a break down in the normal oscillatory processes that govern movement and motor control. A β rhythm that normally disinhibits motor control is overactive in the disease, and may contribute to the motor dysfunction that is observed (Levy et al., 2002). The success of deep brain stimulation in treating the condition has been theorised to be due to its ability to diminish the aberrant beta frequency activity (Bronte-Stewart, 2012).

Schizophrenia can be conceived of as a disease characterised by abnormalities in the expression of coordinated activity in the brain, resulting from a dysconnection between functional networks and breakdown in communication. In turn, neural oscillations and the synchronisation of neuronal outputs can play a role in information processing and cortical communication; allowing us to conceptualise schizophrenia as a disease of dysfunctional oscillations. γ frequency oscillations in particular have been proposed as the mechanism through which coordinated neural activity is generated and controlled (Fries, 2005), leading to the conception of γ as a mechanism through which the symptoms of schizophrenia may arise (Uhlhaas and Singer, 2010). This hypothesis presents a powerful framework to understand how

schizophrenia may manifest and could potentially lead to improvements in our ability to treat and prevent it.

1.5.1 Studying Oscillations

The most common way to study neural oscillations in humans is through scalp recorded EEG, this recording technique captures the broad electrical activity of large populations of neurons. However, oscillatory activity patterns can be seen all the way down to the level of individual neurons for which different recording techniques and methods can be utilised to capture different levels and types of information.

Magneto/Electroencephalogram (MEG/EEG) – Scalp recorded magnetic or electrical activity, the signals measured by MEG and EEG represent activity from large ensembles of cells with fairly crude spatial resolution, although modern analysis techniques are improving the ability to separate out and identify distinct current sources.

Electrocorticogram (ECoG) – Electrical recordings made directly from the cortical surface these provide stronger and clearer signals than scalp recorded EEG. Due to the incredibly invasive nature these recordings are only performed on humans who require cranial surgery. Can also be performed in non-human primates and rodents for the study of neuronal activity. Both the EEG and ECoG are thought to only record the activity of cortical layer neurons.

Local field potentials (LFP) – Recordings made from electrodes inserted directly into brain tissue, allowing precise targeting of relevant brain regions for study. LFP recording allows the study of the electrical activity of cortical and subcortical structures that are unavailable to EEG/ECoG recordings. It is generally believed that the LFP signal reflects the electrical currents associated with synaptic

activity and intrinsic membrane properties in a local population of neurons around the electrode (Buzsaki et al., 2012). LFP recordings can capture the spiking activity from multiple or individual neurons as well as the surrounding electric field.

Single and multi-unit recordings – The lowest level at which oscillatory γ frequency activity can be detected is in the firing pattern of individual neurons. This can be achieved by single or multi-unit recordings in animals and again is rarely seen in humans. Typically, γ frequency activity is observed as spiking patterns that occur every 10–30 ms and can be analysed by auto or cross correlating the spike timings or capturing concurrent LFP to analyse the spike timing with regard to LFP phase.

In addition to the multiple recording techniques, each targeting different scales on neuronal activity, when studying neuronal oscillations there are at least four distinct types of oscillatory activity that should be considered (Figure 1.3):

Ongoing – Ongoing or baseline oscillations are spontaneously occurring rhythms that are not tied to any specific stimulus or activity. Also know as resting or pre-stimulus oscillations, ongoing oscillations are spontaneously generated by cortical, hippocampal and other structures (Merker, 2013). They are dominant during the desynchronised state of the electroencephalogram (Ahmed and Cash, 2013; Jasper, 1936).

Evoked – Oscillations directly triggered via a sensory stimulus are called evoked oscillations. Oscillatory activity that is time-locked (in phase) with a stimulus can be averaged across many trials to generate an evoked response. Any oscillations that are not time-locked to the stimulus average to zero over many trials leaving a solely evoked waveform. Evoked oscillations generally occur soon after the stimulus and are thought to reflect early sensory processing (Haenschel and Linden, 2011).

Steady State – Elicited through repeated sensory stimulation, steady state oscillations represent neural 'entrainment' to a repeated stimulus at a set frequency. Steady-state paradigms are utilised to assess the ability of neuronal networks to generate and sustain oscillatory activity in different bands, through varying the stimulus frequency. Auditory stimuli are most commonly used although visual and tactile paradigms exist.

Induced – Oscillations that are related to sensory stimuli (or cognitive tasks) that are not precisely time locked to a stimulus. Induced oscillations vary in time on each trial and are thus not seen with simple averaging techniques. Induced oscillations are thought to be related to object and perceptual representation and higher order cognition (Tallon-Baudry and Bertrand, 1999).
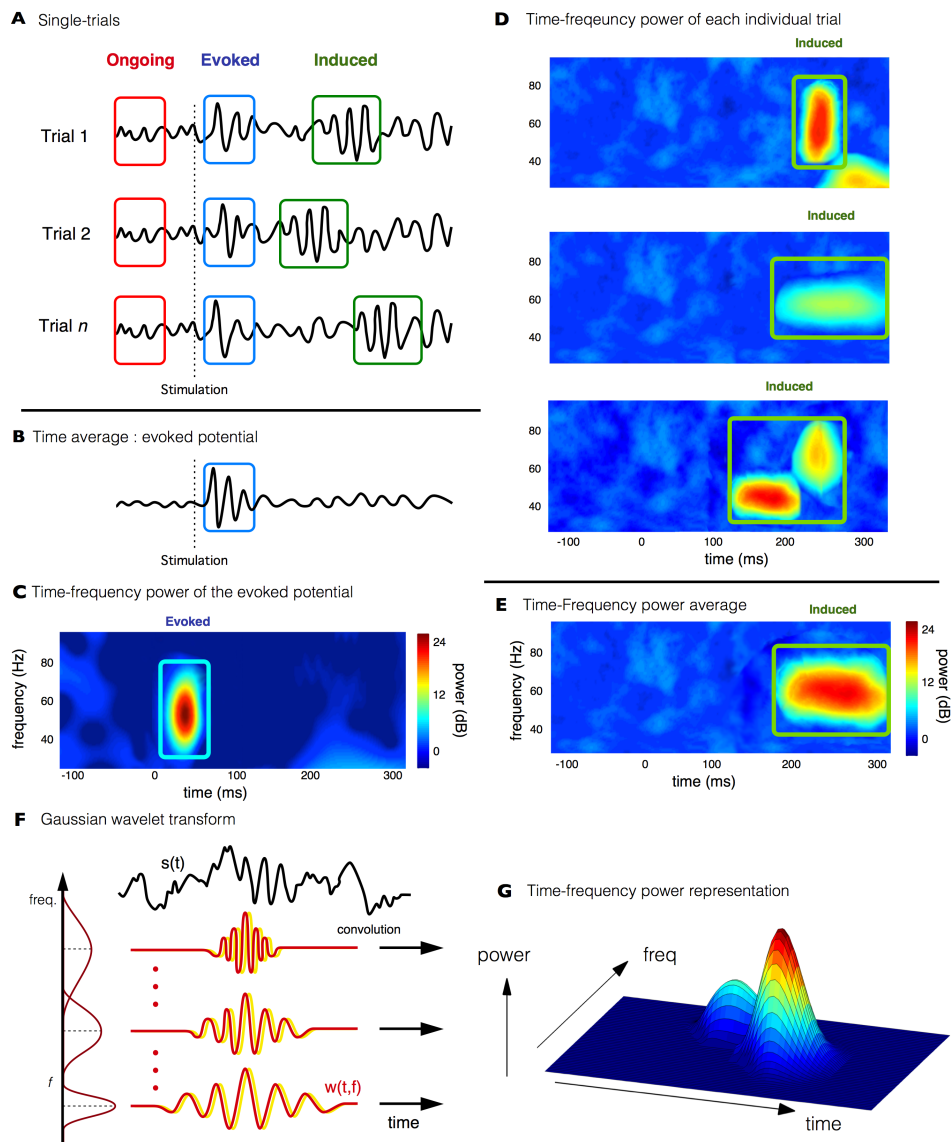
Figure 1.3 – Illustration of the Different Categories of Neural Oscillations and Methods of Analysis

A) Multiple EEG traces in a sensory task (simulated data), the dotted line represents the time that a sensory stimulation is presented. Ongoing or baseline activity is marked in red boxes, the evoked response occurs at the same latency in every trial and is marked in blue. Induced oscillations (marked in green) are triggered by the stimuli but are not time locked, appearing at different latencies in each trial. **B)** Averaging across a large number of trials cancels out the non-time locked activity (both ongoing and induced) leaving a purely evoked response. **C)** A heatmap showing the power of the evoked response across time (x-axis) and across different frequencies (y-axis), the non time-locked activity has been removed through averaging. **D)** Calculating time-frequency data for each individual trial reveals the induced activity for each trial. **E)** When the individual trial data is then averaged the induced gamma response is revealed. **F)** Time-frequency analysis is performed by convolving a family of Morlet wavelets with the EEG signal – s(t). This series of wavelets have differing peak frequency responses and time dimensions. **G)** By utilising a series of wavelets across a sliding time window analysis of both time and frequency can be made with high resolution. *Adapted from (Tallon-Baudry and Bertrand, 1999)*

1.5.2 Gamma Oscillations

γ frequency oscillations have been associated with a wide range of cognitive processes, leading to a growing conception of γ frequency activity as an essential function of information processing in the brain (Fries, 2009). γ oscillations came to prominence as a phenomenon related to perceptual processes due to a series of studies in the late 1980's and 90's that proposed they served as a solution to the 'binding problem' – how the numerous structures and functions of the brain subserving memory, emotion, attention and perception combine their outputs into the single unitary experience we describe as consciousness (Revonsuo, 1999).

A study was performed in the cat visual cortex, where the authors found that in response to moving bars of light, the multi-unit activity in cat visual cortex showed synchronous activity at γ frequencies (Gray and Singer, 1989). This unit activity was accompanied by and in synchrony with prominent γ oscillations in the LFP. A second, similar experiment was performed that demonstrated a similar result in widely separated regions of the visual cortex; units fired in synchrony with almost no lag despite having no direct anatomical connections (Gray et al., 1989). These observations led to the hypothesis that γ frequency synchrony enacts the temporal binding of disparate perceptual features into a coherent whole (Singer, 1993). The γ binding hypothesis posits that neurons in diverse regions can synchronously encode different features of a percept (such as colour, shape or motion) by firing together in a single γ cycle. This synchronous firing across regions is thought to 'bind' the encoded features together into a unified perception of an object (Ahmed and Cash, 2013).

This work in the visual system and the notion of temporal binding initiated enormous interest in the area and a huge body of work has followed. Subsequently, the synchronisation of neuronal spiking has been demonstrated across multiple species and for nearly every perceptual domain: visual, auditory, olfactory and

somatosensory (Engel and Singer, 2001). The animal work regarding γ synchronisation in visual perception has also been replicated in humans (Müller et al., 1996). This role for synchronisation in neural circuits for the representation of incoming sensory information was also rapidly expanded to encompass other domains of cognition (Salinas and Sejnowski, 2001). One of the first cognitive properties found to be correlated with γ frequency oscillations was attention (Tiitinen et al., 1993). This was quickly expanded to encompass nearly the full spectrum of human cognitive processes, including: memory (Basar et al., 2000; Gruber and Müller, 2006; Jensen et al., 2007; Kaiser and Lutzenberger, 2005; Sederberg et al., 2007), object representation (Tallon-Baudry and Bertrand, 1999), perceptual awareness (Martinovic and Busch, 2011; Ohla et al., 2007; Rodriguez et al., 1999; Schurger et al., 2006), language (Bastiaansen and Hagoort, 2006; Crone et al., 2001; Eulitz et al., 1996; Pulvermüller et al., 1996) and physical movement (Pesaran et al., 2002).

In recent years theories of binding and neuronal synchronisation have grown to be considered as neurological explanations for phenomenological consciousness (Engel et al., 2001; Thompson and Varela, 2001). This is still a highly disputed and unproven area, but a hypothesis has been formed wherein dynamically linked networks of synchronised neurons are the substrate in which conscious perception is instantiated (Uhlhaas et al., 2009). γ oscillations have been associated with all the key cognitive domains that are disrupted in schizophrenia, as well as (tentatively) consciousness itself. Schizophrenia is a disease of perception and consciousness, which raises the interesting question of what relationship γ oscillations have to schizophrenia. As will be detailed next, γ oscillations are substantially disrupted in patients with schizophrenia and the molecular and cellular mechanisms of their generation intersects with the NMDAr hypofunction hypothesis of schizophrenia. This positions γ oscillations as the mechanism that results in the dysconnection that

is hypothesised to characterise this illness; establishing a framework which provides a strong foundation to improve our understanding of schizophrenia as well as the neural dynamics of both health and disease.

1.5.3 Gamma Oscillations in Schizophrenia

1.5.3.1 Perceptual Related Gamma

The cognitive functions that have been demonstrated to be associated with γ frequency activity are also disrupted in patients with schizophrenia (Woo et al., 2010). The dysfunction of γ activity in the brain provides an explanation of the varied perceptual and cognitive abnormalities seen in schizophrenia (Uhlhaas and Singer, 2010). Given that hallucinations and delusions are the most dramatic and compelling symptom of schizophrenia, much research has concentrated on perceptual differences in schizophrenia and abnormalities in their associated EEG response (Woo et al., 2010).

The γ frequency response to auditory stimuli is a widely used paradigm due to the ease of implementation. In such tests, subjects have EEG recording electrodes affixed and then listen to a series of tones and the resulting neural responses are analysed. The most widely studied and replicated γ frequency abnormality in patients with schizophrenia is the 40 Hz auditory steady state response (ASSR). In this paradigm a series of audio tones of 40 Hz frequency are played to a subject undergoing EEG recording and there is a concurrent increase in 40 Hz EEG activity, and the auditory triggered neural activity is said to be 'entrained' to the stimulation frequency (Picton et al., 2003). Numerous studies have demonstrated that patients with schizophrenia do not display the same EEG 'entrainment' as seen in healthy subjects (Brenner et al., 2003; Hong et al., 2004b; Kwon et al., 1999; Light et al., 2006). Instead, patients with schizophrenia typically

show a reduction in the power of γ responses and reduction in the degree of synchronisation to the imposed auditory signal (Hong et al., 2004a).

Other studies examining evoked responses and involving simple auditory stimuli have investigated the earliest evoked γ frequency responses, thought to represent the first stages of percepetual processing (Pantev et al., 1991). These studies comprehensively catalogue reductions in auditory triggered γ responses in patients with schizophrenia (Basar-Eroglu et al., 2009; Krishnan et al., 2009; Lee et al., 2001; Leicht et al., 2011; 2010; Roach and Mathalon, 2008). A smaller number of studies have studied later (≥ 200ms) auditory γ responses and also found reductions in patients (Gallinat et al., 2004; Gordon et al., 2001; Haig et al., 2000).Studies examining visually-evoked responses in patients also show similar deficits in the early γ perceptual responses. (Spencer et al., 2004), used a visual perception task that involved responding to the presence of a gestalt image. Not only was visually-evoked γ power and phase locking diminished in patients, there was a significant correlation between phase locking values and the positive symptoms of schizophrenia. These data suggests that perceptual abnormalities can be reflected in γ measures, which in turn reflect symptom dimensions. There have also been studies demonstrating reduced visual steady-state responses in patients (Brenner et al., 2009; Krishnan et al., 2005), in agreement with the auditory findings.

Studies have also examined somatosensory perception (Arnfred et al., 2011) and transcranial magnetic stimulation (Ferrarelli et al., 2008) evoked γ responses, again showing a reduction in the evoked response. Interestingly reduced audio-evoked γ frequency oscillations have been recorded in first-degree relatives of patients with schizophrenia, as well as in unaffected siblings in monozygotic twin pairs discordant for schizophrenia (Hall et al., 2011a; 2011b). This indicates that there is a substantial genetic component to the presence of aberrant evoked γ

frequency activity. This was also replicated in a separate study of unaffected relatives, showing similar deficits to schizophrenia patients (Leicht et al., 2010).

There is a solid body of evidence demonstrating that perceptual stimuli related γ oscillations are disrupted in patients with schizophrenia, providing an electrophysiological correlate to the existing evidence of perceptual abnormalities in patients (Uhlhaas and Mishara, 2007). These findings suggest that abnormal perceptual evoked γ frequency responses reflect a broad dysfunction in the generation of γ frequency synchronisation and oscillations in patients with schizophrenia.

1.5.3.2 Cognition Related Gamma

Cognitive tasks typically generate γ responses that are not time-locked to a specific stimulus and occur later in time than evoked responses. These induced γ responses are thought to reflect high-order cognitive processes in contrast to the early perceptual processing that evoked γ oscillations are associated with (Haenschel and Linden, 2011; Tallon-Baudry and Bertrand, 1999). There is a substantial body of evidence that indicates dysfunction of induced γ frequency responses associated with higher order processes such as memory and attention in patients with schizophrenia. Working memory and executive control abnormalities are representative of these core cognitive deficits in schizophrenia, providing a useful theoretical explanation for these abnormalities and lending further support to the hypothesis that γ frequency abnormalities underlie these symptoms.

Working memory presents an especially relevant cognitive process to study in the context of schizophrenia and γ oscillations. Working memory impairments, particularly in terms of visual and spoken memory are the most consistent cognitive deficits in schizophrenia (Harvey and Keefe, 2001; Heinrichs and Zakzanis, 1998;

Keefe and Harvey, 2012). Furthermore, the magnitude of cognitive impairments as the disease progresses has been associated with poor functioning and lower quality of life (Green, 1996; Leeson et al., 2009). Working memory deficits may themselves lead to poorer outcomes as the cognitive deficits impede patients' ability to cope with the challenges of psychotic symptoms.

Working memory is also particularly pertinent in the context of γ oscillations; a computational model of working memory explains the limitations of working memory in terms of nested γ and θ oscillations (Lisman and Idiart, 1995). The 'magic number' of 7 ± 2 publicised by (Miller, 1956), is thought of as the limit on the number of discrete items able to be maintained in working memory. In Lisman and Idiart's model the number of γ cycles per θ wave is the reason for this computational limit, and there is considerable experimental evidence that supports this both in terms of θ /γ coupling (Axmacher et al., 2007; Lisman and Buzsaki, 2008) and γ oscillations correlating with working memory load (Howard et al., 2003).

The correlation between working memory load and the magnitude of γ oscillations is absent in patients with schizophrenia. Patients display an abnormally large γ response that does not scale with task difficulty (Basar-Eroglu et al., 2007). There is also evidence that points to deficits in specific aspects of working memory; deficits in working memory performance can potentially arise from deficits in the perception, encoding, maintenance or retrieval of the stimuli (Haenschel and Linden, 2011). As discussed previously patients with schizophrenia exhibit deficits in fundamental perceptual processes (Butler et al., 2008) and have corresponding γ frequency abnormalities, which is potentially a confounding factor when studying working memory tasks. However, a study by (Haenschel et al., 2009) examined cortical oscillatory activity during each individual stage of a working memory task: encoding, maintenance and retrieval, between schizophrenia patients and healthy

controls. During encoding, successful memorisation was predicted by the induced θ, α, and β oscillations in control subjects, where patients exhibited substantial reductions in the evoked activity in these frequency bands. During the maintenance period of the task patients showed a load-dependent increase in γ activity that was comparable to the control group. However induced γ was lower in patients during retrieval, which corresponded to poorer performance in the task. These findings suggest that patients have impairments at each stage in the working memory task and they may reach the 'capacity limit' of their working memory system at earlier stages. It is also interesting to note that several studies of working memory have report elevated levels of baseline γ activity in schizophrenia despite a lack of task-specific activation (Barr et al., 2010; Basar-Eroglu et al., 2007), this would seem to support the inference that working memory in patients is more easily filled and they may effectively be functioning as if they were under significant cognitive strain at baseline levels.

There have been studies of other areas of cognitive function that are altered in schizophrenia that also report aberrant γ oscillations. (Kissler et al., 2000) studied patients and healthy controls while performing a complex mental arithmetic task. Similarly to working memory results patients performed worse and also displayed abnormal induced γ oscillations. The healthy subjects showed an increase in γ power over left frontal areas, while the patients showed no task effect at all in a low γ band (30-45 Hz) and displayed a reversal in a higher band (45 - 71 Hz), with increases over the right frontal areas.

A study looking at cognitive control also found reductions in induced γ band activity as well as more complex deficits, (Cho et al., 2006). Patients had similar levels of performance as controls in the 'easy' condition, but performed significantly worse in the harder task. Healthy subjects showed a corresponding increase in induced γ activity as the task difficulty increased, indicating that induced

γ power may represent the current cognitive load. Patients did not show this increase in relation to task difficulty, indicating that there is a deficit in the ability to coordinate synchronised neural activity in reaction to task demands. This result was replicated in another study looking at unmedicated, first-episode patients who displayed similar deficits in induced γ.

Another study examined executive function by utilising the Wisconsin card sorting task – a neuropsychological test that is designed to examine cognitive flexibility, the ability to learn and adapt to changing rules and reinforcement (Monchi et al., 2001). Patients performed extremely poorly on this test, in line with earlier results (Cuesta et al., 1995) which is an exceedingly complex task and presents significant challenges to patients with schizophrenia. When healthy controls were performing the task they exhibited broad increases in all frequency bands, except α where decreases were observed. Patients showed similar increases except in the γ frequency band, again indicating a deficiency in the ability to generate or 'ramp up' γ oscillations in response to cognitive loads.

Overall results from studies looking at cognitive function and induced γ oscillations in patients with schizophrenia reveal a more complex interplay between γ oscillations and cognitive function than the broad reductions in evoked γ in perceptual tasks do. Although the general results support an idea that patients with schizophrenia exhibit lower levels of γ oscillatory activity in response to external stimuli, there are important subtleties to consider. There is evidence that patients lack the ability to appropriately modulate γ activity in response to cognitive demands, the task dependent changes in oscillatory activity that define a normal response to cognitive challenges is absent or abnormal. Complementary to this is some evidence that patients have increased baseline γ activity in relation to cognitive tasks, indicating their cognitive workload may be limited in comparison to healthy controls. Whether this is a causal relationship – deficient mechanisms in

generating and modulating γ oscillations result in reduced working memory, attention and executive function – or these γ abnormalities reflect the already existing cognitive deficits is still unknown and presents an intriguing field of research.

1.5.3.3 Ongoing Gamma

As well as the extensive literature pertaining to deficits and abnormalities in γ oscillatory measures generated through the presentation of stimuli or in cognitive tasks there is also considerable evidence regarding the properties of ongoing or baseline γ activity in schizophrenia. Two separate types of data contribute to the literature on ongoing oscillations, studies specifically examining the characteristics and properties of baseline activity – sometimes called 'resting state' paradigms, to contrast with active studies. The other is obtained by analysing the baseline or 'pre-stimulus' period in sensory stimuli or cognitive task based designs. By examining the EEG activity recorded prior to the presentation of stimuli the 'baseline' activity can be extracted and analysed for differences.

Several studies of the resting state EEG were done in the earlier decades of research in neural oscillations, as a result they do not always examine broadband γ (30-80 Hz), as recording paradigms and equipment available were often limited to a maximum frequency of 40-50 Hz. This is still high enough to capture some of the γ range and inform us of the characteristics of baseline γ oscillations in patients with schizophrenia. In a study dating back to 1942, specific mention is made of the "choppy and disorganised, for want of a better term" oscillations that dominate the EEG of patients with schizophrenia (Davis, 1942). This choppy activity ranged from 26-50 Hz and is highly reminiscent of what we now think of desynchronised EEG activity, which is typically dominated by γ activity. Although this study lacks

modern quantitative analysis, the author notes some 61% of patients with schizophrenia had baseline activity dominated with choppy, or γ activity compared to just 5% of manic-depressive patients. A similar study performed in the 1940's noted that the presence of "high-voltage rapid frequency" (20-40 Hz) activity in up to 75% of psychotic, 35% of schizophrenia and just 5% of 'normal' patients. More applicable to modern EEG analysis techniques several studies using spectral analysis reported elevated high β (26-34 Hz) amongst patients with schizophrenia compared to controls (Giannitrapani and Kayton, 1974; Itil et al., 1972). Other modern studies have reported similar results (Kissler et al., 2000; Krishnan et al., 2005), with (Venables et al., 2009), finding increased baseline power in a 20-50 Hz band in both patients, a result that extended to first-degree unaffected relatives.

Interestingly, excessive baseline γ activity seems to be associated with a specific symptom dimension. Baldeweg et al. (1998), reports a case of a schizophrenia patient experiencing severe somatic hallucinations, accompanied by a dramatic increase in baseline γ power. This relationship to symptom dimensions has been seen in other studies, Lee et al. (2003), reported an overall reduction in frontal γ synchrony in patients undergoing an auditory stimuli task. However, when the patients were categorised by their dominant symptoms, patients that exhibited positive symptoms (primarily disorganisation and reality distortion) were found to exhibit increased γ synchrony. Other studies also reflect this result: in contrast to the typical finding of decreased evoked γ power, patients with more severe positive symptoms are found to exhibit greater levels of γ activity (Hirano et al., 2008; Spencer et al., 2009; Spencer, 2008; Spencer et al., 2004; Uhlhaas et al., 2006) .

As well as the results of resting state studies, several studies report the characteristics of 'pre-stimulus' baseline γ amongst patients with schizophrenia. Two such studies that utilised audio evoked paradigms reported increased pre-stimulus γ power in patients as compared to healthy controls (Winterer et al.,

2004). This raises a potential methodological problem, nearly all evoked stimuli methodologies involve baseline correction of the captured data (Urbach and Kutas, 2006). If there is a pathological increase in ongoing high frequency oscillations in the schizophrenia population this could be masked by the conventions of baseline correcting data and reporting the corrected evoked results. As a result, this may end up overstating the magnitude of sensory-evoked deficits in schizophrenia by comparing them to the already elevated baseline. A particularly pertinent example of this is a study by Spencer, (2011), that reanalysed a previously published (and baseline corrected) study that had found a decrease in the γ response to ASSR (Spencer et al., 2009). The revised results looking at baseline γ found increased power in the patients with schizophrenia.

When examining studies that do not baseline correct the data generated we see other similar results. A study examining audio-evoked responses and utilising non-baseline corrected measures values of γ synchrony found increased levels of γ synchrony amongst first-episode schizophrenia patients (Flynn et al., 2008). The same group repeated this result in a visual task study (Williams et al., 2009), leading them to theorise that this excessive γ activity reflects 'overbinding' of non-relevant stimuli, which in turn leads to the reductions in task-specific γ typically seen. It is also interesting to note that several studies of working memory in schizophrenia report elevated baseline γ, concurrent with deficient task-related γ (Barr et al., 2010; Basar-Eroglu et al., 2007; González-Hernández et al., 2003). These studies agree with the 'overbinding' hypothesis that excessive pathological γ activity may be present at a baseline, or ongoing level that then interferes with the generation and utilisation of 'normal' task-related γ activity.

With the prevalence of disrupted sensory and cognitive task-related γ oscillation in schizophrenia, and the array of baseline γ abnormalities as well, it is possible that this aberrant basal γ activity is modulating task-specific γ (Hakami et

al., 2009). Several reports consider increases in ongoing γ oscillations as a source of excessive 'noise' in neural networks (Baldeweg et al., 1998; Gandal et al., 2012b; Hakami et al., 2009; Rolls et al., 2008). It is hypothesised that this increase in aberrant network γ activity could mask and interfere with genuine sensory-evoked responses thereby impairing sensory information processing (Gandal et al., 2012b; Hakami et al., 2009), resulting in a reduced signal-to-noise ratio in the computation capabilities of the brain. This hypothesis has been suggested by multiple sources (Flynn et al., 2008; Gandal et al., 2012b; Hakami et al., 2009; Kissler et al., 2000; Krishnan et al., 2011; Williams et al., 2009; Winterer et al., 2004) and there is increasing evidence from animal models (Kulikova et al., 2012; Saunders et al., 2012) and human studies (Spencer, 2011; Suazo et al., 2012) that supports the hypothesis that elevations in baseline γ activity represent increased 'noise' in neural networks, and that this may impair γ-mediated sensory-evoked responses and information processing.

There is considerable data to suggest that ongoing or baseline γ oscillations are elevated in patients with schizophrenia, which may be specifically associated with the positive symptoms of the disease (Baldeweg et al., 1998; Lee et al., 2003). There is also a large and still growing body of evidence that the sensory and cognitive task related γ oscillations are disrupted in patients with schizophrenia. These multitude of different γ abnormalities points to the existence of diffuse network noise in the neural circuits that generate and sustain γ activity and a corresponding reduction in the ability to generate and/or distinguish sensory triggered γ oscillations. This presents a theoretical explanation for the broad dysconnectivity that defines schizophrenia, which is potentially the result of the aberrant cellular and molecular pathways already identified.

1.5.4 Mechanisms of Gamma Oscillations

The mechanisms that generate oscillatory activity that typifies neuronal tissue have been intensely studied over the past several decades and provides an important connection between the observed γ oscillation deficits and the known pathophysiological traits found in schizophrenia. GABAergic inhibition is an efficient way of synchronising the activity of large numbers of pyramidal neurons (Gonzalez-Burgos and Lewis, 2008; Whittington et al., 2000). GABA-expressing neurons that target the perisomatic region of pyramidal cells, such as PV+ interneurons are strongly inhibitory, as they synapse near the proximal axon, where action potentials are usually triggered (Cobb et al., 1995; Miles et al., 1996). PV+ cells are particularly important to the generation of γ frequency activity because of their fast-spiking characteristics and the short time constants of synaptic interactions mediated by these cells (Bartos et al., 2007). As reviewed in Section 1.3.3, GABAergic deficits, particularly in the PV+ interneuron class, are one of the most robust findings in histological studies of post-mortem brain tissue. The inhibitory post synaptic potential generated through even a single GABAergic neuron can be sufficient to synchronise the firing of a large number of postsynaptic cells (Cobb et al., 1995). Shortly following GABA-induced inhibitor potentials the targeted pyramidal cells are highly likely to fire, meaning all the cells targeted by a single PV+ GABAergic neuron are silenced for a period and then likely to fire in synchrony. The major mechanisms that generate γ frequency oscillations have been found to depend on the action of inhibitory PV+ interneurons that directly innervate the perisomatic regions of vast numbers of excitatory neurons (Figure 1.4) (Bartos et al., 2007; Mann and Paulsen, 2007). The precise mechanism that governs the generation of γ oscillations in PV+ and pyramidal cell networks is still unclear, and two separate models have been proposed to explain the observed neural dynamics.
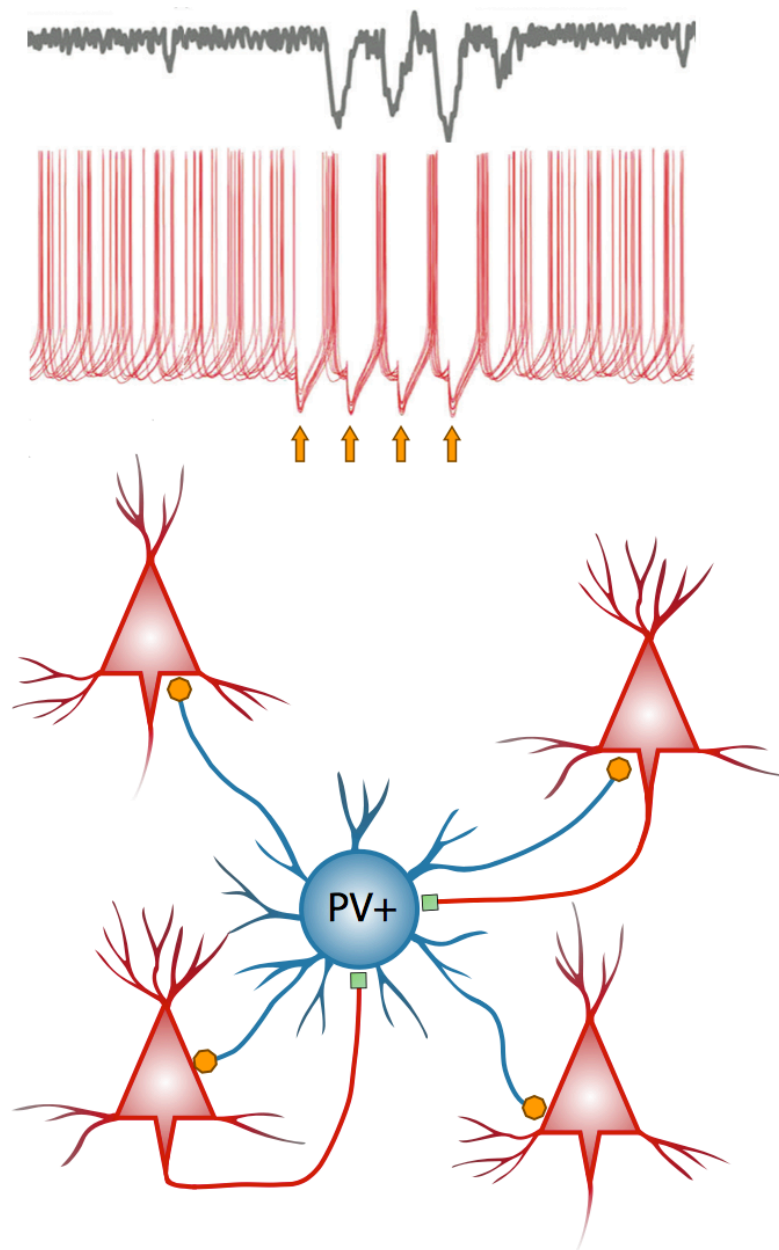
Figure 1.4 – A Schematic Representation of the Pyramidal - Interneuron Gamma (PING) Model

GABAergic PV+ interneurons are efficient at synchronising neuronal activity in cortical networks. The top panel represents a local field potential (LFP) recorded in the vicinity of pyramidal neurons reflects the synchronisation of pyramidal cell activity. Th negative (downward) oscillations of the LFP, coincide with periods of spike synchronisation as shown in the idealised unit activity shown below in red. The bottom panel displays a theoretical PV+ interneuron (in blue) with perisomatic GABAergic synapses (in orange) onto multiple pyramidal neurons (in red). Simultaneous GABAergic release onto the pyramidal neurons produces hyperpolarizing inhibitory postsynaptic potentials (IPSPs) that transiently inhibit spike firing and produce synchronous 'rebound' firing following the inhibition. In the PING model of gamma generation excitatory glutamatergic synapses from pyramidal cells (in green) serve to drive the GABAergic output leading to network wide oscillations. *Adapted from (Gonzalez-Burgos and Lewis, 2008)*

The interneuron network γ activity (ING) model posits that reciprocally connected GABAergic neurons generate rhythmic activity and are synchronised by their mutual inhibitory inputs, while the pyramidal interneuron network γ (PING) model proposes that γ oscillations arise from the interplay between pyramidal cells and GABAergic neurons. In the PING model interneuron activity is driven through glutamate released by the pyramidal cells, which are in turn rhythmically synchronised by the interneurons activity (Whittington et al., 2000).

In both the ING and PING models of γ oscillations, excitatory drive to GABAergic neurons is essential to the maintenance of synchronised activity (Gonzalez-Burgos and Lewis, 2008). As detailed in Section 1.4.1, the main excitatory input in neural networks is glutamatergic. The importance of excitatory input into interneuron-pyramidal networks for the generation of γ oscillations incorporates the other major hypothesis of schizophrenia – NMDAr hypofunction, as a potential mechanism underlying the γ frequency abnormalities we observe. Interneurons in general receive considerable excitatory input through glutamatergic receptors, and PV+ interneurons display a dramatically higher excitatory synapse density than other interneuron sub types (Gulyàs et al., 1999). Results from *in-vitro* studies of PV+ interneuron synaptic properties suggest that the NMDAr contribution to EPSCs in these cells is relatively small, compared to that in pyramidal cells (Hull et al., 2009; Rotaru et al., 2011). This contrasts with several studies demonstrating that GABAergic neurons are substantially more sensitive to the effects of NMDAr antagonism than pyramidal cells, with some studies reporting a 10-fold increase in sensitivity (Greene et al., 2000; Grunze et al., 1996; Olney, 1995).

Studies of neuronal firing in the cortex of awake rodents have also demonstrated that NMDArs may be a crucial driver of inhibitory interneuron activity (Homayoun and Moghaddam, 2007). In this study NMDAr antagonist

administration reduced the activity of GABAergic interneurons while simultaneously disinhibiting the firing of pyramidal cells. Consistent with these results, when NMDAr antagonist was applied to *in-vitro* brain slice preparations, a decrease in IPCSs onto pyramidal cells was observed (Li et al., 2002). It has also been demonstrated that PV+ cells have larger NMDAr currents, while juvenile, and these gradually disappear over adolescence (Wang and Gao, 2009). This indicates a potential environmental sensitivity in PV+ cells specific to NMDArs. The precise role of NMDA and other glutamate inputs into PV+ cells and the generation of γ oscillations is still undetermined, this is an active area of research and presents an opportunity to learn more about how altered synaptic function in schizophrenia can lead to the dramatic network and in turn cognitive abnormalities seen. See Section 1.6.2 for an examination of NMDAr antagonists in models for schizophrenia.

As well as arising through the activity of local cortical networks, γ oscillations can be generated through long range projections (Llinás et al., 2005). For instance, γ oscillations are robustly generated by thalamic cells (Steriade et al., 1993) and can propagate between thalamic and cortical circuits via recurrent thalamocortical interactions (Contreras and Steriade, 1995; Jones, 2001). Of particular interest is the role of the thalamic reticular nucleus (TRN), a thin shell of GABAergic neurons that surrounds the dorsal thalamus and receives glutamatergic innervation from both thalamocortical (TC) and corticothalamic (CT) fibres (Guillery and Harting, 2003; Pinault, 2004). The TRN has intrinsic γ frequency pacemaker properties (Pinault and Deschênes, 1992) and stimulation of the TRN causes bursts of γ frequency activity in the related sensory cortex (Macdonald et al., 1998). It is thought that the TRN serves to modulate and 'gate' information flow between the thalamus and cortex and deficits in this structure could explain the pathological deficits in perception and cognition seen in the disorder. Given the

specific perceptual and sensory abnormalities in schizophrenia and the fact they are associated with abnormal γ oscillations (Spencer et al., 2008) the role of the TRN in the (dys)function of γ oscillations is a key target for future research in neural dynamics and schizophrenia.

It is clear that PV+ interneurons play a key role in the generation and pathology of γ oscillations. In recent years we have gained the ability to manipulate cell specific activity through optogenetic techniques. A study performed PV+ specific manipulations, both inhibiting and driving the activity of these interneurons. Inhibiting PV+ cells caused a suppression of γ frequency activity *in-vivo*, whereas driving them at 40 Hz caused a network-wide increases in γ frequency activity and remarkably an increase in the efficiency of information transfer in these circuits (Sohal et al., 2009). Studies such as this, coupled with the importance of PV+ interneurons in generating γ oscillatory activity have given rise to the idea that the balance of excitatory and inhibitory activity across a network is essential for the maintenance of healthy cognitive functioning (Uhlhaas, 2013).

As well as connecting with the NMDAr hypofunction hypothesis, a proposed mechanism behind the generation of γ oscillations also involves the actions of a key schizophrenia risk gene − *Nrg1*. The receptor for Nrg1, ErbB4, is highly expressed on PV interneurons (Fazzari et al., 2010) and alters the intrinsic excitability of these cells (Li et al., 2011). It has also been shown that upregulating the expression of Nrg1 modulates the development of GABAergic and glutamate synapse development (Fazzari et al., 2010). Nrg1-ErbB4 signalling promotes the development of excitatory synapses onto PV+ interneurons and perisomatic inhibitory synapses from them onto pyramidal cells. This provides a genetic link to the balance of excitatory inhibitory function in neural circuits and warrants further research into its importance to γ disturbances in schizophrenia.

The cellular and network mechanisms that generate γ oscillations neatly intersect with a number of important elements that constitute the pathophyisology of schizophrenia. There is a large body of evidence that demonstrates γ oscillations are disrupted in schizophrenia, providing a neurophysiological account that explains the wide ranging perceptual and cognitive deficits of the disease. The most robust and widely replicated histological finding from patients is a reduction in the specific cell type thought to play the most important role in generating γ frequency activity. The substantial evidence that supports the NMDAr hypofunction hypothesis provides a neurochemical explanation for abnormal γ frequency activity. Key genetic factors implicated in schizophrenia also directly affect the systems relevant for generating γ oscillations. This evidence provides a compelling hypothesis to explain the pathophysiology of schizophrenia and research into γ oscillations should be a useful paradigm to increase our understanding of the disease. There are still many unanswered questions concerning the nature of the γ oscillation deficit – how it is manifested through NMDAr dysfunction, and how it can be treated. γ oscillations present a particularly appealing paradigm for investigation in translational models as electrophysiolgical recordings can be made in animal models and readily compared to EEG data acquired in the clinic, with standardised analysis techniques allowing comprehensive comparisons. The molecular and cellular targets identified in the proceeding sections enables the development of focused and relevant translational models to investigate these changes. As such research into translational animal models presents a compelling path towards a more complete understanding of schziophrenia as well as the neural dynamics that underlie cognition.

1.6

Animal Models for Schizophrenia

Schizophrenia is a fundamentally human disease; the phenomenological aspects such as hallucinations and delusions, the cognitive changes, and the lack of objective physiological markers for diagnosis make it largely impossible to comprehensively model the disease in animals. Despite these clear limitations the use of animal models has a long history in the study of schizophrenia (Van den Buuse et al., 2005), and has led to considerable insight into the disease and important refinements to our understanding of schizophrenia. Current animal models are not intended to model the entire human disease, instead serving to test mechanisms and hypothesis about the underlying processes relevant to specific elements of schizophrenia (Marcotte et al., 2001). We are able to recapitulate select symptoms and aspects of the disease by utilising models that are informed and guided by the underlying biology and pathology of schizophrenia and hopefully gain insight into the pathological mechanisms of the disease. There are a wide variety of animal models that are used in the study of schizophrenia, utilising a variety of methodologies to generate abnormal behaviours and cognitive deficits similar to those seen in humans, in a neurobiologically plausible fashion (Mouri et al., 2013). These include pharmacological models: utilising amphetamine to generate hyper-dopaminergic states, or ketamine to create NMDAr hypofunction. Other models include neurodevelopmental models that use lesions or insults early in life to interfere with the development of the nervous system and genetic models that manipulate gene expression to cause schizophrenia-like behaviour.

Below I will review the evidence of behavioural, molecular and physiological changes that occur in the two models utilised in this thesis: NMDAr antagonism and the Nrg1 transmembrane heterozygous mouse model (Nrg1 TM HET). These two models are particularly relevant for the study of γ oscillations in schizophrenia;

there is evidence of disrupted PV+ activity (del Pino et al., 2013; Fazzari et al., 2010; Morrow et al., 2007; Rujescu et al., 2006) and existing literature that connects these models to alterations in γ oscillations (Fisahn et al., 2009; Hakami et al., 2009; Pinault, 2008). An important principle for animal models of disease is that it should be both reliable and valid (Van den Buuse et al., 2005).

Reliability in a formal sense means that we are able to make accurate and repeatable observations utilising the model that are consistent across time, location and appropriate physiology. Validity is a more complex concept and has multiple aspects to be considered including: face validity - which requires the symptoms and pathology be similar to the clinical condition, construct validity - are the mechanisms involved biologically plausible, and predictive validity – does the model inform about the success or failure of therapeutic agents (McGonigle, 2014). The models used in this thesis have different strengths: NMDAr antagonists have strong face validity as their use in humans causes behavioural changes phenomenologically similar to schizophrenia (Adler et al., 1999), while the neuregulin model has strong construct validity being generated from a clinically identified genetic association (Stefansson et al., 2002). Animal models of the abnormal γ frequency activity seen in schizophrenia are in their infancy and the validity and reliability of such models is not yet established. The research presented in this thesis aims to increase our knowledge about the electrophysiological characteristics seen in animal models for schizophrenia and further the development of useful animal models to study this aspect of schizophrenia.

Currently rodent models for schizophrenia are typically characterised by behavioural changes such as hyperlocomotion and social interaction deficits, cognitive deficits in working memory and attention, and a range of physiological traits that are known to be disrupted in humans with schizophrenia such as MMN and ERPs (Mouri et al., 2013). Adding the study of neural oscillations to this array

of existing measures is of great potential benefit to the study of schizophrenia and understanding of the disease. As detailed throughout this literature review there are several biological pathways that can plausibly be connected to the altered γ oscillations observed in schizophrenia (Gonzalez-Burgos and Lewis, 2012; Uhlhaas and Singer, 2010; Woo et al., 2010), providing construct validity to the measure. γ oscillations have also shown genetic heritability (Hall et al., 2011a; 2011b; Leicht et al., 2010) indicating γ oscillatory deficits may represent an endophenotype of schizophrenia. Furthermore, γ oscillation deficits provide a powerful and far ranging theoretical explanation for the diverse perceptual and cognitive symptoms of schizophrenia – deficits in γ oscillations lead to widespread dysconnectivity across the brain which manifests as the heterogenous symptomatology of schizophrenia (Stephan et al., 2009; Uhlhaas, 2013). This provides γ oscillations considerable explanatory power and by increasing our understanding of their function in both health and disease could reveal important insight into the physiological processes that underlie perception and cognition.

1.6.1 Neuregulin 1 Models for Schizophrenia

Neuregulin 1 (*NRG1*) is a prominent risk gene for schizophrenia (Harrison and Law, 2006; Stefansson et al., 2002; Williams et al., 2003). *NRG1* encodes neuregulin 1 (Nrg1), a protein essential for normal development of nervous system (Riley and Kendler, 2006). Nrg1 is a trophic factor that plays a role in the guidance of thalamocortical afferents (López-Bendito et al., 2006), interneuron migration (Flames et al., 2004; Yau et al., 2003), myelination (Nave and Salzer, 2006) and synapse development and maintenance (Fazzari et al., 2010; Li et al., 2007; Woo et al., 2007). Nrg1 mRNA and protein expression has been found to be altered in schizophrenia (Hashimoto et al., 2004; Law et al., 2006b). A number of different *NRG1* mutant mouse models exist (Duffy et al., 2010), with subtle differences in

behavioural and molecular phenotypes. Mice genetically engineered to be heterozygous for the transmembrane domain of the Nrg1 protein (Nrg1 TM HET mice) possess a behavioural phenotype relevant to schizophrenia, exhibiting hyperlocomotion, increased anxiety, impairments in working memory and social interaction deficits (Chesworth et al., 2012; Duffy et al., 2010; Karl et al., 2007; Stefansson et al., 2002). These animals have already been shown to display schizophrenia relevant electrophysiological abnormalities, with reductions in ERPs and MMN reported (Ehrlichman et al., 2009b). The model also displays predictive validity with clozapine treatment rescuing some of the behavioural and physiological abnormalities (Stefansson et al., 2002).

The mechanisms by which the pathological schizophrenia-like behaviour emerges is not clear, but it appears that it may involve interactions with glutamatergic and GABAergic systems. The receptor for Nrg1 – ErbB4 (which is itself a risk gene for schizophrenia (Silberberg et al., 2006)) is highly expressed on GABAergic interneurons and it has been shown that upregulating the expression of Nrg1 modulates the development of GABAergic and glutamate synapse development (Fazzari et al., 2010). Of particular interest is work showing that ablation of ErbB4 specifically on PV interneurons leads to a range of abnormal synaptic and circuit level activities, including altered γ frequency oscillations (del Pino et al., 2013; Wen et al., 2010) and that administration of exogenous Nrg1 can influence γ oscillations (Fisahn et al., 2009). Abnormal Nrg1 expression presents a potential genomic mechanism that may promote altered GABAergic interneuron and NMDAr function, in turn generating abnormal γ oscillations.

1.6.2 NMDA Receptor Antagonist Models for Schizophrenia

As reviewed in detail in Section 1.4, NMDAr hypofunction is a prominent hypothesis for the origin of schizophrenia. The schizophrenia-related behaviours

observed in humans following NMDAr antagonist treatment are also seen in animals and numerous animal models have been developed utilising NMDAr antagonists such as PCP, ketamine and MK-801 (Bubeníková-Valešová et al., 2008; Jentsch and Roth, 1999). NMDAr antagonists cause a wide range of behavioural deficits in rodents relevant to schizophrenia, including hyperlocomotion (Nagai et al., 2003; Sturgeon et al., 1979), social deficits (Sams-Dodd, 1995; Wang et al., 2007), PPI deficits (Martinez et al., 1999; Tanaka et al., 2011), working memory abnormalities (Campbell et al., 2004; Marquis et al., 2003), impairments in attention (Egerton et al., 2005), learning (Mouri et al., 2007; Noda et al., 2001) and memory deficits (Tanaka et al., 2011). Repeated PCP treatment can lead to long lasting changes in hyperlocomotion and increased immobility in the forced swim test (Mouri et al., 2012; Nagai et al., 2003). This has led to the proposal that repeated PCP exposure may better model the long term effects of schizophrenia, while a single treatment may be more applicable to an acute psychotic episode (Bubeníková-Valešová et al., 2008; Jentsch and Roth, 1999; Mouri et al., 2007). Studies of brain metabolic activity support this hypothesis, with ketamine and MK801 resulting in an increase of cortical glucose uptake, indicating increased activity (Duncan et al., 1999). This matched a study in human patients presenting with unmedicated positive symptoms (Soyka et al., 2005).

Repeated administration of NMDAr antagonists in animals also produces GABAergic deficits reminiscent of schizophrenia. Decreased expression of GAD67 has been seen following continual subcutaneous infusion of MK-801 (Qin et al., 1994). Daily injections of PCP or MK801 also produce reductions in the numbers of PV+ interneurons (Morrow et al., 2007; Rujescu et al., 2006). Sub-chronic ketamine administration has been shown to deplete PV+ interneurons as well (Behrens et al., 2007), attributed to increases oxidative stress in the prefrontal cortex, hippocampus and thalamus. NMDAr antagonist models display considerable predictive validity for current antipsychotics (Miyamoto et al., 2012; Mouri et al.,

2013). In a wide range of preclinical animal studies, pre-treatment with antipsychotics, particularly the atypical drugs can attenuate the behavioural and physiological effects of NMDAr antagonists (Bakshi and Geyer, 1995; Corbett et al., 1995; Duncan et al., 1998; Jones et al., 2012). Unfortunately the mechanisms through which antipsychotic treatment is able to attenuate NMDAr antagonists psychotomimetic effects is poorly understood. It is possible long-term treatment with antipsychotics is modulating the number, density or phosphorylation states of NMDAr receptors. However, to date numerous animal studies have produced inconsistent results, showing an increase, decrease or no changes in several brain regions and for various glutamate receptors. (Giardino et al., 1997; McCoy et al., 1998; Ossowska et al., 1999; Spurney et al., 1999). In summary acute administration of NMDAr antagonists produces a wide variety of abnormal behaviours that are relevant to the study of schizophrenia, this fact and the pharmacological relevance of NMDAr activity to the generation of gamma oscillations makes them a useful model to study the role of gamma oscillations in schizophrenia.

1.6.3 Animal Models and Gamma Oscillations

Gamma oscillations have been well characterised in clinical investigations, with multiple studies reporting altered γ activity in a range of cognitive and perceptual paradigms. Research into γ oscillations in animal models for schizophrenia has not been as fully developed and there is a limited existing literature. As described previously, both Nrg1 and NMDAr antagonists affect biological pathways relevant to γ oscillations, making them potentially useful models to study this phenomenon.

The existing research on γ oscillations in Nrg1 models is sparse, but there is some indication of electrophysiological abnormalities. (Ehrlichman et al., 2009b) reported reductions in MMN and audio-evoked potentials, while not utilising

frequency specific analyses, this indicates a relevant disrupted electrophysiological phenotype. There has also been a report of Nrg1 disrupting inhibitory signalling via a cannabinoid pathway (Du et al., 2013) and more directly related, a study that reporting that exogenous Nrg1 increases the power of γ oscillations in *in vitro* hippocampal slice experiments (Fisahn et al., 2009). When considered in the context of the existing changes to GABAergic pathways and the functional role of Nrg1 in neuronal development, the Nrg1 TM HET mouse is a relevant model for the study of γ oscillations in schizophrenia. Research into this animal model will help develop a biologically relevant genetic neurodevelopmental model with which to study the role of γ oscillations in schizophrenia.

The study of γ oscillations in NMDAr antagonist models for schizophrenia has been further developed and it has been demonstrated as a useful paradigm to investigate electrophysiolgical abnormalities relevant to the disease. A number of studies have reported increases in ongoing γ oscillations following NMDAr antagonist administration (Hunt and Kasicki, 2013; Kocsis et al., 2013). A proposed mechanism to explain NMDAr antagonists increasing γ oscillations is that blockade of NMDArs leads to a reduction of excitatory input into PV+ GABAergic interneurons, leading to a disinhibition of pyramidal cells leading to a hyperglutamatergic state and resulting in pathological γ activity (see Figure 1.5) (Olney et al., 1999).
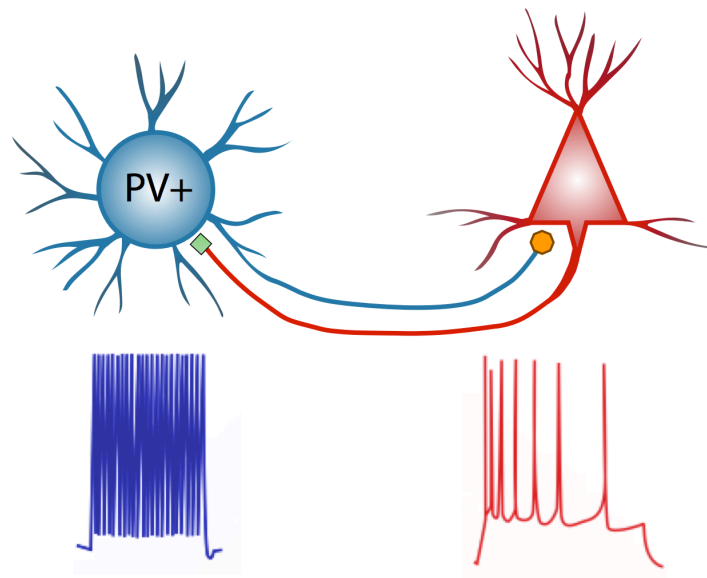
Figure 1.5 A – PV+ Interneuron and Pyramidal Cell Interaction Under Physiological Conditions

Parvalbumin expressing Interneurons have GABAergic synapses onto pyramidal neurons, which act to inhibit their firing. Pyramidal neurons make glutamatergic synapses onto interneurons to excite them through activation of non-NMDA and NMDA receptors. The typical firing properties of each type of neuron are represented by examples underneath. PV+ fast-spiking interneurons have high firing rates. Pyramidal neurons have broader action potentials and fire at much lower rates.
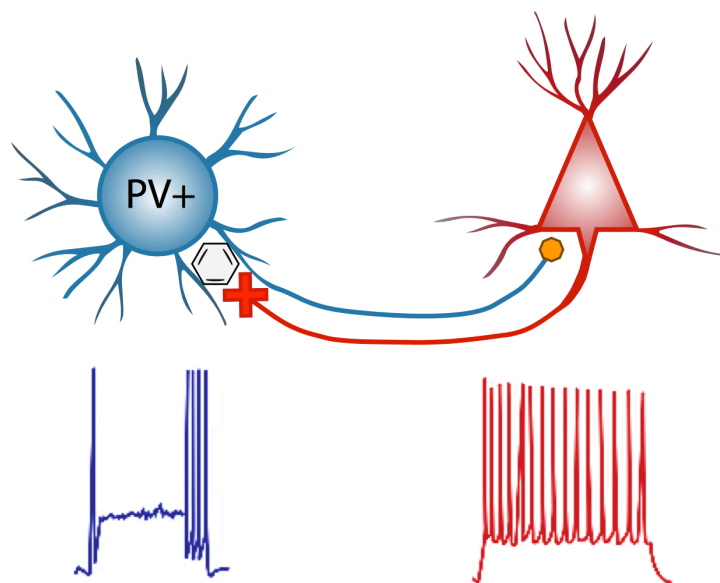
Figure 1.5 B – PV+ Interneuron and Pyramidal Cell Interaction During NMDAr Blockade

Ketamine may block NMDA receptors in interneurons more effectively than it blocks the same receptors in pyramidal neurons. This is hypothesised to result in a disinhibition of pyramidal cells resulting in an increase in neuronal firing. *Adapted from (Seamans, 2008)*

This mechanism is supported by some studies showing opposite effects of NMDAr antagonists on pyramidal and inhibitory neurons (Homayoun and Moghaddam, 2007) and evidence of hyperactivity in pyramidal neurons and excessive cortical glutamate release following NMDAr antagonist administration (Jackson et al., 2004; Suzuki et al., 2002), but further research is needed to verify this hypothesis and understand the precise mechanism involved. This NMDAr antagonist-induced increase in γ power has been shown to be dose-dependent (Pinault, 2008), with systemic ketamine and MK801 causing dose-dependent increases in ECoG recordings from the frontal cortex in freely moving rats. Further studies have demonstrated that this effect is found across the cortex with NMDAr antagonists-induced increases being reported in most cortical regions (Páleníček et al., 2011; Phillips et al., 2012).

ECoG recordings have also been supplemented with LFP recordings from other structures that have also been shown to produce altered γ oscillations following NMDAr antagonist administration including: prefrontal, motor and somatosensory cortex, striatum, amygdala, nucleus accumbens, and thalamus (Ehrlichman et al., 2009a; Hakami et al., 2009; Lazarewicz et al., 2010), indicating NMDAr blockade leads to pathological γ oscillations across the brain. Other studies have reported simultaneous increases in ongoing γ activity and decreases in sensory-evoked γ oscillations in rodent models utilising NMDAr antagonists. Rats receiving somatosensory stimulation of the vibrissae have been shown to have increased baseline γ oscillations following ketamine administration, and a simultaneous reduction in evoked γ power, resulting in a decreased γ signal to noise ratio (Kulikova et al., 2012). Similarly, mice performing an auditory evoked potential task and administered MK801 or ketamine display an increase in ongoing γ oscillations and a decrease in the magnitude of evoked γ (Saunders et al., 2012). These findings have led to the hypothesis that NMDAr antagonists induce a

pathological γ frequency 'noise' throughout disparate neural networks. Increased aberrant ongoing γ oscillations could interfere with normal physiological role of γ in sensory processing, masking incoming sensory-evoked γ and leading to a decreased signal to noise ratio in sensory processing (Gandal et al., 2012b; Kulikova et al., 2012). This mirrors some reported alterations seen in patients (Flynn et al., 2008; Krishnan et al., 2009; Williams et al., 2009) and indicates that NMDAr antagonists may model complex changes in electrophysiological signal-to-noise patterns similar to those seen in schizophrenia. This hypothesis is an active area of research in clinical studies and a working animal model would be a useful translation tool to aid in understanding the mechanisms that may lead to disruptions in γ signal-to-noise.

Investigations into the predictive validity of animal models of γ oscillations deficits have been minimal to date. A study has reported no effect of haloperidol on evoked γ oscillations (Ehrlichman et al., 2009a). Another study from our laboratory has revealed that haloperidol, clozapine and the metabotropic glutamate receptor agonist LY379268, all reduce ongoing γ power in rats, indicating a possible relationship between antipsychotic efficacy against positive symptoms and γ oscillations (Jones et al., 2012). Further research into the effects of antipsychotic medication on γ oscillations will both enhance the predictive validity of animal models for gamma oscillation disturbances in schizophrenia, but will also aid in the development of potential biomarker of antipsychotic efficacy and hopefully aid in the development of novel treatments.

In conclusion NMDAr antagonist and Nrg1 animal models for schizophrenia are viable paradigms for the study of γ oscillation disturbances relevant to the disease. They are based on biological pathways that are relevant to the pathogenesis of schizophrenia and the physiological mechanisms that generate γ oscillations. Improving our understanding of how γ oscillations are disrupted will shed light on

the mechanisms that cause the symptoms of schizophrenia. Therefore it is the goal of the research presented in this thesis to characterise γ oscillations animal models for schizophrenia to aid in the development of robust, reliable and valid animal models to help further research into the disease.

1.7
Thesis Aims

The goal of this thesis is to expand and enhance the characterisation of γ oscillations in rodent models for schizophrenia. It is anticipated that this would increase the utility of such models for translational studies of γ oscillations and their involvement in the pathophysiological processes that underlie schizophrenia. Three separate studies were conducted, each of which explore clinically relevant pharmacological or genetic manipulations and the related changes in γ oscillations:

Aim 1 – Chronic Administration of Antipsychotics and the Behavioural and Electrophysiological Effects of Ketamine

Antipsychotic medications are the current main treatment option for schizophrenia but less than half of patients respond to them and they show limited efficacy in the negative and cognitive symptom domains (Leucht et al., 2008). As such, the development of novel antipsychotic medications is a key goal in improving treatment of this disease. Translational biomarkers of antipsychotic efficacy will greatly aid in the development of such novel medications. Non-competitive N-Methyl-D-aspartate (NMDA) receptor antagonists induce increases in the power of ongoing γ oscillations in rodents concurrently with dose-dependent increases in hyperlocomotor activity (Hakami et al., 2009), a commonly employed model of acute psychosis (Van den Buuse et al., 2005). Animal studies link drug-induced

hyperlocomotor activity with increases in the power of ongoing γ activity in rodents and clinical results demonstrate that abnormalities in γ activity in schizophrenia patients is linked to psychotic symptomatology (Gordon et al., 2001; Spencer, 2009). These findings suggest that NMDAr antagonist-induced increases in the power of γ oscillations represent a useful electrophysiological biomarker of an acute psychotic-like state (Lee et al., 2003). Previous work from our laboratory (Jones et al., 2012) demonstrated that typical and atypical antipsychotics given acutely modulate the power of γ oscillations and reduce ketamine-induced behavioural effects. The studies detailed in this thesis aim to investigate the efficacy of conventional pharmacological treatments and a novel metabotropic glutamate receptor (mGluR) agonist (LY379368) in a dosing paradigm that reflects clinical treatment, to enhance the clinical relevance and validity of this model.

Aim 2 – Aberrant Gamma Frequency Neural activity and NMDA Receptors in Neuregulin 1 Mutant Mice

Nrg1 is a leading candidate for a schizophrenia 'risk' gene (Stefansson et al., 2002), with Nrg1 mRNA and protein expression being altered in schizophrenia (Hashimoto et al., 2004; Law et al., 2006b). Although the pathophysiological mechanisms by which altered Nrg1 expression results in vulnerability to develop schizophrenia is not known, several lines of evidence link Nrg1 and NMDAr function (Geddes et al., 2011). Nrg1 signalling is important for the development of PV interneuron circuits, and these fast-spiking interneurons contribute to the generation of γ oscillations and ablation of ErbB4 (the Nrg1 receptor) on PV interneurons leads to a range of abnormal synaptic and circuit level activities, including altered γ frequency activity (del Pino et al., 2013; Wen et al., 2010). Mice genetically engineered to be heterozygous for the transmembrane domain of the Nrg1 protein (Nrg1 TM HET mice) possess a behavioral phenotype relevant to

schizophrenia, exhibiting hyperlocomotion, increased anxiety, impairments in working memory and social interaction deficits (Chesworth et al., 2012; Duffy et al., 2010; Karl et al., 2007; Stefansson et al., 2002). This study examined γ frequency activity and expression of NMDArs in Nrg1 TM HET mice with the aim to characterise the electrophysiological characteristics of this genetic model for schizophrenia.

Aim 3 – Contribution of the Corticothalamic Pathway to Ketamine-Induced Disruption of Information Processing

There is a growing body of literature indicating involvement of dysfunctional thalamic networks in the pathophysiology of schizophrenia, including disturbances in function-related γ frequency oscillations (Cronenwett and Csernansky, 2010; Woodward et al., 2012). A postulated mechanism of these impairments is reduced NMDAr activation at glutamatergic synapses on GABAergic interneurons (Carlen et al., 2012). We previously demonstrated that a single sub-anaesthetic dose of ketamine (NMDAr antagonist with psychotomimetic effects) increased the power of ongoing γ oscillations and decreased that of sensory-evoked γ oscillations in the rat somatosensory thalamocortical (TC) network (Kulikova et al., 2012), reducing the γ signal-to-noise ratio (defined as the sensory-evoked γ response above the baseline γ noise). Both the glutamatergic TC neurons and the GABAergic thalamic reticular nucleus (TRN) neurons are highly innervated by glutamatergic corticothalamic (CT) axons originating from layer VI. The goal of the present study was to assess the contribution of the CT pathway in the ongoing and sensory-evoked γ oscillations in the thalamus.

CHAPTER 2

EFFECTS OF CHRONIC ADMINISTRATION OF ANTIPSYCHOTICS ON KETAMINE-INDUCED BEHAVIOURAL AND ELECTROPHYSIOLOGICAL ABNORMALITIES

Abstract

Non-competitive N-Methyl-D-aspartate receptor (NMDAr) antagonists elicit many of the symptoms observed in schizophrenia in healthy humans, and induce a behavioural phenotype relevant to psychosis in animals. These compounds also elevate the power and synchrony of high frequency gamma (γ, 30-80Hz) neural oscillations. Acute doses of antipsychotic medications have been shown to reduce ongoing γ power and to inhibit NMDAr antagonist-mediated psychosis-like behaviour in rodents. This study aimed to investigate how a chronic antipsychotic dosing regimen affects ongoing cortical γ oscillations, and the electrophysiological and behavioural responses induced by the NMDAr antagonist ketamine.

Male Wistar rats were chronically treated with haloperidol (0.25 mg/kg/day), clozapine (5 mg/kg/day), LY379268 (0.3 mg/kg/day) or vehicle for 28 days, delivered by subcutaneous (s.c.) osmotic pumps. Weekly electrocorticogram (ECoG) recordings were acquired. On day 26, ketamine (5 mg/kg, s.c.) was administered, and ECoG and locomotor activity were simultaneously measured. These results were compared with data generated previously following acute treatment with these antipsychotics.

Sustained and significant decreases in ongoing γ power were observed during chronic administration of haloperidol (64%) or clozapine (43%), but not of LY379268 (2% increase), compared to vehicle. Acute ketamine injection concurrently increased γ power and locomotor activity in vehicle-treated rats, and these effects were attenuated in rats chronically treated with all three antipsychotics. The ability of haloperidol or clozapine to inhibit ketamine-induced elevation in γ power was not observed following acute administration of these drugs.

These results indicate that modulation of γ power may be a useful biomarker of chronic antipsychotic efficacy.

2.1

Introduction

Schizophrenia is a debilitating psychiatric disorder that is characterised by a range of symptoms, including disordered thought, hallucinations, delusions and diverse cognitive deficits (Harrison, 1999). In recent years there has been a convergence of various lines of evidence suggesting that the underlying pathophysiology of schizophrenia involves disruptions of neural synchrony (Uhlhaas et al., 2008). In particular, it has been demonstrated that high frequency γ (30-80 Hz) oscillations are disrupted in patients with schizophrenia (Herrmann and Demiralp, 2005; Krishnan et al., 2009; Lee et al., 2003; Light et al., 2006; Maharajh et al., 2010; Spencer et al., 2004; Uhlhaas et al., 2006). The functional role of γ oscillations has been linked to a range of higher-order brain functions, including cognition (Engel et al., 2001), working-memory (Howard et al., 2003; Tallon-Baudry et al., 1998) and sensory perception (Gross et al., 2007; Herrmann and Demiralp, 2005; Krishnan et al., 2009; Lee et al., 2003; Light et al., 2006; Maharajh et al., 2010; Spencer et al., 2004; Uhlhaas et al., 2006). These same cognitive processes are disrupted in schizophrenia, an effect that may be mediated by hypofunction of NMDArs (Gonzalez-Burgos and Lewis, 2012).

Ketamine and other NMDAr antagonists induce hallucinations in healthy humans, and exacerbate psychotic symptoms in schizophrenic patients (Krystal et al., 1994). This and other evidence has led to the development of the NMDAr hypofunction hypothesis of schizophrenia; which posits that reduced activity at NMDArs leads to the expression of schizophrenia symptoms. We (Hakami et al., 2009; Pinault, 2008) and others (Ehrlichman et al., 2009a; Lazarewicz et al., 2010) have previously demonstrated that NMDAr antagonists dose-dependently increase the power of ongoing γ oscillations in rodents and also recapitulate complex electrophysiological abnormalities seen in schizophrenia (Kulikova et al., 2012;

Saunders et al., 2012). We further developed this model by examining the effects of antipsychotic compounds on ongoing γ oscillations, and in response to a ketamine challenge (Jones et al., 2012). We tested a typical (haloperidol) and atypical (clozapine) antipsychotic and a preclinical metabotropic glutamate 2/3 receptor (mGluR$_{2/3}$) agonist (LY379268) with antipsychotic properties (Imre, 2007). Our results demonstrated that, on their own, antipsychotic medications reduce the power of ongoing cortical γ oscillations in freely moving rodents. This result has since been replicated in an in-vitro model (Schulz et al., 2012), which provided evidence that dopamine D$_3$ receptors may play a role in inhibiting ongoing γ oscillations. It was also demonstrated that a range of typical and atypical antipsychotics could modulate higher frequency oscillations (100-180Hz) in the nucleus accumbens of rats(Olszewski et al., 2012) , supporting the relevance of neural oscillation modulation to antipsychotic efficacy. In our earlier study acute treatment with conventional antipsychotics did not impact the ability of ketamine to increase γ oscillations, a finding in contrast to the effects of these drugs on ketamine-induced hyperlocomotor activity (Jones et al., 2012)

This result is relevant regarding the study of the relationship between γ oscillations and schizophrenia. The ability of antipsychotic medications to modulate γ oscillations could greatly impact results seen in studies of clinical populations. Although some studies have compared drug-free and medicated patients (Gallinat et al., 2004) or examined first onset patients in order to control for the effects of medication (Symond et al., 2005), this remains an understudied area with important potential ramifications.

Given the wide spread prevalence of γ frequency alterations in schizophrenia, the ability of antipsychotic medications to modulate neuronal oscillations may be central to their efficacy, with the potency of modulation of γ activity representing a potential new biomarker of antipsychotic efficacy. Previous

studies have only examined acute antipsychotic drug treatment, whereas in the clinic patients are chronically administered antipsychotic mediations; the effects of which typically take several weeks to manifest (Gelder et al., 2000). This study aims to further develop rodent models for schizophrenia incorporating γ oscillations exploring a more clinically relevant and realistic model of chronic antipsychotic administration.

2.2
Materials and Methods

2.2.1 Animals

Male Wistar rats aged 10-12 weeks old (weighing 250-350g) were used (N = 32). Animals were bred and housed (3-4 per cage) in the Biological Research Facility of the Department of Medicine, Royal Melbourne Hospital, University of Melbourne with a 12 hour light-dark cycle (lights on at 0600) and maintained at 22 ± 1° C. Animals were housed in opaque plastic cages (59 x 31 cm) with wire lids and woodchip bedding with access to food and water ad libitum. All experiments were approved by the University of Melbourne Animal Ethics committee (Ethics #1011868) and adhered to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.2.2 Surgery

Animals were anaesthetised by inhalation of isoflurane (5% induction, 1.5–2.5% maintenance) in equal parts of medical air and oxygen and implanted with an osmotic mini-pump (Model 2ML4, Alzet, USA) in the dorsal thoracic region via a single incision midway between the scapulae. Animals were then positioned in a stereotaxic frame as described previously (Hakami et al., 2009) for implantation of

electrocorticograph (ECoG) electrodes. Briefly, a single midline incision was made over the scalp and six holes were drilled through the skull with stereotaxic guidance (Paxinos and Watson, 1998) [2 mm anterior and 2 mm lateral to bregma bilaterally (active electrodes); 2 mm posterior and 2 mm lateral to bregma bilaterally (ground electrodes); and 2 mm posterior and 2 mm lateral to lambda bilaterally (reference electrodes)]. Electrodes were then screwed into the skull without breaching the dura, and dental cement applied to the skull to fix the electrodes in place. After recovery from anaesthesia, animals were housed individually for the duration of the experiment.

2.2.3 Drugs and Vehicles

Clozapine, haloperidol and LY379268 were obtained from Tocris Bioscience (Bristol, UK). Ketamine was obtained from Parnell Laboratories (Australia). Isoflurane was purchased from Abbott Pharmaceuticals (USA). Haloperidol and ketamine were diluted in 0.9% sterile saline, clozapine was dissolved in 10% acetic acid in sterile water with pH adjusted to 6.0 using 10M NaOH. Control pumps were loaded with 10% acetic acid (n=5) or sterile saline (n=3): there were no significant differences in the outcome of these treatments so the two vehicle control conditions were combined for analysis. All antipsychotic treatment groups consisted of n=8 animals. Pumps were weighed before and after filling and residual volume was checked at the end of the experiment to ensure adequate delivery (> 95% expected volume) of drug. Clozapine was administered to give an approximate dose of 5 mg/kg/day, haloperidol at 0.25 mg/kg/day and LY 79268 at 0.3 mg/kg/day, based on the predicted weight of animals at day 28. Dosages were selected on two criteria: to maintain a clinically relevant plasma concentration (for haloperidol and clozapine) (Kapur et al., 2003) and to have an equivalent effect on γ power (based on acute dosing (Jones et al., 2012)).

2.2.4 Assessment of ECoG Power and Locomotor Activity

Animals underwent 'baseline' ECoG recordings at 7, 14 & 21 days post surgery. Animals had a recording cable attached whilst in their home cages and after a 30 min acclimatisation period, 30 min of ECoG activity was recorded. On day 26 or 27 of the experiment animals underwent a ketamine 'challenge' between 1100 – 1600 hours. Animals were brought into the Behavioural Testing Facility in the Department of Medicine at least 30 min prior to the start of the study to allow habituation to the environment. Rats were then individually placed into an open arena while attached to an ECoG recording cable suspended from the ceiling. The apparatus used was a circular arena (100cm diameter) enclosed by 30cm high walls, made of black Perspex. Lighting in the centre of the arena was approximately 100 lux from indirect lighting sources. Each rat was allowed to acclimatise to the arena for 30 min at which point ECoG recording began. Following a 30 min baseline recording animals received an injection of ketamine (5 mg/kg s.c.) and recorded for another 60 min. Throughout the ECoG acquisition animals locomotor activity was video-tracked and objectively assessed with Ethovision software (Noldus, The Netherlands), total distance travelled was calculated for each 2 min interval.

2.2.5 ECoG Acquisition and Analysis

ECoG was acquired and analysed with a Synamp amplifier and SCAN v4.5 software (Compumedics, Australia), ECoG was sampled at 2000 Hz with a bandpass of 0.5 – 1000 Hz. Drug effects on the power of different frequency bands was assessed as previously described (Hakami et al., 2009). Briefly, recordings were manually assessed for artifacts and discarded if contaminated by noise, all remaning recordings were treated as independent trials. Raw ECoG was then sectioned into 2.048 s epochs, fast Fourier transformations were performed to determine the average power in different frequency bands (θ – 4-8 Hz; α – 8-12 Hz; β – 13-29

Hz; γ – 30-80 Hz) and average power was then calculated for each 2 min interval and averaged over 30 minutes. For the ketamine challenge experiments, to quantify both γ power and locomotor activity, the first 15 data points (representing the 30 min prior to ketamine) are averaged to give a baseline activity for each recording, all recorded values are then expressed as percentage of this activity.

2.2.6 Acute Dosage Data

Data from an acute dosage experiment (Jones et al., 2012) was reanalysed to compare the effects of different dosage paradigms. ECoG data from the acute experiment was acquired with a MacLab amplifier and Chart v. 3.5 software (AD Instruments, Australia). 50 Hz line noise was eliminated from the signal using selective eliminators (Humbugs ; Digitimer, UK). Data used are from equivalent single dosages i.e. clozapine 5 mg/kg, haloperidol 0.25 mg/kg and LY379268 0.3 mg/kg. ECoG and locomotor data was acquired in the same behavioural facility using a protocol equivalent to the ketamine 'challenge' recording with an added 30 minute post-antipsychotic recording: 30 min acclimatisation period, 30 min baseline recording followed by s.c. injection of antipsychotic, 30 min post drug recording, followed by injection of ketamine and a final 60 min recording. Data from the acute experiment was normalised to be expressed as a percentage of the post-antipsychotic period average.

2.2.7 Statistical Analyses

Differences between treatment groups in spectral power was assessed by averaging the 15 data points from the 30 min baseline recording and then comparing these values using the Kruskal-Wallis test, with Dunn's multiple comparisons post-hoc test. Both the electrophysiological and locomotor response to a ketamine challenge was assessed with two-way repeated measures ANOVA comparing each drug

treatment to vehicle controls. The effects of ketamine on γ power was quantified by analysing the area under the curve for each animal, normalised to the mean γ power in the 20 min preceding ketamine injection. Comparisons between acute and chronic treatments were made with two-way repeated measures ANOVA with Bonferroni's post-hoc test.

2.3

Results

2.3.1 Haloperidol And Clozapine Reduce the Power of Ongoing Gamma Oscillations, LY379268 Increases Low Frequency Power

Chronic treatment with the conventional antipsychotics haloperidol and clozapine caused pronounced and persistent decreases across the power spectrum (Figure 1A). Conversely LY379268 treated animals displayed power comparable to vehicle treated animals. These changes were stable over the 4-week experimental period with the decrease in θ, α and γ power caused by clozapine and haloperidol persisting throughout. The most robust differences were observed in the γ frequency band, two-way repeated measures ANOVA showed a significant effect of drug treatment ($F_{(3,105)}$ = 11.92, p < 0.0001). Post-hoc tests showed significant differences between haloperidol and vehicle treated animals at all time points (p < 0.01) while clozapine was different to vehicle at all but week 2 (p <0.05). LY379268 treated animals did not have significantly different γ power at any time-point. Theta and alpha power bands were also shown to be altered by antipsychotic treatment (two-way repeated measures ANOVA, theta: $F_{(3,105)}$ = 3.161, p = 0.036, alpha: $F_{(3,105)}$ = 8.696, p = 0.0002) while beta was not $F_{(3,105)}$ = 0.706, p = 0.555. Tables 1-4 give a detailed summary of the power levels in each band across the experimental period.
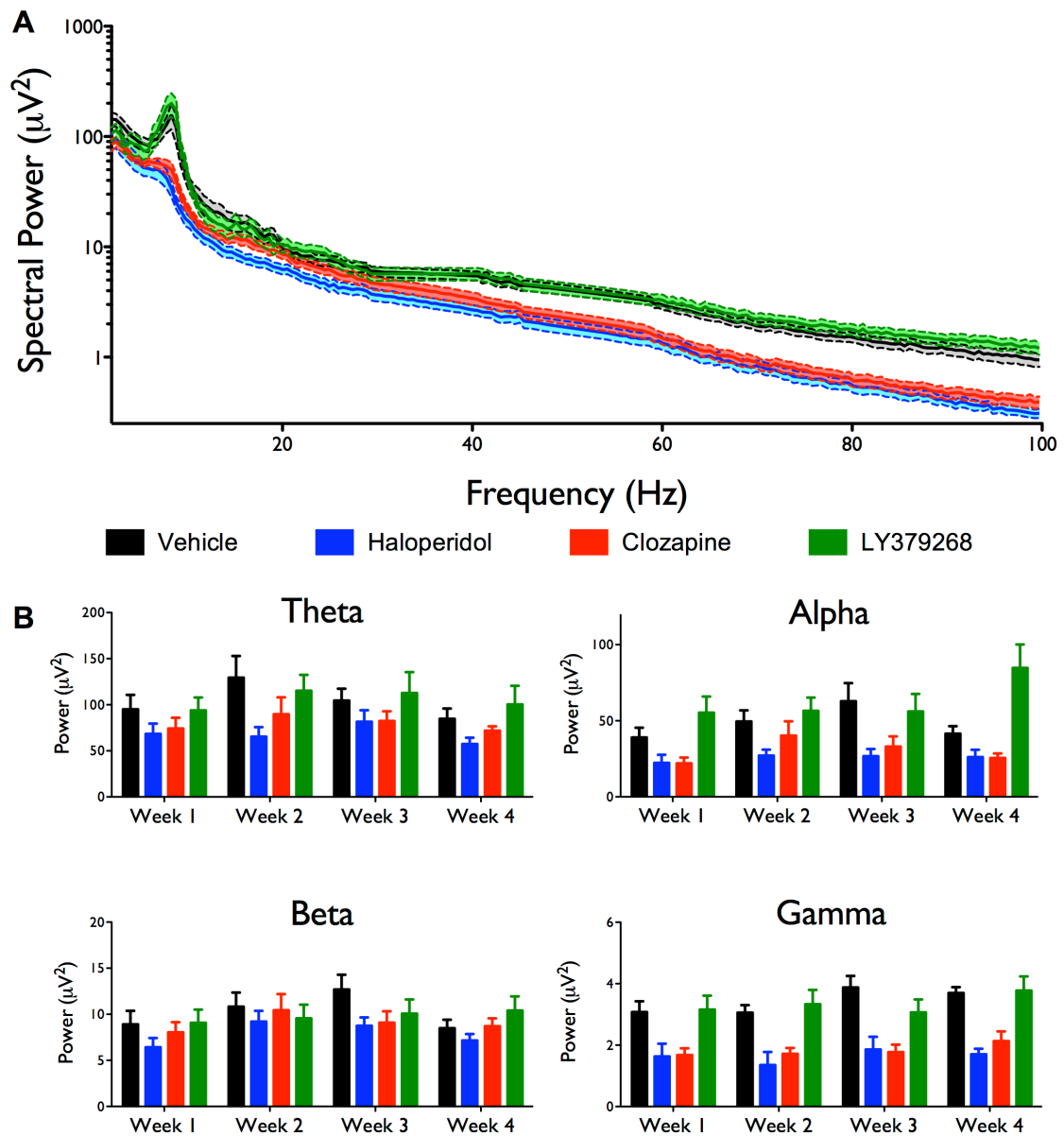
Figure 2.1 – Average Spectral Power from ECoG of Rats Undergoing Chronic Antipsychotic Treatment

The graph in panel A shows average spectral power from all animals recorded on week 3 of treatment. Panel B quantifies spectral power in discrete frequency bands taken during each week of treatment. LY379268 treated animals have elevated alpha power, but no change in γ power compared to vehicle. Clozapine and haloperidol treated animals display reduced θ, α and γ power as compared to vehicle treatment. Data represent mean ± SEM n = 9-11/group.

Table 1: Weekly θ Spectral Power

	Vehicle		Haloperidol		Clozapine		LY379268	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Week 1	95.03	15.73	52.88	14.89	63.56	12.76	93.97	13.88
Week 2	129.40	23.57	45.36 *	14.13	84.43	20.33	115.29	17.11
Week 3	104.57	12.83	73.76	14.84	77.89	11.44	112.69	22.78
Week 4	84.71	11.25	47.48	8.80	61.56	5.97	100.41	20.18

Table 2: Weekly α Spectral Power

	Vehicle		Haloperidol		Clozapine		LY379268	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Week 1	39.02	6.45	15.12	3.73	22.06	3.80	55.40	10.58
Week 2	49.50	7.36	17.01	5.06	40.27	9.43	56.59	8.67
Week 3	62.87	11.85	23.69 *	5.35	33.07	6.70	56.17	11.47
Week 4	41.51	4.91	17.97	2.06	25.58	2.96	84.72 *	15.43

Table 3: Weekly β Spectral Power

	Vehicle		Haloperidol		Clozapine		LY379268	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Week 1	8.91	1.46	5.32	1.18	8.04	1.09	9.07	1.43
Week 2	10.82	1.54	4.88	1.38	10.44	1.74	9.56	1.47
Week 3	12.70	1.59	6.97	1.37	9.09	1.23	10.08	1.53
Week 4	8.50	0.92	6.05	0.54	8.71	0.85	10.42	1.52

Table 4: Weekly γ Spectral Power

	Vehicle		Haloperidol		Clozapine		LY379268	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Week 1	3.08	0.35	1.63 *	0.41	1.68 *	0.22	3.16	0.45
Week 2	3.06	0.25	1.35 *	0.42	1.72 *	0.19	3.33	0.47
Week 3	3.88	0.38	1.86 *	0.42	1.77 *	0.25	3.07	0.42
Week 4	3.70	0.19	1.70 *	0.18	2.13 *	0.32	3.78	0.46

* represents significantly different from vehicle-treatment at that time point.

Tables 2.1-4 – Average Spectral Power in Discrete Frequency Bands from ECoG of Rats Undergoing Chronic Antipsychotic Treatment

Tables 1 through 4 show mean spectral power in the theta, alpha, beta and gamma frequency bands. Data are taken from animals in a quiet resting state and represent the average power in each frequency band in a 30 minute recording period data, n = 9-11 per group

2.3.2 Chronic Treatment with Haloperidol or Clozapine Significantly Attenuates the Ketamine-Induced Rise in Gamma Power

The electrophysiological response to the ketamine challenge injection (5 mg/kg s.c.) manifested as a rapid and large increase in the power of ongoing γ oscillations, with vehicle-treated animals having an average maximum response of 237 ± 14% relative to baseline occurring at 10 minutes post-injection. This effect of ketamine was inhibited in the chronically treated animals, with all treatments reducing ketamine's effect. In the clozapine- and LY379268-treated animals, the power of ongoing γ oscillations returned to baseline levels sooner than vehicle treated animals (Figure 2A). Peak responses for the three treatment groups were haloperidol: 187 ± 13% occurring at 10 minutes post injection; clozapine: 213 ± 9% at 10 minutes post injection; and LY379268: 177 ± 7% at 12 minutes post injection. ANOVA showed that treatment had a statistically significant effect on γ power for haloperidol ($F_{(1,20)}$ = 6.57, p = 0.0185), clozapine ($F_{(1,24)}$ = 25.30, p < 0.0001) and LY 379268 ($F_{(1,22)}$ = 37.83, p < 0.0001).

In order to compare the effects of chronic treatment with antipsychotic drugs to those induced by a single acute dose, we reanalysed our previously published data (Jones et al., 2012), examining single acute doses of the same drugs. When comparing the time course of effects of acute antipsychotics, only LY379268 significantly reduced the electrophysiological effect of ketamine ($F_{(1,24)}$ = 52.68, p < 0.0001) compared to vehicle treated animals (Figure 2B). The electrophysiological response to ketamine was quantified by calculating the area under the curve $0 - 60$ mins post ketamine injection (Figure 2C), and responses compared between the acute and chronic treatment paradigms. ANOVA showed that drug treatment (p < 0.0001), treatment regime (p < 0.0001) and the interaction between these variables (p = 0.0032) were significant. Bonferroni post-hoc tests showed that for both clozapine and haloperidol, only chronic treatment

significantly reduced the effect of ketamine on γ power ($p < 0.05$), whereas LY379268 significantly affected this outcome after both chronic and acute treatments ($p < 0.001$).
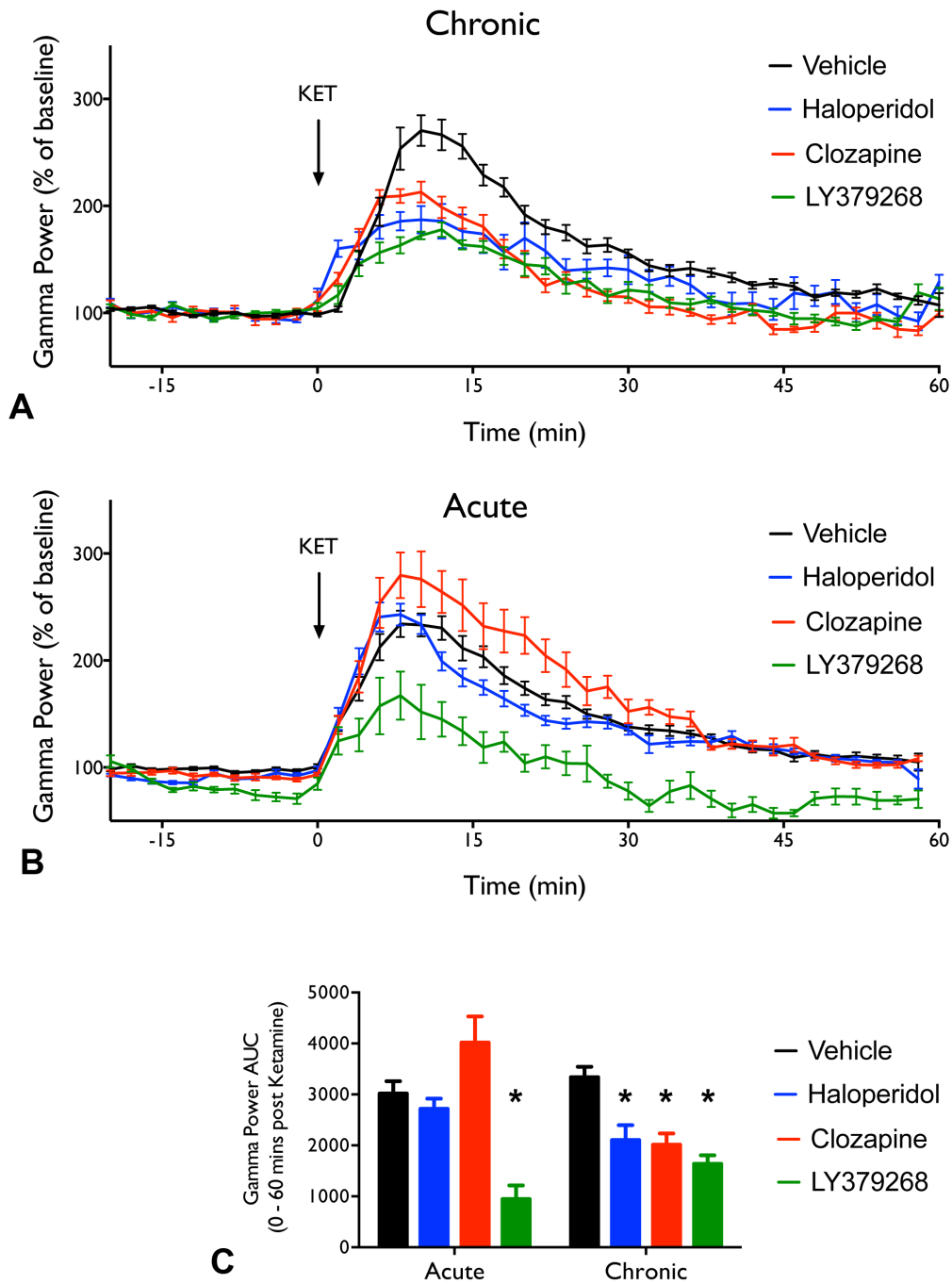
Figure 2.2 – Effects of Antipsychotic Treatment on Ketamine-Induced Increases in Gamma Power

Chronic treatment (top panel) with all three drugs attenuated the ketamine induced rise in γ power, compared to vehicle treatment. In contrast, in the acute treatment paradigm (B), only LY379268 significantly attenuated ketamine's effect. Direct comparison of the effects of chronic versus acute antipsychotic treatment on ketamine induced γ power (C) found that chronic treatment with haloperidol and clozapine reduced the magnitude of ketamine-induced increases in γ power when compared to acute treatments. However, both acute and chronic treatment with LY379268 induced similar magnitude of effects. Data represents mean ± SEM; n = 9-14/group. * indicates $p < 0.05$ compared to vehicle.

2.3.3 All Three Antipsychotics Attenuate Ketamine-Induced Hyperlocomotion

The effects of chronic antipsychotic treatment on the power of ongoing γ oscillations were mirrored in the outcome of the locomotor response to ketamine injection. Ketamine caused a hyperlocomotor response in all injected animals; in vehicle treated animals, we observed a maximum response of 932 ± 229% occurring at 2 min post injection (Figure 3A). This response was significantly, but not completely, reduced in all three chronic treatment groups with maximum locomotor activities for haloperidol: 441 ± 92% at 8 min post injection, clozapine: 494 ± 151% at 2 min and LY379268: 313 ± 105% at 2 min post injection. ANOVA showed that chronic treatment had a significant effect on the locomotor response for haloperidol ($F_{(1,13)}$ = 4.69, p = 0.0496), clozapine ($F_{(1,14)}$ = 8.28, p = 0.0122) and LY379268 ($F_{(1,12)}$ = 9.96, p = 0.0083). Acute treatment (Fig 3B) evoked similar results, with haloperidol ($F_{(1,14)}$ = 22.29, p = 0.0003), clozapine ($F_{(1,13)}$ = 8.12, p = 0.0137) and LY379268 ($F_{(1,11)}$ = 5.68, p = 0.0362) all significantly inhibiting the locomotor response of ketamine as compared to vehicle treated animals.

In contrast to their effects on the electrophysiological response to ketamine, when assessing the area under the curve (Fig 3C), there were no differences between the acute and chronic treatment regime (p > 0.05), such that all treatments were equally effective at reducing the locomotor response to ketamine.
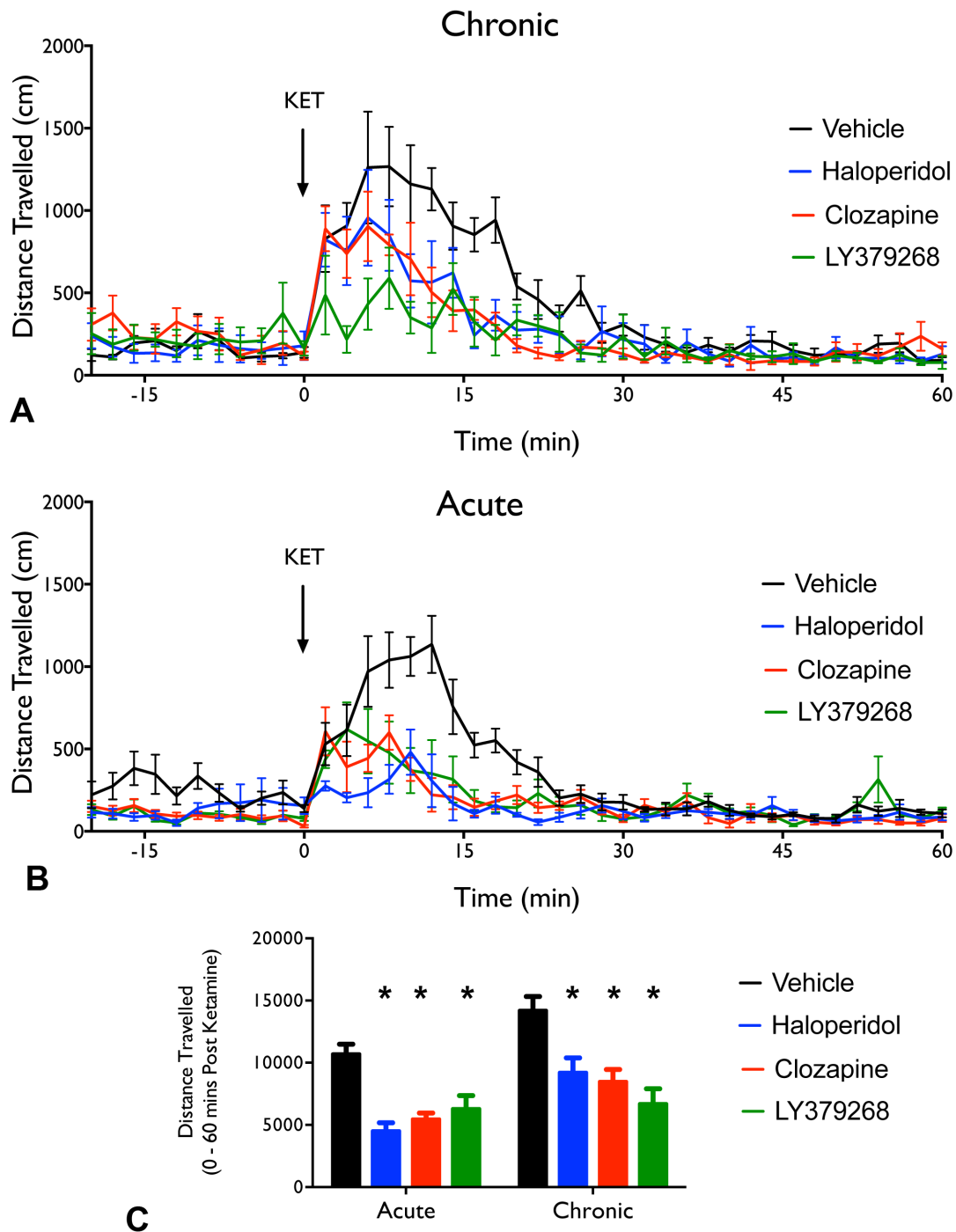
Figure 2.3 – Effects of Antipsychotic Treatment on Ketamine-Induced Locomotion

Chronic treatment (A) with all three different drugs attenuated the ketamine induced locomotor activity as compared to vehicle treatment. Similar effects on locomotion where seen with acutely treated animals (B) where all three medications attenuated the hyperlocomotion effects of ketamine. For all drugs, these two treatment regimens were equally effective at reducing the ketamine-induced locomotor effect (C). Data represent mean ± SEM, n = 6 – 8/group. * indicates $p < 0.05$ compared to vehicle.

2.4

Discussion

The primary finding of this study is that chronic treatment with the antipsychotic medications haloperidol and clozapine, and the pre-clinical drug LY379268, attenuate the effects of an acute ketamine challenge, as measured by electrophysiological and behavioural responses. Chronic treatment with haloperidol and clozapine attenuated the ketamine induced increase in γ power, an effect not seen with single acute dosages. The mGluR$_{2/3}$ agonist LY379268 reduced ketamine-induced locomotor and γ hyper-activities under both acute and chronic treatment paradigms. Furthermore LY379268 did not alter the power of ongoing physiological γ oscillations during the 4-week treatment, while the conventional antipsychotics, clozapine and haloperidol, caused a pronounced depression of the ongoing background γ activity that was sustained throughout the treatment period.

The observation that chronic treatment for 4 weeks with the conventional antipsychotics was effective in attenuating the effects of ketamine to increase cerebral γ activity, while acute single dosing was not (Jones et al., 2012), is a potentially important finding. In clinical practice, the efficacy of antipsychotic treatment is typically not seen until 2-3 weeks after treatment has been instituted (Gelder et al., 2000). While some recent studies have questioned this (Agid et al., 2003), it is clear that in clinical settings antipsychotic treatment displays increasing efficacy over time. The findings of this study suggest that the cerebral γ activity response to ketamine may represent an endophenotype of the efficacy of chronic antipsychotic treatment, which is practical for use in drug screening in animal models. It is important to point out that our study was conducted in healthy animals, displaying physiologically normal γ oscillations, not the chronic pathological abnormalities seen in schizophrenia. This limits any interpretations made; the acute NMDAr antagonist treatment should be thought of at best, as

similar to an acute psychotic event. Further work performed in genetic or pharmacological chronic models for the schizophrenic state should be conducted to verify the present results. Nonetheless, the findings presented in this study – that chronic treatment with antipsychotic medications strongly attenuates the ketamine-induced increase in γ power – supports the hypothesis that γ frequency abnormalities are a significant feature of schizophrenia that is recreated in the NMDAr model.

There is a large body of evidence reporting altered γ frequency oscillations in patients with schizophrenia, with studies reporting both decreases in evoked γ (Ford and Roth, 2004; Kwon et al., 1999; Spencer et al., 2003) and increases in ongoing γ (typically associated with psychotic events or positive symptoms) (Becker et al., 2009; Behrendt, 2010; Gordon et al., 2001; Spencer et al., 2009). It has been proposed that these findings represent different aspects of an overall disruption in γ signal-to-noise in cortical information processing (Flynn et al., 2008; Gallinat et al., 2004; Krishnan et al., 2009; Williams et al., 2009, see Gandal et al., 2012b for a recent review). This conceptualises that the increase in ongoing γ power triggered by NMDAr antagonists represent a diffuse network noise, disrupting cortical processing and leading to perceptual and cognitive deficits reminiscent of those seen in schizophrenia. The findings of this study support the proposition that modulation of γ power represent a key property of antipsychotic medications, with both clozapine and haloperidol's capacity to inhibit aberrant γ oscillations potentially reflecting their efficacy against positive symptoms.

It is interesting to note the disassociation between the effects of antipsychotic medications on ketamine-induced hyperlocomotion and those on γ oscillations. Both acute and chronic treatment with all three different antipsychotic compounds had similar effects, attenuating the ketamine-induced hyperlocomotion seen in the treated animals. This finding supports previous work published by us demonstrating

that the increase in γ oscillations seen following NMDAr antagonist administration can be dissociated from the behavioural measure of hyperlocomotion (Pinault 2008). Investigating the physiological mechanisms behind this disconnection was beyond the scope of this study but a potential mechanism for this result is the downstream dopamine release triggered by NMDAr antagonists (Moghaddam et al., 1997; Schulz et al., 2012). This mechanism could also explain the effectiveness of dopaminergic antagonist antipsychotics in preventing hyperlocomotion while sparing the gamma increasing effects of ketamine. This result supports the possibility that γ oscillations may be a more effective marker of the cognitive symptoms of schizophrenia (Light et al., 2006), than the positive symptoms.

The differential effects of LY379268 on spontaneous (ongoing) γ activity compared to the established clinical antipsychotic drugs, haloperidol and clozapine, is noteworthy. LY379268 is a $mGluR_{2/3}$ agonist, while most clinical antipsychotic drugs are thought to primarily act via dopamine D2 receptors (although clozapine has less effects at this receptor that haloperidol) (Seeman, 2002). The $mGluR_{2/3}$ agonists have been shown to decrease the release of glutamate and inhibit excitatory synaptic activity (Battaglia et al., 1997; Lovinger and McCool, 1995; Yoshino et al., 1996). Ketamine (and other NMDAr antagonists) trigger release of glutamate in the prefrontal cortex (Adams and Moghaddam, 1998) and LY379268 pre-treatment can block this glutamate release following PCP administration (Lorrain et al., 2003), providing a plausible mechanism of action of how this drug may combat the behavioural and electrophysiological effects of ketamine observed here. Furthermore, while LY379268 causes a transient decrease in ongoing γ power following acute administration (Jones et al., 2012), this effect was not seen with chronic treatment. In contrast the two clinical antipsychotic drugs resulted in sustained decreases in ongoing γ activity, yet only chronic treatment with them attenuated the magnitude of ketamine-induced γ activity. $mGluR_{2/3}$ receptor agonists

have been shown in clinical trials to have a lower cognitive side effect profile (Adams et al., 2013). It is possible that the lack of effect of LY379268 on the physiological ongoing γ may be related to its reduced cognitive side effects, given the role they are thought to play on cognitive processing. Haloperidol has been shown to induce negative symptoms (Veselinovic et al., 2013), and a comprehensive assessment in healthy subjects showed a general effect of impairing attention and speed of processing caused by antipsychotic administration (Ramaekers et al., 1999)

It is important to point out that our study was conducted in healthy animals, displaying physiologically normal γ oscillations, not the chronic pathological abnormalities seen in schizophrenia. This limits any interpretations made, with the acute NMDAr antagonist treatment considered an acute psychotic precipitant. Further work performed in genetic or chronic pharmacological models for the schizophrenic state should be conducted to validate the relevance of the findings of this study. Nonetheless, the findings of this study – that chronic treatment with antipsychotic medications attenuates the ketamine-induced increase in γ power – supports the hypothesis that γ frequency abnormalities are a significant feature of the psychotic-like state that is induced in the NMDAr model.

In conclusion this study has demonstrated chronic antipsychotic administration causes a strong attenuation of the psychotomimetic effects of ketamine, as assessed through hyperlocomotion and ECoG γ hyperactivity. The modulation of γ frequency oscillations by NMDAr antagonists and antipsychotic medications provide insights into the role that γ frequency abnormalities may play in schizophrenia, and have potential utility as a measure of antipsychotic efficacy and cognitive side effects.

CHAPTER 3

ABERRANT GAMMA FREQUENCY NEURAL ACTIVITY AND NMDA RECEPTORS IN NEUREGULIN 1 MUTANT MICE

Abstract

Neuregulin 1 (Nrg1) is a protein coded by a replicated schizophrenia 'risk' gene. Nrg1 signalling is important for the development of parvalbumin (PV) interneuron circuits, and these fast-spiking interneurons contribute to the generation of γ (γ, 30-80 Hz) oscillations. Gamma oscillations are disrupted in schizophrenia, and by NMDAr antagonists. In order to link Nrg1 gene mutations to neurobiological change, we examined if γ frequency activity and expression of NMDAr are altered in mice containing a heterozygous mutation in the transmembrane domain of Nrg1. To measure the power of ongoing and auditory-evoked γ oscillations, female Nrg1 mutants and wild-type controls underwent locomotor and electrocorticogram recordings before and after subcutaneous administration of the NMDAr antagonist ketamine (10 mg/kg) or saline. mRNA expression of NMDAr subunits, as well as NR2B phosphorylation levels, were measured in the prelimbic cortex. Nrg1 mutant mice displayed an increase in the power of spontaneously occurring cortical γ oscillations compared to wild-type mice (p<0.0001), significantly reduced sensory-related γ activity (p<0.0001) as well as a dampened γ power response to ketamine. Nrg1 mice exhibited increased baseline locomotor activity compared to WT, but a similar response to ketamine. ErbB4 and NMDAr subunit mRNA expression was unaffected by Nrg1 genotype, but NR2B phosphorylation levels were significantly lower in Nrg1 mutant mice. Nrg1 TM HET mice display an altered γ oscillation phenotype, which may be relevant to the pathophysiology of schizophrenia.

Reduced phosphorylation of the NMDAr NR2B subunit may play a role in the generation of these abnormal γ rhythms.

3.1

Introduction

Gamma frequency (30-80 Hz) oscillations support the dynamics of large-scale neural assemblies involved in global brain operations (Engel et al., 1997), and have been associated with various cognitive functions, including attention, memory and perception (Fries et al., 2001; Melloni et al., 2007). These same cognitive domains are disrupted in patients with schizophrenia, who also display alterations in γ oscillations (Uhlhaas et al., 2008; Woo et al., 2010). GABAergic interneurons expressing parvalbumin (PV) contribute to the generation of γ oscillations (Freund, 2003), and postmortem studies of patients with schizophrenia have identified changes in molecular markers of their function (e.g., (Fung et al., 2010; Hashimoto et al., 2008)). These findings lead to the hypothesis that abnormal γ oscillations in schizophrenia result from a dysfunction of perisomatic inhibition of pyramidal neurons mediated by basket cells expressing PV. Several lines of clinical and experimental evidence suggest that this synaptic dysfunction involves a hyporegulation of NMDArs (Bitanihirwe et al., 2009; Wang et al., 2008a; Woo et al., 2010), although the precise mechanisms involved remain unclear (Gonzalez-Burgos and Lewis, 2012).

Neuregulin 1 (Nrg1) is a protein coded by a leading candidate for a schizophrenia 'risk' gene (Harrison and Law, 2006; Stefansson et al., 2002). Nrg1 mRNA and protein expression is altered in schizophrenia (Chong et al., 2008; Hashimoto et al., 2004; Law et al., 2006a), and the haplotype of single nucleotide polymorphisms in non-coding regions of Nrg1 has been linked to changes in Nrg1 mRNA expression (Law et al., 2006a; Weickert et al., 2012), although the pathophysiological mechanism by which altered Nrg1 expression results in vulnerability to develop schizophrenia is not known. Interestingly, ablation of ErbB4 (the Nrg1 receptor) specifically on PV+ interneurons leads to a range of

abnormal synaptic and circuit level activities, including altered γ frequency oscillations (del Pino et al., 2013); furthermore, exogenous Nrg1 has been shown to influence γ oscillations (Fisahn et al., 2009). A number of different Nrg1 mutant mouse models exist (Duffy et al., 2010), with subtle differences in behavioural and molecular phenotypes. Mice with a heterozygous deletion of the transmembrane domain of Nrg1 (Nrg1 TM HET mice) possess a behavioural phenotype relevant to schizophrenia, exhibiting hyperlocomotion, increased anxiety, impairments in working memory and social interaction deficits (Duffy et al., 2010; Karl et al., 2007; Stefansson et al., 2002). The transmembrane domain mutation is downstream of the biologically active EGF-like domain of Nrg1, which may impact on both the amount of soluble Nrg1 available for extracellular signalling, and on intracellular signalling by the cytoplasmic portion of Nrg1 (Bao et al., 2003). We hypothesised that abnormal Nrg1 expression would alter GABAergic interneuron and NMDAr function, and be detected as abnormal γ oscillations. The receptor for Nrg1, ErbB4, is highly expressed on PV+ interneurons (Fazzari et al., 2010) and alters the intrinsic excitability of these cells (Li et al., 2011), and Nrg1-ErbB4 signaling controls GABAergic synaptic development (Fazzari et al., 2010; Woo et al., 2007). There is also evidence that Nrg1 signalling can affect glutamatergic synapse development (Li et al., 2007) and NMDAr function; ErbB4 attaches to the scaffolding protein PSD-95 in the same location as NMDAr NR2 subunits (Huang et al., 2000) and can alter receptor phosphorylation (Bjarnadottir et al., 2007). This suggests that NMDAr activity (and related γ oscillations) may be affected by alterations in Nrg1 signalling.

Ketamine is a non-competitive NMDAr antagonist, and its administration to rodents has been used as a pharmacological model of psychosis/schizophrenia, modeling NMDAr hypofunction (Bubeníková-Valešová et al., 2008). Previous work demonstrates that administration of low-dose ketamine increases the power of

ongoing γ frequency oscillations, representing a potential electrophysiological correlate of an acute psychotic state (Hakami et al., 2009; Kocsis, 2011; Pinault, 2008). In the present study we investigated how the Nrg1 transmembrane domain mutation affects cortical γ oscillatory activity, both in control conditions and through interaction with ketamine, and the expression of NMDAr subunits using Nrg1 TM HET mice.

3.2
Materials and Methods

3.2.1 Animals

The generation of Nrg1 transmembrane domain heterozygous mutant mice (C57BL/6J background) was described previously (Stefansson et al., 2002). Female mice heterozygous for a mutation in the transmembrane domain of Nrg1 (total Nrg1 TM HET, n = 29) and female wild type-like littermate controls (WT, n = 31) were bred and housed at Australian BioResources (Moss Vale, New South Wales, Australia). For electrophysiology (n=20 Nrg1 TM HET; n=22 WT) and locomotor studies mice were transported to the Department of Medicine, Royal Melbourne Hospital, University of Melbourne. Animals were housed (3-5 per cage) in the Biological Research Facility of the Department of Medicine, Royal Melbourne Hospital, University of Melbourne, with a 12 hour light-dark cycle (lights on at 0600) and maintained at $22 \pm 1°$ C. Animals were housed in opaque plastic cages with wire lids and woodchip bedding with access to food and water ad libitum. All experimental procedures were approved by the University of Melbourne Animal Ethics Committee (#1011868) or by the University of New South Wales Animal Care and Ethics Committee (#10/98B) and carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

3.2.2 Electrode Implantation Surgery

Female mice (~36 weeks of age) were implanted with cranial recording electrodes for the acquisition of surface electrocorticograms (ECoG). Briefly, animals were anaesthetised using isoflurane (Abbott Pharmaceuticals, USA) and placed in a stereotactic frame. A single midline incision was made, four holes were drilled in the skull and epidural brass recording electrodes were implanted and held in place with dental cement.

3.2.3 Electrophysiology Recordings and Analyses

ECoG recordings of ongoing parietal cortical activity in a cohort of mice (n=12 Nrg1 TM HET; n=8 WT) were undertaken in a quiet, dimly lit behavioural testing suite as previously described (Hakami et al., 2009). Animals were habituated to the environment for 30 min, at which time they were in a state of quiet wakefulness. A 30 min baseline ECoG recording was then acquired, which was followed by injection with either ketamine (Parnell Laboratories, Australia, 10 mg/kg subcutaneous (s.c.)) or saline (10 ml/kg s.c.) and a subsequent 60 min of recording. A second cohort of mice (n=8 Nrg1 TM HET; n=14 WT) underwent recordings to assess auditory-evoked activity; recordings were done in SR-Lab startle chambers and stimuli generated with SR-Lab software (San Diego Instruments, CA, USA). Stimuli consisted of 85 dB white noise pulses, 10 ms in duration with an inter-stimulus interval of 6 s, background white noise was maintained at 70 dB. This stimulus is below the threshold for animals of both genotypes to elicit a startle response. In both cohorts, ECoG recordings were acquired using a Powerlab 4/30 amplifier and A-D converter and LabChart 7 software (AD Instruments, Australia), recordings were sampled at 2000 Hz, and band pass filtered offline at $0.5 - 500$ Hz.

Data was exported to MATLAB software, visually inspected for movement artefacts, then spectral power and response to auditory stimuli were analysed. Ongoing γ activity was measured using fast Fourier transformations (Hamming window, 0.48 Hz resolution) for each 2-minute interval of the recording. Total relative power in each frequency band was calculated as the ratio of the raw power of the band divided by the total power (1-100 Hz), with the average relative power defined as the relative power per Hz. e.g for the theta band (4-8 Hz) $P_{theta} = P_{(4\text{-}8\ Hz)}/P_{(1\text{-}100\ Hz)}$. Epochs spanning $\pm$ 500 ms from the auditory stimulus were extracted and event related spectral activity was calculated using EEGLAB toolbox (Delorme and Makeig, 2004). Power was calculated using Morlet wavelets ranging from 3 to 10 cycles across 20-200 Hz.

3.2.4 Locomotor activity

Locomotor effects of ketamine were also assessed. The same cohort of animals that underwent auditory-evoked recordings were utilised prior to the ECoG experiemtns. Animals were individually placed in photocell tracking chambers (Med Associates, St Albans, VT, USA) to measure spontaneous baseline activity and after 30 min were administered ketamine (10mg/kg, s.c) or saline and allowed to explore the chamber for a further 60 min. Quantification of the distance travelled both before and after injection was objectively assessed using Activity Monitor software (Med Associates, St Albans, VT, USA).

3.2.5 Tissue Collection

For mRNA analyses, the prelimbic cortex was dissected from drug naive male and female Nrg1 TM HET and WT littermates at PND 161. Total RNA (N = 18, female 9, male = 9; Nrg1 TM HET = 8, WT = 10) was extracted for qPCR

analysis using Trizol (Invitrogen, Carlsbad, California) according to the manufacturer's instructions. The quality of total RNA was determined using the Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, California). 100-200 ng RNA was applied to an RNA 6000 Nano LabChip, without heating prior to loading. The RNA integrity number (RIN) was used as an indicator of RNA quality, ranging from 1-10 (lowest – highest quality). The mean RIN was 8.81 ± 0.32. For Western blot analyses, animals from the ongoing cortical ECoG cohort were randomised to receive an injection of either ketamine (10mg/kg s.c) or saline (10 ml/kg s.c). 10 min later animals were culled via cervical dislocation and brains rapidly removed and manually dissected to extract the frontal cortex including the prelimbic cortex. Samples were snap-frozen in liquid nitrogen and stored at -80°C.

3.2.6 qPCR Assessment of NMDA Receptor Expression

cDNA was synthesised in two reactions of 0.5 μg of total RNA using the Superscript III First-Strand Synthesis Kit (Invitrogen) according to the manufacturer's protocol. Pre-designed TaqMan Gene Expression Assays (Applied Biosystems, CA, USA) were chosen for the genes encoding the NMDAr subunits of interest (Table 1). qPCR was performed with an ABI Prism 7900HT Fast real-time PCR system with a 384-well format.

Table 3.1 – Applied Biosystems TaqMan Gene Expression Assay Numbers

Gene Name	Gene Symbol	Taqman Assay
Genes of Interest		
Glutamate receptor, ionotropic, NMDA1	Grin1 (NR1)	Mm00433790_m1
Glutamate receptor, ionotropic, NMDA2A	Grin2a (NR2A)	Mm00433802_m1
Glutamate receptor, ionotropic, NMDA2B	Grin2b (NR2B)	Mm00433820_m1
Glutamate receptor, ionotropic, NMDA2C	Grin2c (NR2C)	Mm00439180_m1
Glutamate receptor, ionotropic, NMDA3A	Grin3a (NR3A)	Mm01341723_m1
Reference Genes		
TATA box binding protein	Tbp	Mm00446973_m1
Ubiquitin C	Ubc	Mm01201237_m1
Eukaryotic 18S rRNA	18S	Hs99999901_s1

Measurements were performed in duplicate and relative quantities determined from a seven-point standard curve. Expression levels were normalised to the geometric mean of three reference genes (Table 1) – the expression of the housekeeping gene mRNAs and 18S rRNA did not change significantly with genotype or sex ($p > 0.05$; data not shown).

3.2.7 Western Blot Assessment of NMDA Receptor Phosphorylation

Tissue samples were ground on dry ice, and the powder dissolved in RIPA buffer (150 mM NaCl, 50mM TRIS, 0.1% SDS, 1% Sodium deoxycholate, 1% Triton X-100, Roche Complete Protease Inhibitor Cocktail and Sigma Phosphatase Inhibitor Cocktail), then spun at 12,000g for 20 min at 4°C and supernatants collected. Protein concentration of the supernatants was determined with a BCA protein assay kit, and adjusted to a concentration of 1mg/ml total protein in SDS loading buffer. After heating at 95°C for 5 min, proteins were separated by 6% SDS polyacrylamide gel and the protein bands electrophoretically transferred to PVDF membranes, and immunoblotted with anti-NMDA NR2B (Invitrogen, mouse monoclonal, 1:2000) or anti-NMDA NR2B pY1472 (Novus Biological, rabbit polyclonal, 1:2000) antibodies. Immunoreactive bands were detected with a chemiluminescent substrate kit and exposed to X-ray film. The blots on X-ray film were scanned and the sum optical density quantitatively analysed by Image J. The immunoreactivities of proteins were normalised against α-tubulin (Sigma, mouse monoclonal, 1:10000). All data were expressed as relative levels of wild type-saline treated mean.

3.2.8 Statistical Analysis

For the electrophysiology studies, spectral power and specific frequency bands were compared using the non-parametric stastical Mann Whitney U-tests (for between

genotype analyses) as the normality of the distribution of spectral power cannot be assumed (Korotkova et al,. 2010). The magnitude of the ketamine-induced increase in γ power was quantified as the area under the curve and compared using Student's t-test. Locomotor activity was assessed in 2-min time bins, and compared between genotypes both before and after ketamine injection using two-way ANOVA, as per our previous work (Jones et al., 2010). For γ frequency Event-Related Spectral Perturbations (ERSP), individual trials were averaged into 2 min blocks (20 trials in each), and comparisons between drug conditions were made using the mean of 100 trials spanning $5 - 15$ min post injection, statistical significance was assessed with one-way, repeated measures ANOVA. The relationship between ongoing γ and ERSP was assessed with Pearson's correlation. For the molecular studies, differences in normalised mRNA expression and NMDAr NR2B subunit protein expression were compared using ANOVA.

3.3
Results
3.3.1 Nrg1 TM HET Mice Have Elevated Ongoing Gamma Frequency Power

Spectral analysis of ongoing ECoG recordings revealed a persistent elevation in the spectral power of the Nrg1 TM HET mutants compared to WT (Figure 3.1A). The abnormal ECoG activity was selectively observed in the higher frequency bands, beginning at 20 Hz (β frequency) and appearing most predominantly in the γ frequency (30-80 Hz) range (Figure 3.1B). Statistically, this was borne out when comparing the relative power of γ (Mann-Whitney $U = 2$, $p = 0.01$) and beta ($U = 4$, $p = 0.03$) bands of Nrg1 mutant and WT mice. No significant differences were observed in the theta (4-8 Hz) or alpha (8-12 Hz) bands between genotypes.
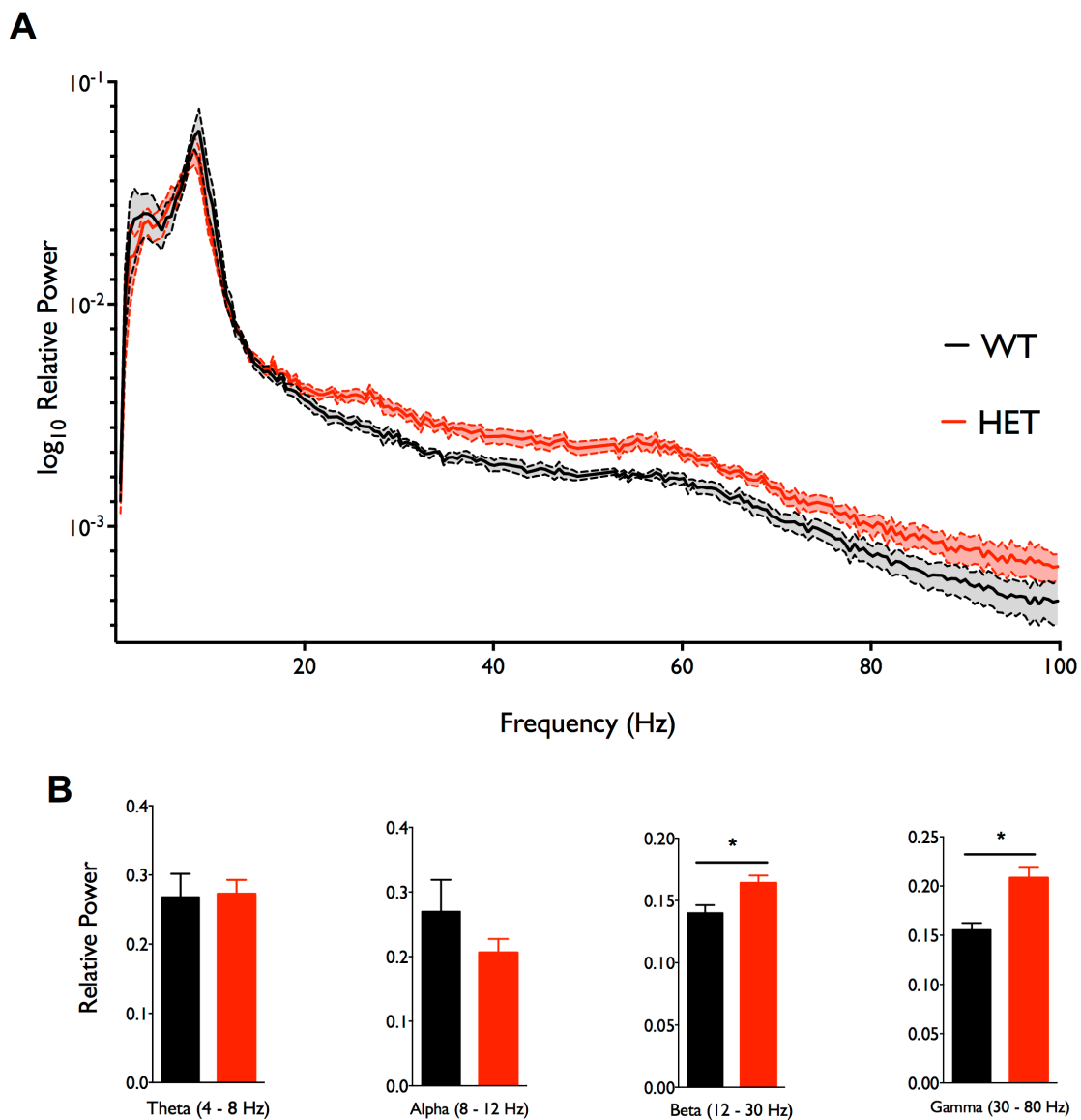
Figure 3.1 – Spontaneous High Frequency Cortical Oscillations are Increased in Nrg1 TM HET Mice

A) Relative power 0.5-100 Hz in Nrg1 TM HET (n = 7) and WT (n = 5) littermates show increased power at higher frequencies. Analysis performed on recordings taken from the 0-30 minute period post saline injection; data displays the average power of 15 individual 2 minute epochs. Spectra significantly differ (p = 0.0012). B) Relative ECoG power in theta, alpha, beta and gamma bands. Power was significantly higher in Nrg1 TM Het animals in the beta and gamma bands (* = p < 0.05, Mann-Whitney test).

3.3.2 Nrg1 TM HET Mice Display Reduced Gamma Frequency Response to Ketamine

We also investigated the cortical oscillatory dynamics of the Nrg1 mutant mice in response to ketamine. All animals exhibited a typical electrophysiological response to ketamine with a pronounced increase in the power of ongoing γ oscillations (Figures 3.2A & B). The magnitude of effect was smaller in Nrg1 mutant mice (maximum change from baseline: 144% vs 156%) and shorter acting (effect returning to baseline at 26 min post-ketamine injection in Nrg1 TM HET compared to 36 min in WT). This response to ketamine was quantified by calculating the area under the curve (Figure 3.2B inset) and this was significantly smaller in Nrg1 mutant mice (t = 2.14, p = 0.048). Saline injection did not change ongoing γ power in either genotype.
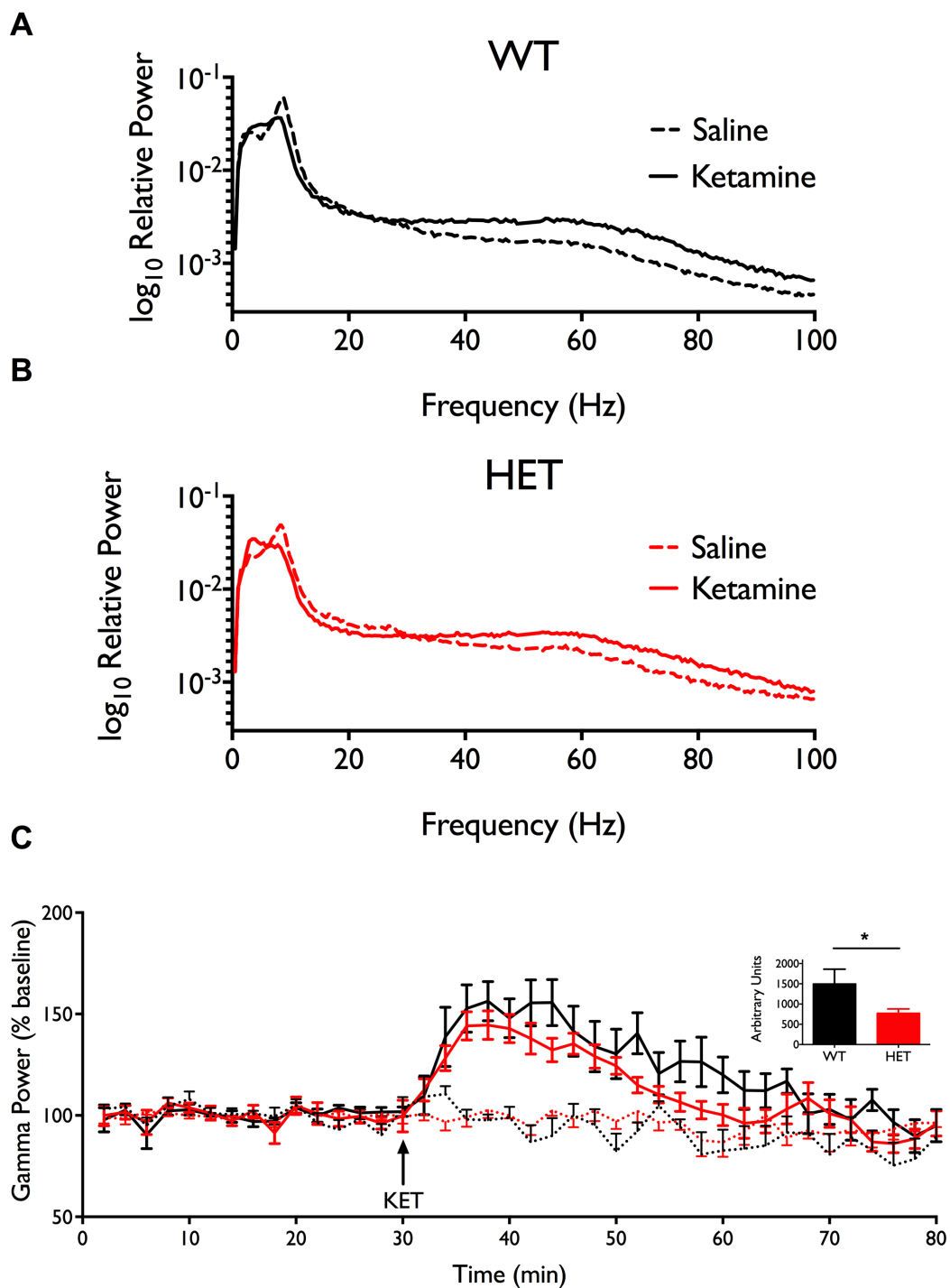
Figure 3.2 – Changes In Spectral Power Following Saline or Ketamine Injections in Nrg1 TM HET Mice and Wildtype Littermates

A-B) Relative spectral power following saline or ketamine injections in Nrg1 TM HET mice and wildtype littermates. **C)** Gamma power following ketamine administration, normalised to percentage of mean power in the 30 min preceding injection. **Inset)** Area under the curve 30 – 60 min, quantifying changes in gamma power. (* = p < 0.05, Mann-Whitney test). All data are presented as mean ± SEM, WT n = 8, HET n = 11.

3.3.3 Nrg1 TM HET Mice Show Reduced Auditory-Evoked Gamma Oscillations

We next examined the ECoG response elicited by auditory stimulation in Nrg1 TM HET and WT mice (Figure 3.3). The electrographic response (Figure 3.3) in control WT mice appeared to have three components: an early, large positive deflection, a late positive short-lasting wave, and a subsequent long-lasting negative wave. Ketamine treatment abolished the late positive short-lasting wave of the evoked potential, and also the subsequent long-lasting negative wave, compared to vehicle treatment. When quantifying the γ power evoked by the stimulus, Nrg1 TM HET animals exhibited reduced sensory-evoked γ power compared to WT controls ($F_{(3,142)}$ = 116.8, p < 0.0001; Figure 3.4 A). Ketamine also reduced the γ response to the auditory stimuli ($F_{(1,32)}$ = 100.71, p = 0.0016), although no interaction was observed between drug and genotype. Correlation analysis showed significant negative correlations between the power of ongoing γ oscillations and the event-related γ signal for both genotypes (r = 0.83 for both WT and Nrg1 TM HET animals; Figure 3.4 B), such that the higher the ongoing γ oscillations, the smaller the evoked γ responses.
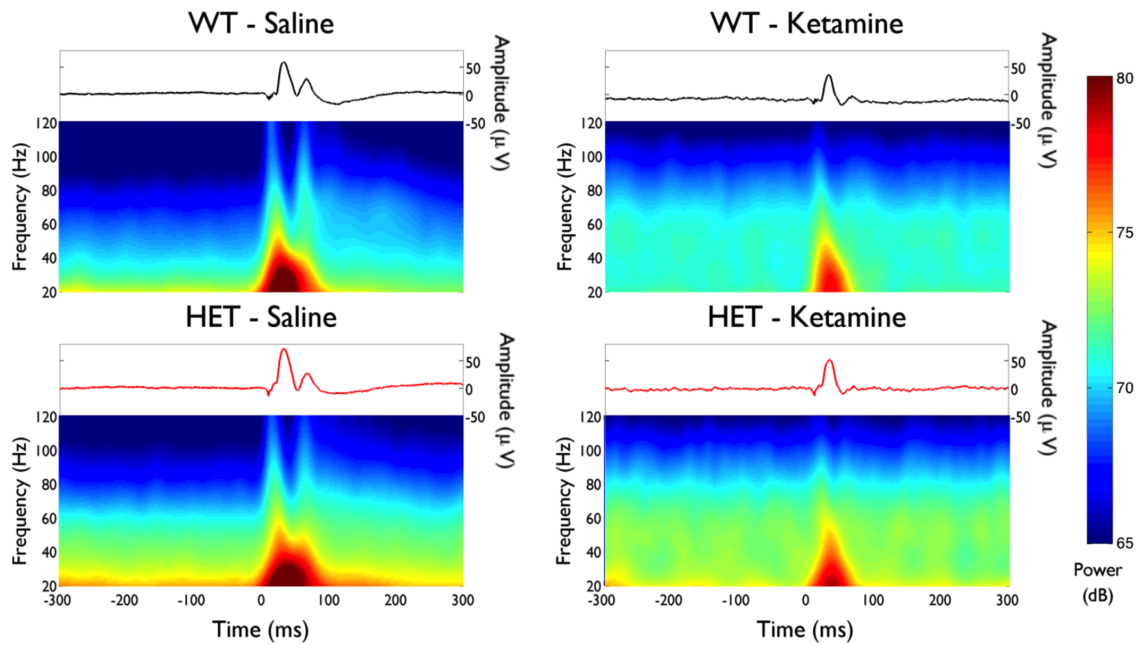
Figure 3.3 – Average Evoked Responses and Time-Frequency Heatmaps of Audio Stimuli ECoG Recordings in Nrg1 Mice And Wildtype Controls

Grand average evoked potentials from wild type and Nrg1 TM HET mice under both saline and ketamine conditions (top panels) and heatmaps representing the event-related spectral perturbation triggered by auditory stimuli (bottom panels). Averages are generated from all trials in the period 2-12 minutes post injection. Note the increased gamma power prior to stimulus in the HET mice relative to WT, and in the ketamine conditions, n = 20 for both genotypes.
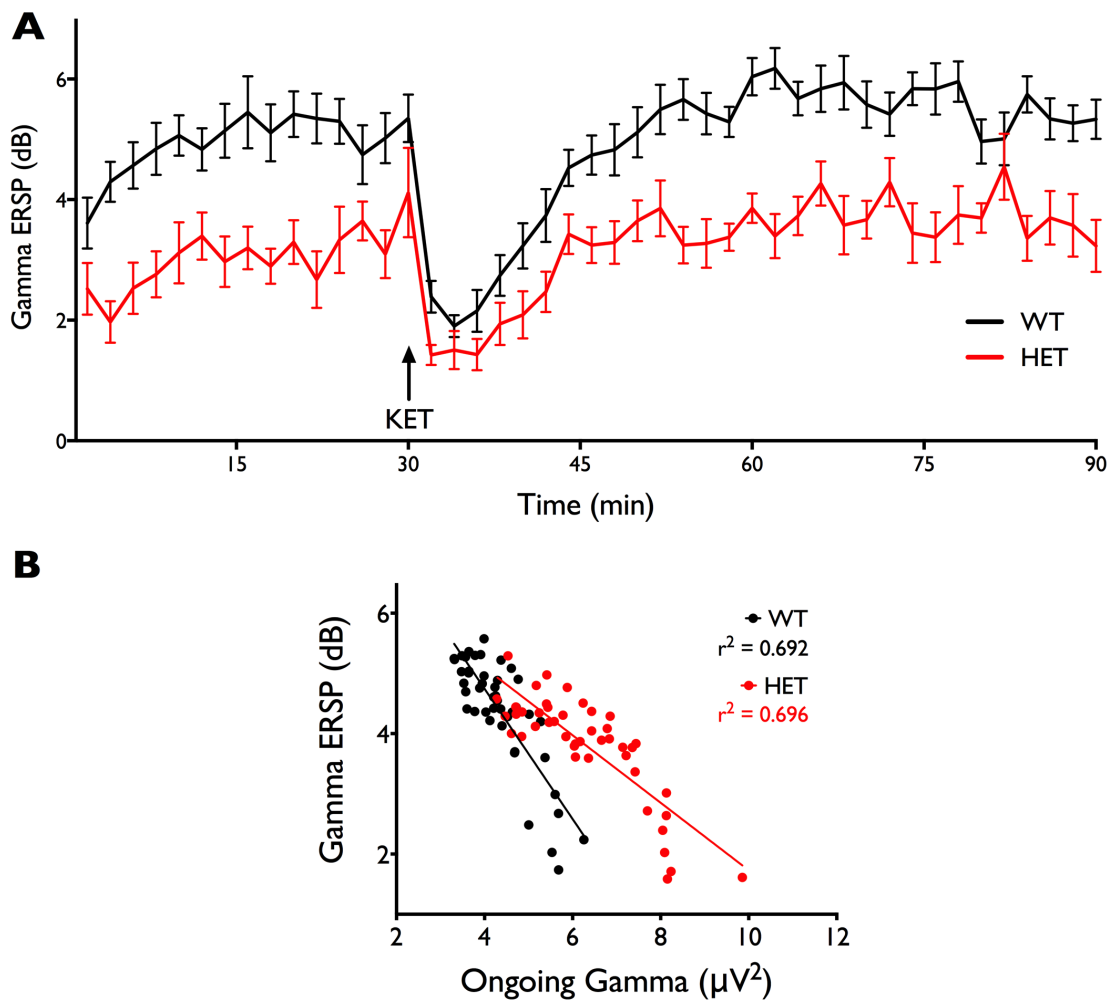
Figure 3.4 – Reduced Event-Related Gamma Activity in Nrg1 Mutant Mice

A) Gamma frequency (30 - 80 Hz) event related spectral perturbation following auditory stimulus (0 – 60 ms post stimuli) over time, data points represent 2 min mean ± SEM. **B)** Correlations between ongoing gamma activity and gamma ERSP, data points are 2 min averages, n = 20 for both genotypes.

3.3.4 Nrg1 TM HET Mice Exhibit Hyperlocomotion, but Genotype Does Not Differentially Impact Behavior Following Ketamine.

Consistent with previous reports, Nrg1 TM HET mice displayed hyperlocomotion upon being introduced to the testing chamber, with significantly greater distance travelled as compared to WT controls (t = 3.886; p < 0.001). This hyperlocomotion was less pronounced in the time bracket following saline administration, but still reached statistical significance (t = 2.133; p < 0.05).

Ketamine administration triggered an increase in locomotor activity in WT and mutant mice as compared to saline ($F_{(1,20)} = 37.22$; $p < 0.0001$) although there was no significant difference in locomotion between the genotypes in the 30 min period after ketamine administration ($t = 0.168$; $p = 0.868$).
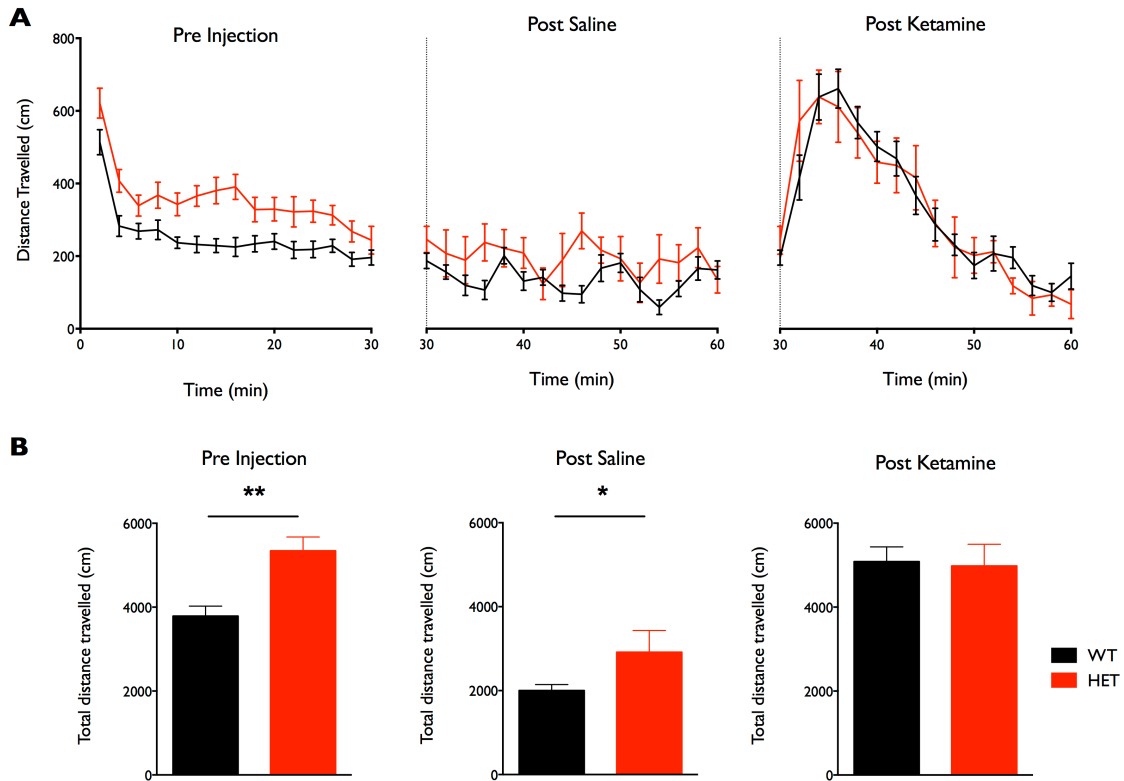
Figure 3.5 – Locomotor Activity of Nrg1 TM HET Mice and Wildtype Controls Over 30 Minute Periods

A) Locomotor activity displayed in 5 min averages: upon introduction to recording chamber, following saline, and following ketamine injections. B) Total distance travelled in the 30 min periods prior to and following ketamine and saline administration, * $p < 0.05$, **$p < 0.001$. Data represent mean ± SEM, WT n = 14, HET n = 8.

3.3.5 NMDA Receptor Subunit Expression in Nrg1 TM HET Mice

Since activation of Nrg1 signalling can cause changes in gene expression we first examined if the mechanisms behind altered γ power in Nrg1 TM HET mice may involve changes in cortical mRNA expression of the NMDAr subunits NR1, NR2A, NR2B, NR2C and NR3A (Figure 3.6) in adult WT and Nrg1 TM HET mice. We observed no significant differences in the expression of mRNA for any genes examined between Nrg1 TM HET and WT mice or between male and female mice (Figure 3.6).
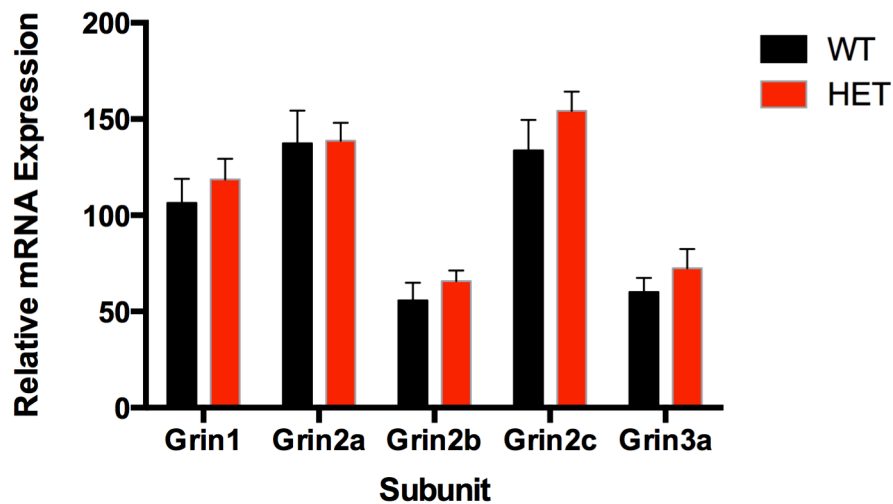
Figure 3.6 – mRNA Expression of NMDA Receptor Subunits in Prelimbic Cortex of Nrg1 TM HET Mice and Wildtype Controls

mRNA expression was determined by qPCR and normalised to the geometric mean of three reference genes. No expression changes in any of the genes were observed. Data represents mean ± SEM, Nrg1 TM HET n = 8, WT n = 10

3.3.6 Cortical NR2B Subunit Phosphorylation is Reduced in Nrg1 TM HET Mice

We next tested if the activation state of NMDAr may be changed by examining the phosphorylation state of the Y1472 residue of NR2B in the frontal cortex (Figure 3.7). We found that Nrg1 TM HET mice displayed reduced phosphorylation of Y1472 compared to WT mice ($F_{(1,20)} = 20.25$, $p < 0.001$). We also found a

significant main effect of ketamine treatment ($F_{(1,20)} = 37.52$, $p < 0.0001$) where ketamine induced phosphorylation of NR2B overall, and a significant genotype × treatment interaction ($F_{(1,20)}=13.61$, $p=0.0015$), such that the saline-treated WT mice showed significantly higher levels of NR2B phosphorylation than the other three treatment groups. We found no changes across the treatment groups in the total expression levels of NR2B receptor protein (effect of genotype: $F_{(1,20)} = 0.79$, $p = 0.39$; effect of treatment: $F_{(1,20)} = 0.09$, $p = 0.76$), consistent with our mRNA expression data.
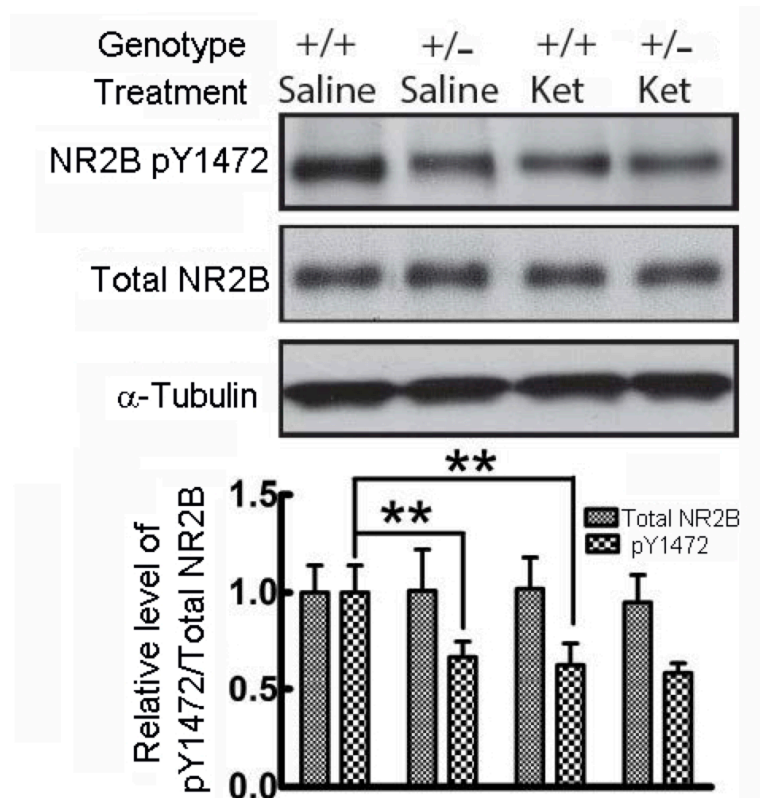
Figure 3.7 – Protein Levels of the NMDA Receptor Subunit NR2B and the Phosphorylated NR2B Y1472

Phosphorylation of NMDAr NR2B subunits is reduced in Nrg1 TM HET mice, but the ketamine-induced reduction in NR2B phosphorylation observed in wild-type mice is not apparent in the mutants. The top panel shows a representative Western Blot image. The bottom panel shows total cortical NR2B protein is not affected by genotype or treatment, but phosphorylation of the NR2B Y1472 residue is reduced in Nrg1 TM HET mice. Ketamine reduces phosphorylation in WT, but not in Nrg1 mutants (** $p < 0.01$). Nrg1 TM HET n=12, WT n=8.

3.4

Discussion

The major findings of this study are that schizophrenia-relevant mutations in Nrg1 result in increased γ power in ongoing cortical oscillations, and reduced sensory-evoked γ responses. These electrophysiological observations were associated with alterations in locomotor behaviour, and reduced phosphorylation of the NMDAr NR2B subunit in Nrg1 mutant mice. This study provides the first evidence of abnormal electrophysiological regulation in the Nrg1 transmembrane domain mutant mouse, providing a neurophysiological correlate of the behavioural abnormalities previously characterised in this model. This also raises intriguing possibilities concerning mechanisms by which mutations in Nrg1 may result in schizophrenia – a disorder linked with disturbances in γ frequency cortical activity.

There is an extensive literature demonstrating that γ frequency neural activity is disrupted in patients with schizophrenia, both at rest and during various cognitive or perceptual tasks (summarised by (Gandal et al., 2012b)). This supports a hypothesis that abnormal γ activity is a measure of disrupted neuronal activity that underlies the various symptoms in schizophrenia. Increasing evidence from animal models (Kulikova et al., 2012; Saunders et al., 2012) and human studies (Spencer, 2011) suggests that elevations in baseline γ activity represent increased 'noise' in neural networks, and that this may impair sensory-evoked γ oscillations and information processing. Our findings lend further support to this hypothesis, with Nrg1 TM HET mice displaying elevated γ frequency power in 'baseline' recordings and concurrent reductions in sensory-driven γ responses. This study supports previous work (Ehrlichman et al., 2009) that found electrophysiological alterations in Nrg1 mutant animals and presents evidence of specific sensory-evoked gamma alterations similar to those seen in NMDAR mutant animals (Gandal et al., 2012).

In addition to the electrophysiological differences observed between the genotypes we also replicated previous findings that the Nrg1 mutant mice exhibit a hyperlocomotor phenotype. However, both genotypes displayed a typical and equivalent hyperlocomotor response to ketamine, a finding consistent with previous reports showing no differential effects of NMDAr antagonists on hyperactivity or prepulse inhibition of startle in Nrg1 TM HET mice (van den Buuse et al., 2009). What causative role alterations in γ activity may play in the behavioural phenotype of these mice is still unclear, although this may be particularly pertinent to the working memory deficits previously identified (Duffy et al., 2010), since γ oscillations have been linked with this behaviour (Howard et al., 2003). Our results demonstrating altered γ oscillations in the Nrg1 TM HET mice establish this genetic model as a suitable one for further exploring the relationship between altered γ oscillations and cognitive abnormalities relevant to schizophrenia.

The mechanism through which altered Nrg1 signalling leads to the abnormal γ frequency activity is not clear. Nrg1 is a trophic factor that plays a role in several developmental processes, such as interneuron migration (Flames et al., 2004), and synapse development and maintenance (Fazzari et al., 2010; Li et al., 2007; Woo et al., 2007); disruptions to any of these processes could conceivably result in neuronal networks which generate disturbed neural oscillations in adulthood. In particular, subtle changes in synaptic composition during different developmental stages could lead to lasting alterations in brain network connectivity. The expression of NMDAr subunits, for example, is under tight regulatory control during development (Monyer et al., 1994) and can be influenced by Nrg1 signalling (Li et al., 2007). Conditional ablation of NMDAr on PV+ interneurons (the cells proposed to underlie the generation of γ frequency activity) results in marked increases in the spectral power of ongoing neural oscillations specific to the γ frequency band (Carlen et al., 2012; Korotkova et al., 2010) − similar to our

observations in the Nrg1 TM HET mice. Deletion of the Nrg1 receptor ErbB4 on PV positive interneurons also causes synaptic abnormalities and altered γ oscillations in mouse models (del Pino et al., 2013), highlighting the role this pathway plays in establishing and maintaining normal excitatory/inhibitory balance.

However, we found no evidence that the Nrg1 TM mutation influences expression of NMDAr subunits in adulthood, suggesting that the altered γ power observed in these mice is not caused by changes in gene expression of NMDAr subunits. This is consistent with the lack of change in MK 801 radioligand binding to NMDAr in Nrg1 TM HET mice (Long et al., 2012). However, we cannot rule out a selective alteration of NMDAr on cortical interneurons, particularly since ErbB4 – the signalling partner of Nrg1 – is largely expressed on these cells (Fazzari et al., 2010).

Considerable evidence suggests that Nrg1 signalling affects NMDAr function: ErbB4 (the Nrg1 receptor) attaches to the scaffolding protein PSD-95 in the same location as NMDArs (Huang et al., 2000). This allows direct physical interaction between the two systems – interactions which have been demonstrated to be altered in schizophrenia (Hahn et al., 2006). Multiple studies have shown that altered Nrg1 signaling can affect NMDAr activity, alter subunit phosphorylation and influence NMDAr internalisation (Bjarnadottir et al., 2007; Hahn et al., 2006). While baseline levels of NMDAr mRNA are unchanged, we found that the Nrg1 TM mutation influenced the functional activation of NMDA NR2B receptors, robustly decreasing cortical phosphorylation of Y1472 (~50%) compared to WT. The Y1472 residue is the major phosphorylation site of the NR2B receptor (Nakazawa et al., 2001), and phosphorylation prevents internalisation of NMDArs, thereby prolonging its synaptic availability for activation and enhancing its cell surface function (Prybylowski et al., 2005).

Our finding of reduced phosphorylation in Nrg1 TM mutant mice, which agrees with a previous study in hippocampus (Bjarnadottir et al., 2007), is suggestive of reduced surface expression and availability of these receptors (Prybylowski et al., 2005), therefore representing a potential mechanism responsible for the dampened electrophysiological response to ketamine in mutant mice. We also observed that, in WT mice, ketamine lowered phosphorylation of the Y1472 residue of NR2B, agreeing with previous reports of the consequence of NMDAr antagonism (Xi et al., 2011), and mimicking the baseline state of Nrg1 TM mutants. Interestingly, Nrg1-ErbB4 signaling is thought to inhibit plasticity at some synapses via suppression of phosphorylation of the NR2B subunit (Pitcher et al., 2011), raising intriguing questions about whether and how Nrg1-ErbB4 signaling is altered by the transmembrane domain mutation.

In conclusion, we have demonstrated increased ongoing, and reduced auditory-evoked γ frequency ECoG activity in the Nrg1 transmembrane domain heterozygous mutant mouse. These findings, which may be related to reduced phosphorylation of NMDAr found in the mutant mice, add to the existing evidence of schizophrenia-relevant molecular, behavioural and cognitive abnormalities in this mutant mouse. This study suggests that alterations in the regulation of γ frequency oscillations may represent a neural mechanism by which altered Nrg1 signalling contributes to the pathogenesis of schizophrenia.

CHAPTER 4

CONTRIBUTION OF THE CORTICOTHALAMIC PATHWAY TO KETAMINE-INDUCED DISRUPTION OF INFORMATION PROCESSING

Abstract

There is a growing body of literature indicating involvement of dysfunctional thalamic networks in the pathophysiology of schizophrenia, specifically in the observed disturbances in function-related gamma (γ, 30-80 Hz) frequency oscillations (Cronenwett and Csernansky, 2010; Pinault, 2011; Woodward et al., 2012). A postulated mechanism of these impairments is reduced NMDAr activation at glutamatergic synapses on GABAergic interneurons (Carlen et al., 2012). We previously demonstrated that a single subanesthetic dose of ketamine transiently increases the power of ongoing γ oscillations and decreases sensory-evoked γ oscillations in the rat somatosensory thalamocortical (TC) network (Kulikova et al., 2012), reducing the γ signal-to-noise ratio; (defined as the power of the sensory-evoked γ response above the power of the baseline γ noise). Both glutamatergic TC neurons and GABAergic thalamic reticular nucleus (TRN) neurons are innervated by glutamatergic corticothalamic (CT) axons originating from layer VI. The goal of the present study was to assess the contribution of the CT pathway in the ongoing and sensory-evoked γ oscillations in the somatosensory thalamus.

To investigate the contribution of the CT pathway to thalamic ongoing and sensory-evoked γ activities we performed a series of in-vivo electrophysiological studies in lightly anaesthetised adult Wistar rats. Local-field potential and multi-unit activities were simultaneously recorded at the three key sites of the somatosensory

TC system. We recorded the activities of 1) the medial part of the ventral posterior nucleus (VPm) – the principal target of trigeminal nerve input to the thalamus; 2) The thalamic reticular nucleus (TRN) – a GABAergic structure with γ pacemaker properties that exclusively innervates the dorsal thalamus and receives innervation from both the VPm and the Layer VI CT pathway (Pinault, 2004); 3) Layer VI of the corresponding somatosensory cortex. Electrical stimulation of the vibrissae was applied to evoke somatosensory activity and electrophysiological measures were recorded under saline and ketamine (2.5 mg/kg, s.c.) conditions. Further experiments were conducted with tetrodotoxin (TTX, a sodium channel blocker) applied directly to the cortical surface to block cortically-generated feedback. Data generated were analysed with time-frequency decomposition using wavelet techniques, this allowed for the qualitative and quantitative assessment of ongoing and sensory-evoked γ activities.

A single injection of ketamine quickly and transiently increased the power of ongoing γ oscillations and simultaneously decreased both the amplitude and the γ power of the sensory-evoked responses in both the thalamus and the cortex. The sensory-evoked response recorded in the VPm and TRN was characterised principally by a short-latency (3.7±0.1 ms) and a long-latency (10.5±0.1 ms) negative component. The late response was hypothesised to be the result of CT feedback. Local application of TTX in the related cortex abolished the amplitude of the late response and decreased the amount of the associated γ power. The amplitude of the late response was highly correlated ($r^2 = 0.956$, $p < 0.0001$), with the power of the sensory-evoked γ oscillations, indicating that the CT pathway significantly contributed to the sensory-evoked γ in the thalamus. Investigating the contribution of the CT pathway in the ongoing and somatosensory-evoked γ oscillations demonstrated that the cortex plays a great role in the generation of

thalamic γ oscillations during information processing and that ketamine reduces the CT-mediated sensory γ signal-to-noise ratio.

4.1

Introduction

Sensory and cognitive deficits are core pathophysiological features in schizophrenia (Ellevåg and Goldberg, 2000; Wobrock et al., 2009). A growing body of clinical and experimental evidence indicates that they are underlain by dysfunction of highly distributed neural networks (Stephan et al., 2009; Uhlhaas, 2013), including corticothalamic (CT) and thalamocortical (TC) systems (Pinault, 2011) which display disturbances of gamma (γ, 30-80 Hz) frequency oscillations. Clinical observations of increased ongoing or baseline γ power in patients with schizophrenia (Kissler et al., 2000; Krishnan et al., 2005), coupled with extensive reports of decreased evoked γ oscillations (Gandal et al., 2012b; Uhlhaas and Singer, 2010) have led to a hypothesis that there is reduced gamma signal-to-noise during cortical information processing in schizophrenia (Kulikova et al., 2012; Winterer et al., 2000). The fundamental mechanisms underlying these deficits are still unknown. The glutamate hypothesis of schizophrenia has come to prominence in recent years and is the subject of ongoing clinical and basic experimental investigations (Gonzalez-Burgos and Lewis, 2012; Moghaddam and Krystal, 2012). In particular NMDAr antagonists such as ketamine have been widely used as pharmacological models for schizophrenia, as they induce hallucinations in healthy humans, and exacerbate psychotic symptoms as well as cause sensory and cognitive deficits in patients with schizophrenia (Krystal et al., 1994; Newcomer et al., 1999). We (Hakami et al., 2009; Pinault, 2008) and others (Ehrlichman et al., 2009a; Lazarewicz et al., 2010) have previously demonstrated that NMDAr antagonists dose-dependently increase the power of ongoing γ oscillations in rodents and also recapitulate the complex electrophysiological abnormalities seen in schizophrenia (Kulikova et al., 2012; Saunders et al., 2012).

We demonstrated that a single systemic low-dose injection of ketamine increases the power of baseline γ oscillations and decreases that of sensory-evoked γ in the rat somatosensory TC pathways (Kulikova et al., 2012). This initial sensory-evoked γ was calculated from a 100-ms long post stimulation epoch, compared to the approximately 10 ms latency of the principal TC-mediated potential. This 100 ms epoch should contain the effective results of multi-synaptic cortico-cortical and corticofugal integration processes, the sort of distributed neuronal integration hypothesised to underlie cognitive function (Uhlhaas, 2013). This finding supports the hypothesis that ketamine-induced psychotomimetic state impairs the ability of sensory TC systems to discriminate incoming sensory signals from background neural activity during information processing (Flynn et al., 2008; Gandal et al., 2012b; Winterer and Weinberger, 2004). In this conception ketamine induces a diffuse aberrant network 'noise', elevating the natural γ frequency oscillations that are prominent during desynchronised cortical activity to an abnormal and excessive level and reducing the 'effective' signal-to-noise ratio of sensory processing systems. These findings leave open the question about the relative contributions of the TC, cortico-cortical and CT pathways in ketamine-induced sensory and cognitive deficits and the related neural information processing.

We also demonstrated that the power of sensory-evoked γ oscillations is strongly positively correlated with the amplitude of a short-latency TC-mediated cortical potential, indicating that sensory-evoked γ oscillations represent a useful index of intra-cortical sensory information processing, which involve cortico-cortical and corticofugal pathways. Therefore the goal of the present study was to estimate the impact of TC-mediated cortical γ oscillations on cortical output under physiological and pathological (ketamine influence) conditions. One of the principal cortical outputs is the CT pathway originating from layer VI. Layer VI CT neurons innervate both the dorsal thalamus and the GABAergic thalamic reticular nucleus

(TRN). In the present study these two anatomically and functionally related structures were used to identify and characterise the sensory-evoked, TC-mediated CT response using multisite cell-to-network recordings under light anaesthesia in rats. Baseline activity of the somatosensory TC-CT system was challenged by sensory stimulation of the vibrissae, which evoked in both the thalamus and the TRN a well-identifiable CT response. We further provide strong evidence that the CT pathway significantly contributes to the generation of thalamic γ oscillations and that ketamine disrupts the function of the CT pathway.

4.2
Materials and Methods

4.2.1 Animals

A total of twenty adult (3–6-month-old) male Wistar rats (280–380 g) were used in accordance with European Union Guidelines (directive 2010/63/EU) and with the approval of the National and Regional Ethics Committee (Comité Regional d'Ethique en Matière d'Experimentation Animale, Université de Strasbourg). Animals were group housed (3-4 animlas per cage) in opaque plastic cages with wire lids and woodchip bedding. Animals had ad libitum access to standard rat chow and water and were housed in a temperature controlled facility ($21 \pm 1°$ C) with a 12 h light-dark cycle (lights on at 0700).

4.2.2 Surgery

Surgical procedures were performed under deep general anaesthesia induced with pentobarbital (40 mg/kg i.p. Sanofi, Libourne, France) and supplemented with ketamine (50 mg/kg i.m. Merial, Lyon, France). The penile vein was catheterised for the i.v. administration of light neurolept anaesthesia. The trachea was

cannulated and animals were connected to a ventilator (SAR-830/P, CWE, Inc., BIOSEB, Chaville, France). Artificial ventilation was maintained with an O_2 enriched mixture (50% air, 50% O_2) at 60 breaths per minute. A rectal temperature probe was inserted and core body temperature was maintained at 37-38 °C using a thermoregulated blanket. Electrocardiogram electrodes were inserted subcutaneously to monitor heart rate. Animals were placed in a stereotaxic frame.

Local anaesthetic was injected under the skin on the head (lidocaine 2%) and a midline incision made. The skin was pinned back and the skull surface revealed and cleared of any tissue. A micro-cranioduratomy (diameter approx. 0.5 mm^2) was performed above the left somatosensory parietal cortex as per (Pinault, 2005). For recordings made in layer VI of the parietal cortex the cranioduratomy was extended laterally to expose the target region. A dental drill (Technobox, Bien Air France SARL, Paris) was used to ablate the skull surface above the recording site until only a thin bone membrane remained. Small openings for parietal cortex ECoG electrodes were also drilled, (without breaching the dura) adjacent to the cranioduratomy site. The bone membrane was kept hydrated through the application of saline (0.9% NaCl) soaked gelatine surgical sponges. A small opening is then made in the bone membrane with forceps. The precise site of recording electrode insertion was stereotaxically marked on the dura surface with a pontamine blue filled glass micropipette. This marked location was then carefully incised with the bent tip of a 26 gauge needle. The brain surface and surrounding area was maintained hydrated and clean through the regular application of saline infused gelatine sponges.

At this point in the procedure light neurolept anaesthesia was induced by continuous intravenous injection of a mixture of fentanyl (2 mg/h), glucose (25 mg/h). Muscle rigidity and tremors were blocked with i.v. D-tubocurarine chloride (0.4 mg/h). Depth of anaesthesia was monitored through

electrocardiogram and ECoG recordings and lidocaine was topically applied to surgical wounds every two hours. At least two hours was given before beginning electrophysiological recordings after neuroplept anaesthesia began.

4.2.3 Recording Procedure

Glass micropipettes were used to record LFPs and multi-unit activity in the VPm, TRN and the ventral lateral nucleus of the thalamus (VL). Control recordings were made in the VL to determine effects specific to the somatosensory pathway, as this nucleus does not receive direct somatosensory input. Micropipettes were prepared from 1.2 mm glass capillaries (A-M systems) on a Kopf model 730 pipette puller (Kopf instruments, Tujunga, CA). Pipettes were filled with artificial cerebral spinal fluid (ACSF) and neurobiotin (1%) and had a tip diameter of 5-10 μm and typical resistance of 10-20 MΩ. A metallic quartz-glass coated platinum-tungsten electrode with a resistance of 0.5MΩ (Thomas Recording GmbH, Giessen, Germany) was used to record multi-unit activity and LFPs in layer VI of the somatosensory parietal cortex in some experiments. ECoG electrodes were made from Ag-AgCl wire and used to record broad cortical activity from somatosensory parietal cortex. Recordings were made under two different experimental designs:

1) Simultaneous recordings from the VPm and TRN made with glass micropipettes and concurrent somatosensory cortical ECoG.

2) Simultaneous recordings from the VPm and VL thalamic nuclei with conjoined glass micropipettes, layer VI of the somatosensory parietal cortex with a platinum-tungsten electrode or glass micropipette and concurrent somatosensory ECoG.

Electrode locations were made with the use of a stereotaxic atlas (Paxinos and Watson, 1998) and verified through electrophysiological characteristics (cell firing, receptive field) and post-mortem histology. Recordings were made at least 2 hours after the induction of light neurolept anaesthesia and monitoring of the cortical ECoG verified that deep anaesthesia had worn off. Electrodes were attached to micro-drivers and slowly lowered into the brain tissue. Cortical electrodes were lowered to 1.7-1.8 mm, TRN electrodes were lowered to approximately 4 mm and TRN recording was confirmed by the presence of fast-spiking cells. VPm and VL micropipettes were lowered to 4 mm depth and then electrical stimulation of the vibrissae was applied. Thalamic electrodes were further lowered until a maximal stimulus-evoked short-latency (<5 ms) response was detected. Once the optimal recording location was determined the current used for stimulation was increased until a maximal response was observed and then 50% of that current was used as the stimulation current, with a typical stimulation current of ≈1 mA.
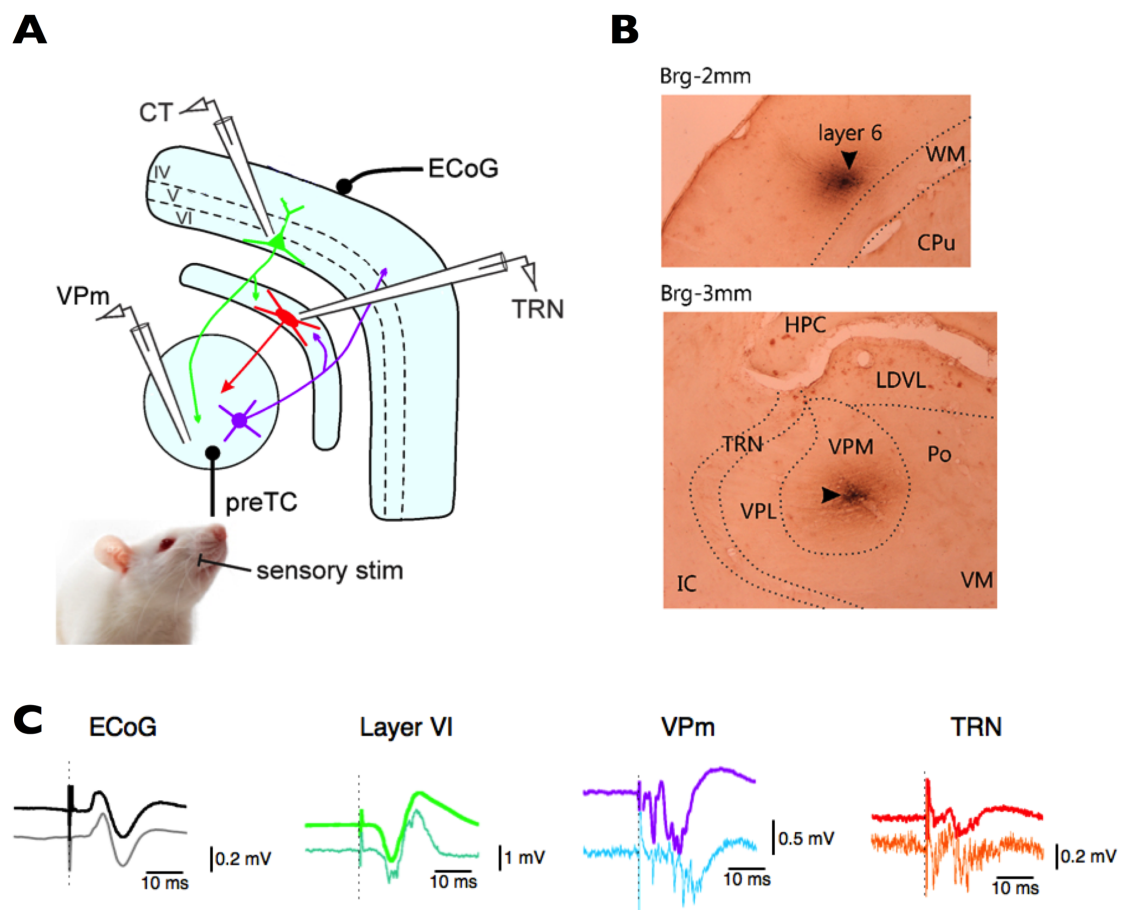
Figure 4.1 – Experimental Design for Simultaneous Recording of the Major regions of the Somatosensory TC-CT Networks

A) This diagram illustrates the experimental design used to investigate the function of TC-CT sensory networks. Glass micropipettes filled with ACSF and neurobiotin (1%, tracer) were inserted into the VPm, TRN and layer VI of the somatosensory cortex. Simultaneous ECoG was captured over the related somatosensory cortex with a Ag/AgCl wire electrode. Superimposed in colour is a diagram of the TC-CT projections in the somatosensory system. **B)** Micrographs made of post-mortem extracellular histological staining, showing regions ionphoretically marked with neurobitotin. These stainings highlight the locations of the primary recording pipettes in layer VI and the VPm. **C)** Sensory-evoked potentials in the primary recording regions. These are representative electrophysiolgical traces showing the average evoked potential (100 trials) in the bold, top trace and a typical single trial in the bottom trace. VPm and TRN traces were simultaneously recorded in a single experiment. ECoG and layer VI LFP were also recorded simultaneously from a separate recording session.

Experimental procedure involved 20-min recording session of spontaneously occurring electrophysiological activity while the rat received an electrical stimulation of the vibrissae every 10 s (Figure 4.1). Recordings were made under one of two drug conditions, saline control (1ml/kg, s.c.) or ketamine (2.5 mg/kg s.c.). A separate series of recordings were made with the topical application on the somatosensory cortex of TTX (100 μM) or ACSF control impregnated gelatine sponges (Figure 4.2).
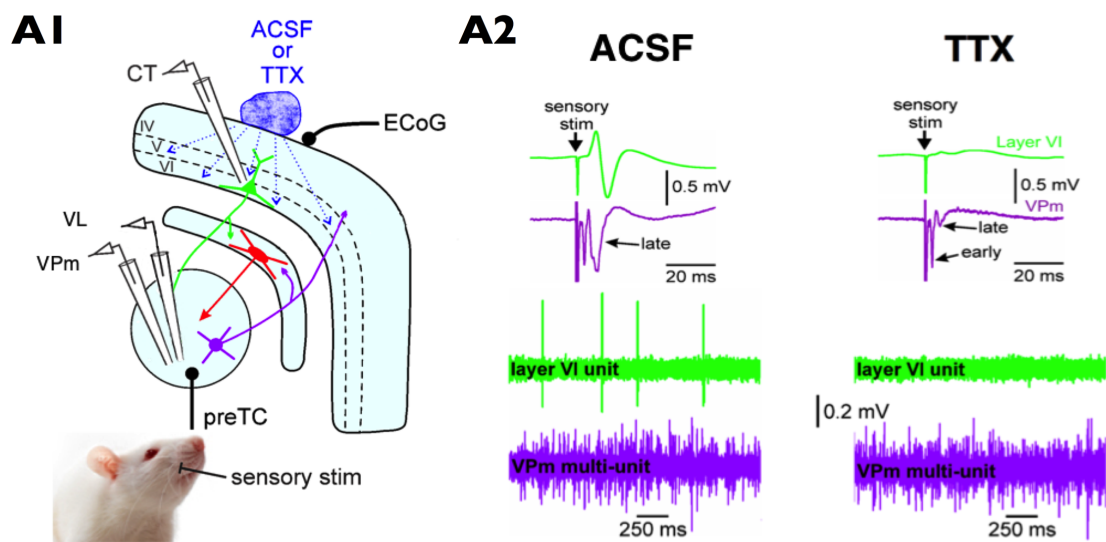
Figure 4.2 – Experimental Design of TTX Experiments for Identification of the CT Component in the Thalamic Sensory-Evoked Potential

A1) This diagram illustrates the experimental design used to investigate the role of the CT pathway on sensory-evoked responses in thalamic nuclei. Glass micropipettes filled with ACSF and neurobiotin (1%, tracer) were inserted into the VPm, VL, and layer VI of the somatosensory cortex. Simultaneous ECoG was captured over the related somatosensory cortex with a Ag/AgCl wire electrode. Superimposed in colour is a diagram of the TC-CT projections in the somatosensory system. Topical application of ACSF of TTX soaked gelatine sponges allowed the silencing of CT feedback. **A2)** TTX application silences cortical neuronal firing and abolishes cortical sensory-evoked potentials. This panel shows representative local field potential traces and average waveforms from layer VI and VPm recording electrodes after local topical application of ACSF (left) or TTX (right) to the parietal cortex surface. These recordings demonstrate that under ACSF conditions there is ongoing neuronal firing and characteristic evoked potentials in the Layer VI and VPm LFP recordings. After TTX administration cortical firing has ceasedin the LayerVI LFP trace while multi-unit activity in the VPm is unaffected; also the evoked response in the cortex has been abolished while only the second component has been affected in the VPm.

4.2.4 Electrophysiology and Histology

Neural activity recorded with glass micropipettes was amplified with ultra-low noise amplifiers. ECoG signals were processed with a bandpass of 1–800 Hz, LFP with a bandpass of 0.1-6 kHz, all signals were sampled at 20 kHz. Potential 50 Hz line noise was eliminated with Humbug selective noise eliminators (Quest Scientific, Hertfordshire, UK). To locate recordings sites, extracellular labelling (Pinault, 1996) using neurobiotin was performed at the end of the experimental sessions. The tracer was iontophoretically expelled from the glass micropipettes using a 600-nA current pulse, 200 ms on-200ms off for 5 min. Animals were than euthanised with an over dose of pentobarbital and trans-cardially perfused with saline (200 ml) followed by 500 ml of 0.1M pH 7.4 phosphate buffered saline (PBS) containing 4% paraformaldehyde and 0.5 % glutaldehyde. Serial coronal brain sections were cut at 100 μm with a vibratome then washed in PBS three times before being incubated in avidin-biotin-peroxidase complex (ABC, Vector labs, CA) overnight. The tracer was visualised using a DAB kit (Vector labs) and slices mounted on gelatine coated slides. Micrographs were taken using a Nikon E600 light microscope (Nikon France, Champigny-sur-Marne, France).

4.2.5 Analysis

Data analysis was performed with Clampfit 10 (Molecular Devices, CA), Matlab (Mathworks, MA) and Sciworks (Datawave Technologies, CO) software packages. Spectral analysis of ongoing spontaneously occurring activity was performed with fast Fourier transformation at a resolution of 2.5 Hz (unless specified otherwise). The power of baseline activity was analysed in four frequency bands: slow (1-14 Hz), β (16-29 Hz), γ (30-80 Hz) and high frequency oscillations (HFO, 81-250 Hz), total power in a band was calculated as the sum of all power within the frequency range. The time-course of γ frequency changes was calculated using

mean γ power in two-minute blocks, then normalising all data to the mean γ power in the 20-min saline control condition and expressing all values as a percentage of this. For the sensory-evoked calculations time-frequency analysis was performed on 1-s long epochs, extracted from the continuous data and centred on the time of stimulation. Epochs were manually inspected for artefacts and slow-wave activity; only trials occurring during a desynchronised state were used for analysis. Amplitude and latency of sensory-evoked responses in the thalamus was calculated by determining the minimal voltage in two windows 1-7 ms post stimulus for the 'early' and 7-15 ms post stimulus for the 'late' response. The same process was used in the cortical recordings using minima and maxima across a single window 3-20 ms post-stimulus. Spectral power was calculated for all trials using the newtimef function from the EEGLAB Matlab toolbox (Delorme and Makeig, 2004). Morlet wavelets were utilised in 80 evenly spaced frequency bands from 20 to 100 Hz, with wavelet cycles ranging from 3 at the lowest frequency to 10 at the highest frequency. Baseline γ power was calculated for each individual trial as the average power in the γ range from -400 to -50 ms before stimulus onset. Evoked γ power was calculated in the span 0 - 100 ms post stimulus. γ signal was defined as the per trial difference between the evoked and baseline γ power i.e. γ signal = evoked γ - baseline γ. γ power measures were averaged into minute blocks (10 data points). Correlation between γ signal and the amplitude of the 'late' thalamic response was calculated using Pearson's correlation between the mean VPm γ signal and the mean late amplitude of all recordings. Drug condition means were calculated using the periods 10-20 min post drug administration (100 data points). Statistical significance of the observed effects was evaluated with ANOVA, Student's t-test and Wilcoxon signed-ranked test performed in Prism 6 (GraphPad Software, CA); spectral power and specific frequency bands were compared using the non-parametric stastical Wilcoxon signed-ranked tests as the normality of the distribution of spectral power cannot be assumed (Korotkova et al., 2010).
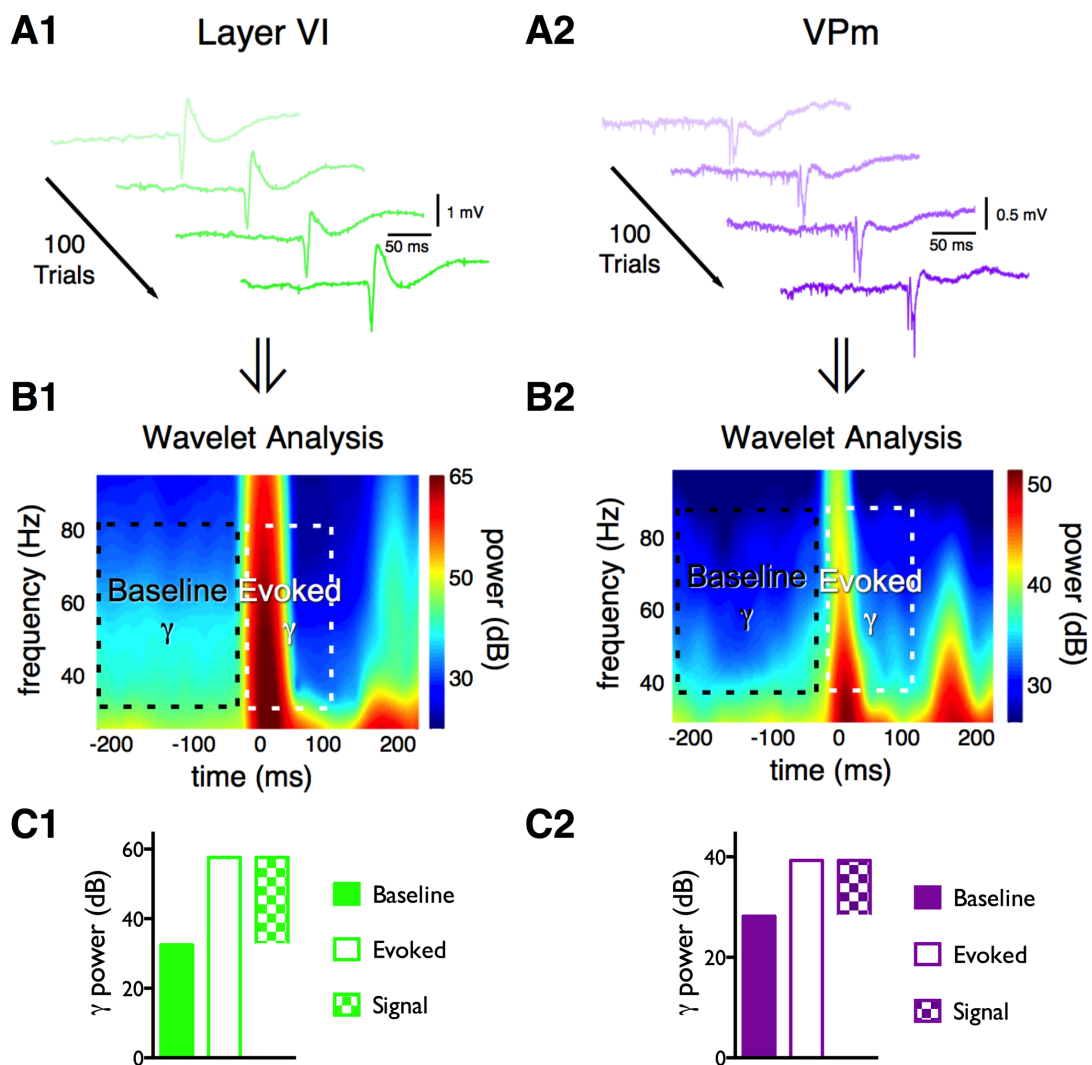
Figure 4.3 – Time-Frequency Analysis Using Morlet Wavelets to Determine the Gamma Signal-to-Noise Ratio

This figure gives a schematic overview of the analysis strategy used to calculate baseline and evoked γ power and the corresponding γ signal. **A1-2)** The sensory pathway is challenged through repeated (0.1 Hz) electrical stimulation of the whisker teguments, causing stereotypical evoked responses in both the related cortex (layer VI) and thalamus (VPm). 1-second epochs centred on the stimulus were extracted for time-frequency analysis. **B1-2)** The extracted epochs were decomposed using a sliding wavelet window to produce a multidimensional time × frequency × trial matrix. From this we were able to extract the γ power in the pre-stimulus baseline and the γ power evoked by the sensory stimulation. **C1-2)** By calculating the baseline and evoked γ power for each individual trial we were able to calculate the average baseline and evoked γ power. By subtracting the difference between the two we calculate an estimation of the γ signal, the ratio of the ongoing noise to the evoked response.

4.3

Results

4.3.1 Ketamine has Opposite Effects on Ongoing and Sensory-Evoked Gamma Oscillations Throughout the TC-CT Somatosensory Pathway

To examine the effects of a psychotomimetic dose of ketamine on oscillatory activity throughout the somatosensory neural circuit, we conducted simultaneous multisite recordings in the VPm, TRN and the related parietal somatosensory cortex. Spectral analysis of these recordings demonstrated that in all cases (ParCx & VPm, n =7; TRN, n =5; Layer VI, n = 3) there was a significant (Wilcoxon matched-pairs test: $p < 0.001$) increase in the total power of γ and HFO bands following ketamine administration. The parietal ECoG recording displayed significant increases in the beta (Wilcoxon matched-pairs test: $p = 0.014$), γ ($p < 0.001$) and HFO ($p < 0.001$) frequency bands.

As well as the distinct frequency bands, analysis of the total spectra showed significant differences under saline and ketamine conditions in each recording site ($p < 0.0001$, Wilcoxon matched-pairs test). This is illustrated in Figure 4.4 B, where pooled data from 3 animals for each recording site are displayed. These results were in line with previous studies we have conducted (Kulikova et al., 2012; Pinault, 2008) showing increases in cortical γ and HFO following systemic NMDAr antagonist administration and replicates and expands upon our previous finding demonstrating the increases in aberrant γ activity throughout subcortical structures (Hakami et al., 2009).
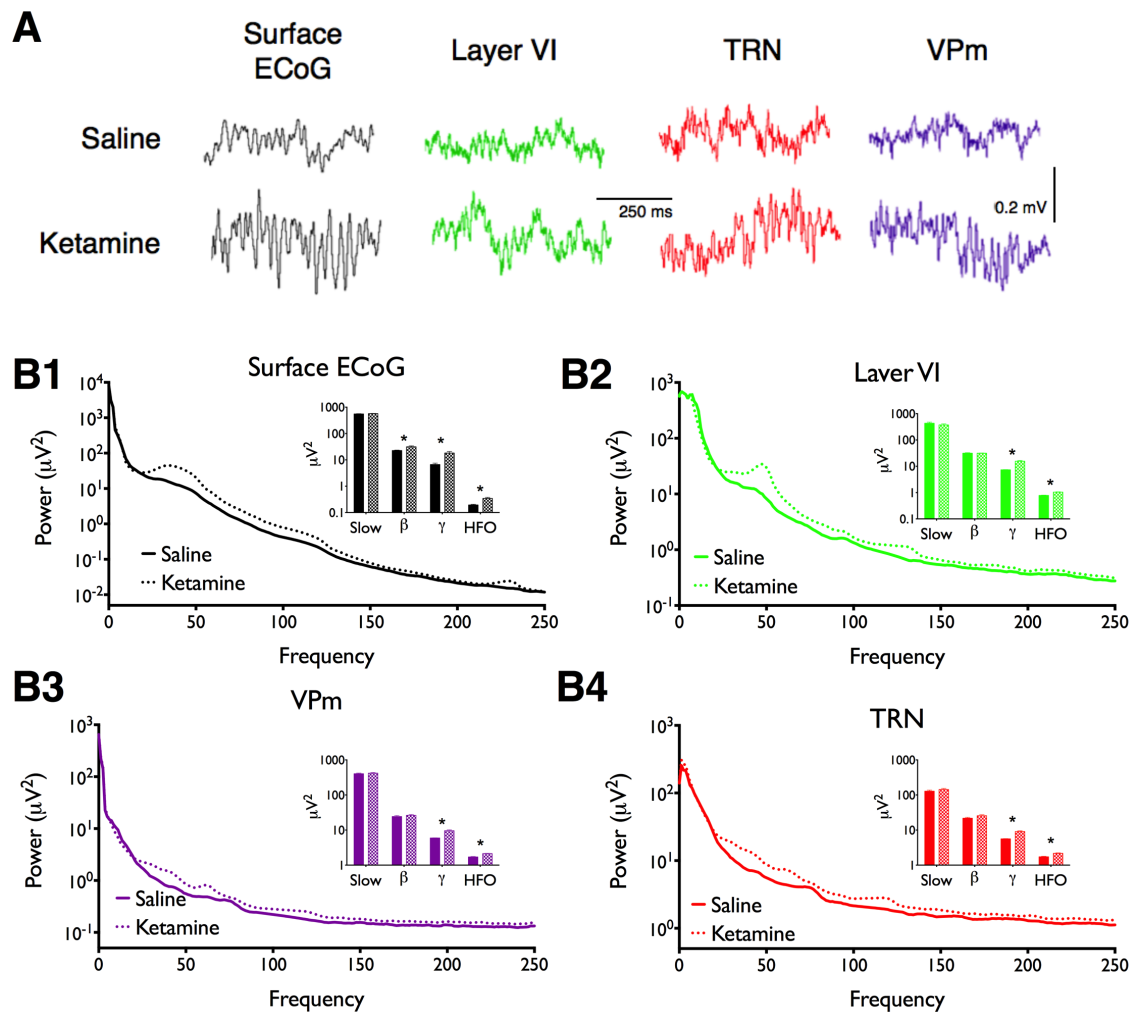
Figure 4.4 – Effects of Ketamine on Ongoing Oscillations in the Somatosensory Cortex and Related Thalamus

A) Representative traces from the different recordings sites of the somatosensory TC-CT network. These traces were taken in the period 10-20 min after administration of either saline (1 ml/kg, s.c.) or ketamine (2.5 mg/kg, s.c). Ketamine increases the amplitude of the bouts of γ oscillations. **B1-4)** Panels B1 through B4 show mean power spectra under both saline (thick line) and ketamine (dotted line) conditions, from the surface ECoG of the parietal somatosensory cortex (vibrissae area), the corresponding layer VI LFP and the related VPm and TRN LFPs. VPm and TRN recordings were made simultaneously and show pooled data from 3 separate animals. Layer VI and parietal cortex ECoG were acquired simultaneously and represent pooled data from 3 different recordings sessions. Each panel shows the mean of a 10-minute recording period, 10 minutes following drug administration, Ketamine injection (2.5 mg/kg, s.c) significantly increased the power at all recording sites (Wilcoxon matched-pairs test: p < 0.0001). The inset histograms show the average power at four frequency bands: slow (1–15 Hz), β (16–29 Hz), γ (30–80 Hz or GFO) and HFO (81–250 Hz); ketamine specifically increased the power of γ and HFO in all recording sites, as well as increasing beta frequency power in the ECoG. (* p < 0.001, Wilcoxon matched-pairs test).

We also investigated the effects of a sub-anaesthetic dose of ketamine on the first stages of sensory information processing throughout the somatosensory circuit. The system was challenged with repetitive (0.1 Hz) electrical stimulation of the vibrissae to generate sensory input and the subsequent evoked oscillations were analysed utilising the time-frequency techniques outlined in Figure 4.3. The baseline or pre-stimulus γ activity mirrored that seen when assessing the ongoing γ with FFT analysis (Figure 4.4). That is, ketamine caused an increase in the power of baseline γ oscillations that returned to pre-drug levels within approximately 45-60 minutes, (Figure 4.6 A & B). This was seen in all recording sites analysed and in all experimental animals (ParCx & VPm n = 9, TRN n = 5, Layer VI n = 3 animals). These effects were broadly similar between the LFP recordings with the VPm, TRN and layer VI showing maximum increases in ongoing gamma power of 227%, 209% and 210% respectively; the surface parietal cortex ECoG recording showed a maximal change of 360%.

As well as increasing the ongoing γ activity throughout the somatosensory pathway ketamine administration leads to a decrease in the γ signal-to-noise ratio, reducing the sensory-evoked γ signal in these regions. This is best illustrated in the representative heatmaps of the time-frequency data from the layer VI and VPm LFP recordings taken from a single experiment (Figure 4.5). These images demonstrate that under saline conditions there is persistent ongoing γ activity and a distinct large burst of evoked response from the sensory stimulation, followed by a decrease in activity. Under ketamine conditions the pre-stimulus baseline activity in the γ frequency band is increased and there is a substantial reduction in the magnitude of the sensory stimulation evoked response. This is best illustrated in the bottom panels, which show the difference between the ketamine and saline conditions. There is a clear increase in baseline γ power and a substantial reduction in the evoked response following the stimulus; at the time of stimulus, the decrease in

power response following the sensory stimulation is also attenuated. Ketamine increases the ongoing γ power and decreased the sensory-evoked γ signal. Figure 4.6 A1 & A2 shows the time-course of these ketamine induced changes and it is clear to see the opposite nature of ketamine's effects on the ongoing and sensory-evoked γ activity. Two-way ANOVA's revealed that the effects of ketamine were significant on both baseline γ ($F_{(1,23)}$ = 106.3, p < 0.0001) and γ signal ($F_{(1,23)}$ = 138.9, p < 0.0001), with post-hoc tests revealing significant differences between saline and ketamine conditions in all recording sites for both baseline γ and γ signal (p < 0.001, Bonferroni's multiple comparisons test, Figure 4.6 C).
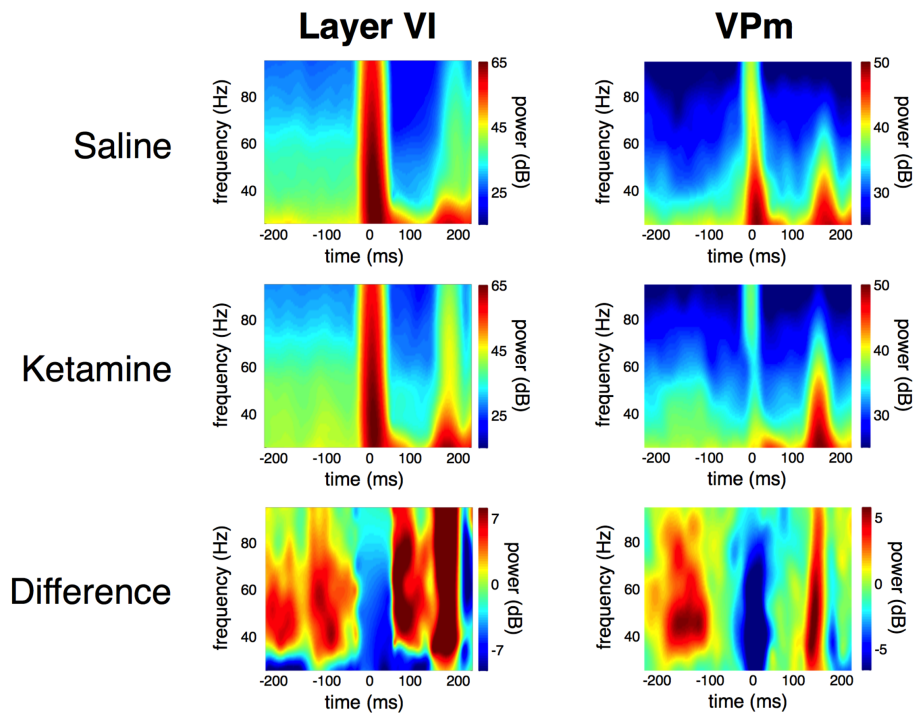
Figure 4.5 – Ketamine has Opposite Effects on Ongoing and Sensory-Evoked Gamma Activity in both Layer VI and the VPm

This figure shows representative heatmaps generated through time-frequency analysis of the sensory-evoked waveforms from layer VI of the parietal cortex and the VPm. The top panels show under saline conditions persistent baseline electrophysiological activity and large evoked responses at time = 0. The middle panels demonstrate that under ketamine conditions the baseline γ activity is increased and the corresponding stimulus triggered response is reduced. This is further highlighted in the bottom panels showing the difference between the ketamine and saline conditions: an increase in pre-stimulus gamma power and decrease in the stimulus triggered response, data taken from 10-20 min after drug administration.
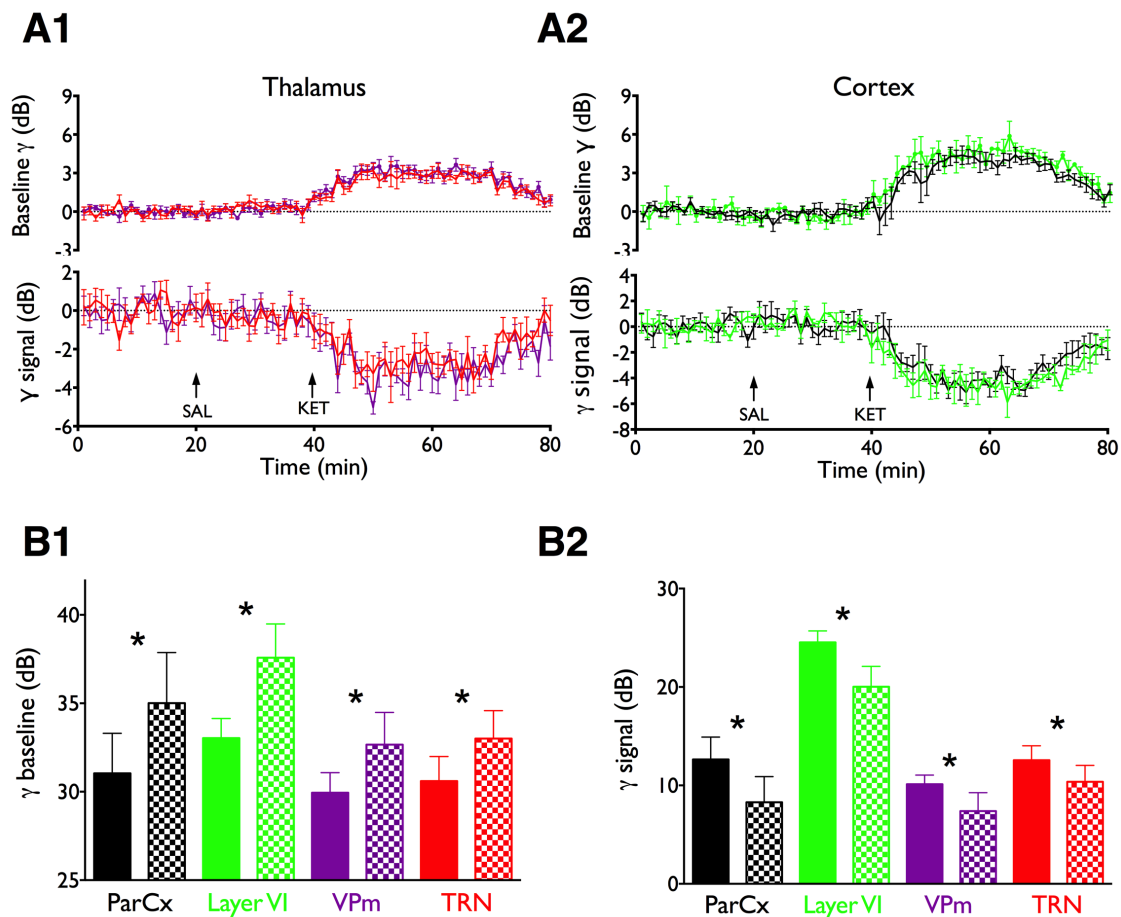
Figure 4.6 – Ketamine has Opposite Effects on the Baseline and Sensory-Evoked Gamma Signal Throughout the TC-CT Somatosensory System

A1-2) Panels A1 and A2 show the time-course in changes to both the γ baseline (top section) and γ signal (bottom section) in the thalamus (A1) and cortical (A2) recording regions. Baseline γ was calculated as the mean power in the frequency range 30 – 80 Hz, 250 – 50 ms preceding the stimulus onset. γ signal was determined by calculating the evoked γ power (mean power 30 – 80 Hz, 0 – 100 ms post stimulus) and subtracting the γ baseline power on a trial by trial basis. **B1-2)** Histograms that represent the mean baseline γ power (B1) and mean γ signal (B2) in the 10 min period following injection with saline (filled bars) or ketamine (checked bars). Ketamine causes significant increases in γ baseline at each recording site and similar decreases in the stimulus elicited γ signal were seen at all recording sites in the somatosensory pathway. * p < 0.01, Bonferonni's multiple comparisons test. In all panels data represents the mean ± SEM of ParCx & VPm n = 9, TRN n = 5, Layer VI n = 3 animals

4.3.2 Latency of Sensory-Evoked Responses Indicate CT Feedback Regulates the Late Thalamic Response

The extended γ evoked response seen in the 100ms post-stimulus period should contain the effective results of multi-synaptic cortico-cortical and corticofugal integration processes; as ketamine administration disrupted the γ signal-to-noise ratio in this period we next decided to investigate the role of CT pathways in the generation of sensory-evoked γ oscillations. We observed that the sensory-evoked response recorded in the VPm and TRN was characterised principally by two distinct negative going components; a short-latency (3.75 ± 0.01 ms) and long-latency (10.5 ± 0.1 ms) component. Qualitative and quantitative analysis of the sensory-evoked response in the both the thalamus and the cortex was undertaken and the 'late' response was hypothesised to be the result of CT feedback arriving in the thalamic nuclei.

The results of this analysis support the hypothesis of the involvement of CT feedback processes, in agreement with (Kublik et al., 2003). Figure 4.7 illustrates typical individual and average responses observed simultaneously from the layer VI and VPm LFP electrodes following sensory stimulation. The two distinct separate responses seen in the thalamus indicated the possibility of two mechanisms of generation; the latency of these responses and those observed in the cortical LFP support the hypothesis that CT activity is driving the second or 'late' response. Quantitative analysis of the latencies of the sensory-evoked responses also supported this hypothesis, Table 4.1 records the latencies of the early and late responses in various recording sites throughout the TC-CT pathway. The early thalamic response preceded the cortical evoked response in every recording made, with the thalamic late response in turn following the cortical activation. An example of population spiking consistent with layer VI firing related to the late thalamic response is shown in Figure 4.7 B.
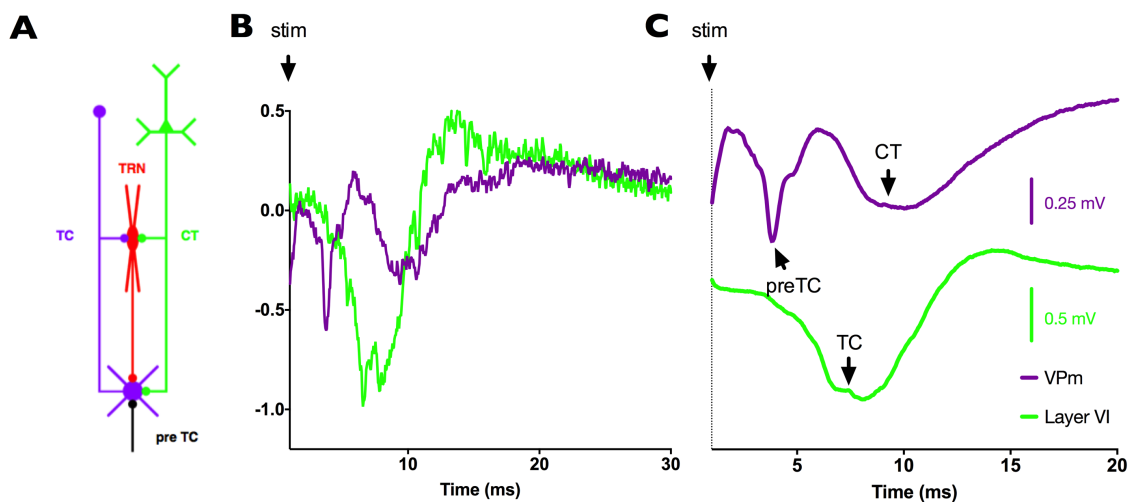
Figure 4.7 – Timing of Sensory-Evoked Response in the Somatosensory TC-CT Circuit

A) Schematic 'wiring' diagram of the somatosensory TC-CT circuit demonstrating the anatomical connections between the separate components of the system. Incoming information relayed via the trigeminal nerve arrives in the VPm nuclei of the thalamus and is relayed to the cortex, where corticofugal fibres originating in layer VI send feedback returning to the thalamus. **B)** Single trial simultaneous recordings in the VPm and Layer VI of the somatosensory cortex. **C)** Average sensory-evoked responses in VPm and layer VI recordings. Panels B and C demonstrated the latencies of sensory-evoked activity in the TC-CT circuit support a hypothesis where pre-thalamic input evoked a population response in the VPM, which in turn triggers population spiking in the somatosensory cortex. CT feedback is relayed via layer VI neurons eliciting a second 'late' response in the thalamus; the proposed origin of the discrete evoked responses in marked by arrows in panel C.

Table 4.1 – Latencies of Sensory-Evoked Potentials Throughout the TC-CT Somatosensory Pathway

	VPm		TRN		Layer VI		Surface ECoG	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Early	3.74	0.007	3.17	0.01	8.22	0.01	9.31	0.07
Late	10.51	0.02	10.90	0.03	15.10	0.01	15.98	0.02

Mean and standard deviations of the early and late sensory-evoked potentials in the thalamus (VPm), TRN and somatosensory cortex (surface ECoG and the related layer VI LFP). The temporal order of the sensory-evoked response supports a hypothesis where CT feedback is responsible for eliciting the second, late response in the VPm and TRN. Mean and standard deviations are produced from of ParCx & VPm n =9, TRN n =5, Layer VI n = 3 animals.

4.3.3 Cortical Application of TTX Alters the Sensory-Evoked Activity in the Thalamus

To determine whether the observed sensory-evoked late response in the VPm and TRN involved the CT pathway, neuronal firing in the somatosensory cortex was blocked using TTX. Gelatine surgical sponges were immersed in either 100 μM TTX or ACSF before being applied directly to the parietal cortex surface. As illustrated in Figure 4.2 TTX application resulted in the cessation of recorded unit firing in layer VI of the somatosensory cortex while not affecting observed multi-unit activity in the VPm. Furthermore the stereotypical evoked response to sensory stimulation observed in the cortex was abolished following TTX applications, whilst ACSF had no discernible effect. In support of our hypothesis that the 'late' response observed in thalamic recording sites was mediated through CT pathways, TTX application also greatly attenuated the amplitude of this long-latency negative wave. The time course of these effects is illustrated in Figure 4.8, in the cortex the layer VI LFP recorded large amplitude early and late evoked responses, both of which rapidly decrease following the topical application of TTX (panel A1).

The parietal cortex ECoG showed a smaller early response than the LFP recordings and an equivalently smaller decline in the first response, the larger second response was diminished much the same as the LFP recordings. Two-way ANOVA of the amplitudes of the evoked response in the period 10 - 20 min after TTX or ACSF application showed significant effects for drug for both early and late responses ($F_{(1,15)}$ = 12.19 p < 0.005 and $F_{(1,15)}$ = 25.1 p < 0.0001 for early and late effects respectively). Post-hoc tests showed significant differences in the layer VI LFP amplitude at both early and late responses and significant changes only in the late response in the parietal cortex ECoG (p < 0.05 Bonferroni's multiple comparisons). When performing TTX experiments simultaneous recordings were also made in the VL nuclei; the VL receives basal ganglia input and should not be affected by manipulations of the somatosensory pathways serving as a control

recording for these experiments. Recorded activity in the VPm displayed two prominent negative going amplitudes, one early (amplitude: 0.52±0.01 mV; latency: 3.7±0.01 ms) and one late (amplitude: 0.83±0.01 mV; latency: 10.5±0.3 ms). In the VL some response to the sensory stimulation was detectable but of a lower amplitude (early amplitude: 0.1±0.01 mV, late amplitude: 0.27±0.01 mV). The mean amplitude of the early, responses showed no discernible effect following TTX application, supporting the hypothesis that their source is pre-thalamic, represent incoming information from the trigeminal nerve, via the brain stem. The 'late' response however showed a clear effect of TTX application in the VPm, but not the VL. Two-way ANOVA showed no effect of drug on the early response, location was statistically significant ($F_{(1,8)}$ = 11.09, p < 0.05). For the late response both drug ($F_{(1,9)}$ = 18.35, p < 0.005) and location ($F_{(1,9)}$ = 6.89, p < 0.05) were significant effects, with post-hoc tests showing the late VPm response was different (p < 0.05 Bonferroni's multiple comparisons).

The time course of these effects is illustrated in Figure 4.8, in the cortex the layer VI LFP recorded large amplitude early and late evoked responses, both of which rapidly decrease following the topical application of TTX (panel A1).   The parietal cortex ECoG showed a smaller early response than the LFP recordings and an equivalently smaller decline in the first response, the larger second response was diminished much the same as the LFP recordings. Two-way ANOVA of the amplitudes of the evoked response in the period 10-20 after TTX or ACSF application showed significant effects for drug for both early and late responses ($F_{(1,15)}$ = 12.19 p < 0.005 and $F_{(1,15)}$ = 25.1 p < 0.0001 for early and late effects respectively). Post-hoc tests showed significant differences in the layer VI LFP amplitude at both early and late responses and significant changes only in the late response in the parietal cortex ECoG (p < 0.05 Bonferroni's multiple comparisons).
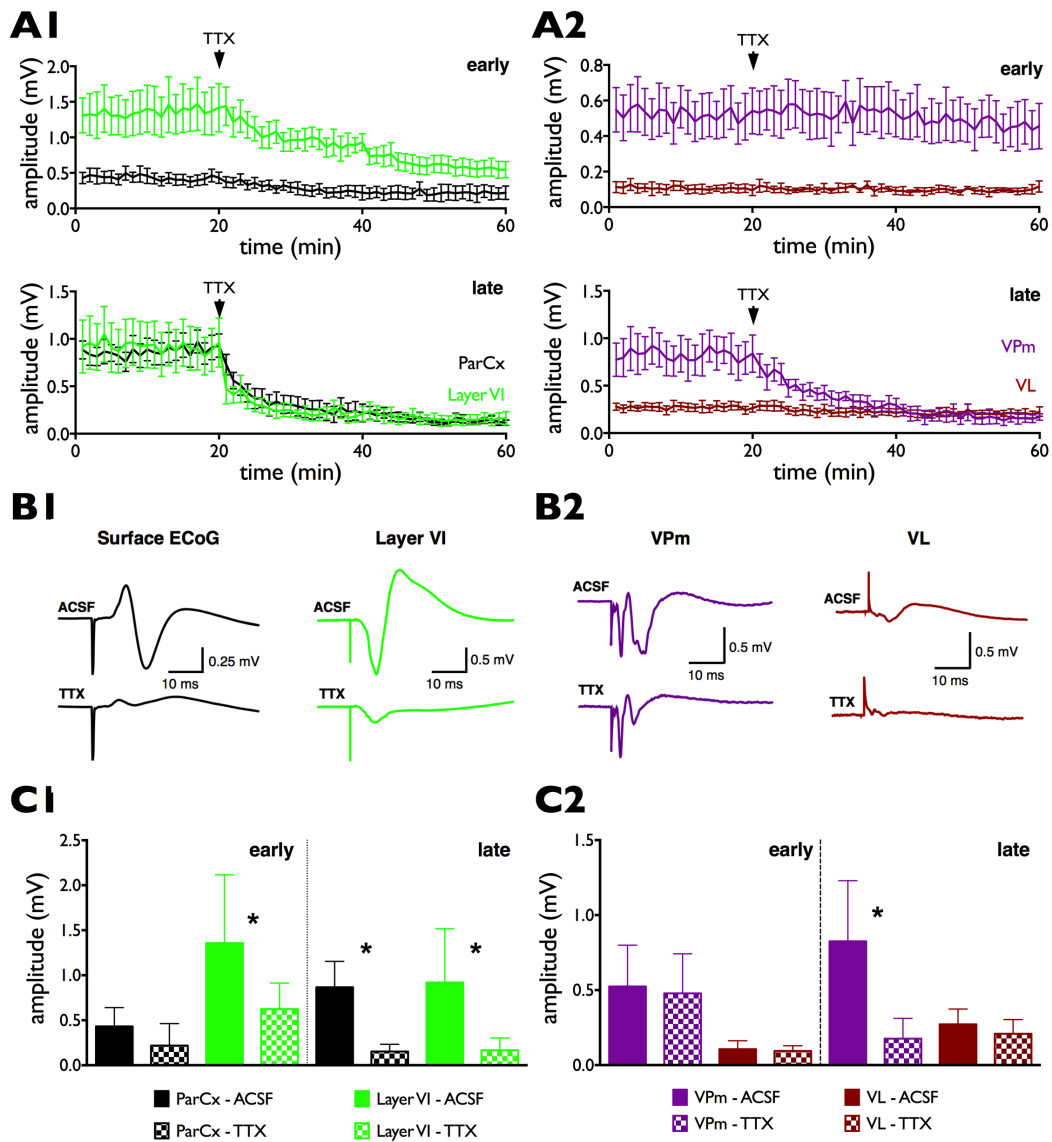
Figure 4.8 – TTX Application to the Cortical Surface Reveals the 'late' Thalamic Evoked Response involves CT Feedback

A1-2) Time course of TTX's effects on cortical (A1) and thalamic (A2) sensory-evoked response amplitudes. Local application of TTX to the cortical surface causes a profound decrease in the amplitude of both the early and late response in ParCx ECoG and Layer VI LFP recordings. In the thalamic recordings TTX has no effect on either the VPm or the control area VL early response, while it does significantly decrease the late amplitude in the VPm alone. Data points represent the average amplitude in each minute of recording (10 trials), data SEM ± mean of n = 7 in ParCx, 6 in Layer VI, 5 in VPm and VL. **B1-2)** Representative sensory-evoked potentials recorded simultaneously from the somatosensory parietal cortex (B1) and thalamic nuclei (B2), after topical application of ACSF (top traces) and TTX (bottom traces). Traces represent the average of 120 trials taken 20 - 40 min after drug administration. **C1-2)** Bars represent mean ± SEM of average amplitude 10 - 20 min post application of ACSF (C1) or 20-30 minutes post TTX (C2). * p < 0.001, Bonferonni's multiple comparisons test. Animal numbers are: ParCx n = 7, Layer VI n = 6, VPm, and VL n = 5.

4.3.4 Reducing CT Feedback Attenuates the Gamma Signal in the Thalamus

We have previously established that the amplitude of the cortical sensory-evoked response is positively correlated with the power of the sensory-evoked γ response (Kulikova et al., 2012). We further investigated the TTX effects on the TC-CT pathways in the somatosensory system through time-frequency analysis of the sensory-evoked activity. Utilising Morlet wavelet decomposition of the LFP and ECoG recordings we assessed the effects of TTX application on sensory-evoked γ signals (Figure 4.9). Unsurprisingly, TTX had a pronounced effect on the γ frequency activity in the somatosensory parietal cortex, causing a rapid decreases in evoked γ signal, seen in both the layer VI LFP and the parietal cortex ECoG (Figure 4.10 A1). In the thalamus TTX application also triggered a decrease in the evoked γ signal recorded in the VPm, while leading to only a slight decrease in the γ signal observed in the VL (Figure 4.10 B2).

Two-way ANOVA of the mean γ signal recorded in in the 10-20 min following TTX or ACSF administration revealed significant effects of TTX ($F_{(1,22)}$ = 107.7, $p < 0.0001$) and a significant interaction between TTX administration and the location recorded from ($F_{(3,22)}$ = 5.82, $p < 0.01$). Post-hoc analysis revealed significant differences between ACSF and TTX conditions in the parietal cortex ECoG, layer VI LFP and VPm LFP ($p < 0.0001$, Bonferroni's multiple comparisons test) (Figure 4.10 B). There was no significant difference in evoked γ signal recorded in the VL between ACSF and TTX conditions. This result further supports the hypothesis that CT feedback projections mediate the sensory-evoked response in the VPm, and that they are essential for evoking γ signal activity. We also demonstrated a strong positive correlation between the amplitude of the 'late' CT mediated evoked response in the VPm (r^2 = 0.957, $p < 0.0001$), this relationship was much weaker for the 'early' evoked response (r^2 = 0.147, $p < 0.005$).
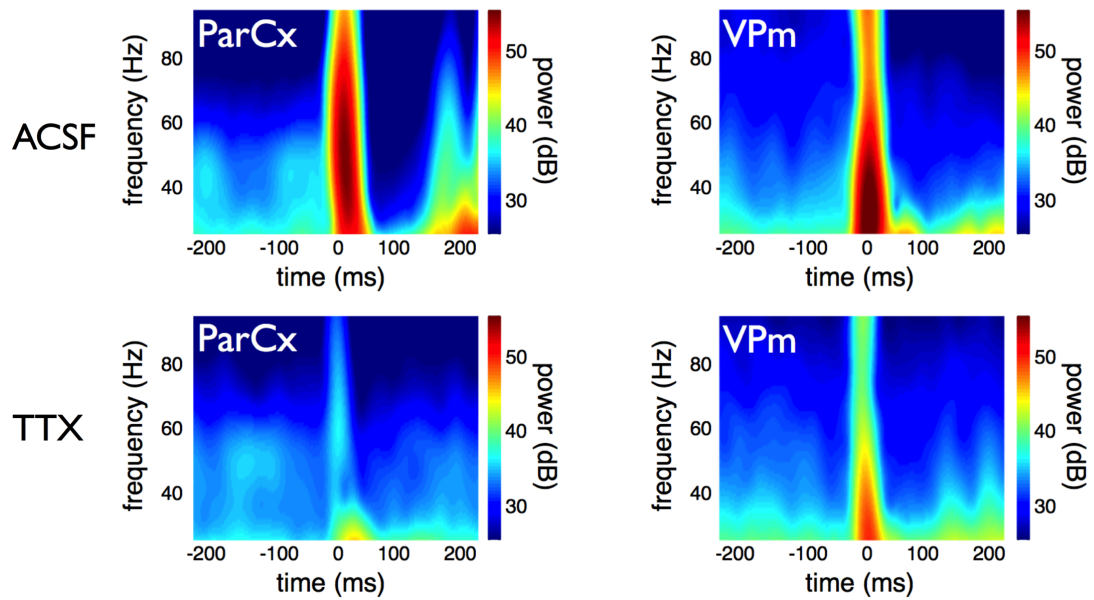
Figure 4.9 – Time-Frequency Analysis of TTX Effects on Sensory-Evoked Responses in the VPm and Layer VI of the Related Somatosensory Cortex

Representative heatmaps generated through time-frequency analysis of the sensory-evoked waveforms from the parietal ECoG and related thalamic (VPm) LFP. The top panels show show ongoing followed by sensory-evoked activities under control condition (local, topical application of ACSF onto the somatosensory cortex). The bottom panels show ongoing followed by sensory-evoked activities after (~20-30 min) TTX application to the cortical surface. The evoked response in the ParCx is greatly diminished and the response in the related VPm is also reduced.
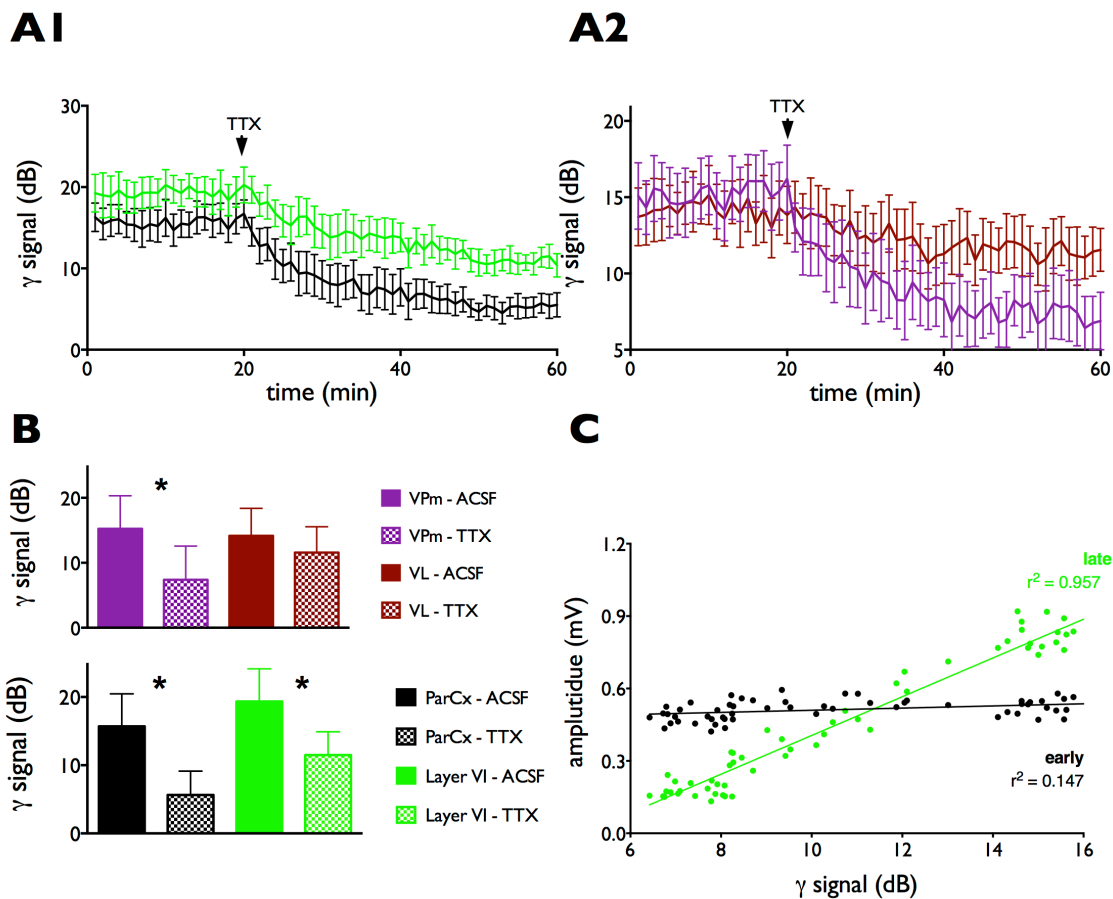
Figure 4.10 – TTX Effects on the Power of Sensory-Evoked Gamma Responses in the VPm and Layer VI of the Somatosensory Cortex

A1-A2) Time course of TTX's effects on cortical (A1) and thalamic (VPm and VL, A2) γ signal. The sensory-evoked γ signal is greatly diminished in the cortex and related VPm. A non-significant slight decrease is recorded in the VL. Each point represents the mean ± SEM of ParCx & VPm n = 7, VL & Layer VI n = 6 animals. **B)** Histograms highlighting drug effects, data are taken from 10-20 min post drug administration and represent 100 trials from ParCx & VPm n = 7, VL & Layer VI n = 6 animals. TTX administration causes a decrease in all areas, with this reaching significance in ParCx, Layer VI and VPm, * = p < 0.05, Bonferonni's multiple comparisons test. **C)** Correlation between the amplitude of sensory-evoked responses in the VPm and the γ signal; data points are from 20 min before through 40 min after TTX application to the cortical surface and represent the mean of 7 animals. Early VPm amplitude has a much weaker relationship to the strength of the γ signal while the second 'late' component is highly correlated to the sensory γ signal, both correlations are significant (p < 0.001, Pearson's correlation)

4.4

Discussion

The major novel result of this study is the finding that the CT pathway significantly contributes to the generation of γ oscillations in the thalamus during information processing of somatosensory input. We demonstrated a specific role of CT feedback in the generation of sensory-evoked γ oscillations in the thalamus by blocking the CT pathway with local application of the sodium channel blocker TTX. Silencing firing in layer VI of the somatosensory cortex led to the attenuation of a distinct 'late' component of the sensory driven response. This CT response was strongly correlated with the magnitude of sensory-evoked γ response in the thalamus, lending support to the hypothesis that CT feedback is driving this γ activity. This was further supported by the fact that activity in the VL nuclei of the thalamus was not significantly affected by the blockade somatosensory driven CT feedback.

This result supports research that posits CT feedback plays a role in enhancing and modulating thalamic responses to perceptual stimuli (Alitto and Usrey, 2003). Thalamic sensory relay neurons have specific receptive fields; areas of stimulation that elicit an excitatory response. In the somatosensory system, VPm neurons react to specific whisker input (Waite, 1973). It is hypothesized that corticothalamic input serves to sharpen and adjust the profile of thalamic receptive fields and evidence for a role of CT feedback in thalamic perceptual processing has been established in visual (Murphy and Sillito, 1987) auditory sensory domains (Suga et al., 2000), and somatosensory domains (Ghazanfar et al., 2001; Krupa et al., 1999). A role has also been suggested for CT feedback in enhancing extraction of salient information from 'noisy' background environments (Sillito and Jones, 2002). Given the now well established role of γ frequency activity in perceptual and sensory processes (Brosch et al., 2002; Gray et al., 1989; Palva, 2005; Ribary et al., 1991; Ribary, 2005; Singer and Gray, 1995; Tallon-Baudry and Bertrand,

1999; Traub et al., 1996) the γ activity we observed is likely related to the early stages of information processing. The disruption caused to this γ activity by blocking CT feedback indicates that the CT feedback loop plays a specific role in generating γ oscillations related to the information processing of sensory input. CT feedback to the thalamus originates in cortical layer VI and can either directly innervate thalamic neurons or activate inhibitory neurons in the TRN that synapse into the thalamus. The TRN is a thin GABAergic layer that interfaces between the thalamus and cortex, modulating TC activity and playing a key role in the integration of multiple diverse cognitive activities (Ferrarelli and Tononi, 2011; Pinault, 2011). The TRN's GABAergic constitution has specific γ frequency pacemaker properties (Pinault and Deschênes, 1992) that further indicate its relevance in γ oscillatory deficits. The present study does not establish whether CT feedback is acting directly or through TRN input to generate γ activity but given the role of the TRN in γ oscillations in TC and CT networks this is a likely target and warrants further investigation.

In the present study we also demonstrated that an acute sub-anaesthetic dose of the psychotomimetic ketamine reduces sensory-evoked γ signal-to-noise ratio in both TC and CT pathways of the somatosensory network. This finding supports our previous study that established NMDAr hypofunction impairs the ability of the somatosensory TC networks to distinguish sensory-evoked γ activity from the ongoing background γ noise (Kulikova et al., 2012). It also expands this result to include not just cortical γ oscillations but also activity in the VPm and TRN of the dorsal thalamus. In this study we demonstrated that the VPm and TRN γ activity is disrupted through the actions of NMDAr antagonists and this could contribute to the sensory and cognitive abnormalities NMDAr blockade engenders. Further study on the role of TRN modulation of TC and CT pathways in sensory processing will

be essential to understanding the dynamics involved and their relationship to schizophrenia.

It is important to note potential limitations of the current study; as it was conducted solely in an anaesthetised preparation this limits the interpretation able to be made. It is known that the use of anaesthesia can affect the spiking activity of neurons in electrophysiological recordings, which could alter the results seen (Massaux, 2004). The use of anaesthesia has also been shown to alter oscillatory dynamics, with deep anaesthesia inducing a state reminiscent of slow-wave sleep (Bennett et al., 2009), which would also alter the results seen here. These confounding factors are somewhat ameliorated by our use of neurolept anaesthesia during our active recording portion of the experiments. This form of anaesthesia allows for more periods of desynchronised activity, reminiscent of the awake state. Furthermore, our previous studies conducted (Kulikova et al., 2012) obtained similar results in both anaesthetised and awake preparations giving confidence that the basic physiological properties underlying these observations persist in both circumstances. However, to be completely confident of the accuracy of these results the experiments would need to be supplemented with recordings made in awake head-restrained animals to validate the results.

A single sub-anaesthetic dose of ketamine was sufficient to increase the baseline γ noise throughout the cortex and sub-cortical areas and reducing the sensory-evoked γ response, resulting in a decreased γ signal-to-noise ratio throughout the TC-CT somatosensory network. These findings further bolster the existing literature that hypothesises the existence of abnormalities and dysconnectivity in TC circuits in schizophrenia (Clinton and Meador-Woodruff, 2004; Ferrarelli and Tononi, 2011; Friston, 2002; Lisman, 2012; Pinault, 2011; Stephan et al., 2006; 2009; Uhlhaas, 2013) and is in line with clinical reports of dysfunctional neuronal oscillations in patients with schziophrenia (Herrmann and

Demiralp, 2005; Krishnan et al., 2009; Lee et al., 2003; Light et al., 2006; Maharajh et al., 2010; Spencer et al., 2003; 2004; Uhlhaas et al., 2006). Increasing evidence from animal models (Kulikova et al., 2012; Saunders et al., 2012) and human studies (Spencer, 2011; Suazo et al., 2012) suggests that elevations in baseline γ activity represent increased 'noise' in neural networks, and that this may impair γ-mediated sensory-evoked responses and information processing. Our findings lend further support to this hypothesis, with the NMDAr antagonist ketamine leading to an elevation in diffuse γ noise throughout the cortical and subcortical structures, including the somatosensory TC-CT system.

This finding raises interesting questions about the mechanisms that lead to the broadly psychotomimetic effects of NMDAr antagonists. NMDAr antagonists cause a wide variety of behavioural deficits, spanning a range of cognitive domains (Bubeníková-Valešová et al., 2008). The role of CT feedback in generating sensory-evoked γ oscillations indicates that this dysfunction may be driven by top down cortical disturbances, rather than bottom up abnormalities in sensory transmission. The present study can neither rule out nor confirm that NMDAr antagonists act through CT driven dysfunction, but the role of CT feedback does indicate that top-down cortical processes are clearly important in sensory γ activity.

The role of CT feedback also provides a potential explanation for the detrimental effects that ketamine has on the sensory γ signal-to-noise ratio. If ketamine is acting to interfere with cortico-cortico and CT communication it could disrupt the necessary feedback loops that are required to generate coherent oscillations (Contreras et al., 1996; Jones, 2001).The mechanisms by which NMDAr blockade leads to such disrupted neural transmission is not yet clear but is thought to be the result of disinhibition of pyramidal neurons (Moghaddam, 2003; Nakazawa et al., 2012). NMDAr blockade can preferentially target GABAergic interneurons (Homayoun and Moghaddam, 2007), which leads to the disinhibition

of pyramidal cells (Jackson et al., 2004) and an increase in glutamate release (Deutch et al., 1987; Verma and Moghaddam, 1996) and markers of increased neuronal activity (Moghaddam et al., 1997). This hyper-gultamatergic state and the disinhibition of pyramidal neurons would interfere with the ability to generate coherent synchronised activity and effectively transmit information through neural networks (Lisman et al., 2008).

These are the same effects of NMDAr antagonism that are hypothesised to cause the increase in ongoing γ oscillations (Hakami et al., 2009; Moghaddam, 2003). Whether the mechanisms through which the sensory-evoked γ signal is disrupted and the excessive γ baseline noise is generated is still unknown. The opposite effects of NMDAr antagonists on the magnitude of ongoing and sensory-evoked γ activity would indicate that they are governed by different mechanisms, as would the involvement of CT feedback loops in the generation of sensory-evoked γ. In this study and previous work (Kulikova et al., 2012) we have demonstrated the NMDAr antagonists administration can clearly interfere with both aspects of γ oscillations in sensory networks, but further research into the distinct mechanisms of sensory-evoked γ is still needed. Our finding of the importance of CT mediated feedback to the generation of thalamic sensory-evoked γ gives an indication that TC-CT and potentially cortico-thalamo-cortical networks will be involved.

A recent review by (Gandal et al., 2012b) suggests that abnormalities in γ frequency oscillations are ubiquitous in schizophrenia. As such, the γ signal-to-noise ratio may be a useful translational biomarker to study the health and function of sensory neural networks in models for schizophrenia. It has been demonstrated in several animal (Kulikova et al., 2012; Saunders et al., 2012) and human studies (Spencer, 2011; Suazo et al., 2012) that elevations in baseline γ activity represent increased 'noise' in neural networks, and that this may impair γ-mediated sensory-evoked responses and information processing. This study acts to further develop

this concept by replicating previous findings of NMDAr antagonist induced abnormal γ signal-to-noise ratios in somatosensory pathways and extending this result to the thalamic structures in the circuit. We further demonstrated that CT mediated feedback plays a role in the generation of thalamic sensory-evoked γ activity. In conclusion, we have demonstrated that abnormalities in the γ signal-to-noise ratio are induced throughout the TC-CT somatosensory pathway through the action of NMDAr antagonists. These abnormalities may have relevance to the understanding of the pathophysiological neural activity that gives rise to the symptoms of schizophrenia. Understanding the neural dynamics involved in sensory processing will inform us on the pathophysiology of psychiatric diseases and help in the generation of effective therapies.

CHAPTER 5

DISCUSSION

5.1

Key Findings

This thesis sought to expand upon our current knowledge of γ oscillatory abnormalities in rodent models for schizophrenia, aid in further improvement of these models as translational tools for the development of novel antipsychotics and research into the fundamental mechanisms governing coordinated neural activity. By utilising genetic and pharmacological rodent models for schizophrenia, the work performed in this thesis has generated the following key novel findings:

Aim 1) Utilising a chronic dosing paradigm in a rodent model to study the effects of antipsychotic medications, it was revealed that antipsychotic drugs had a significant impact on the characteristics of ongoing γ oscillations and their alteration in response to NMDAr blockade. Chronic administration of the typical antipsychotic haloperidol and the atypical drug clozapine produced significant and sustained decreases in the power of ongoing γ oscillations throughout the course of a 28-day treatment. Chronic treatment with the novel pre-clinical antipsychotic, an mGluR$_{2/3}$ agonist, LY379268, did not produce continual changes in the power of ongoing γ oscillations, despite having pronounced effects on this property acutely. The effects of acute ketamine injection (significantly increased γ power and locomotor activity) were greatly attenuated by chronic administration of all three drugs compared to control animals.

When compared to the effects of single acute antipsychotic administration, the chronic delivery of both haloperidol and clozapine was able to significantly

reduce the electrophysiological effects of ketamine injection. This result is in line with the known clinical treatment profile of antipsychotic medications, which take time to produce their clinical effects (Gelder et al., 2000). This result increases the face validity of this model; the observed results match with the current clinical expectations and understanding. These findings also indicate increased support for the predicative validity of measuring γ oscillations in an acute NMDAr antagonist model for schizophrenia. The results with the pre-clinical drug also support current human studies utilising mGluR$_{2/3}$ agonists in the treatment of schizophrenia, showing equivalent or greater efficacy as the conventional drugs. The differences in the mGluR$_{2/3}$ agonists γ oscillation profile also potentially provides insight into how a reported beneficial side-effect profile may be the result of the relative sparing of ongoing activity. In summary the study of chronic antipsychotic treatment both verifies and expands our previous work demonstrating that antipsychotic medications affect γ oscillations. This result validates a potential biomarker of antipsychotic efficacy and further increases the relevance of γ oscillations to the schizophrenia disease state.

Aim 2) In a genetic model for schizophrenia based on widely replicated schizophrenia association studies (Stefansson et al., 2003; 2002), we utilised mice that were heterozygous for a mutation in the Neuregulin 1 gene. Nrg1 TM HET mutant mice display a range of behavioural and physiological abnormalities that are relevant to the schizophrenia disease state (Duffy et al., 2010; Karl et al., 2007). The study reported in this thesis provides the first evidence of γ oscillations abnormalities that coincide with their schizophrenia relevant behaviour. Nrg1 TM HET mice displayed an increase in the power of ongoing cortical γ compared to wild-type mice, a result similar to the effects of the psychotomimetic NMDAr antagonists on γ oscillations.

Studying sensory-evoked γ oscillations in these animals revealed further neural oscillations deficits relevant to schizophrenia. Nrg1 TM HET mice displayed a reduction in sensory-evoked γ activity; coupled with the presence of increased baseline γ this resulted in a substantially disrupted γ signal-to-noise ratio in these animals. This altered sensory related γ signal-to-noise finding is relevant to the pathophysiology of schizophrenia; supporting theoretical conceptions of the perceptual and cognitive abnormalities in the disease as a result of aberrant noises and reduced sensory signal discrimination in neural networks (Gandal et al., 2012b; Kulikova et al., 2012; Winterer et al., 2000). ErbB4 and NMDAr subunit mRNA expression was unaffected by Nrg1 genotype, but NR2B phosphorylation levels were significantly lower in Nrg1 mutant mice. The reduced phosphorylation of the NMDAr NR2B subunit presents a potential physiological mechanism in the generation of these abnormal γ rhythms and adds further support for the involvement of NMDAr dysfunction in abnormal γ oscillations and schizophrenia. In summary, the presence of distinct γ frequency electrophysiological abnormalities in Nrg1 mutant animals is the first evidence of an abnormal γ oscillatory phenotype in the Nrg1 transmembrane domain mutant mouse, providing a neurophysiological correlate of the behavioural abnormalities observed in this model. It provides further evidence of the signal-to-noise decreases in schizophrenia models and is an important extension of our γ oscillation model into a genetic foundation.

Aim 3) In a study utilising the NMDAr antagonist ketamine to probe the cell-to-network level effects of NMDAr blockade on γ oscillations in the TC-CT somatosensory neural pathway, it was demonstrated that a single injection of ketamine simultaneously increased the power of ongoing γ oscillations and decreased both the amplitude and the γ power of the sensory-evoked responses in both the thalamus and the cortex. This result both replicates and expands upon our previous findings (Hakami et al., 2009; Kulikova et al., 2012) that NMDAr

blockade reduces sensory-evoked γ signal-to-noise in the cortex. As well as replicating these existing results, this study reported the novel finding that this disruption of sensory information transfer occurs throughout the TC-CT somatosensory pathway.

Further investigation of the thalamic response to somatosensory stimulation revealed the presence of a putative CT feedback response, a 'late' latency response at approximately 10 ms after the stimulation. We were able to successfully demonstrate that this late response was dependent on CT neuronal firing, indicating it is CT feedback to the sensory stimulation. Furthermore, the magnitude of this response strongly correlated ($r^2=0.9$, $p<0.001$) with the power of the sensory-evoked γ oscillations in the thalamus, indicating that the CT pathway significantly contributed in the sensory-evoked γ in the thalamus. Investigating the contribution of the CT pathway in the ongoing and sensory-evoked γ oscillations demonstrated that the cortex plays a significant role in the generation of thalamic γ oscillations during information processing and that ketamine reduces the CT-mediated sensory signal-to-γ-noise ratio.

As well as the specific findings in each study, the combined work adds to the growing impression that the study of neural dynamics will be critical to further our understanding of schizophrenia and other psychiatric illnesses (Uhlhaas, 2013). Utilising multiple rodent models for schizophrenia, this thesis demonstrated the presence of multiple γ oscillatory abnormalities across these different models and in a variety of perpetual and behavioural paradigms. Disruptions and changes in γ frequency activity were shown to be pervasive in both acute and chronic pharmacological interventions, as well as chronic genetic alterations based on clinically relevant mutations.

The prevalence of these alterations throughout our rodent models gives reassurance that γ oscillations are important neurological properties relevant to psychiatric illness as well as encouragement that rodent models will be useful tools for their study. The work presented here extends our understanding of the involvement of γ alterations in rodent models for schizophrenia, demonstrating how cellular and synaptic changes (be they pharmacological or genetic) can lead to network level modifications in neuronal dynamics. These findings expand the scope and relevance of these rodent models and will help guide future work in translational models of γ oscillations and schizophrenia.

5.2

Rodent Models as Translational Tools for Understanding Gamma Oscillations in Schizophrenia

There is a growing consensus that disruptions in neural oscillations, particularly γ oscillations, play a significant role in the pathophysiology of human psychiatric disease (Herrmann and Demiralp, 2005; Sun et al., 2011). The result of this research has led to a modern conception of schizophrenia as a disease of dysconnection (Stephan et al., 2009) that is underpinned by disruptions in communication caused by faults in neural synchrony and disruptions to γ oscillations (Uhlhaas, 2013). The research presented in this thesis expands upon our existing knowledge of γ oscillation abnormalities in rodent models for schizophrenia and further develops models that have strong translational potential for the investigation of the neural dynamics that give rise to psychiatric illness.

There is strong evidence for the NMDAr hypofunction hypothesis of schizophrenia across a broad range of neuropsychological, pharmacological and genetic fields of research (Goff and Coyle, 2001; Moghaddam, 2003), however it is

only recently that studies have examined γ frequency activity in these models. The research presented in this thesis fully supports the existing understanding of the effects of γ oscillations in rodent models for schizophrenia. This is not an insignificant point, as the existing literature is relatively limited and establishing these are robust and replicable effects is important to establishing the reliability and validity of these models. Furthermore, the work in this thesis establishes that the effects of NMDAr antagonist treatment on ongoing γ oscillations are broadly applicable across a range of behavioural conditions, treatment paradigms and species. In addition to the replication and support of our existing understanding of γ oscillations in rodent models, the work presented in this thesis crucially expands the scope of γ oscillation abnormalities in rodent models. The study conducted in Nrg1 TM HET mice made the novel finding that these mutant mice display increased γ power in baseline cortical oscillations, and reduced sensory-evoked γ responses. These electrophysiological observations were associated with reduced phosphorylation of the NMDAr NR2B subunit. This study provides the first evidence of an abnormal γ oscillatory phenotype in the Nrg1 transmembrane domain mutant mouse, providing a γ oscillation correlate of the behavioural abnormalities previously observed in this model (Duffy et al., 2010; Karl et al., 2007).

This finding validates and extends the existing literature on γ oscillations in rodent models for schizophrenia, in particular other studies demonstrating opposite effects on ongoing and baseline γ oscillations (Kulikova et al., 2012; Saunders et al., 2012). This study is useful in demonstrating the potential for animal models to be informed by clinical research, demonstrating that a clinically relevant genetic mutation related to schizophrenia can induce substantial γ oscillation abnormalities in a rodent model. This finding also supports the hypothesis that γ oscillations are integral to the pathophysiology of schizophrenia; a mutation in a gene identified as a

risk factor for schizophrenia leads to the recapitulation of complex electrophysiological disturbances that are also observed in patients. Secondly, this result provides important support for the utility of animal models for schizophrenia. We now have a neurodevelopmental, and environmentally realistic model animal that possesses innate γ oscillatory deficits. This animal model can provide a basis for studies looking at interventions and treatments, specifically geared towards rescuing electrophysiological abnormalities. It also provides a unique animal model for examining the pathology underlying these abnormal γ oscillations. By studying the cellular and molecular differences in Nrg1 mutant mice, we will be able to reveal potential causes of the similar pathological neural dynamics in humans.

The results of the existing study do not provide a mechanistic explanation for the observed γ abnormalities, although they do provide support for the currently existing hypotheses of NMDAr hypofunction and PV+ GABAergic interneuron dysfunction (Gonzalez-Burgos et al., 2010; Woo et al., 2010). Considerable evidence suggests that Nrg1 signalling affects NMDAr function: ErbB4 (the Nrg1 receptor) attaches to the scaffolding protein PSD-95 in the same location as NMDArs (Huang et al., 2000). Although we saw no alteration in the expression NMDAr subunits, we cannot rule out a selective alteration of NMDArs on cortical interneurons, particularly since ErbB4 is highly expressed by these cells (Fazzari et al., 2010). Also, we did see alterations in NMDAr phosphorylation, supporting previous reports (Bjarnadottir et al., 2007) and providing some evidence that glutamatergic dysfunction is involved. Recent studies that utilised the conditional ablation of NMDArs on PV+ interneurons found marked increases in the spectral power of ongoing neural oscillations specific to the γ frequency band (Korotkova et al., 2010) supporting the hypothesis that NMDAr input to PV+ cells is essential for normal γ oscillatory function.

Our study into the TC-CT somatosensory circuit and the effects of NMDAr antagonism on sensory transmission demonstrates the utility of such animal models. Ketamine provides a viable model of the electrophysiological abnormalities seen in schizophrenia (Gandal et al., 2012b) and by utilising this effect we were able to investigate the pathophysiological processes in detailed anatomical specificity. Using this model we demonstrated that detreimental changes in the γ signal-to-noise ratio were present throughout the TC circuit that relays somatosensory information. Furthermore we showed that top-down CT feedback is essential for sensory-evoked γ responses in the VPm. These results indicate that sensory-evoked γ oscillations, (which are known to play a role in perceptual and sensory processes (Gray et al., 1989; Singer and Gray, 1995; Tallon-Baudry and Bertrand, 1999; Traub et al., 1996)) rely on CT pathways for generation in the thalamus. While these findings are still at a preliminary stage they will help guide future research. For instance these results indicate that the top-down cortical influence on thalamic sensory processing plays a significant role in sensory-evoked γ, indicating that this process would be a useful target for investigating the pathological mechanisms of schizophrenia. The use of NMDAr antagonists in anaesthetised animal preparations enables the investigation of detailed cellular and circuit mechanisms underlying the pathophysiological γ oscillations relevant to schizophrenia and other psychiatric disease.

Our research into the effects of antipsychotic treatment on γ oscillations also demonstrates the potential translational utility of such animal models. A previous study performed in our laboratory demonstrated that the acute administration of antipsychotic medications had a substantial impact on the power of ongoing γ oscillations (Jones et al., 2012). Treatment with haloperidol, clozapine or LY379268 caused a rapid and sustained decrease in γ oscillations. Given the widespread prevalence of γ abnormalities observed in the disease (Gandal et al.,

2012b; Uhlhaas and Singer, 2010) the finding that antipsychotic medications alter γ activity indicates a potential novel mechanism for their efficacy. This study established a potential translational model of antipsychotic efficacy; NMDAr antagonists induced pathological γ activity and antipsychotic medications opposed this. Interestingly, although the conventional antipsychotics reduced the power of ongoing γ oscillations and so attenuated ketamine's overall effect on γ activity, they did not reduce the proportional effects of ketamine. The study reported in this thesis sought to replicate this original study and validate its results as well as expand the model into a more clinically realistic dosing paradigm.

In clinical practice it has long been held that the response to antipsychotic treatment is typically delayed 2-3 weeks before effects are seen (Gelder et al., 2000) and so we aimed to model this time frame of antipsychotic effects. The primary finding of the study was that chronic treatment with the conventional antipsychotics haloperidol and clozapine strongly attenuated the psychotomimetic effects of an acute ketamine challenge, as measured through electrophysiological and behavioural activity. This effect was substantially different to that seen in acute dosing. Under the chronic treatment paradigm the conventional antipsychotics substantially attenuated the proportional effects of ketamine, reducing its impact on γ oscillations. This finding matches the expected clinical results and so increases the face validity of this new translational model. As well as displaying validity with respect to existing antipsychotic medications the methods used in this paradigm give potential insights into the mechanisms that underlie antipsychotic efficacy. The drugs we tested have highly varied pharmacological profiles yet had similar effects on animal behaviour and electrophysiology, indicating that changes in γ oscillations may be relevant to the antipsychotic efficacy. This animal model also yielded potential insight into the cause of antipsychotic side effects, $mGluR_{2/3}$ agonists are reported to be effective antipsychotics with beneficial side-effect profiles (Adams et

al., 2013), which may be reflected in the lack of persistent effects on ongoing γ. These results would need further investigation, but demonstrate the utility of such animal models. A γ oscillation rodent model of antipsychotic efficacy could be used to evaluate novel antipsychotic compounds as well as investigate the mechanisms that underlie neural dynamic deficits and their potential pharmacological amelioration.

5.3

Gamma Signal-to-Noise Abnormalities

The findings presented in this thesis relate to the hypothesis that the observed increase in ongoing γ oscillations represents a pathological, excessive neural 'noise' and that the electrophysiological abnormalities in schizophrenia result in an overall decrease in the γ signal-to-noise ratio in information processing circuits. This idea has been proposed multiple times (Flynn et al., 2008; Gandal et al., 2012a; Hakami et al., 2009; Kulikova et al., 2012; Williams et al., 2009; Winterer et al., 2000; 2004) citing evidence from clinical studies (see Section 1.5.3 for a detailed summary, also reviews by (Gandal et al., 2012b; Uhlhaas and Singer, 2010)). This literature demonstrates that γ frequency neural activity is disrupted in patients with schizophrenia, both at rest and during various cognitive or perceptual tasks.

The results reported in this thesis offer support for this hypothesis, as well as demonstrating that animal models for schizophrenia present a reliable and valid method to study this phenomenon. Our research provides support across multiple paradigms and techniques indicating a commonality of sensory γ signal-to-noise abnormalities in rodent schizophrenia models. To briefly summarise the findings that are relevant to the neural 'noise' hypothesis, it was established that NMDAr antagonists disrupt γ signal-to-noise ratios in both rat and mouse models for

schizophrenia, across pharmacological and genetic models, and in multiple different sensory domains. The overall results of the studies that constitute this thesis support a conception of pathological γ activity in schizophrenia, including aberrant ongoing γ noise and a subsequent disruption of the γ signal-to-noise ratio in information processing circuits of the brain. While not providing mechanistic explanations for these electrophysiological abnormalties, we have demonstrated that these rodent models are viable paradigms for investigations of γ oscillation defiicts relevant to schizophrenia.

Our finding that Nrg1 TM HET mice display elevated γ frequency power in 'baseline' recordings and concurrent reductions in stimulus-driven γ responses is a good example of the potential utility of such rodent models. This finding expands the existing animal models that manifest the complex electrophysiological characteristics seen in patients with schizophrenia from just pharmacological models into genetic neurodevelopmental models as well. This is an important advance in the capabilities of animal models of electrophysiological deficits in schizophrenia, demonstrating that pharmacological intervention is not the only method to elicit γ signal-to-noise deficits. The fact that a mutation that presumably results in the development of aberrant neuronal networks (Fazzari et al., 2010) can also result in similar electrophysiological characteristics is an important addition to our understanding of these deficits and increases the face validity of such models. The similarities between the Nrg1 model and the NMDAr antagonist models could also shed light into the mechanisms involved, although further studies into the anatomical and cellular pathologies associated with the Nrg1 mouse model are still needed.

In the third results chapter of this thesis, the study of TC and CT circuits in the somatosensory pathway was explicitly designed to elucidate the effects of NMDAr antagonist administration on the γ signal-to-noise ratio in sensory

processing. The findings replicated the previous results of (Kulikova et al., 2012), and expanded upon these, demonstrating that ketamine has opposite effects on ongoing and sensory-evoked γ oscillations and disrupts the γ signal-to-noise ratio throughout the somatosensory pathway. This adds to our existing understanding of γ abnormalities in animal models for schizophrenia, demonstrating that γ signal-to-noise are present at a precise anatomical level throughout the somatosensory pathway. This study has also provided insight into the mechanisms that may drive the pathological γ processes in sensory processing. Our results demonstrated an important role for CT feedback in generating sensory-evoked γ oscillations. This indicates that dysfunctional γ generated in sensory pathways may be driven by top-down cortical disturbances. The present results can neither rule out nor confirm that NMDAr antagonists act through CT driven dysfunction, but they do indicate that top-down cortical processes are essential to sensory γ activity. The role of CT feedback also provides a potential explanation for the detrimental effects that ketamine has on the sensory γ signal-to-noise ratio. If ketamine is acting to interfere with cortico-cortical and CT communication it could disrupt the necessary feedback loops that are required to generate coherent oscillations (Contreras et al., 1996; Jones, 2001)

It is important to note some important limitations in our understanding of γ signal-to-noise deficits; we do not have a complete understanding of the mechanisms that lead to these abnormal oscillations and we also do not understand the relationship between ongoing γ activity and sensory-evoked γ oscillations. γ oscillations are generated through the peri-somatic inhibition of pyramidal cells (Gonzalez-Burgos and Lewis, 2008; Whittington et al., 2000), which is mediated via the action of PV+ interneurons (Bartos et al., 2007; Cobb et al., 1995). NMDAr blockade is theorised to lead to a reduction of excitatory input to GABAergic interneurons and result in a disinhibition of pyramidal cells (Behrens et

al., 2007; Breier et al., 1998; Homayoun and Moghaddam, 2007), which in turn produces a hyper-glutamatergic state. This excessive glutamate release is purported to cause the increase of γ frequency activity seen in-vivo following NMDAr antagonist administration (Hakami et al., 2009; Lazarewicz et al., 2010; Pinault, 2008). Although widely supported this theory is not conclusive (Kulikova et al., 2012) and ketamine's 'off target' actions at muscarinic, serotonergic, dopaminergic and GABAergic receptors could all conceivably play a role (Flood and Krasowski, 2000; Irifune et al., 2000; Kapur and Seeman, 2002). Further investigation into the mechanisms that lead to NMDAr antagonist-induced γ oscillations is needed to further refine and develop animal models for γ oscillation abnormalities in schizophrenia.

Another important issue is that the relationship between ongoing γ oscillations and those elicited through sensory and cognitive tasks. The nature of these two types of γ is not clearly understood and two competing hypotheses exist. In one conception the 'additive' model, sensory-evoked activity reflects bottom-up processing of sensory stimulus that is superimposed on top of the random background 'noise' of ongoing oscillations (Allison et al., 1991). The competing view is that evoked potentials result from the phase locking or reset of basic underlying oscillations as a response to external input (Basar, 1980). In this second phase locking theory, the state of the ongoing neural oscillations could dramatically affect the evoked and induced responses to sensory input. Reduced phase locking has been reported in schizophrenia, in the context of a reduction of γ signal-to-noise ratio (Winterer et al., 2000). NMDAr antagonist-induced pathological ongoing γ 'noise' will alter the overall γ signal-to-noise ratio in different ways depending on the precise mechanisms involved in the generation of sensory-evoked responses. At present we do not have conclusive data as to which mechanism is involved in sensory-evoked γ, nor how NMDAr blockade affects this. This is an

area that should be the subject of future research with the goal of understanding sensory abnormalities in schizophrenia. The models developed throughout this thesis are viable paradigms for the further investigation of such processes.

In conclusion, the research presented in this thesis provides evidence that the γ frequency signal-to-noise ratio is disrupted in pharmacological and genetic rodent models for schizophrenia. This measure may be a suitable marker for the use of evaluating sensory neural circuit function in both animals and human, serving as a translational measure in models for schizophrenia (Gandal et al., 2012b; Kulikova et al., 2012; Winterer et al., 2000). We have presented findings that a mutation in the Nrg1 can result in dysfunctional sensory processing circuits in mice, leading to reductions in the γ signal-to-noise ratio. This is similar to the effects induced by NMDAr antagonists (Kulikova et al., 2012) and also mirrors the complex electrophysiological abnormalities seen in patients with schizophrenia (Gandal et al., 2012b; Winterer et al., 2000). We also demonstrated that these abnormalities exist throughout TC and CT pathways in somatosensory networks and further investigation of these pathways will hopefully yield insights into the pathophysiological processes that underlie schizophrenia. The animal models we have developed in these studies should prove to be useful tools for investigating perceptual and cognitive deficits in schizophrenia and treatments to mitigate these deficits.

5.4

Limitations and Future Directions

The goal of this thesis was to expand and enhance our understanding of γ oscillations in rodent models for schizophrenia and the work presented here has successfully grown our knowledge regarding γ activity in pharmacological and

genetic models for schizophrenia. The goal of such animal models is to improve our understanding of the human disease and ultimately improve the well-being of patients. Extrapolating any results to the human condition must be done with great care and the limitations of animal studies must be acknowledged. Schizophrenia in particular is a uniquely human disease and no animal model can ever hope to capture the full spectrum of its effects. However if we can restrict our models to specific aspects of the disease and ensure the validity and relevance of the biological mechanisms involved we can gain useful insight into the pathophysiological mechanism involved with these specific aspects of the disease. In the case of rodent models for schizophrenia and the specific measure of electrophysiological characteristics we can have a high degree of confidence that our findings will be comparable to the human condition. Rodents have a similarly structured cortex to humans, including a six layered organisation and topographically aligned sensory and motor areas (Lui et al., 2011). Mice and rats also share the specific neuronal subtypes regarded as important for the generation and coordination on neural oscillations and in many ways the human brain can be considered a 'scaled-up' version of other primates and mammals' brains (Herculano-Houzel, 2009). Of specific relevance to this thesis, studies of the temporal characteristics of the multiple brain rhythms that constitute neural oscillations show a high degree of similarity across practically all mammals (Buzsaki et al., 2013). As a result we can assume with a high degree of confidence that the results of rodent studies into neural oscillations do have relevance to the human condition.

One of the foremost limiations in the work that constitutes this thesis is in regard to the study of chronic antipsychotic administration. This study expanded upon previous work that demonstrated antipsychotics could alter the power of ongoing γ oscillations in rats. We increased the scope of this model to include a more clinically relevant dosing paradigm that covered several weeks of treatment, which attenuated a ketamine-induced increase in the power of ongoing γ

oscillations. However this also reveals a key limitation of the study; while the treatment received more accurately reflects the clinical situation, the model used did not. This study was conducted in healthy animals, displaying physiologically normal γ oscillations, not the chronic abnormalities seen in schizophrenia. This places clear limits on the interpretations that can be made. As discussed earlier (Section 1.4 on NMDAr hypofunction) there is reason to believe that acute NMDAr antagonist treatment may reflect the early-onset of schizophrenic illness representing the psychotic symptoms of the disease (Bubeníková-Valešová et al., 2008; Jentsch and Roth, 1999; Mouri et al., 2007). The increased efficacy of chronic antipsychotic treatment versus the single acute dose administered in (Jones et al., 2012), is a useful advancement of the model, but further studies in paradigms that model the chronic nature of schizophrenia are essential to the further development of these models.

Sub-chronic administration of NMDAr antagonists have already been demonstrated to successfully capture some elements of chronic schizophrenia, producing persistent changes in hyperlocomotion and increased immobility in the forced swim test (Mouri et al., 2012; Nagai et al., 2003) as well as anatomical deficits in markers of GABAergic health (Morrow et al., 2007; Qin et al., 1994; Rujescu et al., 2006), suggesting these may be suitable models to investigate the chronic disease. Neurodevelopment models would also be a useful addition; in that context the Nrg1 TM HET model utilised in Chapter 3 would be ideal. We have already demonstrated specific γ oscillation abnormalities in this model and there is existing research that demonstrates its responsiveness to antipsychotic treatment (Bjarnadottir et al., 2007; Moghaddam and Jackson, 2003; Stefansson et al., 2002). Finally, the addition of electrophysiological measures to clinical studies of novel antipsychotics would be an essential step to truly demonstrate the translational utility of such model. Another limitation of this study is that it does not address the molecular mechanisms that underlie the effects of antipsychotic treatment on γ

oscillations. It is possible that long-term treatment with antipsychotics is modulating the number, density or phosphorylation states of NMDAr receptors. However, to date numerous animal studies have produced inconsistent results. Showing increases, decreases and no changes in various brain region and for various glutamate receptors. (Giardino et al., 1997; McCoy et al., 1998; Ossowska et al., 1999; Spurney et al., 1999). The original study also revealed the interesting finding that an acute dose of antipsychotics resulted in long-lasting (~ 24 hour) depressions in ongoing γ power (Jones et al., 2012). This is well beyond the time that these drugs would have effective plasma concentrations; indicating that down-stream processes are involved in their modulatory effect on γ power. Future research will need to address both the molecular mechanisms and the neural dynamics involved in order to understand these processes.

The link between γ oscillations and behaviour in the experimental animal models investigated in this thesis is another limitation. The results of the studies reported in chapters 2 and 3 as well as previous studies from our laboratories (Hakami et al., 2009; Jones et al., 2012; Pinault 2008) provide evidence that there exists a disconnection between the γ oscillations and hyperlocomotor activity induced by psychotomimetic NMDAr antagonists such as ketamine and MK-801. We have specifically demonstrated that the increase in power of ongoing γ oscillations does not depend upon concurrent locomotor activity (Pinault 2008) and that antipsychotic medications can attenuate the locomotor effects of ketamine while not affecting the magnitude of increase in γ oscillations (Jones et al., 2012; Chapter 2). Investigating the physiological mechanisms behind this disconnection was beyond the scope of the studies presented in this thesis, but a likely pathway is the downstream dopamine release triggered by NMDAr antagonists (Moghaddam et al., 1997). This mechanism can potentially explain the effectiveness of dopaminergic antagonist antipsychotics in preventing hyperlocomotion while

sparing the γ increasing effects of ketamine. Further studies to specifically examine the link between NMDAr antagonism, γ oscillation changes and hyperlocomotor effects will be needed in order to understand this phenomeneon.

This also highlights the need for more complex and robust behavioural paradigms to be developed and investigated in order to understand the links between altered γ oscillations and behaviour. Understanding of the mechanisms underlying the negative and cognitive symptoms in schizophrenia is currently an underserved need in the disease and γ oscillations provide a potential pathway for doing so. γ oscillations are robustly linked to cognitive processes in human studies (Engel et al., 2001; Herrmann et al., 2004; Uhlhaas and Singer 2010) and disruptions to neural oscillations may give rise to the cognitive symptoms seen in schizophrenia. The studies presented in this thesis have not investigated the cognitive and negative symptoms of schizophrenia and the main behavioural paradigm used (locomotion) fails to give insight into any potential links between these factors.

Future research will need to develop new paradigms and behavioural measures that simultaneously examine γ oscillations and aspects of cognition such as memory, attention and perception in order to increase our understanding of the link between these domains and their relevance to schizophrenia. There already exists numerous cognitive testing paradigms for rodent models that have been demonstrated to be relevant and valid for assessing schizophrenia relevant behavioural outcomes in animals (Young et al., 2009); adapting these tests to incorporate simultaneous measures of neural oscillations will lead to the development of useful behavioural paradigms to measure both cognitive outcomes and neural oscillatory activity relevant to the schizophrenia disease state.

Chapters 3 and 4 reported reductions in the γ signal-to-noise ratio, which has been proposed as a marker of neuronal circuit dysfunction (Gandal et al., 2012b; Kulikova et al., 2012; Winterer et al., 2000). This is an important step towards developing effective animal models for the study of γ oscillations in schizophrenia, but further development of novel behavioural paradigms will be essential to understand the role that γ oscillations play in sensory and cognitive processing. For example, in our study in the somatosensory circuit we demonstrated reduced γ signal-to-noise in the TC and CT pathways. In order to understand the functional results of this disruption it will be essential to develop behavioural paradigms that can reveal the outcome of disrupted γ oscillations. Perceptual tasks that involve responding to threshold level sensory information is one potential route, conducted under control and NMDAr blockade conditions, γ oscillations can be recorded in the sensory pathway and correlations between the level of task performance can inform us of the function of these oscillations. Similar tasks exist for memory paradigms (Tort et al., 2008) and there are a range of well-validated animal behavioural tasks that can measure elements of schizophrenia pathology (Young et al., 2009). As well as correlative measures of the effect of γ oscillations more direct manipulation of neural dynamics is now possible through the use of optogenetic and pharmacological targeting of specific neuronal subtypes (Armbruster et al., 2007; Deisseroth et al., 2006). It has already been demonstrated that γ frequency optogenetic stimulation of PV+ interneurons is sufficient to generate γ oscillations (Sohal et al., 2009). Furthermore, this enhancement of γ frequency activity was found to enhance information theoretic measures of signal transmission *in vivo* (Carlen et al., 2012; Sohal et al., 2009). The ability to target specific cell-types coupled with the development of novel behavioural paradigms will allow for the precise elucidation of the functional role of γ frequency activity in a range of perceptual and cognitive tasks. The pharmacological and genetic animal models of altered γ oscillations developed in

this thesis should be useful tools in the development of future paradigms and hopefully lead to new insights into the cognitive and negative symptoms of schizophrenia and their treatment.

5.5
Final Conclusions

Gamma oscillations have been demonstrated to play an important role in the various cognitive processes that constitute healthy brain function. There is increasing evidence that the cognitive abnormalities and symptoms seen in schizophrenia are related to dysfunctional γ frequency activity and that deficits in the brain's ability to coordinate synchronised neuronal networks may underlie the pathophysiology of the disease. Animal models for schizophrenia that capture the complex neural dynamics and pathological γ oscillations seen in schizophrenia will be essential to the understanding and eventual treatment of schizophrenia. The findings in this thesis have demonstrated that a variety of animal models for schizophrenia can recapitulate specific γ oscillation abnormalities relevant to the disease state and that these models may have utility for future translational studies into the pathophysiology of schizophrenia.

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Oscillations dans la bande de fréquence gamma dans des modèles de rongeurs pour la schizophrénie

Résumé

La schizophrénie est un trouble mental débilitant qui se caractérise par des perturbations de la pensée, des émotions et de la cognition. Ces processus d'intégration fonctionnelle sont généralement associés à des oscillations bioélectriques cérébrales synchrones dans la bande de fréquence gamma (30-80 Hz), lesquelles sont aussi altérées chez des patients souffrant de schizophrénie. Ce travail de thèse vise à développer des méthodes et des outils pour étudier les mécanismes neuronaux sous-tendant les altérations de ces oscillations physiopathologiques. Pour ce faire, nous avons développé des modèles de rongeurs de laboratoire pour la schizophrénie. Nous avons démontré que des modifications génétiques ou pharmacologiques conduisent à des perturbations des oscillations gamma et que des médicaments antipsychotiques peuvent les moduler.

Mots-clés: Schizophrénie, Gamma Oscillation, Antagoniste du récepteur NMDA, Des modèles de rongeurs, Stimulation sensorielle, thalamocortical

Résumé en anglais

Schizophrenia is a debilitating mental disorder that is characterised by a breakdown in normal thought processes, blunted emotional responses and a variety of cognitive difficulties. Gamma frequency (30 – 80 Hz) oscillations are associated with many processes that are disrupted in people with schizophrenia memory, perception and attention. This thesis aimed to develop methods and tools to investigate the basic mechanisms that underlie the alterations in gamma frequency brain activity that are observed in patients suffering from schizophrenia. To do this we developed a variety of experimental animal models for schizophrenia. We successfully demonstrated that both genetic and pharmacological changes lead to alterations in gamma oscillations and that antipsychotic medications can modulate them.

Keywords : Schizophrenia, Gamma Oscillation, NMDA receptor antagonist, Rodent Models, Sensory Stimulation, Thalamocortical