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Medial forebrain bundle deep brain stimulation in a rodent model of depression:

modulation of sleep abnormalities and mechanisms of anti-depressant effects

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Medial forebrain bundle deep brain stimulation in a rodent model of depression: modulation of sleep abnormalities and mechanisms of anti-depressant effects

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For my mum and my dad.

Introductory materials

Table of Contents i
Acknowledgements iii
Déclaration sur l'honneur v
Summary vi
Zusammenfassung x
Resumé xv
Abbreviations xix
List of figures xxiii

Table of Contents

1. Introduction 1

1.1 Depression and medial forebrain bundle deep brain stimulation 1

1.1.1 Depression 1

1.1.2 Modelling depression in animals.....10

1.1.3 Treatment of depression and the problem of treatment resistance 14

1.1.4 The medial forebrain bundle as a target for deep brain stimulation 19

1.2 The role of dopamine in depression and treatment response 24

1.2.1 Dopamine and depression 24

1.2.2 Dopaminergic interactions with other neural mechanisms of depression 35

1.3 Sleep and depression 40

1.3.1 Normal Sleep 40

1.3.2 Sleep disturbances in depression.....45

1.3.3 Sleep in rodents 50

1.3.4 Connecting depressive pathology with sleep disturbance 52

1.4 The anti-depressant mechanisms of mfb-DBS 55

1.4.1 The action and mechanisms of deep brain stimulation 55

1.4.2 The effects of mfb-DBS 60

1.5 The Flinders Sensitive Line rat as a model of depression 68

1.5.1 Face validity: depressive-like phenotypes in the FSL.....68

1.5.2 Construct validity: physiological abnormalities in the FSL 69

1.5.3 Predictive validity: anti-depressant response in the FSL.....71

1.5.4 Aetiological validity: the onset and course of the phenotype 72

1.5.5 Selection of the model 73

2. Summary and aims 75

3. Materials and methods.....77

3.1 Animals 77

3.2 Behavioural testing 78

3.3 Electrodes 80

3.4 Surgery.....82

3.5 Deep brain stimulation 83
3.6 Electrophysiology....85
3.7 Tissue collection 89
3.8 Quantitative real-time PCR 91
3.9 High Performance Liquid Chromatography 93
3.10 Statistical analysis....95
4. Slow wave sleep deficits in the FSL model of depression: modulation by medial forebrain bundle deep brain stimulation 96
4.1 Introduction....96
4.2 Materials and methods 98
4.3 Results 101
4.4 Discussion....116
5. Modulation of dopaminergic receptor expression and induction of plastic mechanisms by medial forebrain bundle deep brain stimulation....122
5.1 Introduction....122
5.2 Materials and methods 125
5.3 Results 127
5.4 Discussion....138
6. Modulation of frontal monoamine concentration by medial forebrain bundle deep brain stimulation in the FSL model of depression 144
6.1 Introduction....144
6.2 Materials and methods 146
6.3 Results 148
6.4 Discussion....154
7. General Discussion 159
7.1 The FSL as a model for depressive-like symptoms: implications for depression ...159
7.2 Effects and potential mechanisms of mfb-DBS....167
7.3 Conclusions and outlook 176
References 178

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Summary

Depression is a major health burden worldwide, and presents a major challenge of both diagnostics and treatment. Diagnosis relies on symptomatology, with no fully established, unique biomarkers for the disease, and a substantial minority of patients fail to respond to conventional treatments. Deep brain stimulation (DBS) of the medial forebrain bundle (MFB, when referring to the structure in humans; mfb, when referring to the structure in rodent models) is an experimental treatment which has seen positive results in severe treatment-resistant patients. MFB-DBS is believed to produce anti-depressant effects by modulating ascending dopaminergic projections related to systems of reward and motivation. However, like the pathophysiology of the disease, the biological mechanisms which underpin this treatment are not well understood, and its effects are likely to be diverse. A growing body of research aims to elucidate the neurobiology of depressive symptoms and the mechanisms of treatment response in order to provide foundations for improved understanding, diagnosis and treatment of depression. In our chosen model of depression, the Flinders Sensitive Line (FSL) rat, several behavioural and physiological phenotypes have been characterised, while pre-clinical studies have also begun to uncover potential mechanisms of mfb-DBS. However, further work is required in this regard to advance our understanding of this treatment.

In this thesis, this broad goal was undertaken with a specific focus on mfb-DBS as a treatment, the neurophysiological systems it is theorised to act upon, and the link between depression and sleep disturbance, a common feature of the disorder. In the first experiment, an investigation was conducted with the aim of providing a detailed characterisation of sleep in the FSL rat and the response to mfb-DBS. Sleep

was assessed via electrophysiological recording of global signal and local activity from deep brain structures implicated in depression, prior to and following mfb-DBS. In a second experiment, the effect of mfb-DBS on dopaminergic signalling and synaptic plasticity was investigated. FSL animals underwent mfb-DBS, and the levels of expression of dopamine receptors and markers of activity-dependant plasticity were assessed. Finally, in a third experiment, the concentration of levels of the monoamines dopamine and serotonin, long implicated in depressive biology and treatment response, were monitored after two varying paradigms of mfb-DBS.

In the FSL, previously unreported sleep deficits were characterised, adding to the validity of the model and highlighting the importance of slow wave sleep (SWS) as dysfunctional in depression. Site-specific dysfunction in the brain during SWS was identified in the nucleus accumbens, further suggesting this structure's importance in depressive physiology. Changes to the time spent in SWS sleep were found, relating to circadian rhythm, while diminished slow wave activity was apparent alongside increased high frequency gamma oscillations compared to controls. We also present evidence supporting the presence of anhedonic-like behaviour in the FSL, a phenotype believed to be absent in the model, but suggested by a small number of recent reports.

Examining the effects of mfb-DBS, our results suggested several changes which may represent mechanisms or markers of anti-depressant effect. Gamma oscillations were suppressed in global signal and in the pre-frontal cortex during SWS, while overall sleep architecture was not notably altered. The modulation of SWS physiology without affecting the timing of sleep suggests an interesting separation of effect on usually interrelated aspects of sleep. mfb-DBS also led to alterations to

monoaminergic transmission at the target sites of the mesolimbic and mesocortical pathways. In the nucleus accumbens (NAc), dopamine and serotonin concentrations were diminished after 24h of mfb-DBS, while in the medial pre-frontal cortex (mPFC), changes in metabolism of these two molecules were suggested. Expression of dopamine receptor types was differentially altered in the NAc and mPFC: in the FSL, dopamine D1 receptors were upregulated in the mPFC, and D2 receptors downregulated in the NAc; in non-depressive-like rats, both D1 and D2 receptors were downregulated in the mPFC and NAc. These observations provide evidence that mfb-DBS modulates ascending monoaminergic systems; specifically, in the NAc these changes may be suggestive of elevated release of dopamine, while in the mPFC expression of D1 receptors may be modulated independently of transmitter release. Although further validation is required of the exact dynamics of these changes and how they may reflect wider modulation of dopaminergic transmission, a strong theoretical basis and similarities to effects observed after other treatments suggest these changes may form part of anti-depressant response after mfb-DBS.

Finally, mfb-DBS led to an increase in expression of the immediate early gene markers of synaptic plasticity, Homer1a and Arc, in the mPFC of FSL rats. The expression of these proteins is related to activity-dependant synaptic remodelling, and has been linked to numerous anti-depressant treatments, with the enhancement of cortical plasticity theorised to be a final common pathway of anti-depressant effect.

The results presented here provide a deeper characterisation of sleep deficits in an established model of depression, and demonstrate a variety of potential effects of mfb-DBS, ranging from modulation of electrophysiological activity during sleep, altering monoaminergic transmission and potentiating mechanisms of cortical plasti-

city. The observed changes have theoretical associations with both the pathophysiology of depression and mechanisms of treatment, suggestive of potential roles in the anti-depressant mechanisms of mfb-DBS. While the effects of mfb-DBS are likely diverse and doubtless extend beyond those demonstrated here, these results provide insight into this modality of treatment, and a platform for deeper research into its mechanisms.

Zusammenfassung

Depressionen sind weltweit eine große gesundheitliche Belastung und stellen eine große Herausforderung sowohl für die Diagnostik als auch für die Behandlung dar. Die Diagnose basiert auf der Symptomatik, wobei keine vollständig etablierten, eindeutigen Biomarker für die Krankheit existieren, und eine beträchtliche Minderheit der Patienten auf konventionelle Behandlungen nicht anspricht. Die Tiefenhirnstimulation (DBS) des medialen Vorderhirnbündels (MFB, wenn man sich auf die Struktur beim Menschen bezieht; mfb, wenn man sich auf die Struktur in Nagetiermodellen bezieht) ist eine experimentelle Behandlungsmethode, welche bei schwer behandlungsresistenten Patienten positive Ergebnisse gezeigt hat. Es wird angenommen, dass MFB-DBS, durch die Modulation aufsteigender dopaminerger Projektionen, die mit Belohnungs- und Motivationssystemen in Verbindung stehen, antidepressive Effekte hervorruft. Nicht nur die Pathophysiologie der Erkrankung, sondern auch die biologischen Mechanismen, die dieser Behandlung zugrunde liegen, sind bis dato nicht gut verstanden, und die hervorgerufenen Wirkungen sind höchstwahrscheinlich vielfältig. Eine wachsende Zahl von Forschungsarbeiten zielt darauf ab, die Neurobiologie depressiver Symptome und die Mechanismen des Ansprechens auf die Behandlung aufzuklären, um Grundlagen für ein besseres Verständnis, eine bessere Diagnose und eine bessere Behandlung der Depression zu etablieren. In dem von uns gewählten Depressionsmodell, der Flinders Sensitive Line (FSL) Ratte, wurden verschiedene Verhaltens- und physiologische Phänotypen charakterisiert, und zugleich mögliche Mechanismen der mfb-DBS erforscht. Den-

noch sind weitere Arbeiten in dieser Hinsicht erforderlich, um unser Verständnis dieser Behandlung zu erweitern.

In dieser Arbeit wurde dieses breite Ziel mit Fokus auf die mfb-DBS als Behandlung, die neurophysiologischen Systeme, auf welche diese einwirkt, und die Verbindung zwischen der Depression und der Schlafstörung, einem häufigen Symptom der Erkrankung, verfolgt. Im ersten Experiment wurde eine Untersuchung mit dem Ziel durchgeführt, eine detaillierte Charakterisierung des Schlafes bei der FSL-Ratte und der Reaktion auf mfb-DBS zu liefern. Der Schlaf wurde mittels elektrophysiologischer Aufzeichnung des globalen Signals und der lokalen Aktivität von tiefen Hirnstrukturen, die bei Depressionen eine Rolle spielen, vor und nach mfb-DBS untersucht. In einem zweiten Experiment wurde der Effekt der mfb-DBS auf die dopaminerge Signalübertragung und die synaptische Plastizität untersucht. FSL-Tiere wurden einer mfb-DBS unterzogen, und die Expressionsniveaus von Dopaminrezeptoren und Markern der aktivitätsabhängigen Plastizität wurden analysiert. Schließlich wurde in einem dritten Experiment die Konzentration der Monoamine Dopamin und Serotonin, die seit langem mit der Pathophysiologie der Depression und dem Ansprechen auf die Behandlung in Verbindung gebracht werden, nach zwei mfb-DBS-Paradigmen überwacht.

Im FSL Ratenmodell wurden bisher nicht berichtete Schlafdefizite charakterisiert, welche zur Validität des Modells beiträgt und die Bedeutung des bei der Depression dysfunktionalen Slow-Wave-Schlafs (SWS) unterstreicht. Eine ortsspezifische Dysfunktion während des SWS im Gehirn wurde im Nucleus accumbens identifiziert, was zudem die Wichtigkeit dieser Struktur in der depressiven Physiologie hervorhebt. Es wurden Veränderungen in der im SWS-Schlaf verbrachten Zeit gefun-

den, die mit dem zirkadianen Rhythmus zusammenhängen, während eine verminderte Slow-Wave-Aktivität neben erhöhten hochfrequenten Gamma-Oszillationen im Vergleich zu den Kontrollen zu beobachten war. Wir präsentieren darüber hinaus Beweise, die das Vorhandensein von anhedonischem Verhalten in der FSL Ratte unterstützen, ein Phänotyp, von welchem man annahm, dass er in diesem Modell nicht vorkommt, der jedoch von einer kleinen Anzahl neuerer Berichte beschrieben wird.

Bei der Untersuchung der Auswirkungen der mfb-DBS zeigten unsere Ergebnisse mehrere Veränderungen, die möglicherweise Mechanismen oder Marker einer antidepressiven Wirkung darstellen. Gamma-Oszillationen wurden im globalen Signal und im präfrontalen Kortex während des SWS unterdrückt, während die gesamte Schlafarchitektur nicht merklich verändert war. Die Modulation der SWS-Physiologie, ohne das Timing des Schlafes zu beeinflussen, deutet auf eine interessante Trennung der Wirkung auf normalerweise zusammenhängende Aspekte des Schlafes hin. mfb-DBS führte auch zu Veränderungen der monoaminergen Übertragung an den Zielorten der mesolimbischen und mesokortikalen Bahnen. Im Nucleus accumbens (NAc) waren die Dopamin- und Serotonin-Konzentrationen nach 24 Stunden mfb-DBS vermindert, während im medialen präfrontalen Cortex (mPFC) Veränderungen im Metabolismus dieser beiden Moleküle vermutet wurden. Die Expression von Dopaminrezeptortypen war im NAc und mPFC unterschiedlich verändert: bei der FSL waren Dopamin-D1-Rezeptoren im mPFC hochreguliert und D2-Rezeptoren im NAc herunterreguliert; bei nicht-depressiven Ratten waren sowohl D1- als auch D2-Rezeptoren im mPFC und NAc herunterreguliert. Diese Beobachtungen liefern Hinweise darauf, dass mfb-DBS aufsteigende monoaminerge

Systeme moduliert; speziell im NAc könnten diese Veränderungen auf eine erhöhte Dopaminfreisetzung hindeuten, während im mPFC die Expression von D1-Rezeptoren unabhängig von der Transmitterfreisetzung moduliert sein könnte. Obwohl die genaue Dynamik dieser Veränderungen und die Art und Weise, wie sie eine breitere Modulation der dopaminergen Übertragung widerspiegeln, einer weiteren Validierung bedürfen, deuten eine starke theoretische Basis und Ähnlichkeiten mit Effekten, die nach anderen Behandlungen beobachtet wurden, darauf hin, dass diese Veränderungen Teil der antidepressiven Reaktion nach mfb-DBS sein könnten.

Schließlich führte die mfb-DBS zu einem Anstieg der Expression der unmittelbaren frühen Genmarker der synaptischen Plastizität, Homer1a und Arc, im mPFC der FSL-Ratten. Die Expression dieser Proteine steht im Zusammenhang mit aktivitätsabhängigem synaptischem Umbau und wurde mit zahlreichen antidepressiven Behandlungen in Verbindung gebracht, wobei die Steigerung der kortikalen Plastizität als letzter gemeinsamer Weg der antidepressiven Wirkung theoretisiert wird.

Die hier vorgestellten Ergebnisse liefern eine tiefere Charakterisierung von Schlafdefiziten in einem etablierten Depressionsmodell und zeigen eine Vielzahl von möglichen Effekten der mfb-DBS, die von der Modulation der elektrophysiologischen Aktivität während des Schlafes über die Veränderung der monoaminergen Übertragung bis hin zur Potenzierung von Mechanismen der kortikalen Plastizität reichen. Die beobachteten Veränderungen haben theoretische Assoziationen sowohl mit der Pathophysiologie der Depression als auch mit den Mechanismen der Behandlung, was auf eine mögliche Rolle in den antidepressiven Mechanismen der mfb-DBS hindeutet. Während die Effekte der mfb-DBS wahrscheinlich vielfältig sind und zweifellos über die hier gezeigten hinausgehen, bieten diese Ergebnisse einen Ein-

blick in diese Behandlungsmodalität und eine Plattform für die weitere Erforschung ihrer Mechanismen.

Resumé

La dépression est un fardeau majeur pour la santé dans le monde entier, et représente un défi à la fois en ce qui concerne le diagnostic et le traitement. Le diagnostic repose sur la symptomatologie, sans qu'il existe de biomarqueurs uniques et bien établis pour la maladie, et une minorité importante de patients ne répond pas aux traitements conventionnels. La stimulation profonde (DBS, de 'deep brain stimulation' en anglais) du faisceau médian du télencéphale (MFB de 'medial forebrain bundle' en anglais; MFB, lorsqu'il s'agit de la structure chez l'homme ; mfb, lorsqu'il s'agit de la structure dans les modèles de rongeurs) est un traitement expérimental qui a donné des résultats positifs chez des patients sévèrement résistants au traitement. La MFB-DBS est censée produire des effets antidépresseurs en modulant les projections dopaminergiques ascendantes liées aux systèmes de récompense et de motivation. Cependant, tout comme la physiopathologie de la maladie, les mécanismes biologiques qui sous-tendent ce traitement ne sont pas bien compris, et ses effets sont susceptibles d'être divers. Un nombre croissant de recherches, notamment au niveau préclinique, vise à élucider la neurobiologie des symptômes dépressifs et les mécanismes de réponse aux traitements afin de fournir les bases d'une meilleure compréhension, d'un meilleur diagnostic et d'un meilleur traitement de la dépression. Dans le modèle de dépression que nous avons choisi, le rat FSL (de Flinders Sensitive Line), plusieurs phénotypes comportementaux et physiologiques ont été caractérisés, tandis que des études précliniques ont également contribué à découvrir les mécanismes potentiels sous-tendant les effets bénéfiques de la mfb-DBS ; cependant, ces connaissances restent parcellaires et il

reste nécessaire d'entreprendre des études afin de faire progresser notre compréhension de ce traitement.

Lors de ma thèse, c'est dans cet objectif que mon travail a été entrepris, en mettant un accent spécifique sur les systèmes neurophysiologiques sous-tendant l'action de ma mfb-DBS, en théorie, et sur le lien entre la dépression et les troubles du sommeil, une caractéristique établie de la maladie. La première expérience a eu pour but de fournir une caractérisation détaillée du sommeil chez le rat FSL, avant et après mfb-DBS. Le sommeil a été évalué suite à l'enregistrement électrophysiologique du signal cortical global (électrocorticogramme, ECoG), et de l'activité locale (potentiels de champs locaux) de structures cérébrales profondes impliquées dans la dépression, telles que l'hippocampe dorsal et le cortex préfrontal médian (CPFm). Dans une deuxième expérience, nous avons étudié les conséquences de la mfb-DBS sur des marqueurs tissulaires (ARN messager), au niveau du noyau accumbens (NAc) et du CPFm, de la signalisation dopaminergique, i.e. récepteurs dopaminergiques D1 et D2 et tyrosine hydroxylase, et de la plasticité synaptique, i.e. gènes précoces Arc, H1a et NPAS4 ; . Enfin, dans une troisième expérience, la concentration des niveaux des monoamines telles que la dopamine (DA) et la sérotonine (5-HT), ainsi que de leurs métabolites, a été analysée suite à deux protocoles de mfb-DBS . Ainsi, nous avons pu caractériser, pour la première fois chez le rat FSL, des déficits des rythmes veille/sommeil encore, notamment une élévation de la puissance des oscillations gamma au niveau du NAc, ajoutant à la validité du modèle et soulignant l'importance du sommeil lent dans la physiopathologie de la dépression, ainsi que l'importance du NAc. Des modifications du temps passé en sommeil lent ont également été constatées,.

Enfin, au niveau comportemental, nous avons pu mettre en évidence chez les rats FSL un comportement de type anhédonique, un phénotype que l'on croyait absent du modèle, mais qui avait été récemment suggéré.

Nos résultats suggèrent plusieurs changements pouvant représenter des mécanismes ou des marqueurs de l'effet antidépresseur de la mfb-DBS. Les oscillations gamma ont été affectées à l'ECoG ainsi que dans le CPFm pendant le sommeil lent, tandis que l'architecture globale du sommeil n'a pas été modifiée de façon notable. Cette modulation de la physiologie du sommeil lent, sans modification additionnelle de l'architecture du sommeil, suggère une séparation intéressante des effets de la mfb-DBS sur des aspects du sommeil habituellement liés. La mfb-DBS a également entraîné des altérations de la transmission monoaminergique aux sites cibles des voies mésolimbique et mésocorticale ; dans le NAc, les contenus en DA et en 5-HT ont diminué après 24h de mfb-DBS, tandis que dans le CPFm, des changements dans le métabolisme de ces deux molécules ont été suggérés. L'expression des types de récepteurs de la DA a été modifiée de manière différentielle dans le NAc et le mPFC : chez le FSL, les récepteurs D1 de la DA étaient régulés à la hausse dans le mPFC, et les récepteurs D2 régulés à la baisse dans le NAc ; chez les rats non dépressifs, les récepteurs D1 et D2 étaient régulés à la baisse dans le mPFC et le NAc. Ces observations fournissent des preuves que la mfb-DBS module les systèmes monoaminergiques ascendants ; plus précisément, dans le NAc, ces changements peuvent suggérer une libération élevée de DA, suite à la mfb-DBS, tandis que dans le mPFC l'expression des récepteurs D1 a pu être modulée indépendamment de la libération de DA. Bien qu'il soit nécessaire de valider davantage la dynamique exacte de ces changements et la façon dont ils peuvent refléter

une modulation plus large de la transmission dopaminergique, une base théorique solide et des similitudes avec les effets observés après d'autres traitements suggèrent que ces changements peuvent faire partie de la réponse antidépressive induite par la mfb-DBS.

Enfin, la mfb-DBS a conduit à une augmentation de l'expression des gènes marqueurs immédiats de la plasticité synaptique, Homer1a et Arc, dans le mPFC des rats FSL. L'expression de ces protéines est liée au remodelage synaptique dépendant de l'activité, et a été associée à de nombreux traitements antidépresseurs, l'amélioration de la plasticité corticale étant théoriquement une voie commune finale d'un effet antidépresseur.

Les résultats présentés ici fournissent une caractérisation plus approfondie des déficits de sommeil dans un modèle établi de dépression, et démontrent une variété d'effets potentiels de la mfb-DBS, allant de la modulation des paramètres électrophysiologiques pendant le sommeil, notamment le sommeil lent, à l'altération de la transmission monoaminergique et à la potentialisation des mécanismes de plasticité corticale. Les changements observés étant non seulement associés à la pathophysiologie de la dépression mais également aux mécanismes des traitements antidépresseurs, ils révèlent comment peut agir la mfb-DBS. Bien que les effets de cette dernière soient probablement plus complexes et s'étendent sans doute au-delà de ceux décrits ici, ces résultats fournissent un aperçu de cette modalité de traitement, et une base solide pour une exploration plus approfondie de ses mécanismes.

Abbreviations

ACC	anterior cingulate cortex
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AMY	amygdala
AP	anterior-posterior
Arbp	acidic ribosomal phosphoprotein P0
Arc	activity-regulated cytoskeleton-associated protein
BDNF	brain-derived neurotrophic factor
BLA	basolateral amygdala
CB	cerebellum
CC	corpus collosum
CDM	chronic despair model
cDNA	complementary DNA
cLH	congenital learned helplessness
CMS	chronic mild (unpredictable) stress
Ctrl	Sprague Dawley non-depressive-like control
COMT	catechol-O-methyltransferase
CSF	cerebrospinal fluid
DA	dopamine
DAT	dopamine transporter
DBS	deep brain stimulation
DOPAC	3,4-dihydroxyphenylacetic acid
DRD1	dopamine D1 receptor
DRD2	dopamine D2 receptor
DRN	dorsal raphe nucleus
DSM-V	Diagnostic and Statistical Manual 5th Edition
DV	dorsal-ventral
ECoG	electrocorticogram
ECT	electroconvulsive therapy
EEG	electroencephalogram

e/i	excitatory/inhibitory
EMG	electromyogram
FRL	Flinders Resistant Line
FSCV	fast scanning cyclic voltammetry
FSL	Flinders Sensitive Line
FST	forced swim test
Glu	glutamate
GABA	gamma-aminobutyric acid
GADPH	glyceraldehydes-3-phosphate dehydrogenase
GWAS	genome-wide association study
HC	hippocampus
HPLC	high-performance liquid chromatography
HVA	homovanillic acid
H1a	Homer1a
ICSS	intracranial self-stimulation
IEG	immediate early gene
ILPFC	infra-limbic pre-frontal cortex
LC	locus coeruleus
LDP	long term depression
LDT	laterodorsal tegmental nuclei
LFP	local field potential
LH	lateral hypothalamus
LHb	lateral habenula
LPT	lateral pontine tegmentum
LTP	long term potentiation
MADRS	Montgomery-Åsberg Depression Rating Scale
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
MDD	major depressive disorder
mfb	medial forebrain bundle (referring to the rodent structure)
MFB	medial forebrain bundle (referring to the human structure)
ML	medial-lateral

mPFC	medial prefrontal cortex
MRN	median raphe nucleus
mTOR	mechanistic target of rapamycin
NA	noradrenaline
NAc	nucleus accumbens
NMDAR	N-methyl-D-aspartate receptor
NPAS4	neuronal PAS domain-containing protein 4
NREM	non-REM
OB	olfactory bulb
OBX	olfactory bulbectomy
OFC	orbitofrontal cortex
OT	olfactory tubercle
PFC	pre-frontal cortex
PPTg	pedunculopontine nucleus
ppVTA	projection pathway of the ventral tegmental area
PrL	pre-limbic cortex
PSG	polysomnography
qPCR	quantitative real-time polymerase chain reaction
REM	rapid eye movement
RMTg	rostromedial tegmental nucleus
RN	raphe nuclei
SCN	subthalamic nucleus
SCT	sucrose consumption test
SDS	social defeat stress
SERT	serotonin transporter
SGC	subgenual cingulate cortex
SHY	synaptic homeostasis hypothesis
slMFB	supero-lateral branch of the medial forebrain bundle
SNRI	selective noradrenaline reuptake inhibitor
SPT	sucrose preference test
SSRI	selective serotonin reuptake inhibitor
SWA	slow wave activity

SWS	slow wave sleep
TCA	tricyclic antidepressant
TH	tyrosine hydroxylase
TMS	transcranial magnetic stimulation
TRD	treatment resistant depression
TST	tail suspension test
vlPAG	ventrolateral periaqueductal grey
vmPFC	ventromedial pre-frontal cortex
VP	ventral palladium
vSUB	ventral subiculum
VTA	ventral tegmental area
WHO	World Health Organisation
WKY	Wistar Kyoto
5-HT	serotonin (5-hydroxytryptamine)
5HIAA	5-hydroxyindoleacetic acid

List of figures

Figure 1.1. A schematic diagram of nuclei connections involved in reward and effect, presented in the rat brain. 8

Figure 1.2. The two principle tests of depressive-like behaviour in rats. 12

Figure 1.3. Graphical representation of implanted electrodes for the implementation of DBS. 17

Figure 1.4. Currently investigated targets of deep brain stimulation for depression. 19

Figure 1.5. A graphical representation of monoaminergic projections of the rodent mfb. 20

Figure 1.6. The human slMFB imaged by diffusion tensor imaging utilising global tractography algorithms. 21

Figure 1.7. Response of treatment-resistant patients to slMFB-DBS. 22

Figure 1.8. Schematic diagram of mesocorticolimbic regulatory afferent circuits influencing the activity of dopaminergic VTA neurons. 27

Figure 1.9. Schematic example of how network dysregulation may result in hypo-functionality of dopaminergic VTA projections. 28

Figure 1.10. An example hypnogram from a healthy human subject. 41

Figure 1.11. Oscillatory activity recorded by human EEG in wake, SWS and REM sleep. 42

Figure 1.12. An example hypnogram from an untreated depressed patient. 46

Figure 1.13. Slow wave activity during slow wave sleep in a healthy adult and depressed patient. ...48

Figure 1.14. Proposed local and distal effects of high frequency (130Hz) deep brain stimulation 56

Figure 1.15 Dopamine (DA) release in the nucleus accumbens (NAc) as measured by fast scanning cyclic voltammetry. 64

Figure 3.1. Example spectrograms taken from ECoG recordings. 88

Figure 3.2. Estimation of electrode placement in the mfb. 89

Figure 3.3. Target tissue of the mPFC for collection after micro dissection. 90

Figure 3.4. Target tissue of the NAc for collection after micro dissection. 91

Figure 4.1. Experimental timeline. 98

Figure 4.2. Deep electrode implantation for rats in the sleep groups. 99

Figure 4.3. Positions of electrodes included in the study. 101

Figure 4.4. Baseline sleep architecture in controls and the FSL. 103

Figure 4.5. Baseline SWS spectral activity in controls and the FSL. 106

Figure 4.6. Baseline REM spectral activity in controls and the FSL. 107

Figure 4.7: Immobility as measured in the forced swim test. 108

Figure 4.8 Sleep architecture in control and FSL rats at baseline, experimental days 1 and 7 111

Figure 4.9: Time course of SWS spectral activity before and after mfb-DBS. 114

Figure 4.10: Time course of REM spectral activity before and after mfb-DBS. 115

Figure 5.1. Experimental timeline. 125

Figure 5.2. Behavioural measures of control and FSL animals at baseline. 127

Figure 5.3. Immobility in control and FSL animals as measured in the FST, pre- or post- 24h sham- or mfb-DBS. 129

Figure 5.4. Sucrose consumption in control and FSL animals, pre- or post- 24h sham- or mfb-DBS. ... 131

Figure 5.5. Expression of dopamine receptors and in response to mfb-DBS in the FSL. 133

Figure 5.6. Expression of dopamine receptors and in response to mfb-DBS in controls. 134

Figure 5.7. Expression of plasticity markers in response to mfb-DBS in the FSL.....136

Figure 5.8. Expression of plasticity markers in response to mfb-DBS in controls. 137

Figure 6.1. Experimental timeline. 146

Figure 6.2. Tissue concentration of dopamine and its metabolites in the nucleus accumbens. 149

Figure 6.3. Tissue concentration of serotonin and its metabolites in the nucleus accumbens.....150

Figure 6.4. Tissue concentration of dopamine and its metabolites in the medial pre-frontal cortex. 152

Figure 6.5. Tissue concentration of serotonin and its metabolite in the medial pre-frontal cortex. ...153

1. Introduction

1.1 Depression and medial forebrain bundle deep brain stimulation

1.1.1 Depression

Major depressive disorder (MDD, depression) is a mental disorder characterised by depressed mood and reduced motivation and pleasure. It represents the largest cause of disability worldwide according to the World Health Organisation (World Health Organisation, 2017). One in six adults will be affected in their lifetime, with the number of sufferers worldwide numbering more than 300 million people. Women are more commonly affected than men; in 2015 the global population population with depression was estimated at 5.1% for females and 3.6% for males. While some variation in prevalence exists, it is considered common in every country across the world (Demyttenaere *et al.*, 2004). The incidence of depression is increasing, with 18% more people living with depression in 2015 than in 2005 (Vos *et al.*, 2016). It ranks highly on all measures of burden including social, economic and medical factors, with years lost to disability due to depression rising by 14% between 2007 and 2017 (James *et al.*, 2018). Alongside these burdens, it carries increased suicide risk, a prominent cause of death worldwide, particularly amongst young people (Kessler, 2012). Depression is marked by its heterogeneity across almost all aspects. Its aetiology is multifactorial, its presentation, course and severity varies between patients and it is co-morbid with a number of other diseases (Bentley, Pagalilauan and Simpson, 2014). It is episodic, with episodes associated with both MDD and other classifications of mental disorder including bipolar disorder. Episodes vary in length and although they typically resolve over time, depression is often a persistent and recurring chronic condition (Palazidou, 2012).

Aetiology of depression: genetic predisposition

The factors leading to the development of depression are highly variable. While an episode is often preceded by a stressful life event (Kendler, Thornton and Gardner, 2001), the level of stress required to trigger depression varies enormously between people. This has been suggested to imply a genetic predisposition to the disease. Studies of familial risk have estimated the heritability of depression at 30-40% (Sullivan, Neale and Kendler, 2000; Colodro-Conde *et al.*, 2018); despite this, both genome wide association studies (GWAS) examining depressed patients and studies of specific candidate genes have provided ambiguous results, failing to clearly identify specific genetic causes of the disease (Shadrina, Bondarenko and Slominsky, 2018). Candidate genes are investigated according to their theoretical relationship to pathophysiology, for example in relation to monoaminergic function (hypotheses of depression will be discussed later in this section). Of candidate genes for depression, *SLC6A4* is probably the most studied, encoding for the serotonin transporter (SERT) which clears serotonin (5-HT) from the synaptic cleft. Although polymorphisms of SLC6A4, along with others linked to monoaminergic transmission, have been associated with increased risk, findings have been inconsistent and sometimes irreproducible; this may be due to small effects and differences in sample populations (Murphy, Maile and Vogt, 2013; Gatt *et al.*, 2015; Oo *et al.*, 2016). GWAS studies have also highlighted potentially implicated genes. An example is the *Homer1*, a gene encoding proteins implicated in glutamatergic transmission and synaptic plasticity (Rietschel *et al.*, 2010). While *Homer1* has proved the basis of fruitful further research, and recent studies have identified further genes of interest (Wray *et al.*, 2018; Howard *et al.*, 2019), GWAS studies in general have failed to consistently identify genetic loci conferring specific predisposition to depression (Shadrina, Bondarenko and Slominsky, 2018).

These ambiguous findings do not preclude the influence of genetic factors on depression. Rather, they reflect the complexity and heterogeneity of depression, indicating that predisposition involves the interaction of multiple genetic variations, with each individual variant making a relatively small contribution.

Aetiology of depression: environmental contribution

The other side of the gene-environment interaction coin, stress constitutes the primary external risk factor in depression. Stress is a natural factor in all lives, and proper behavioural reaction to stress serves as an adaptive response to the environment which may be protective from harmful situations. Neither stress alone, nor hereditary factors suggested above, definitely leads to depression. Rather it is the interaction between the two which leads to pathology. Depression can therefore be considered a maladaptive response to stress, primed to some degree by underlying biological influence (Krishnan and Nestler, 2008). Although some forms of depression have been termed 'endogenous' (i.e., without external influence), reviews of this classification have suggested stress still plays a role (Mazure, 1998). It may be however, that in cases of extreme susceptibility, what would be normally considered mild stress is enough to induce depression. This 'stress-diathesis' model describes a complex and fluid interaction between personal characteristics, genetics, and different modalities and contexts of stress (Hammen, 2005). Long-lasting effects of stress, particularly in early life, demonstrate how changes imparted by the environment contribute to future susceptibility, including via epigenetic changes (Han and Nestler, 2017; Park *et al.*, 2019). A previous depressive episode is itself the biggest predictor of future episodes, with the risk of recurrence increasing with each successive episode (Solomon *et al.*, 2000). The influence of stress on biology will be explored further during **section 1.2**.

Symptoms and diagnosis

Depressive episodes are diagnosed solely based on symptomatology, due to the lack of identified depression-specific biomarkers (Hacimusalar and Eşel, 2018). Two diagnostic guides are commonly used diagnosis of depressive disorders: the US Diagnostic and Statistical Manual, 5th edition (DSM-V), and the International Statistical Classification of Diseases and Related Health Problems, 10th edition (ICD-10). According to the DSM-V (American Psychiatric Association, 2013), diagnosis of MDD requires five or more of the following symptoms in the same two week period:

- depressed mood
- loss of interest or pleasure ('anhedonia')

- change in weight or appetite
- insomnia or hypersomnia
- psychomotor retardation or agitation
- loss of energy or fatigue
- worthlessness or guilt
- impaired concentration or indecisiveness
- thoughts of death or suicidal ideation/attempt

The core symptoms of depression are affective: depressed mood (feelings of sadness, emptiness, hopelessness) and anhedonia (the lack of interest or pleasure taken in previously pleasurable activities), and at least one of these two must be present among the above for diagnosis (Otte *et al.*, 2016). The other symptoms may be variously present, creating a wide variety of possible presentations, with severity of individual symptoms another point of variability (Bentley, Pagalilauan and Simpson, 2014). This massive heterogeneity within the disorder creates complications for diagnosis, research and treatment; to combat this, various 'subgroups' of depression are often described, to differentiate common symptom clusters, onset and course (Rush, 2007). The most common subtypes are melancholic depression, characterised by persistent low mood and anhedonia, psychomotor retardation, and somatic symptoms; atypical depression is defined by more reactive mood, agitation and fatigue without sustained low affect; anxious depression characterised by co-occurring anxiety behaviours; and psychotic depression which has delusional or hallucinatory features relating to mood (Rush, 2007; Uher *et al.*, 2011). These subgroups help to guide diagnosis and treatment for a disorder in which two patients may only share a single symptom. However, the groups are not strictly delineated or mutually exclusive, and their clinical usefulness as been questioned (van Loo *et al.*, 2012).

While diagnostically separate, somatic and psychological symptoms are multi-dimensional and interact with one another. Sleep disturbances are a somatic change, but are psychologically distressing for patients; and fatigue and appetite changes can have a large impact on psychological aspects such as self-esteem and overall quality of life (Katz and McHorney, 2002; Nutt, Wilson and Paterson, 2008). Overall,

the symptomatology of depression is varied and complex. Some have questioned the sense of classifying such variety in a single disorder; the conception of subgroups gives some direction, but heterogeneity remains a challenge in diagnosis, treatment and research of depression (van Loo *et al.*, 2012).

Sleep and depression

Sleep shares an almost fundamental relationship with depression. Outside of key diagnostic symptoms, sleep disturbances are perhaps the most significant aspect of the disease. Insomnia is the most common disturbance, in 80% of patients, with hypersomnia in 15-35% (Yates *et al.*, 2007; Steiger and Pawlowski, 2019); this overlap illustrates that some patients suffer from both hypersomnia and insomnia during the same episode (Jindal and Thase, 2004). Disturbed sleep often precedes other symptoms and represents a significant risk factor (Baglioni *et al.*, 2011). This bi-directional relationship has seen sleep disturbance described as a prodromal, predictive symptom (Alvaro, Roberts and Harris, 2013; Fang *et al.*, 2019). Although not strictly prerequisite, it has been suggested that diagnosis of depression without sleep problems should be made with caution, such is the link between the two (Jindal and Thase, 2004). Furthermore, sleep symptoms often persist beyond the depressive episode, and are predictive of future episodes (Nutt, Wilson and Paterson, 2008; Pandi-Perumal *et al.*, 2020). Disruption of sleep is highly distressing for patients, and is reported as one of the most significant factors affecting quality of life (Katz and McHorney, 2002; Mayers, van Hooff and Baldwin, 2003), which may suggest a causal link to subsequent episodes.

The strong association between sleep and depression has a broad theoretical basis, as various systems involved in the physiology of normal sleep also contribute to the regulation of mood, motivation and other systems deeply implicated in depression. These systems, their normal functioning and their pathophysiology in depression will be explored in depth in **section 1.3.**

Linking symptoms to biological dysfunction

The resolution of symptoms, whether through treatment or spontaneous remission, does not necessarily occur for all symptoms simultaneously. Residual symptoms are strong predictors of future episodes (Carney *et al.*, 2007; McClintock *et al.*, 2011; Zajecka, Kornstein and Blier, 2013). This may be due to stress caused by these residual symptoms acting as a trigger, but may also reflect unresolved pathophysiologies specific to certain symptoms. The relationships between symptoms and pathophysiology are not completely understood. A growing body of evidence links specific symptoms and pathologies, but heterogeneity and complexity in these relationships leaves many questions open. This has been likened to diabetes, which was previously symptomatically and categorically diagnosed (elevated blood glucose, with or without obesity, for example), until advancing understanding of the pathophysiology allowed meaningful classification (insulin deficiency vs insulin receptor sensitivity), improving diagnostics and treatment (Kupfer, 2002). Almost twenty years on since this comparison, and despite advances in our understanding of the pathophysiology of depression, diagnosis remains based on symptomatology, as no reliable, non-invasive and quantitative test unique to depression has yet been found (Hacimusalar and Eşel, 2018). Despite this, numerous potential biomarkers have been identified. Measurable in blood serum tests, depressed patients tend to show metabolic changes (Pan *et al.*, 2012), higher pro-inflammatory markers (Haapakoski *et al.*, 2015), and overactive endocrine function relating to the stress-reactive hypothalamic-pituitary-adrenal (HPA) axis (Horowitz and Zunszain, 2015). Other candidate biomarkers relate more directly to neural functioning. Of great interest are neurotrophic factors, proteins relating to the growth and maintenance of neurons and their connections, therefore crucial to the health and proper function of neural tissue. Brain-derived neurotrophic factor (BDNF), a key marker and functional element in neural plasticity, is reportedly decreased in depressed patients (Molendijk *et al.*, 2014). Changes in neuroimaging measures, such as grey matter volume, are also promising markers (Brand, Moller and Harvey, 2015). Despite strong evidence for some of these biomarkers, the heterogeneity of depression and methodological inconsistencies have so far prevented their establishment in clinical practice. Further understanding of the role of these potential biomarkers, and their practical im-

plementation could be crucial to better defining the complexity within depression and leading to better treatment (Strawbridge, Young and Cleare, 2017).

Brain structures implicated in depressive symptoms

The heterogeneity evident in almost all aspects of depression is a manifestation of enormous complexity in the neurobiology underpinning the disease. This is reflected in the genetic and aetiological variety, the range of symptoms and their severity, the diversity of potential biomarkers and the difficulty in establishing their role. Several pathways and systems are implicated in depression. The localisation of depressive pathology is somewhat hard to conceptualise. In addressing the question, "where in the brain is depression," Pandya and colleagues (2012) concluded that depression "seems to lie in many brain regions as well as nowhere in particular". Others have described the disorder as a "functional lesion" rather than a specifically localised one (Mayberg, 2003). Depression is now more commonly thought of as a network disorder, associated with dysfunction at and between nodes of communication reaching across the brain (Pandya *et al.*, 2012).

Core symptoms of low mood, anhedonia and motivational deficit implicate systems of reward and affect (Russo and Nestler, 2013); these systems involve a diffuse network of reciprocally connected nuclei. Brain stem nuclei, the ventral tegmental area (VTA) and raphe nuclei (RN; including the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN)), send ascending dopaminergic and serotonergic projections, respectively, to limbic structures, including the nucleus accumbens (NAc) and amygdala (AMY), and to areas of the pre-frontal cortex (PFC), which contains various subregions including the orbitofrontal frontal cortex (OFC), anterior cingulate cortex (ACC) and subgenual cingulate gyrus (SGC). Limbic and frontal areas communicate with the hippocampus (HC), as well as projecting back to brainstem nuclei. Finally, the brainstem nuclei form circuits with the lateral habenula, which acts to inhibit the activity of both the VTA and RN (figure 1.1; these circuits will be examined in more detail in **section 1.2**) (Der-Avakian and Markou, 2012). Dysregulation of sleep, appetite and arousal is suggested by somatic and neurovegetative symptoms (Nutt, Wilson and Paterson, 2008; Otte *et al.*, 2016). Several neurochemical systems ascending from the brainstem control arousal and sleep, including projections from

the VTA, RN and the noradrenergic locus coeruleus (LC) (Eban-Rothschild, Appelbaum and de Lecea, 2018). Cognitive deficits implicate various cortical and subcortical mechanisms, with theorised roles of the hippocampus, thalamus and areas of the PFC (Grahek *et al.*, 2019). Substantial overlap exists in both the anatomical and neurochemical substrates of systems implicated in various depressive symptoms. On one hand, this may help explain the connection and interaction between symptoms; yet differential emergence of symptoms further complicates the picture.

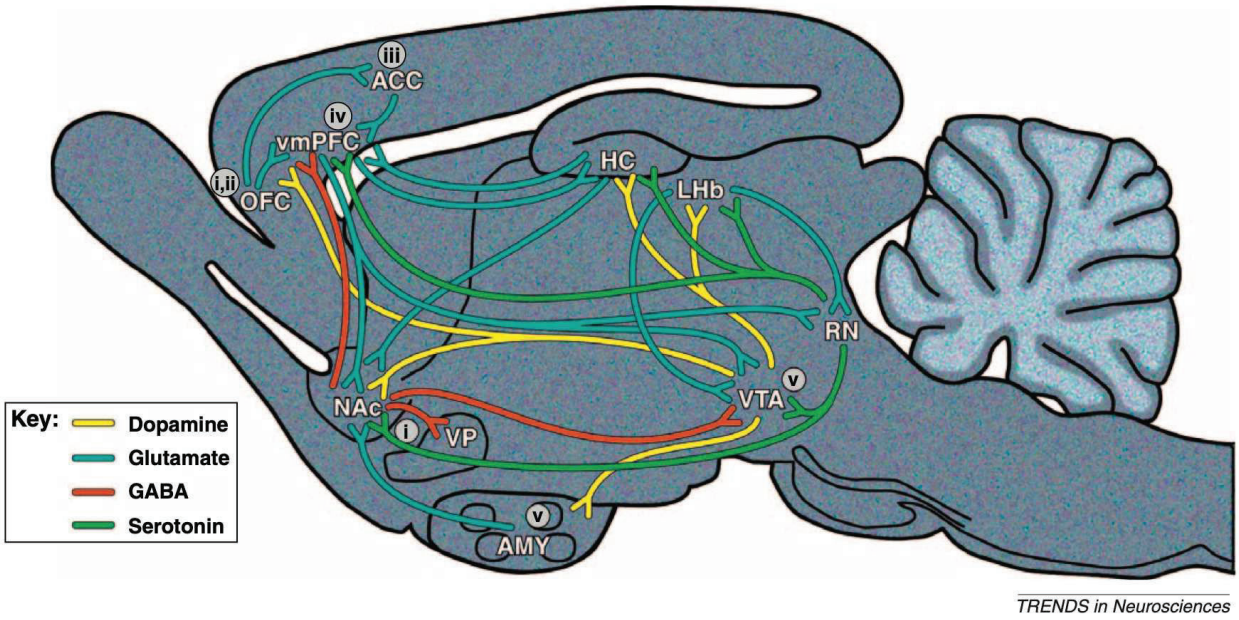

***Figure 1.1**. A schematic diagram of nuclei connections involved in reward and effect, presented in the rat brain.*
ACC: anterior cingulate cortex; AMY: amygdala; HC: hippocampus; LHb: lateral habenula; NAc: nucleus accumbens; OFC: orbitofrontal cortex; RN: raphe nuclei; vmPFC: ventromedial prefrontal cortex; VP: ventral palladium; VTA: ventral tegmental area. The vmPFC of the rat is regarded as approximately homologous to the SGC of the human. Reproduced with permission from Der Avakian and Markou, 2012.

Dysfunctional systems in depression

Crucial to the networks outlined above, the principal monoamines – neuromodulatory transmitters dopamine (DA), serotonin (5-HT) and noradrenaline (NA) – have long been implicated in depression. The actions of early anti-depressant pharmaceuticals, found to elevate synaptic availability of 5-HT, NA and DA, led to the post-hoc theory of their involvement in the original pathology (Pereira and Hiroaki-Sato,

2018). If enhancing the activity of the transmitters was anti-depressant, it was presumed this was normalisation of a pathological deficiency. It has since been shown that although depletion can reverse the effects of treatment in remitted patients, it does not induce symptoms in healthy subjects, suggesting that reduced monoaminergic activity is not causative of depression (Delgado *et al.*, 1993, 1999; Miller *et al.*, 1996). Despite the oversimplification of this original monoamine hypothesis, monoaminergic function is thought to play an important role in the varied mechanisms of depressive biology (Elhwuegi, 2004).

Depression is now more commonly understood as the common outcome of various related pathologies; while this is a strong foundation for better understanding of the disease, the specific mechanisms and how they interact remains broadly to be explained.

Theoretically, it is attractive, and intuitive, to neatly tie a specific symptom to a specific deficit. Depression could therefore be considered the cumulation of several co-existing but separate pathologies—a cluster of related syndromes rather than a single disease (Nestler *et al.*, 2002). This approach is attractive when considering those patients who share few common symptoms – only the dysfunctions present in each patient manifest in the disease. It risks failing, however, to account for shared substrates between symptoms, and to truly explore the relationships between them. For instance, dysfunction of mesolimbic dopaminergic projections to the NAc is implicated in amotivational symptoms (Nestler and Carlezon, 2006) and improper sleep regulation (Eban-Rothschild *et al.*, 2016); considering them as neatly separate syndromes may disguise critical overlap in mechanisms, even if the dysfunction is not directly communal. While it cannot be excluded that depressive symptoms are truly separate, a contrasting approach would see fundamental cellular and molecular deficits converging on common pathways to produce circuit-level mechanisms (Thompson *et al.*, 2015). In our example of mesolimbic projections, hypo-function of DA receptors in the NAc may cause blunted motivational response during wake, and improper regulation of arousal during sleep. This may better illustrate interplay between symptoms with common substrate, but perhaps fails to acknowledge the extent of heterogeneity – absence or bi-directionality of symptoms (hypersomnia vs

insomnia, psychomotor retardation vs agitation, for example) between patients, or even experienced by a patient within the same episode (Jindal and Thase, 2004).

Depression as a matrix of pathologies

Drawing from both of these interpretations is the idea of depression as a "matrix" of related pathologies (Watt and Panksepp, 2009; Dean and Keshavan, 2017). Each pathology is a node on this matrix, capable of influencing the network. Some common molecular mechanisms may cause primary pathologies in multiple systems, while secondary abnormalities may arise as downstream consequences of impaired neural transmission or compensatory effects. The primary pathology for each patient may be be different, and nodes affected to varying degrees, producing the range of presentations of the disease. Predispositions, via heritable factors, early life stress or previous episodes of depression, may prime the response of certain nodes to stress, while acute and chronic stress may be sufficient to induce some pathologies, in fitting with the diathesis-stress model outlined above.

This unified, matrix model of depression provides a framework in which specific pathologies identified by experimental evidence can be understood. Among these pathologies are abnormalities in the serotonergic, noradrenergic and dopaminergic systems; in glutamatergic and GABAergic systems (the principle excitatory and inhibitory systems of the brain, respectively); in the HPA axis, central to the stress response; and in mechanisms of plasticity, the brains adaptive response to its environment (Willner, Scheel-Krüger and Belzung, 2013; Dean and Keshavan, 2017). In **section 1.2**, some of these mechanisms will be explored in greater detail, with a focus on the dopaminergic system and its interactions with other depressive mechanisms, and the relationship with anti-depressant treatment response.

1.1.2 Modelling depression in animals

The majority of work discussed in this thesis will be relate to pre-clinical investigations of the neurobiology of depression and the response to treatment. Despite the inherently human nature of depression, investigating the underlying biology in patients remains difficult. Although advances in neuroimaging have emerged as a

valuable research tool, the majority of our biological understanding has come from animal models of depressive-like phenotypes. Despite the difficulties of modelling depression in animals, and the subsequent limitations of such models, they have and continue to contribute significantly to research (Czéh and Simon, 2020).

Depressive-like behaviours in animal models

Two principal depressive-like behaviours are modelled in rodents (Belovicova *et al.*, 2017). The first is measured in the forced swim test (FST, figure 1.2A) (Porsolt, Le Pichon and Jalfre, 1977). In the FST, the animal is placed in a cylinder of water from which it cannot escape. Immobile, floating behaviour (i.e., not struggling to escape) is taken as representative of a depressive-like phenotype, sometimes described as a passive stress response or as "behavioural despair" (Slattery and Cryan, 2012; Yankelevitch-Yahav *et al.*, 2015). Although it does not directly resemble human behaviour, immobility in the FST is associated with lack of energy, reduced motivational state and response to aversive stimuli, and is responsive to known anti-depressant treatments (Czéh *et al.*, 2016; Scheggi, De Montis and Gambarana, 2018). A variation on this test is the tail suspension test (TST), used in mice, in which the animal is suspended by the tail in an inescapable and mildly stressful position, with lack of escape behaviour representative of depressive-like behaviour (Steru *et al.*, 1985). Also considered a measure of behavioural despair is the learned helplessness paradigm, in which animals are exposed to inescapable foot shock stress, resulting in susceptible animals responding passively when given the opportunity to escape avoidable shocks (Vollmayr and Henn, 2001).

The second principal depressive-like behaviour measured is an anhedonia-like phenotype. The term anhedonia (or loss of interest or pleasure) applied as a diagnostic tool in humans can describe a broad range of behavioural changes, reflecting several aspects of reward or motivational behaviour (Scheggi, De Montis and Gambarana, 2018). Aside from a blunted response to pleasure, deficits in the anticipation or prediction of reward, assessment of reward value, determination of effort required, integration of risk/reward judgement, the motivation to act and reward-learning could all separately lead to anhedonia; these mechanisms are neurobiologically varied and distinct from pleasure (Der-Avakian and Markou, 2012).

Measuring anhedonia-like phenotype in animals is therefore complex, and the aspect of reward being measured must be carefully considered (Treadway and Zald, 2011). The most common test, the sucrose preference test (SPT, figure 1.2B) measures an animal's consumption of a sweet solution compared to consumption of water (Overstreet, 2012; Rizvi *et al.*, 2016). Preference is generally considered a measurement of the 'liking' (i.e., hedonic) aspect of reward; it has been suggested that absolute sucrose consumption in the test is more representative of the 'wanting' (i.e. motivational salience) aspect of reward, and shown that the two can be differentially affected by experimental manipulations (Meyerolbersleben, Winter and Bernhardt, 2020). The element of choice (between bottles) in the SPT has also been suggested to introduce the aspect of reward learning (Schneider, Heise and Spanagel, 2010). Reduction of sucrose preference or consumption are therefore taken as a measure of anhedonia-like behaviour, but may represent varying deficits of reward functioning.

Beyond these two principal measurements, a variety of tests exist relating to the range of symptoms commonly present in depression. Monitoring of locomotion can be used to assess psychomotor retardation, anxiety-like behaviours may be measured by social interaction/avoidance or time spent in open vs sheltered areas (File and Seth, 2003; Belovicova *et al.*, 2017) and cognitive deficits in learning and memory tasks (Czéh *et al.*, 2016).

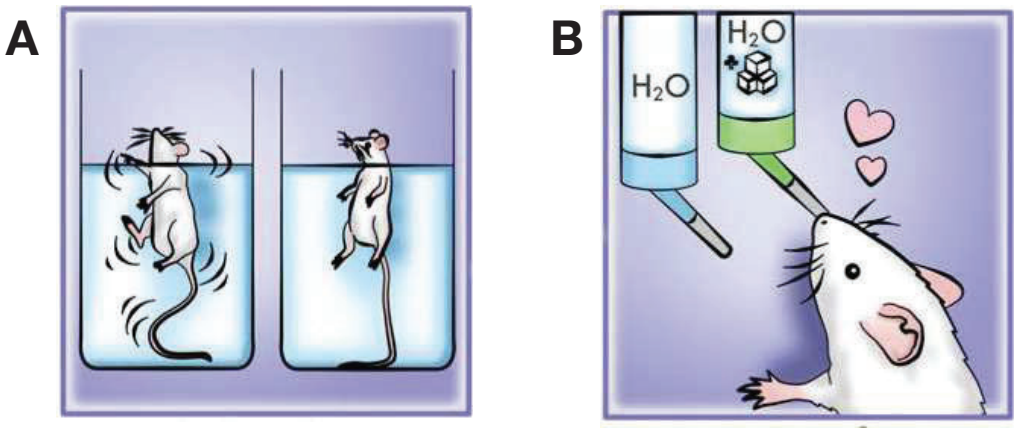

***Figure 1.2**. The two principle tests of depressive-like behaviour in rats. In the forced swim test (A), depressive-like behaviour is represented by passive floating during inescapable stress. In the sucrose preference test (B), anhedonic-like behaviour is represented by a reduced preference for or consumption of a sweet solution in contrast to water. Adapted from Planchez et al., 2019.*

The validity of animal models

An animal model of depression can be assessed across three main categories of validity: face validity, the resemblance of the behaviour of the animal to human symptoms; predictive validity, the amelioration of phenotype by clinically effective treatments, and a lack of response to clinically non-effective treatments; and construct validity, a theoretical and experimentally validated relationship between the pathophysiology of the model and the human disease (Willner, 1984; Belzung and Lemoine, 2011). A proposed supplementary criteria relating to face validity, aetiological validity, represents the likeness to the onset and course of the illness (Czéh *et al.*, 2016; Czéh and Simon, 2020). While all animal models are limited and tend to model only certain aspects of depression, their validity continues to be considered with increasing research into their phenotype, allowing insight into the different facets of human depression they model (Willner and Belzung, 2015).

Established animals models for depression

Several methods have been used to induce depressive-like phenotypes in rodents, based on different forms of validity. The diathesis-stress framework, described above, provides the foundation for an aetiological approach. For example, various paradigms of stress can be used to induce depressive-like phenotype in healthy rodents. The most common are the chronic mild stress (CMS) model, in which a variety of mild stressors are applied over time, (Willner, 2017); the learned helplessness model, induced by repeated inescapable stress (Vollmayr and Henn, 2001); and social defeat stress (SDS), where animals are paired with dominant social partners (Golden *et al.*, 2011). These models have strong aetiological validity, as they mirror the triggering of symptoms by stress in humans. Other rats are selectively bred according to a phenotype to produce a strain with a predisposition for depressive-like behaviour. Models of predisposition to depression include the Flinders Sensitive Line (FSL) rat (Overstreet and Wegener, 2013), the Wistar Kyoto (WKY) (Aleksandrova, 2019) and the congenital learned helpless (cLH) model (Vollmayr *et al.*, 2004). These models often have high face validity, as they are selected for their phenotype. Vulnerability to stress can also be considered aetiologically valid, al-

though spontaneous depression fails to model common onset in humans. However, whether their biological substrates match those in humans with depression is a point of uncertainty. Finally, direct manipulations may be used to induce a depressive-like phenotype. In the olfactory bulbectomy (OBX) model, the olfactory bulb is surgically removed, producing depressive-like behaviour (Song and Leonard, 2005). Genetic manipulations are also common in mice; for example, a knockout of the SERT gene produces depressive-like behaviour (Haenisch and Bönisch, 2011). In other cases, pharmacological, optogenetic or lesion-based manipulations can be used to induce symptoms. For example, Parkinsonian models may involve chemical lesioning of dopamine-producing nuclei, leading to depressive-like symptoms (Furlanetti, Coenen and Döbrössy, 2016); direct modulation of dopaminergic neurons via optogenetics can also induce depressive-like behaviours (Tye *et al.*, 2013). These direct manipulations tend to have high specific construct validity, as they typically target a system implicated in human depression. However, this construct validity is partial, as it likely does not reflect the poly-modal nature of depression, aetiological validity is poor and face validity may be narrowly symptom-specific (Planchez, Surget and Belzung, 2019).

While no model provides strong validity in all categories, several have been well established experimentally, and provide the foundation of research into depression that would be impossible in human patients (Czéh and Simon, 2020). The phenotype and validity of the FSL rat, as the model used in the experimental work of this thesis, will be further discussed in **section 1.5.**

1.1.3 Treatment of depression and the problem of treatment resistance

Treating depression

Mirroring the diagnostic process, the treatment of depression is focused on the resolution of symptoms, as opposed to the monitoring of biomarkers. Conventional treatment is based on pharmacological and psychological interventions. Although

various anti-depressant drugs are prescribed, they fall into three broad categories (first or second generation, and atypical antidepressants) and all major pharmacological anti-depressants act on the monoamine systems, DA, 5-HT and NA (Pereira and Hiroaki-Sato, 2018). Although well studied, the mechanisms by which these drugs effect anti-depressant response is not well understood. Improvement of symptoms occurs typically after 2-4 weeks, despite the acute biological effects occurring within hours; despite this lag, clinical effect is indeed associated with monoaminergic transmission, as impeding 5-HT, NA or DA transmission can blunt the effect of specific drugs (Miller *et al.*, 1996; Delgado *et al.*, 1999; Willner, Hale and Argyropoulos, 2005). Like the underlying pathophysiology, treatment response is now hypothesised to be multifactorial. Potentiation of monoamine transmission is one mechanism which may lead to remission, likely through inducing downstream plastic responses in other systems via direct or indirect modulation (Willner, Scheel-Krüger and Belzung, 2013; Rincón-Cortés and Grace, 2020).

Treatment resistance

Conventional anti-depressants, alongside cognitive therapies, lead to remission in approximately 70% of patients (Rush *et al.*, 2006). However, the course of treatment is not always straightforward, with residual symptoms a common problem. The figure of 70% is representative of cumulative remission, with patients who do not respond to a first treatment treated with a second, up until a 4th treatment step. Rates of remission decrease with each step, from 37% in the first to just 13% in the fourth. Most importantly, a substantial minority of around 30% of patients do not respond after successive treatment, and can be classed as suffering from treatment-resistant depression (TRD) (Rush *et al.*, 2006). There is no single accepted definition of TRD (Bartova *et al.*, 2019), and other estimates vary depending on factors such as the chosen threshold of non-response. For example, figures of 53% and 60% percent have been cited considering non-response to single treatments (Dold and Kasper, 2017). Other important nuances relate to factors such as patient non-compliance due to intolerable side effects, and the heterogeneity and incomplete understanding of depression itself (Demyttenaere and Van Duppen, 2018). For

the purposes of this thesis, a broad definition of TRD will be used: the failure to obtain an "acceptable" outcome in the treatment of depression.

This problem of resistance and the inadequacies of conventional anti-depressant treatments is a serious concern, illustrated by the fact that 23% of suicide victims in the United States were taking anti-depressant medication at the time of their death (Thompson *et al.*, 2015). It is unclear whether TRD may be biologically different from typical depression, or whether the heterogeneity of the disorder and lack of specificity of treatment adequately accounts for non-responders. However, the need for improved understanding of depressive pathology, mechanisms of treatment response, and subsequent advancement on currently used medications is clear. Pharmacologically, ketamine, a primarily glutamatergic agent, has opened new avenues of research after its discovery as a powerful, fast-acting anti-depressant (Berman *et al.*, 2000; Pereira and Hiroaki-Sato, 2018; Krystal *et al.*, 2019). Other treatments involve non-pharmacological neuromodulation, via electrical or magnetic stimulation (Benabid *et al.*, 2005; De Raedt, Vanderhasselt and Baeken, 2015): deep brain stimulation (DBS), delivering targeted intracranial electrical stimulation (Schlaepfer *et al.*, 2011) and transcranial magnetic stimulation (TMS), a non-invasive form of neuromodulation (Klomjai, Katz and Lackmy-Vallée, 2015), are experimental treatments; electroconvulsive therapy (ECT) is an established therapy, often used as an intervention in severe cases and otherwise treatment-resistant patients (Pagnin *et al.*, 2004).

Deep brain stimulation as a treatment for TRD

DBS is a method of delivering targeted neuromodulation, the manipulation of neural activity, via electrodes surgically implanted into selected nuclei, as represented in figure 1.3. Implanted electrodes connected to a pulse generator emit electrical current, typically at high frequency (100-185Hz) (Benazzouz and Hamani, 2020). The therapeutic use of DBS stretches back more than 30 years in the treatment of Parkinson's disease, and although a serious invasive intervention, the long-term implantation of DBS devices is a safe and established procedure (Benabid *et al.*, 1987; Hariz, 2017). With depression increasingly viewed as a diffuse disorder impacting networks across the brain rather than single regions, DBS emerged as a potential

therapy for modulating activity across such dysfunctional networks. Alternate forms of neuromodulation have produced promising results in severe, treatment-resistant cases of depression, including ablative brain surgery, in which a targeted lesion is made, and non-invasive stimulation such as ECT and TMS (Pagnin *et al.*, 2004; Klomjai, Katz and Lackmy-Vallée, 2015; Volpini *et al.*, 2017). DBS offers certain advantages over these treatments. Ablative brain surgeries create permanent lesions, meaning any adverse effects are likely to be permanent too. On the other hand DBS is considered reversible; effects diminish with cessation of treatment, and adverse responses have been successfully managed by changing stimulation parameters (Mayberg *et al.*, 2005; Lozano *et al.*, 2008; Bewernick *et al.*, 2010; Volpini *et al.*, 2017). Compared to external stimulation methods, DBS can be specifically targeted, which may allow the delivery of more tailored treatments for patients as the understanding of the mechanisms of depression and DBS grow. In general, DBS can be seen to provide more flexible and precise neuromodulation than other treatment options, for a similar or improved risk/benefit ratio (Schlaepfer *et al.*, 2011).

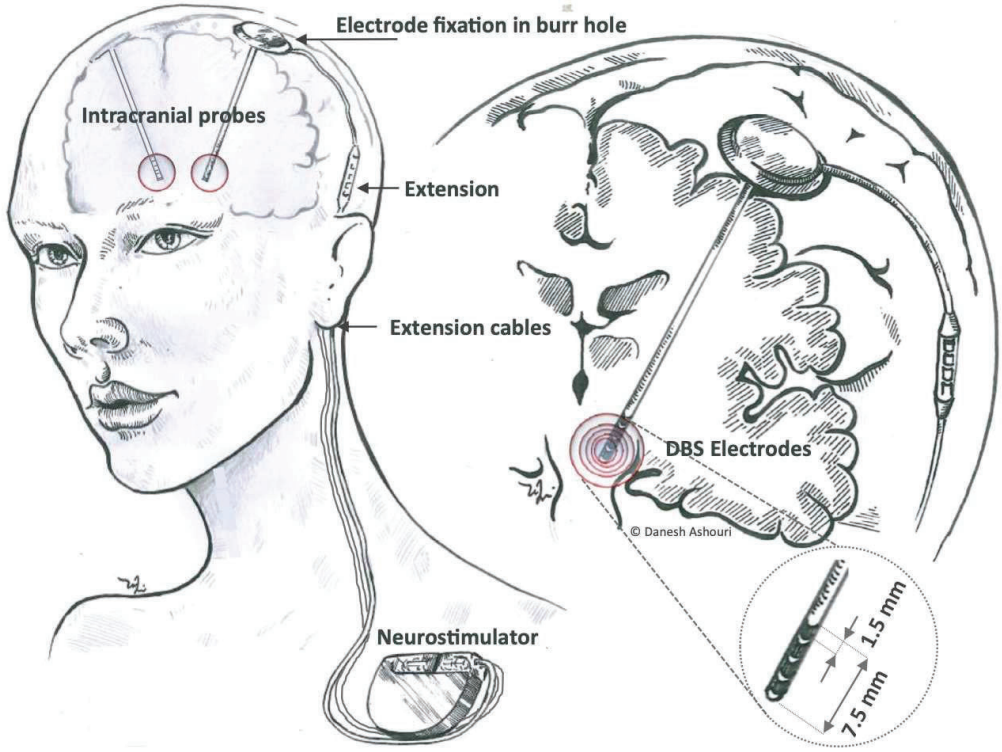

***Figure 1.3**. Graphical representation of implanted electrodes for the implementation of DBS. Adapted from Ashouri Vajari et al., 2018.*

Targets of DBS in depression

For the treatment of TRD, several implicated structures have been targeted in clinical and pre-clinical investigations. The target site of DBS is clearly crucial for treatment success, and candidate areas have been selected on the basis of their theoretical involvement in the pathophysiology of depression. The optimal target for DBS is yet to be truly established, and indeed may differ according to individual patients, given the wide variability of the depressive phenotype and various potential mechanisms of action (Drobisz and Damborská, 2019). The first implemented target for DBS in depression was the SGC, with improvements seen in treatment-resistant patients (Mayberg *et al.*, 2005; Lozano *et al.*, 2008). Since these first promising results, multiple targets, comprising part of or communicating with the limbic system, have been investigated (figure 1.4). These include the nucleus accumbens (Schlaepfer *et al.*, 2008; Bewernick *et al.*, 2010), ventral striatal areas (Malone *et al.*, 2009), inferior thalamic peduncle (Jiménez *et al.*, 2007), the lateral habenula (Sartorius and Henn, 2007) and the medial forebrain bundle (Schlaepfer *et al.*, 2013; Bewernick, Kayser, Gippert, Switala, *et al.*, 2017; Volker Arnd Coenen *et al.*, 2018). The anti-depressant effects achievable via modulation at multiple sites supports the idea of a network-level response, which may be more effectively evoked by some targets than others. The medial forebrain bundle (presented here as the MFB when referring to the human structure; as the mfb when referring specifically to the rodent structure), is a fibre tract containing projections communicating with all of the aforementioned structures targeted in DBS, potentially representing an important site for modulating a wider network involved in depression.

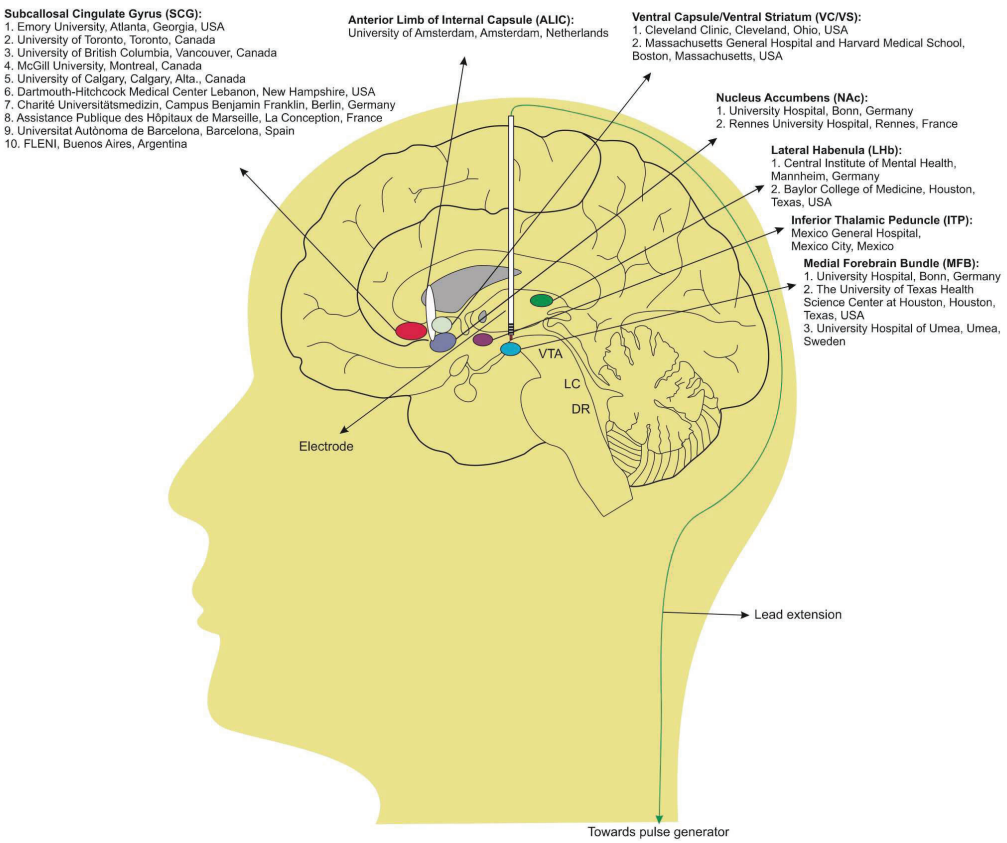

Figure 1.4. *Currently investigated targets of deep brain stimulation for depression. Reproduced with permission from Dandekar et al., 2018.*

1.1.4 The medial forebrain bundle as a target for deep brain stimulation

Anatomy of the MFB in the rodent and human

The MFB is diverse and complex tract, constituting a major connection between midbrain and forebrain areas, first described in the rodent (Nieuwenhuys, Geeraedts and Veening, 1982; Veening *et al.*, 1982; Geeraedts, Nieuwenhuys and Veening, 1990a, 1990b). It contains ascending and descending fibres of multiple neurotransmitter systems, including myelinated and unmyelinated projections of the major monoamines (figure 1.5), the glutamatergic and GABAergic systems. Contained in its complex network are the ascending dopaminergic projections from the VTA in the mesolimbic pathway, to the ventral striatum, NAc, AMY and HC, and in the

mesocortical pathway, to the frontal and pre-frontal cortical areas (Dunlop and Nemeroff, 2007).

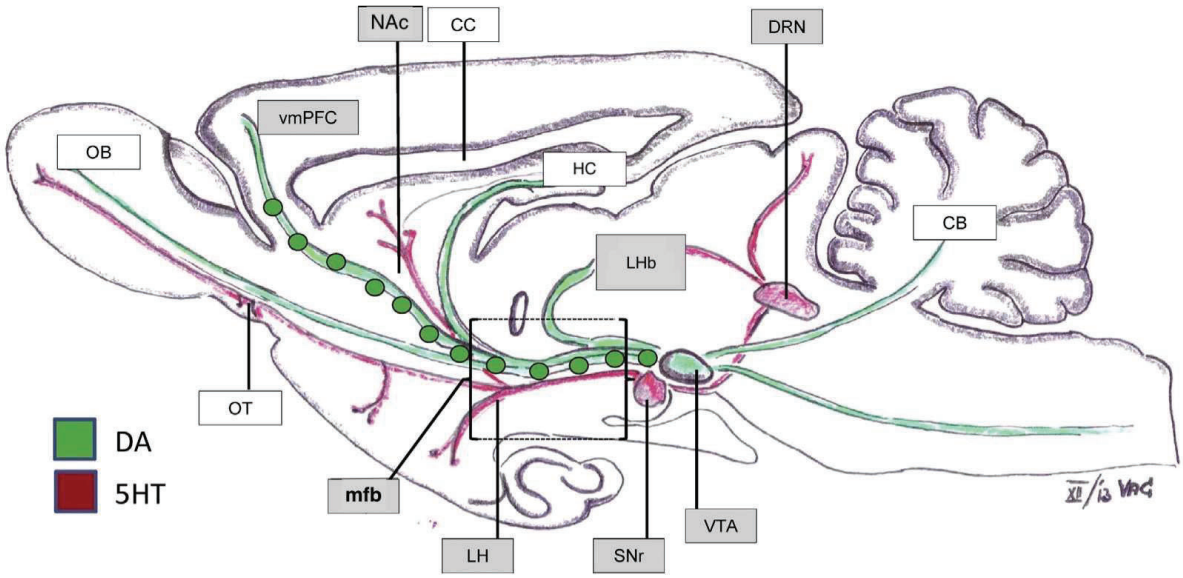

Figure 1.5. *A graphical representation of monoaminergic projections of the rodent mfb.*
Green circles highlight the mesolimbic and mesocortical projections of the VTA dopaminergic system. Shaded boxes represent areas targeted by DBS in pre-clinical investigations. Black square highlights the targeted portion of the mfb in mfb-DBS. DA: dopaminergic projection; 5HT: serotonergic projection; CB: cerebellum; CC: corpus callosum; DRN: dorsal raphe nucleus; HC: hippocampus; LH: lateral hypothalamus; LHb: lateral habenula; mfb: medial forebrain bundle; NAc: nucleus accumbens; OB: olfactory bulb; OT: olfactory tubercle; SNr: substantia nigra; vmPFC: ventromedial pre-frontal cortex; VTA: ventral tegmental area. Adapted with permission from Döbrössy et al., 2015.

In the human, these projections of the VTA form the recently described supero-lateral branch of the MFB (slMFB) or projection pathway of the VTA (ppVTA) (figure 1.6) (Coenen *et al.*, 2012, 2020; Volker Arnd Coenen *et al.*, 2018). These pathways are implicated in depression, forming a critical part of the brain's reward circuitry (Russo and Nestler, 2013; Heshmati and Russo, 2015). Although often misnomered as 'pleasure' systems, these pathways mediate more generalised motivation (Watt and Panksepp, 2009). The core depressive symptoms of anhedonia and amotivation are therefore strongly linked to the dysregulation and dysfunction of these dopaminergic

circuits (Belujon and Grace, 2017). In addition, the purported presence of glutamatergic, GABAergic, serotonergic and noradrenergic fibres (Nieuwenhuys, Geeraedts and Veening, 1982), each also implicated in depression and treatment response (Sanacora, Treccani and Popoli, 2012; Hamon and Blier, 2013; Thompson *et al.*, 2015; Fakhoury, 2016; Fogaça and Duman, 2019), suggests a wide modulatory therapeutic potential of MFB-DBS.

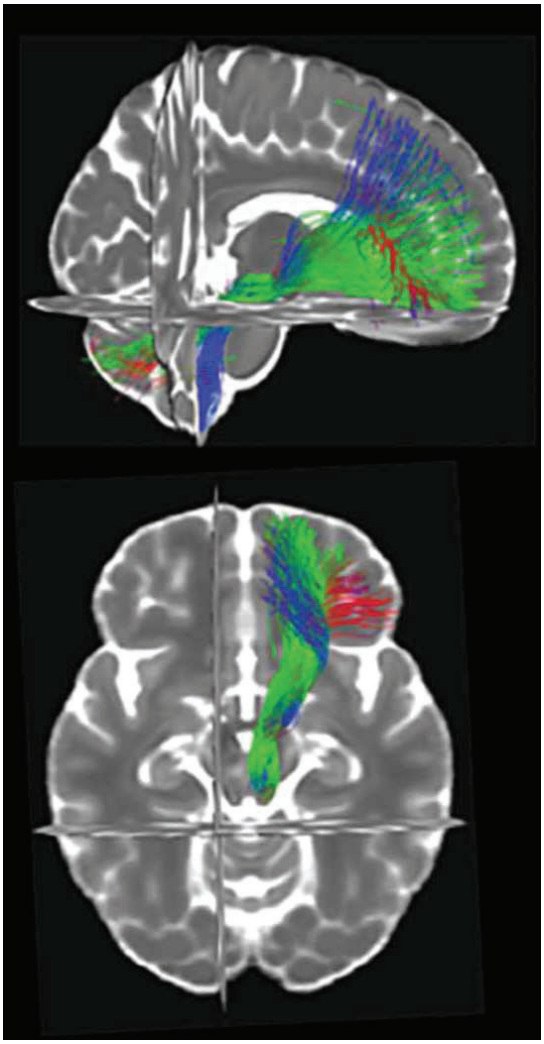

Figure 1.6. *The human slMFB imaged by diffusion tensor imaging utilising global tractography algorithms.*
Ascending projections to the superior frontal gyrus (blue), middle frontal gyrus (green) and lateral orbitofrontal region (red) are shown in the saggital (top) and axial (bottom) planes. Adapted from Coenen et al., 2018.

Clinical application of slMFB-DBS

Identification of the human anatomy of the ppVTA as the slMFB was followed by a series of clinical trials of bilateral, continuous, high frequency (130Hz) slMFB-DBS in treatment-resistant patients (Schlaepfer *et al.*, 2013; Bewernick, Kayser, Gippert, Switala, *et al.*, 2017; Coenen *et al.*, 2019). Rapid-onset anti-depressant effects were observed in the majority of patients, as measured by the Montgomery-Åsberg Depression Rating Scale (MADRS) (figure 1.7), with remission sustained up to 4 years after the initiation of treatment in 50% of patients. These outcomes have been replicated up to 1 year after implantation in trials at different centres (Fenoy *et al.*, 2016, 2018).

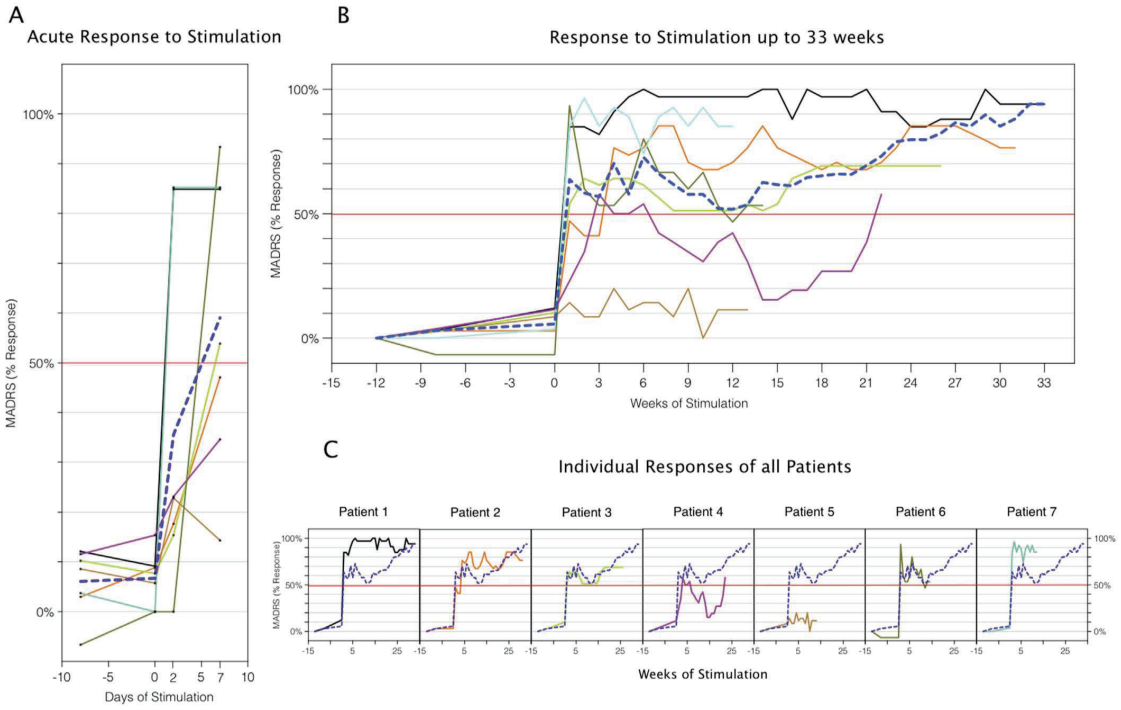

Figure 1.7. *Response of treatment-resistant patients to slMFB-DBS. Response as measured by percent reduction on the Montgomery-Åsberg Depression Rating Scale (MADRS), (A) within one week of stimulation and (B) over 33 weeks of stimulation. Coloured lines represent individual patients, shown separately in (C). Dotted blue line represents average response. Replicated with permission from Schlaepfer et al., 2013.*

Despite a strong theoretical background and the promising results of clinical trials of slMFB-DBS, numerous important questions remain unanswered. Fundamentally, the physiological effects of MFB-DBS remain to be uncovered, as do the mechanisms by which these anti-depressant response manifests. In addition to the incomplete understanding of the neurobiology of depression, two factors contribute to the complexity of elucidating these mechanisms: the non-specific nature of electrical stimulation, and the heterogeneity of the slMFB itself. Due to the limitations of studying biological mechanisms in human patients, these questions necessitate pre-clinical investigations. The mechanisms by which DBS can affect biological tissue are diverse, and pre-clinical research of DBS at various sites has demonstrated a wide variety of cellular, molecular and electrophysiological effects at local and distal sites (McCracken and Grace, 2009; Torres-Sanchez, Perez-Caballero and Berrocoso, 2017). While modulation of the dopaminergic system is theorised to be a crucial mediator of the effects of slMFB-DBS, stimulation likely interacts with a number of systems contained within the MFB (Döbrössy *et al.*, 2021). Pre-clinical work addressing the potential mechanisms of DBS and the specific effects of mfb-DBS in rodent models will be discussed in detail in **section 1.4.**

Summary

Depression is a major health burden, with serious personal health, social and economic repercussions. It affects a substantial proportion of the population. As a disorder, it is largely heterogeneous, and an incomplete understanding of its neurobiology means there are still no specific biomarkers for the condition. Furthermore, this lack of understanding contributes to the lack of progress in the treatment of depression, which leaves a substantial minority of patients as non-responders. For many, depression must be considered a chronic and disabling condition in which recovery is “the exception not the rule” (Verduijn *et al.*, 2017). The problem of TRD necessitates both improved understanding of the neurobiology of depression and novel treatments which improve outcomes for patients.

As a theoretically fundamental component of depressive pathology and the actions of slMFB-DBS, the following **section 1.2** will focus on dopamine and its interactions within relevant circuits, in the context of depression and treatment response.

1.2 The role of dopamine in depression and treatment response

1.2.1 Dopamine and depression

Dopamine can almost be considered the forgotten monoamine of the original monoamine hypothesis. Even though it was known that early generation anti-depressants, tricyclic anti-depressants (TCAs) and monoamine oxidase inhibitors (MAOIs), acted not only on 5-HT and NA but also DA, the role of the latter was not considered critical to the pathology or treatment (Willner, Scheel-Krüger and Belzung, 2013; Pereira and Hiroaki-Sato, 2018). Indeed, advances in pharmaceutical treatment focused primarily on narrowing the effects onto 5-HT and NA, but succeeded only in reducing unwanted side effects rather than improving efficacy (Willner, Scheel-Krüger and Belzung, 2013). Meanwhile, insight into the dopaminergic system, in particular the role of the mesolimbic pathway in reward and motivation, and the mesocortical pathway as a bridge between cortical and sub-cortical regions, provide a strong theoretical basis for a role of DA in the disease. Limbic DA projections to the NAc assign appetitive value, predict reward and guide motivated behaviour, specifically implicating the system in motivational deficits and anhedonia (Nestler and Carlezon, 2006; Yadid and Friedman, 2008; Alloy *et al.*, 2016). Importantly, TRD is closely associated with anhedonia, which is often unresponsive to conventional treatments, and can be a predictor of poor overall outcomes (Dunlop and Nemeroff, 2007; Treadway and Zald, 2011; Vrieze *et al.*, 2013).

In addition to limbic targets, projections of the midbrain to the PFC regulate various aspects of emotion- and reward-related behaviour and cognition via a complex interplay of DA D1 and D2 receptors (DRD1s and DRD2s) (Floresco, 2013; Hare and Duman, 2020).

The medial PFC (mPFC) is the primary target of DA projections in the frontal cortices (Berger, Gaspar and Verney, 1991). Within the mPFC, the ACC, and in particular its SGC portion are considered key to mood regulation (Mayberg *et al.*, 1999). In humans with depression, neuroimaging and post-mortem studies have suggested reduced grey matter volume and hypo-metabolism in the SGC (Drevets *et al.*, 1997;

Drevets, Savitz and Trimble, 2008), while treatment with the commonly prescribed class of anti-depressant, the selective serotonin re-uptake inhibitor (SSRI), ECT and DBS have been demonstrated to modulate these measures (Mayberg *et al.*, 2000, 2005; Nobler *et al.*, 2001). In the rodent, these areas are generally reported as the mPFC or ventral medial PFC (vmPFC), with the human SGC thought to be largely homologous to the vmPFC, containing the infralimbic (IL) and prelimbic (PrL) subregions, though there is some debate and inconsistency in the literature (Heilbronner *et al.*, 2016; Hare and Duman, 2020).

Finally, a growing recognition is that, despite NA being the monoamine most associated with the stress response, the DA system is also deeply involved, particularly in the concept of resilience and in pathological response (Belujon and Grace, 2015). Glucocorticoids, released as a function of the HPA stress response, modulate mesocortical and limbic DA firing and contribute to stress-related damage to PFC and hippocampal neurons (Arnsten, 2015).

Dopamine's role in conventional pharmacological treatments

The role of DA modulation in anti-depressant treatment was initially considered secondary to effects on 5-HT and NA (Willner, Scheel-Krüger and Belzung, 2013). However, later evidence suggested a more important role of DA modulation not only in TCA and MAOI drugs, but also in later generation anti-depressants which more specifically target 5-HT and NA. DA receptor antagonism was shown to block the response of serotonergic and noradrenergic drugs (Muscat, Sampson and Willner, 1990; Muscat, Papp and Willner, 1992), and induce relapse in remitted patients, just as 5-HT antagonism did (Willner, Hale and Argyropoulos, 2005). Modulation of DA receptors can itself have anti-depressant effects, and can be used to augment conventional treatments (Yadid and Friedman, 2008; Cusin *et al.*, 2013). Following the growing appreciation of the role of DA in conventional anti-depressant effects, studies utilising methods of electrophysiology and optogenetics have provided further evidence for DA dysfunction in depressive-like behaviour (Friedman *et al.*, 2007; Chaudhury *et al.*, 2013; Tye *et al.*, 2013; Moreines *et al.*, 2017). These evidence suggest the VTA's projections via the mesocortical and mesolimbic pathways and their

target regions as sites of dysfunction. Functionally, the interactions between the VTA, PFC, NAc and numerous modulatory regions are altered, centring the DA system as a major node in the matrix of depressive pathophysiology (Belujon and Grace, 2017).

Normal function of the mesolimbic and mesocortical dopamine projections

At any one time, a proportion of VTA DA neurons are spontaneously active, with irregular tonic activity and phasic (burst) firing (Floresco et al., 2003). The remainder are silent as the result of GABAergic inhibition, regulated by various systems. The LHb exhibits strong control over the monoaminergic systems; in the case of DA, excitatory efferents of the LHb innervate the rostromedial tegmental nucleus (RMTg), which in turn inhibits the VTA via GABAergic projections (Jhou *et al.*, 2009). Further inhibitory control comes from multi-synaptic connections from the VTA's upstream mesolimbic and cortical targets, including the basolateral AMY (BLA), NAc and mPFC (Figure 1.8) (Floresco *et al.*, 2003; Patton, Bizup and Grace, 2013). The resulting balance of active/inactive cells provides a baseline of tonic firing and subsequent DA release in target structures, including the NAc and mPFC. This tonic baseline is environment-dependant, with more DA neurons inhibited in a restful state, and more neurons tonically active in an alert state (Belujon and Grace, 2017). Phasic bursts, spontaneous or evoked by salient stimuli via the pedunculopontine tegmental nucleus (PPTg), can only be produced by tonically active neurons, and are differentially stimulated by conditioned or unpredictable rewards as well as by aversive and behaviourally salient stimuli (Zweifel *et al.*, 2009, 2011; Lammel *et al.*, 2011). As a result, environmental state primes the magnitude of response to salient stimuli: an alert state, with high tonic activation, leads to a burst response from a greater population of neurons, greater DA release in target structures, and an appropriate behavioural response (Belujon and Grace, 2017). The activity and physiology of these pathways have been repeatedly shown to be sensitive to stress and variably altered in depressive-like models.

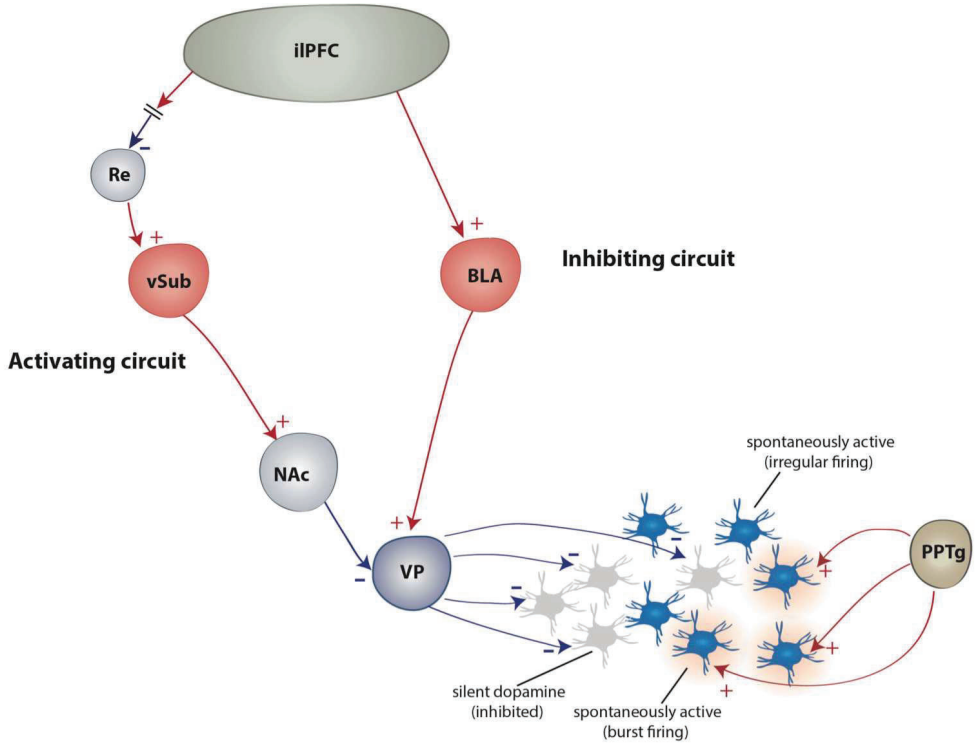

***Figure 1.8**. Schematic diagram of mesocorticolimbic regulatory afferent circuits influencing the activity of dopaminergic VTA neurons.*
IIPFC: infralimbic prefrontal cortex; vSUB: ventral subiculum; NAc: nucleus accumbens; VP: ventral palladium; BLA: basolateral amygdala; PPTg: pedunculopontine tegmental nucleus. Individual cells represent dopaminergic neurons of the ventral tegmental area (VTA). Reproduced from Belujon and Grace, 2017.

Dysfunction of VTA circuits in depressive-like models

Dysfunction of the VTA's projections and target structures involves altered electrophysiological activity of neurons, synthesis and release of transmitters, and regulation of receptors. DA receptors are homeostatically regulated in response to neural activity and DA release; increased synthesis and abnormal firing patterns may be compensatory responses to responsiveness of the system, caused by abnormal receptor regulation (Floresco *et al.*, 2003; Yadid and Friedman, 2008). The numerous feedback mechanisms and reciprocal nature of these (and other interconnected) circuits makes identifying the primary pathology a challenge; variation in the initial

dysfunction and extent of wider changes likely contributes to heterogeneity seen in both animal models and patients. Hyperactivity of the LHb has been hypothesised in human patients and animal models (Shumake, Edwards and Gonzalez-Lima, 2003; Sartorius and Henn, 2007; Lawson *et al.*, 2017), suggesting exaggerated subsequent inhibition of dopaminergic VTA neurons. Alternatively, dysregulation in mesolimbic and cortical circuits could lead to altered balance of the top-down excitatory/inhibitory feedback (figure 1.9) (Belujon and Grace, 2017). These abnormalities are not mutually exclusive, and the interconnectivity inherent in these circuits provides further substrate for common dysregulation. The final common result appears to be hypo-functionality of the DA systems.

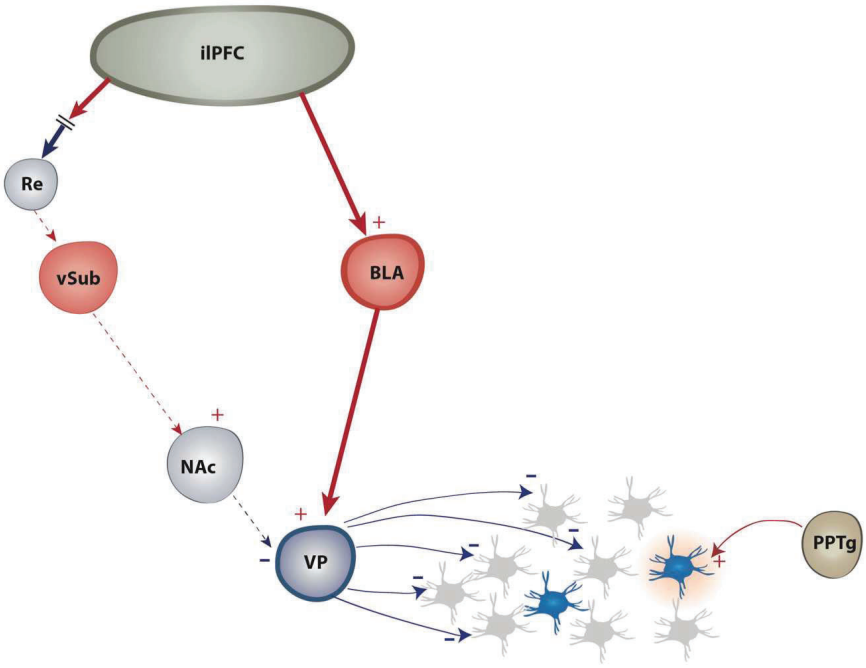

Figure 1.9. *Schematic example of how network dysregulation may result in hypo-functionality of dopaminergic VTA projections.*
Hyperactivity in the IIPFC leads to increased inhibition of VTA DA population. IIPFC: infralimbic prefrontal cortex; vSUB: ventral subiculum; NAc: nucleus accumbens; VP: ventral palladium; BLA: basolateral amygdala; PPTg: pedunculopontine tegmental nucleus. Individual cells represent dopaminergic neurons of the ventral tegmental area (VTA). Reproduced from Belujon and Grace, 2017.

Pre-clinical work has supported the hypothesis of altered functionality of the mesolimbic and mesocortical projections in depressive-like behaviours. Tye and colleagues (2013) demonstrated, using optogenetic techniques, that inhibition of VTA neurons induces multiple depressive-like behaviours. The FSL rat exhibits altered VTA burst firing, with typically shorter trains of impulses than controls, contributing to an altered electrophysiological profile (Friedman, Friedman, *et al.*, 2008; Friedman *et al.*, 2012). In the NAc, FSL rats have significantly higher DA tissue content (Zangen, Overstreet and Yadid, 1999) but lower concentrations in extracellular space (Zangen *et al.*, 2001; Friedman *et al.*, 2007). This implies normal or elevated synthesis but impaired release, which may be linked to D2 autoreceptor dysfunction, or impaired glutamatergic and GABAergic modulation mediated by postsynaptic DRD2s (Anzalone *et al.*, 2012). Zangen and colleagues (2001) showed a lack of 5-HT-induced DA release in the NAc, highlighting how interactions with other systems may be involved in abnormal release. DRD2 expression is reduced in the NAc in FSL rats when socially isolated (Bjørnebekk, Mathé and Brené, 2007), suggesting relatively mild stress is enough to trigger these changes in this susceptible model, which in many cases may include normal laboratory practices (Rea *et al.*, 2014). More severe stress in healthy animals also produces dopaminergic dysregulation in a variable fashion, dependant on the type, duration and context of the stressor. After chronic stress, the tonic activity of DA neurons is reduced, an effect also seen after inescapable stress in the 'learned helplessness' paradigm (Chang and Grace, 2014; Moreines, Owrutsky and Grace, 2017). Conversely, repeated social defeat stress increased both tonic and burst firing of the VTA (Krishnan *et al.*, 2007; Cao *et al.*, 2010), and optogenetic upregulation of VTA-NAc activity induced such social defeat behaviours (Chaudhury *et al.*, 2013). Maternal separation increased later-life VTA DA neuron excitability (Spyrka *et al.*, 2020). Acute stressors have long been known to induce DA release in mesolimbic and cortical targets (Finlay and Zigmond, 1997), while pre-exposure to mild inescapable stress was shown to dampen this response (Valenti, Gill and Grace, 2012). Like the FSL, healthy animals may also exhibit NAc DRD2 changes. This was reported after inescapable stress, and after 7 or 8, but not three, weeks of CMS, despite all timescales inducing depressive-like

symptoms (Willner *et al.*, 1991; Papp, Klimek and Willner, 1994; Dziedzicka-Wasylewska, Willner and Papp, 1997; Kram *et al.*, 2002). One week of CMS reduced tissue DA content and increased turnover in the NAc and PFC (Ahmad *et al.*, 2010), while 3 weeks increased NAc tissue content (Willner *et al.*, 1991). In the NAc, animals resilient to social stress displayed improved synaptic strength of DRD1-containing neurons, and the activity of these neurons prior to stress was predictive of resilience (Khibnik *et al.*, 2016; Muir *et al.*, 2018). Stress-induced anhedonia was found to depress the excitatory inputs onto these neurons (Lim *et al.*, 2012).

Evidence from stress responses suggests hypo- and hyper-functionality can contribute to dysfunction. In general, it seems that active management of stress increases the activity of ascending VTA projections, while chronic stress results in dampening of activity (Douma and de Kloet, 2020). This increased dopaminergic activity during initial stress response may in fact promote plasticity, facilitating maladaptive changes (Grace, 2016). It is therefore likely that abnormal response in either direction can be maladaptive and result in depressive endotype (Polter and Kauer, 2014).

Modulation of dopaminergic circuits in anti-depressant response

Two converging lines of evidence demonstrate how dysfunction in these circuits is modulated by anti-depressant action. Firstly, various existing treatments have been shown not only to interact with the DA system, as described above, but also to modulate specific dysfunctions observed. In the FSL, the TCA desipramine and atypical antidepressant nefazodone have been shown to normalise the diminished release of NAc DA (Dremencov *et al.*, 2005; Friedman *et al.*, 2007), although not all serotonergic drugs have this effect (Yadid and Friedman, 2008). The blunted response of 5-HT-induced DA release in the FSL was potentiated by both desipramine and the SSRI paroxetine (Zangen *et al.*, 2001), while desipramine also normalised the reduced burst-firing of the FSL VTA (Friedman, Friedman, *et al.*, 2008).

In healthy rats, SSRI fluoxetine and desipramine treatment can reverse stress-induced DRD2 reduction in the NAc (Papp, Klimek and Willner, 1994; Dziedzicka-Wasylewska, Willner and Papp, 1997; Ainsworth *et al.*, 1998), while NAc DRD1 expression was upregulated by the SSRI citalopram and selective noradrenaline re-uptake

inhibitor (SNRI) oxaprotiline but downregulated by the TCA imipramine (Dziedzicka-Wasylewska *et al.*, 1997). DRD1 agonism, either via systemic application or direct infusion into the mPFC has anti-depressant effects, and blocking this receptor blunts the efficacy of the TCA imipramine (D'Aquila *et al.*, 1994; Hare *et al.*, 2019). The SSRIs citalopram and escitalopram have been shown to reduce the firing of VTA DA neurons, likely through increased activity of 5-HT inhibitory inputs (Dremencov, El Mansari and Blier, 2009). Conversely, agomelatine, a melatonin receptor agonist and 5-HT2C antagonist with anti-depressant properties, was found to increase tonic and burst firing of the VTA (Chenu, El Mansari and Blier, 2013). These effects of serotonergic modulators on VTA firing were observed in healthy rats, however, which may not be representative of their actions in a depressive-like system. However, it is clear traditional serotonergic and noradrenergic anti-depressants are capable of modulating these dopaminergic circuits, with may be a feature of anti-depressant action.

Adding to these data are the effects of direct experimental manipulation of the mesolimbic and mesocortical pathways. In the FSL, electrical stimulation of the VTA was able to normalise the profile of mesolimbic projection burst firing while alleviating depressive-like behaviour (Friedman *et al.*, 2012). Optogenetic inhibition of VTA bursts, thought to be affecting the mesolimbic pathway, was found to be pro-depressive, and stimulation anti-depressant in stressed but not normal animals (Tye *et al.*, 2013). However, upregulation of phasic firing in the same pathway was also linked to increased social stress susceptibility, with inhibition conferring resilience (Chaudhury *et al.*, 2013); yet driving activity of DRD1 containing NAc neurons has also been shown to rescue social and anhedonic-like behaviour after social defeat (Francis *et al.*, 2015).

Inactivation of the mPFC – and it's top-down inhibition of the VTA – reverses stress-induced reductions in VTA burst firing (Moreines, Owrutsky and Grace, 2017). Direct optogenetic and pharmaceutical activation of DRD1s in the mPFC also has an anti-depressant effect (Scornaiencki *et al.*, 2009; Hare *et al.*, 2019), while inhibiting VTA-mPFC projections increased susceptibility to social stress (Chaudhury *et al.*, 2013). Acute DRD2 antagonism increased VTA firing in normal, but not stressed rats; con-

versely chronic DRD2 antagonism increased VTA firing in stressed but not healthy rats (Moreines *et al.*, 2017). These various manipulations demonstrate how shifts in dopaminergic transmission in these circuits can have decisive pro- or anti-depressant effects, or render an animal more or less sensitive to stressors. Like the impact of stress, directionality of changes may not be straightforward, and is dependant on the functional state of the network and the form of manipulation, with the probable involvement — adaptive or maladaptive – of compensatory alterations.

The role of plasticity in depression

A crucial wider concept in understanding both healthy and aberrant stress response is that of cortical plasticity. Plasticity describes, on various levels, the ability of neural systems to adapt to their environment. As such, it is fundamental to adaptive behaviour, including appropriate reactions to stress and reward. The morphology and stability of dendritic spines, and their strength, number and integrity of their synaptic networks are crucial mediators of plastic function (Holtmaat and Svoboda, 2009; Yoshihara, De Roo and Muller, 2009). In depression, improper maintenance and regulation of synapses, disruption of their adaptive function and morphology, ultimately leads to wider dysfunction in circuitry relating to mood and cognition (Duman *et al.*, 2016). Stress is known to alter dendritic morphology, particularly in the PFC and HC (Goldwater *et al.*, 2009; McEwen *et al.*, 2012), while heritable factors may confer deficits in regulation, leading to synaptic instability and vulnerability to maladaptive response. For example, GWAS studies have identified variations in the *Homer1* gene as a potential risk factor (Rietschel *et al.*, 2010); the Homer1a (H1a) protein it encodes plays a role in glutamate (Glu)-dependant synaptic reorganisation, critical in regulating glutamatergic transmission and activity-related plasticity (de Bartolomeis and Iasevoli, 2003; Clifton *et al.*, 2019). A consequence of these gene-environment interactions, the reduced grey matter volume in the PFC and HC of depressed patients is thought to be a related to reduced synaptic density (Drevets, Price and Furey, 2008; Duman *et al.*, 2016).

DA and the other monoamines modulate processes of plasticity, alongside glutamatergic and GABAergic signalling (Meunier, Chameau and Fossier, 2017) in conjunc-

tion with synaptic regulators including neurotrophins, such as BDNF (Martinowich, Manji and Lu, 2007); activity-dependant proteins such as the aforementioned H1a (Clifton *et al.*, 2019); the GABAergic synapse regulator, neuronal PAS domain-containing protein 4 (NPAS4) (Bloodgood *et al.*, 2013; Jaehne *et al.*, 2015); and synaptic strength modulator activity-regulated cytoskeleton-associated protein, Arc (Korb and Finkbeiner, 2011). It has been suggested that the main action of all anti-depressants may in fact be to create a more plastic environment to correct maladaptive processes (Castrén, 2013). The delayed therapeutic response to drugs elevating 5-HT may indicate that the anti-depressant mechanism is the eventual plastic responses to the drug's acute effects, while the action of rapid-acting treatments such as ketamine is theorised to relate to the more immediate induction of plasticity (Belujon and Grace, 2014; Rincón-Cortés and Grace, 2020). Supporting this, the elevation of BDNF expression, a marker of increased plastic function, has been linked to positive response after chronic treatment of conventional anti-depressants including fluoxetine (Molteni *et al.*, 2006), and after novel treatments such as agomelatine (Molteni *et al.*, 2010; Martinotti *et al.*, 2016) and ketamine (Silva Pereira *et al.*, 2017); H1a induction has also been linked to the anti-depressant response after chronic imipramine and acute ketamine treatment (Serchov *et al.*, 2015).

The role of the dopamine D1 receptor in plasticity

The activity of the DRD1 regulates numerous functional mechanisms of Glu-dependant plasticity, including long term depression and potentiation (LTD/LTP) and synaptic remodelling (Wolf, Mangiavacchi and Sun, 2003; Iasevoli *et al.*, 2014), and anti-depressant response to DRD1 activation in the mPFC has been linked to activation of signalling pathways related to synaptogenesis (B. Zhang *et al.*, 2017). DRD1 activity contributes to maintenance of dendritic morphology in normal circumstances (Huang *et al.*, 2004), and mediates response to stress in a variable fashion. During restraint stress, DRD1 activity mediates dendritic retraction, and after chronic stress downregulation and subsequent reduction in activity of DRD1s inhibits synaptic remodelling (Goldwater *et al.*, 2009; Lin *et al.*, 2015). DRD1-mediated dendritic growth was observed in mice resilient to social defeat stress; repetitive social defeat stress reduced DRD1 expression and reduced dendritic length (Shino-

hara *et al.*, 2018). Inhibition of VTA-mPFC projections and knockdown of DRD1 expression induce vulnerability to social stress (Chaudhury *et al.*, 2013; Shinohara *et al.*, 2018), and increased stress resilience may underlie the anti-depressant and anxiolytic effects of DRD1 activation (Hare *et al.*, 2019). Responding to glutamatergic BLA inputs, the mPFC also inhibits the dopaminergic response to stress in the NAc via DRD1s, antagonism of which leads to an exaggerated NAc response (Doherty and Gratton, 1996; Stevenson and Gratton, 2003). In the NAc itself, rats resilient to inescapable stress exhibited elevated DRD1 expression (Kram *et al.*, 2002). These results collectively highlight the role of DA, and in particular DRD1s, in functional plasticity and adaptive response. Dysregulation of the VTA projections, particularly mesocortical, may underlie maladaptive responses and thus contribute to vulnerability, whether through hereditary abnormalities or previous stress response (Radley *et al.*, 2015; Douma and de Kloet, 2020). DRD1s are differentially responsive to strong and weak activation by DA (Seamans and Yang, 2004; Floresco, 2013), a feature which may contribute to their pathological function in response to both hyper- and hypoactivity in VTA DA neurons.

Summary

In summary, the role of mesolimbic and mesocortical dopaminergic projections in the development of a depressive phenotype is well supported, likely relating to blunted reward processing and dysfunction of top-down regulation by the mPFC. Furthermore, modulation of these pathways appears important, though perhaps not fundamental, to anti-depressant response. The directionality of effect is complex, with both up- and down-regulation of activity having each been associated with pro- and anti-depressant conditions. This is likely due to the heterogeneity of pathological mechanisms which share a common outcome of altered dopaminergic functionality, and the subsequent interactions with other neural systems.

1.2.2 Dopaminergic interactions with other neural mechanisms of depression

Despite the outlined central role of dopaminergic projections from the VTA, it would be reductionist and incorrect to ignore the importance of interactions between these circuits and other mechanisms implicated in depression. Many critical processes discussed above rely on such interactions, from overlapping functions in regulating plasticity, to reciprocal modulation of the systems themselves.

Interactions between dopamine and serotonin

The activity of the DA and 5-HT systems is functionally interconnected, and altering one inevitably affects the other (Dean and Keshavan, 2017). It has been suggested that in conventional anti-depressant treatments, the near-ubiquitous increase of extracellular 5-HT may be the first domino in producing downstream responsive changes, including those to dopaminergic signalling (Rincón-Cortés and Grace, 2020). The delayed onset of effect is thought to reflect this, with an initial increase of 5-HT inhibition of VTA output leading eventually to a compensatory increase in DA function (Dremencov, El Mansari and Blier, 2009; Belujon and Grace, 2017). FSL rats show an impairment of 5-HT-induced DA release in the NAc (Zangen *et al.*, 2001). This may be the result of hyper-functionality of the 5-HT2C receptor, a post-synaptic receptor which inhibits DA, 5-HT and NA activity and is overexpressed in the FSL PFC (Dremencov *et al.*, 2005, 2011; du Jardin, Müller, *et al.*, 2017). The rapid anti-depressant response to nefazodone, a 5-HT2C antagonist, is linked to improved 5-HT-induced DA release (Dremencov *et al.*, 2004), a mechanism also seen after chronic TCA treatment (Zangen *et al.*, 2001). This suggests that the rapid effect is mediated by a change that chronic treatment produces via long-term compensatory plasticity. The importance of these interactions is further supported by the augmentation of SSRI response by dopaminergic drugs, including by anti-psychotics (Thase *et al.*, 2007; Kato and Chang, 2013). Counter-intuitively, these drugs are typically DA receptor antagonists; the broad DA receptor blocker quetiapine was found to increase VTA DA neuron activity in CMS rats, leading to the suggestion that

a low-level receptor blockade may lead to a homeostatic upregulation of activity via compensatory mechanisms (Moreines *et al.*, 2017).

Interestingly, Moreines, Owrutsky and Grace (2017) showed that inactivation of the mPFC increased VTA burst firing, while inactivation of the LHb, a strong dampener of DA activity, did not. Conversely, Zhang and colleagues (2018) demonstrated that inhibiting LHb output by specifically silencing DRN-LHb excitatory input is anti-depressive after CMS. By inhibiting the LHb, an increase in VTA bursts may be prevented by dysregulation of the DRN and RMTg, increasing inhibitory inputs onto the VTA (Jhou *et al.*, 2009; Bourdy and Barrot, 2012). Meanwhile lesions of the DRN may increase VTA firing by releasing the 5-HT side of this inhibition (Guiard *et al.*, 2008). Together, these results emphasise the complexity of 5-HT-DA interactions. Different pathologies may converge on similar phenotypes, and various modalities of treatment may achieve anti-depressant response through different interactions with these networks.

Interactions with glutamate and activity-dependant plasticity

The role of Glu in depression is a relatively recent proposal, deriving from the rapid-onset and sustained effects of ketamine (Berman *et al.*, 2000; Krystal *et al.*, 2019). Ketamine is an antagonist of the Glu NMDA receptor (NMDAR), which increases PFC Glu transmission dependant on the AMPA receptor (AMPAR) (Murrough *et al.*, 2013). The primary action of ketamine is thought to be either by NMDAR-mediated dampening of interneuron activity, therefore disinhibiting cortical neurons, or by the activation of cortical neurons via the upregulation of excitatory synaptic inputs (Miller, Moran and Hall, 2016), but its anti-depressant effects take effect after metabolism, and have thus been linked to secondary effects. As with other anti-depressants, the crucial mechanisms are theorised to be related to Glu-dependant plasticity, mediated by BDNF and regulatory proteins (Duman, Deyama and Fogaça, 2021).

Dopaminergic modulation has been linked to these secondary effects (Belujon and Grace, 2014; Rincón-Cortés and Grace, 2020). As discussed in **section 1.2.1**, the DRD1 is specifically implicated in the functional regulation of Glu-dependant plastic mechanisms. While DRD2s appear to play a less direct role, disrupted Glu trans-

mission of DRD2-containing neurons has also been linked to depressive-like behaviours (Seo *et al.*, 2017). Ketamine increases the activity of VTA DA neurons in chronically stressed and learned helpless animals, a response proposed to contribute to induced plasticity in target regions and not seen in healthy animals (Belujon and Grace, 2014; Carreno *et al.*, 2016; Rincón-Cortés and Grace, 2017). Antagonism of both DRD1 and DRD2s has been reported to block the effects of ketamine (Li, Zhu, *et al.*, 2015a; Hare *et al.*, 2019).

Acute ketamine and chronic SSRI treatment both increase cortical expression of BDNF (Molteni *et al.*, 2006; Iadarola *et al.*, 2015); direct injection of BDNF increases local 5-HT and DA activity and has an antidepressant effect (Siuciak *et al.*, 1996, 1997). Induction of the H1a protein in the mPFC by various treatments has been suggested as a "final common pathway" for anti-depressant effect (Serchov *et al.*, 2015). Activity-related H1a induction and its mediation of Glu receptor organisation is thought to be critical to adequate stress response through control of both Glu and DA sensitivity (Lominac *et al.*, 2005; Datko *et al.*, 2017). In turn, DA also modulates H1a induction: in the PFC, DRD1 antagonism prevented H1a induction (Ghasemzadeh *et al.*, 2009), which leads to pro-depressive effects (Datko *et al.*, 2017).

Dopamine-glutamate interactions influence excitatory/inhibitory balance

Another crucial facet of glutamatergic activity is its interaction with GABAergic interneurons. The proportional contributions of local glutamatergic-GABAergic circuits form a balance of excitatory and inhibitory activity (e/i balance), fundamental to various processes and thought to be disrupted in depression (Fee, Banasr and Sibille, 2017; Page and Coutellier, 2019). In depressed human patients, post-mortem studies have suggested elevated levels of glutamate and reduced GABA in areas including the ACC (Godfrey *et al.*, 2018), and imaging studies suggest dysregulated Glu metabolism in the PFC (Colic *et al.*, 2019), and hyperactivity in areas of the PFC, BLA and hippocampus (Hamilton *et al.*, 2011). In animals, repeated and chronic stress has also been linked to reduced PFC GABA function and interneuron numbers (Otero Losada, 1988; Shalaby and Kamal, 2009; Czéh *et al.*, 2018). NPAS4, which regulates BDNF expression and inhibitory synapse formation, is reduced after stress (Bloodgood *et al.*, 2013; Zhang *et al.*, 2014; Lin *et al.*, 2015), and its inhibition

induces depressive-like phenotypes (Jaehne *et al.*, 2015). SSRIs have been shown to increase GABA in the cerebrospinal fluid (Goren, Berkman and Terzioglu, 2007), and although the acute effect of ketamine is thought to inhibit GABAergic interneurons, this may cause a long-term adaptive response restoring balance (Ghosal, Hare and Duman, 2017). DA regulates GABAergic inhibition on PFC neurons via opposing actions of DRD1 and DRD2s, with DRD1s increasing the excitability of GABA interneurons and DRD2s reducing GABA release probability (Seamans *et al.*, 2001). Ketamine's anti-depressant effect in animals has been separately reported as blocked by either DRD1 (Hare *et al.*, 2019) or DRD2/D3 antagonism (Li, Zhu, *et al.*, 2015a), which may suggest varying modalities of reaching a final anti-depressant effect.

Gamma oscillations as a potential marker of glutamatergic abnormality

A final intriguing link between depression and glutamatergic/GABAergic activity comes from electrophysiological recordings. In broad scale recordings (i.e., from large populations of neurons), interconnected Glu-GABA circuits generate high frequency oscillations in the gamma range (typically reported as 30-120Hz or 30-200Hz, with variation between reports) (Bartos, Vida and Jonas, 2007; Buzsáki and Wang, 2012). There is some debate about the physiological role of these oscillations: their amplitude may be a marker of generalised neural activity, but their presence has been more specifically associated with inhibitory signalling, dendritic plasticity and e/i balance, as well as a variety of cognitive processes (Traub *et al.*, 1998; Fee, Banasr and Sibille, 2017). Changes in amplitude (or 'power') may be reflective of dysfunctional mechanisms underlying related processes; it has been proposed that a state-dependant 'optimal' level of gamma may exist, which elevated or diminished power representative of poor regulation of local neuronal activity (Voytek and Knight, 2015; Nugent *et al.*, 2019). The influence of DA on gamma oscillations is suggested by its modulation of Glu and GABA signalling, as described above. Indeed, stimulation of the VTA and agonism of mPFC DRD2s have each been shown to augment the amplitude of gamma oscillations (Ott, Westendorff and Nieder, 2018; Lohani *et al.*, 2019), while blocking either DRD1 or DRD2s prevented the gamma-generating effects of methamphetamine in the HC (Li *et al.*, 2019).

Abnormal gamma activity, whether as an emergent signal or contributing factor, has been recently proposed as a marker for pathophysiology in depression (Fitzgerald and Watson, 2018). The amplitude of gamma oscillations has been shown to be abnormal in depressed patients during rest, emotional and spatial tasks (Pizzagalli *et al.*, 2006; Strelets, Garakh and Novototskii-Vlasov, 2007; Lee *et al.*, 2010; Liu *et al.*, 2014). Abnormalities have also been reported in the FSL (Gazit *et al.*, 2015; Voget *et al.*, 2015), social defeat and chronic stress models (Khalid *et al.*, 2016; Iturra-Mena *et al.*, 2019), with normalisation associated with behavioural improvement. Both reduction and elevation of gamma have been reported, depending on behavioural state and recording site. Varying abnormalities indicates dysfunction of underlying processes fundamental to several states, such as mechanisms of plasticity and e/i balance (Fitzgerald and Watson, 2018). A variety of anti-depressant treatments have been shown to modulate gamma power, including monoaminergic actors (Hajós *et al.*, 2003; Méndez *et al.*, 2012; Akhmetshina *et al.*, 2016) and ketamine (Hunt, Raynaud and Garcia, 2006; Nugent *et al.*, 2019).

Summary

The mesolimbic and mesocortical dopaminergic systems likely represent critical pathways in the pathophysiology of depression. Their association with adaptive functions, such as plastic mechanisms and stress response, suggests a role in both hereditary and environmental vulnerability, and their regulation of motivational and reward behaviours implicates them in key symptoms. Reciprocal interaction with other implicated systems – the other monoamines, Glu and GABA circuits – suggests DA dysfunction is likely involved in a variety of depressive pathologies.

Evidence also suggests modulation of these circuits is important in a variety of anti-depressant mechanisms and represents a promising target for novel anti-depressants. Amelioration of dopaminergic function may represent a crucial anti-depressant mechanism in itself or an access point to facilitate downstream effects, such as enhancing plasticity. While the mesolimbic and mesocortical circuits may not be uniquely implicated in depression and treatment (due to the inherently heterogenous nature of the disease), they clearly represent a significant node in the matrix of depressive pathophysiology.

1.3 Sleep and depression

Sleep and depression are so closely associated that understanding sleep and its disorders has been described as fundamental to truly understanding depression (Thase, 2006). The vast majority of patients with depression experience sleep-related symptoms, which also have a strong connection to the onset, recovery and potential relapse of the disease. In this section, the relationship between sleep disturbance, depressive pathophysiology and treatment will be discussed.

1.3.1 Normal Sleep

The architecture of sleep

Sleep is a fundamental behavioural state, which despite its uncertain function, is highly conserved across all known animals. Sleep is necessary for normal cognitive and biological function, and its disturbances are therefore associated with a wide range of mental disorders (Baglioni *et al.*, 2016; Scammell, Arrigoni and Lipton, 2017). Normal human sleep can be broadly divided into two stages: REM (rapid eye-movement) sleep and non-REM (NREM) or slow wave sleep (SWS), which cycle throughout the duration of sleep; SWS sleep can be subdivided by depth of sleep (figure 1.10). These stages can be identified via polysomnography (PSG), the recording of electroencephalogram (EEG) via electrodes placed on the scalp, with wake, SWS and REM containing distinct activity of varying frequency ranges (figure 1.11) (Carskadon and Dement, 2011). NREM sleep is dominated by high amplitude slow wave activity (SWA, in the delta frequency range, 0.5-4Hz) and unique waveforms called sleep spindles and k-complexes. REM sleep is characterised by activity which resembles, but is distinct from the EEG seen in wake, with oscillations in the theta range (4-8Hz), as well as higher frequency activity such as gamma oscillations (30-200Hz), thought to be related to local and network processes such as memory consolidation in the hippocampus.

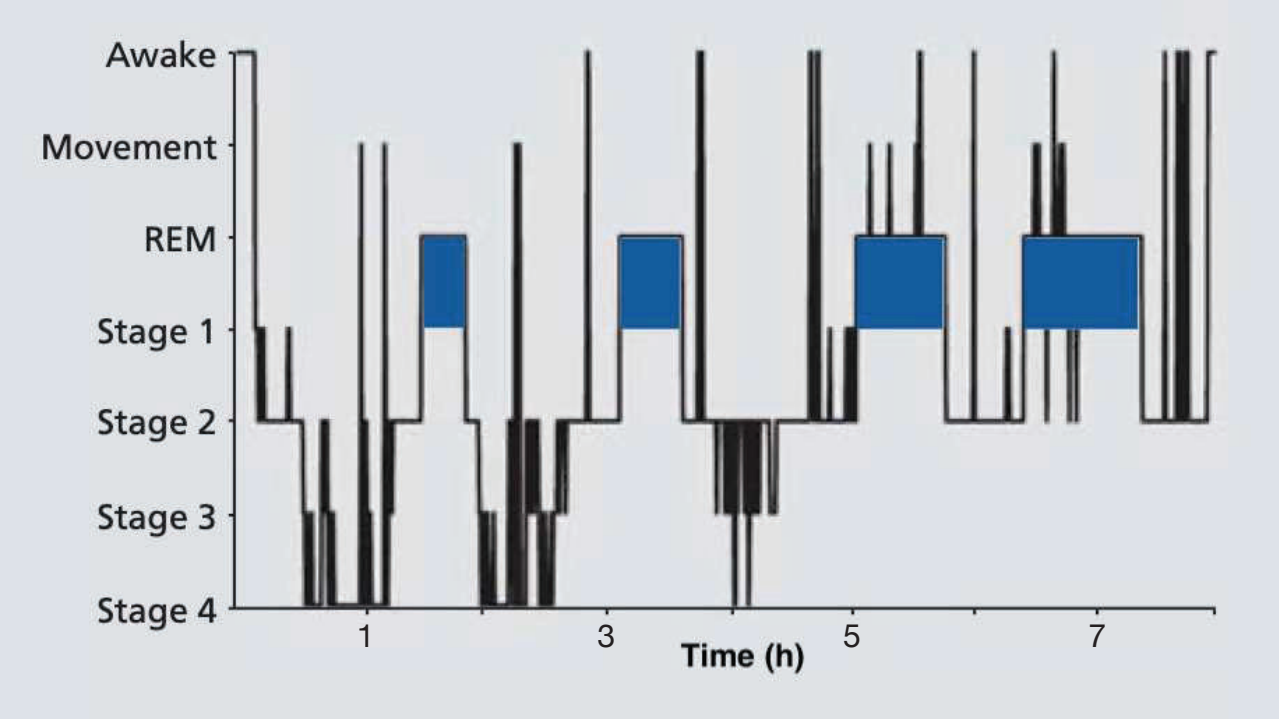

Figure 1.10. *An example hypnogram from a healthy human subject. REM sleep is represented by blue boxes; slow wave sleep is divided into stages by depth. Adapted from Nutt et al., 2008.*

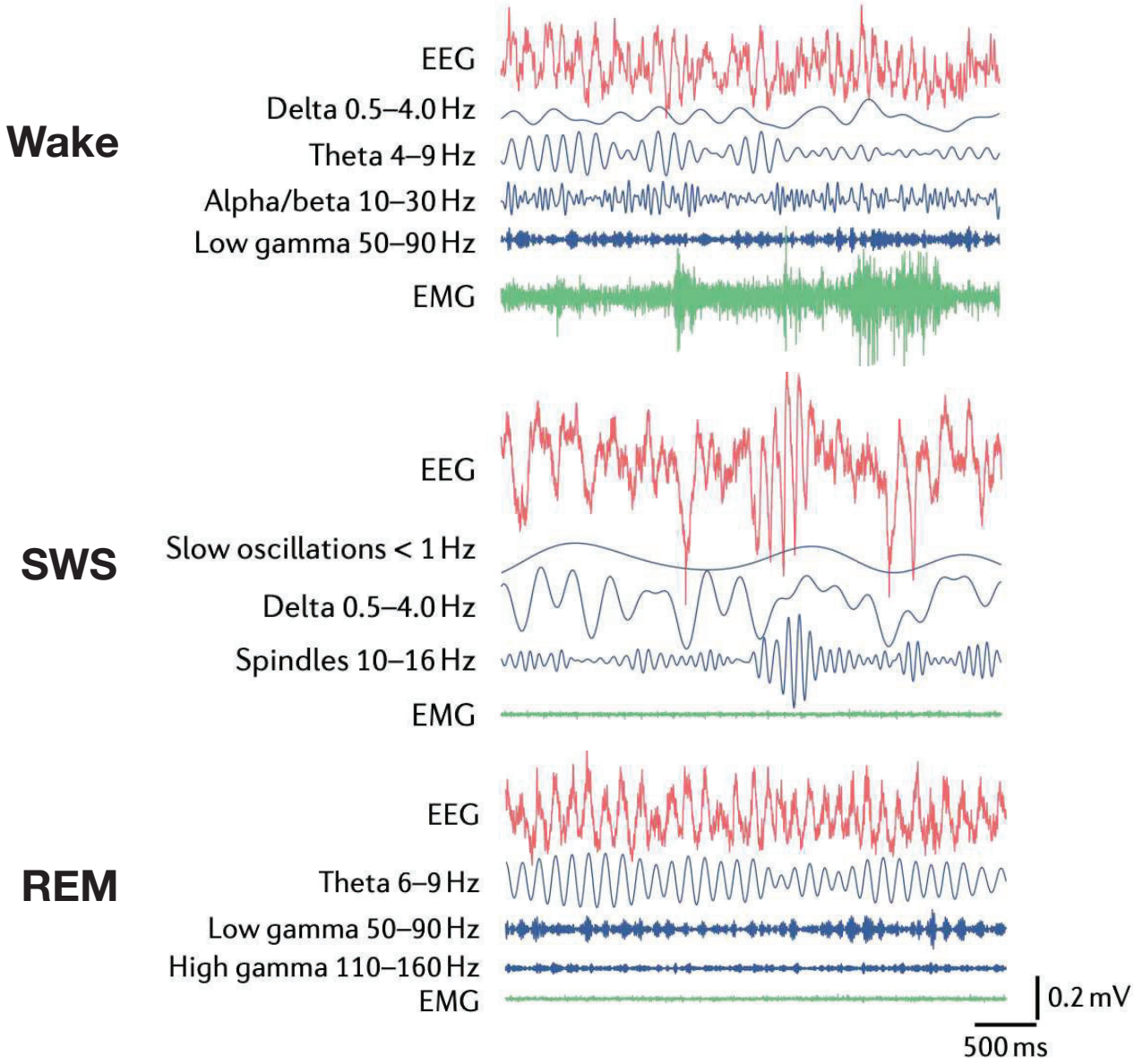

Figure 1.11. *Oscillatory activity recorded by human EEG in wake, SWS and REM sleep. Overall EEG signal (red) and isolated constituent frequencies (blue) are shown. EMG (green) shows muscular activity. Adapted with permission from Adamantidis et al., 2019.*

The two process model of sleep regulation

Sleep and its architecture are regulated by the interaction of two independent systems, known as process S and process C, first described by Borbély in 1982. Pro-

cess S describes the homeostatic drive to sleep, or sleep pressure, which builds over a period of wake; process C describes circadian regulation, a series of cellular and molecular mechanisms linked to a roughly 24h cycle under the control of a central pacemaker 'clock', the suprachiasmatic nucleus (SCN) of the hypothalamus. The mechanisms of these concurrent processes define the quantity and quality of sleep.

Process S can be thought of as the accumulation and reversal of 'sleep debt'. This debt increases during wake and decreases during sleep. At its highest point, it facilitates sleep-promoting mechanisms, and at its lowest point facilitates wake-promoting mechanisms. The primary marker of process S is SWA during SWS. During SWS early in the night, SWA is at its highest, and falls during later periods of SWS; elevated SWA can be induced by sleep deprivation, confirming its link to process S and the homeostatic drive for sleep (Borbély *et al.*, 1981, 1984).

Process C promotes sleep and wake according to a circadian (from the Latin, 'circa diem', meaning "approximately one day") cycle. The central pacemaker of the SCN exerts control over peripheral pacemakers in neural and other tissue, entraining them to the cycle. The cycle is endogenously generated and self-sustaining, but can be influenced by environmental cues including light and temperature, called Zeitgebers (from the German, "time giver") (Albrecht, 2012).

Sleep-wake behaviours (that is, the timing of arousal, activity, rest and sleep) can be considered functions of both processes. The two processes modulate one another, but are also separate: lesions of the SCN do not affect homeostatic mechanisms, for example, and altering sleep-wake behaviour can shift homeostatic drive away from circadian rhythm (Borbély *et al.*, 2016).

Neural basis of sleep-wake regulation

The neural regulation of sleep-wake behaviour includes various overlapping circuits involved in heterogenous activity (Scammell, Arrigoni and Lipton, 2017). Ascending projections from the LC, DRN and VTA are all wake-promoting, alongside cholinergic projections from the parabrachial nucleus, and the PPTg and laterodorsal tegmental nuclei (LDT). The wake-promoting behaviours of these nuclei are co-ordinated by the hypocretin and histaminergic neurons of the lateral hypothalamus (LH)

(Eban-Rothschild, Appelbaum and de Lecea, 2018). The onset of NREM sleep is believed to be mediated by GABAergic inhibition of wake promoting nuclei, primarily from the ventrolateral pre-optic area and parafacial zone in the midbrain, while the basal forebrain inhibits frontal areas (Scammell, Arrigoni and Lipton, 2017). Descending GABAergic projections from the NAc to the LH, VTA and LC have also recently been implicated in inhibiting wakefulness (Luppi, Peyron and Fort, 2017; Oishi, Xu, *et al.*, 2017). REM sleep is controlled primarily by nuclei in the pons, including the PPT, LDT and sublaterodorsal nucleus (SLD), which can be categorised as 'REM-on' and 'REM-off'. The PPT and LDT activate the REM-on SLD, which reciprocally inhibits the ventrolateral periaqueductal grey and lateral pontine tegmentum (vlPAG and LPT), REM-off areas. This on-off "flip-flop" switch is under further modulatory control from several areas, including the LH, LC and DRN (Peever and Fuller, 2017; Scammell, Arrigoni and Lipton, 2017).

Glutamatergic and GABAergic transmission, modulated by the monoamines, play a major role controlling sleep/wake states, although direct neural substrates of processes S and C are not firmly established (Saper and Fuller, 2017; Eban-Rothschild, Appelbaum and de Lecea, 2018). The major monoaminergic pathways are active in wake, less so in SWS and near-silent in REM (Scammell, Arrigoni and Lipton, 2017). In the mesolimbic DA projections, however, bursting activity during REM sleep resembles that of wake, reward-oriented behaviour (Dahan *et al.*, 2007). Motivational state represents a significant modulator of arousal, and recent work has implicated the mesolimbic VTA projections in sleep regulation. Activation of dopaminergic projections during high sleep pressure maintains wake, while inhibition induces sleep even in the presence of salient reward stimuli (Eban-Rothschild *et al.*, 2016; Oishi, Suzuki, *et al.*, 2017).

The function of sleep

Despite its importance in biological function, implied by its near universal conservation across species, the function of sleep is still not well understood. There are three broad theories: the regulation of energy metabolism; immune function; and the maintenance of neural plasticity and synaptic homeostasis (Porkka-Heiskanen, Zitting and Wigren, 2013). The importance of sleep in synaptic plasticity is supported

by the enhancement of learning and memory processes after sleep, and the impairment of cognitive function by extended periods of wake (Diekelmann and Born, 2010). Sleep provides an important opportunity – with the exclusion of external stimuli – for the "offline" processing and regulation of adaptive responses to the environment (Adamantidis, Gutierrez Herrera and Gent, 2019). The synaptic homeostasis hypothesis (SHY), proposed by Tononi and Cirelli (Tononi and Cirelli, 2003, 2006), proposes global downscaling of synapses during sleep, particularly SWS. During wake, synapses are primarily formed and strengthened through activity-mediated processes such as LTP. This accumulation, and subsequent maintenance, of synapses is metabolically expensive, and must be homeostatically controlled. Global downscaling of the strength of recently potentiated synapses preserves encoded information, but recovers the potential for synaptic reorganisation and strengthening during future wake (M.-Q. Zhang *et al.*, 2017). Slow waves during SWS are proposed to be directly involved in synaptic downscaling; as predicted by SHY, amplitude of SWA has been shown to reflect synaptic strength and number (Esser, Hill and Tononi, 2007; Riedner *et al.*, 2007; Vyazovskiy *et al.*, 2007; Olcese, Esser and Tononi, 2010), and to increase locally in response to activity during wake (Hanlon *et al.*, 2009). Increased SWA accordingly leads to increased LTP in subsequent periods of wake, and the disrupted expression of mRNAs related to synaptic formation blunts SWA (Huber *et al.*, 2004; Cirelli *et al.*, 2005). Exploratory behaviour during wake has been shown to lead to increased SWA and expression of plasticity related genes including BDNF, Homer and Arc, reflecting increased synaptic potentiation and subsequent SWA rebound (Huber, Tononi and Cirelli, 2007). Homeostatic sleep pressure may therefore be linked to the increasing energy demand of synaptic maintenance during prolonged wake.

1.3.2 Sleep disturbances in depression

The vast majority of patients with depression experience disturbed sleep, a symptom which uniquely effects quality of life and the course of disease. Many researchers have suggested sleep disturbance as a fundamental feature of depression, such is the relationship between the two (Jindal and Thase, 2004; Nutt, Wilson and Pa-

terson, 2008; Mendlewicz, 2009; Fang *et al.*, 2019). As well as subjective disruption, PSG reveals changes to sleep in depressed patients (figure 1.12): sleep is more fragmented, with more awakenings, longer sleep latency (time to fall asleep) and early morning awakenings; with REM latency is reduced (time to enter REM sleep), more time is generally spent in REM overall, and density of eye movements increased; time spent in deep SWS is reduced, with reduced SWA, particularly at the start of sleep when sleep pressure is highest (Borbély *et al.*, 1984; Benca *et al.*, 1992; Pillai, Kalmbach and Ciesla, 2011; Baglioni *et al.*, 2016).

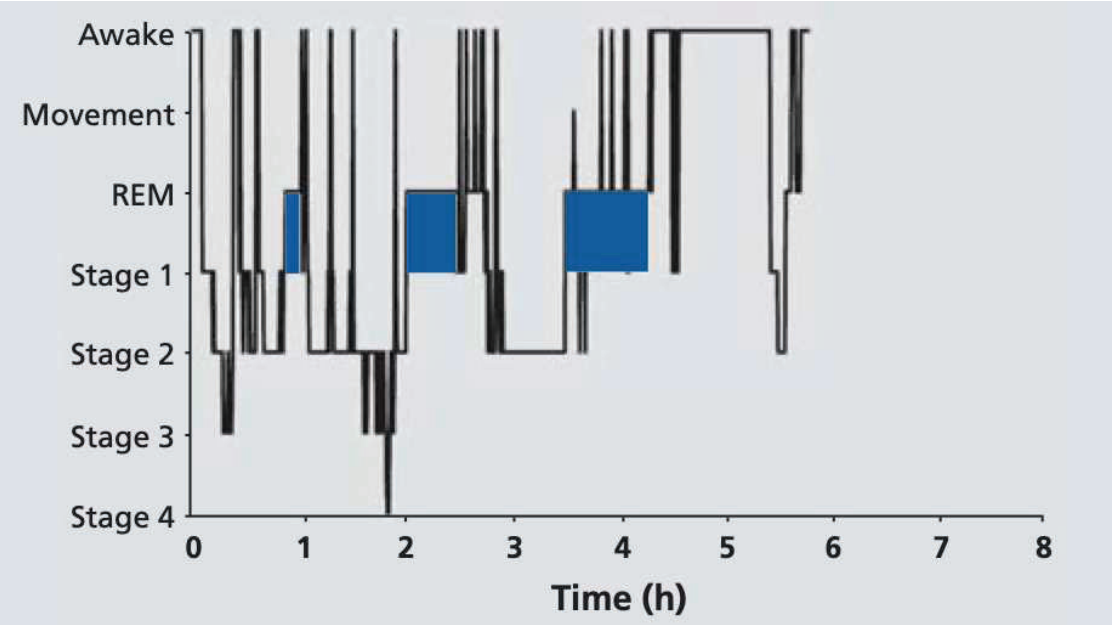

Figure 1.12. *An example hypnogram from an untreated depressed patient. REM sleep is represented by blue boxes; slow wave sleep is divided into stages by depth. Compared to healthy controls, the sleep of depressed patients is typically more fragmented and time take to enter REM sleep is reduced. More sleep time is spent in REM, and less time spent in deeper stages of slow wave sleep. Adapted from Nutt et al., 2008.*

REM sleep deficits have the strongest association between sleep and depression (Palagini *et al.*, 2013; Baglioni *et al.*, 2016). REM deficits are often prodromal before depressive episodes, residual after, and may predict treatment response (Clark *et al.*, 2000; Mendlewicz, 2009). In rats, specific deprivation of REM sleep is able to induce depressive-like symptoms (Ma *et al.*, 2019).

Changes to the architecture of SWS are less commonly associated with depression, and have received less attention. Early meta-analyses of psychiatric conditions associated duration of SWS with depression (Benca *et al.*, 1992; Pillai, Kalmbach and Ciesla, 2011), while a later analysis disputed this link (Baglioni *et al.*, 2016). Within SWS, several studies have linked reduced amplitude and altered distribution of SWA to depression. The typical pattern of high SWA early in the night, when the homeostatic drive is strong, is significantly altered in depressed patients: SWA is typically reduced and spread more evenly across the night, implicating mechanisms of sleep homeostasis (figure 1.13) (Borbély and Wirz-Justice, 1982; Borbély *et al.*, 1984). The extent of the early-night reduction has been linked to depression severity (Nissen *et al.*, 2001; Landsness *et al.*, 2011; Goldschmied *et al.*, 2019) and treatment response (Borbély *et al.*, 1984; Kupfer *et al.*, 1990; Ehlers, Havstad and Kupfer, 1996). However, in some patients, particularly young women, SWA is instead elevated compared to controls, suggesting complexity of the relationship (Frey *et al.*, 2012; Plante *et al.*, 2012). Perhaps as a result of these mixed results and the strong focus on REM sleep-related abnormalities, SWS abnormalities in depression have received relatively little attention; for instance, SWA changes were not assessed in the recent meta-analysis of EEG studies by Baglioni and colleagues (2016).

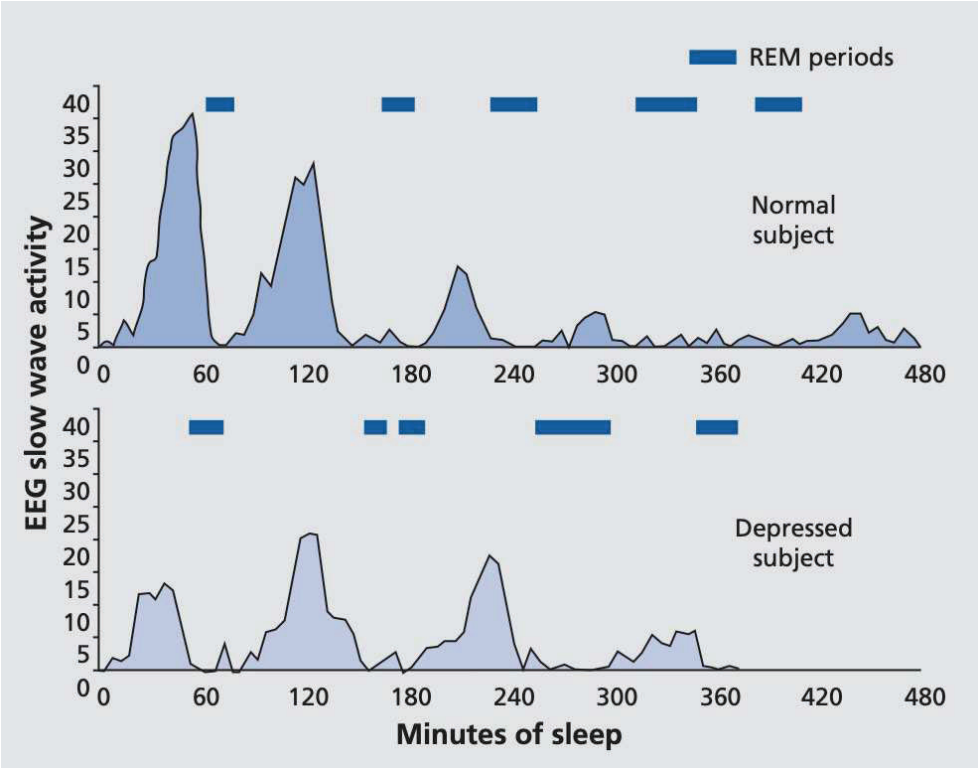

Figure 1.13. *Slow wave activity during slow wave sleep in a healthy adult and depressed patient. The typical pattern of strong slow wave activity at the start of sleep, diminishing throughout the night is not seen in depressed patients. Adapted from Nutt et al., 2008.*

The complex influence of sleep on other aspects of depression has lead to the relationship being described as bi-directional (Alvaro, Roberts and Harris, 2013). A significant source of distress for patients, sleep problems often precede and persist after a depressive episode, and are predictive of both relapse and suicide risk (Ağargün, Kara and Solmaz, 1997; Mayers, van Hooff and Baldwin, 2003; McClintock *et al.*, 2011). Slow wave sleep reductions preceding an episode have been suggested as a marker of depressive risk, and alternatively described as prodromal features of the disease (Lauer *et al.*, 1995; Modell *et al.*, 2002; Fang *et al.*, 2019).

Other evidence of the complex interaction comes from the fact that sleep deprivation acts as a powerful anti-depressant (Borbély and Wirz-Justice, 1982). However, the effects are not practical for clinical treatment, as they are typically reversed by the first period of sleep after deprivation. Sleep deprivation evokes strong periods of

SWA, with the response correlated with the rebound of SWA (Nissen *et al.*, 2001; Landsness *et al.*, 2011) suggesting that its anti-depressant effects may be linked to the stimulation of processes related to sleep homeostasis; after a period of sleep, these mechanisms may be once again diminished (Duncan and Zarate, 2013). These mechanisms are heavily linked to plasticity, which will be discussed in more detail in **section 1.3.4**.

The effect of anti-depressants on sleep

The response of sleep symptoms to treatment is also not straightforward. It has been suggested that to improve sleep is to improve depressive outcomes (Manber *et al.*, 2008; McCall *et al.*, 2010); however co-treatment of insomnia and depression has not produced consistent results (Asarnow, 2020). Depressed patients perceive their sleep to be worse than controls, even when disturbances in PSG are similar (Perlis *et al.*, 1997; Mayers, van Hooff and Baldwin, 2003), which may be linked to features of the PSG beyond architecture, such as the presence of high frequency oscillations (Perlis *et al.*, 2001). When treated, patients may report improved sleep even when no objective changes occur (Mayers and Baldwin, 2005).

Conventional anti-depressants almost all suppress REM sleep in both depressed patients and in healthy controls (Steiger and Pawlowski, 2019). Most major drug classes have been shown to suppress REM, including SSRIs (Shipley *et al.*, 1984; von Bardeleben *et al.*, 1989), TCAs (Dunleavy *et al.*, 1972) and MAOIs (Landolt *et al.*, 2001). The commonality of this effect led to the suggestion that REM suppression may be a key mechanism of anti-depressant action (Vogel *et al.*, 1975). However, REM-suppression occurs within hours of treatment, whereas effect on mood typically takes weeks (Grözinger, Kögel and Röschke, 2002; Thase, 2006). Further, some anti-depressants do not suppress REM: the TCA trimipramine has no suppressant effect, while bupropion can elevate REM while acting as an anti-depressant (Nofzinger *et al.*, 1995; Ott *et al.*, 2004). Longitudinal studies have suggested that when remitted patients stop treatment, REM deficits return while the patients remain remitted (Rush *et al.*, 1986; Steiger *et al.*, 1989). These findings suggest that while common, REM suppression is not necessary for anti-depressant response; it

is likely that rather than a causal relationship, there may be shared mechanisms in the regulation of REM and depressive symptoms (Steiger and Pawlowski, 2019).
The effects of anti-depressants on SWS is more variable. TCAs can both increase and decrease the time spent in SWS (Chen, 1979; Steiger, 1988; Sonntag *et al.*, 1996); SSRIs generally do not affect SWS, but may disrupt sleep continuity (Saletu *et al.*, 1991; Sharpley *et al.*, 1996; Pandi-Perumal *et al.*, 2020). However the SSRIs paroxetine and vilazodone have been found to increase early-night SWA (Murck *et al.*, 2001; Argyropoulos *et al.*, 2009), an effect also seen with the TCA clomipramine (Ehlers, Havstad and Kupfer, 1996). The rapid-acting effects of ketamine have also been associated with increases in SWA (Duncan, Sarasso, *et al.*, 2013).

1.3.3 Sleep in rodents

Comparing sleep in rodents and humans

Although PSG allows insight into the physiology of sleep in humans, experimental research using rodent models still provides vital information. PSG is applied in rodents using electrodes implanted directly onto the cortex and is thus known as electrocorticogram (ECoG). The broad sleep stages – SWS and REM sleep – are present and identifiable in rodents, and recent research suggests may even be more similar to their human equivalents than previously thought (Lacroix *et al.*, 2018). Sleep architecture in rodents is superficially very different from human sleep; rather than single, consolidated periods of sleep and wake, rats exhibit polyphasic sleep, with relatively short cycles. Despite this difference, homeostatic drive and circadian influence are still readily observable (Yasenkov and Deboer, 2012; Osorio *et al.*, 2020).
In studies of depression, rodent models allow the investigation of the relationship between sleep disturbances and biological mechanisms of the disease. The presence of sleep disturbances, identified in several models of depression, both confirms the fundamental link between the two, and suggests validity of the models themselves.

Sleep in models of depression

Sleep disturbances resembling those in human patients with depression have been shown to emerge alongside other depressive-like symptoms in several models. Different durations of chronic stress produce changes to REM sleep. After 2 or 3 weeks CMS, the duration of REM sleep was increased at the expense of both wake and SWS, with onset of disturbance emerging at the same point as anhedonia-like symptoms (Cheeta *et al.*, 1997; Grønli *et al.*, 2004). Acute stress produces more variable sleep disruption: while a short (1h) period of immobilisation stress increases subsequent REM, longer periods (4h and 4 days repeatedly) reduces REM duration (Rampin *et al.*, 1991; Bonnet *et al.*, 1997; Marinesco, Bonnet and Cespuglio, 1999; Papale *et al.*, 2005). Repeated footshock stress initially reduced total sleep time, with a greater loss of REM sleep, but this returned to baseline levels before the end of the 14-day stress protocol. However, the circadian pattern (more sleep in the light cycle, less in the dark) was significantly weakened, an effect which lasted beyond the recovery of sleep duration (Jean Kant *et al.*, 1995). Similarly, Papale and colleagues (2005) found 4 days of footshock stress decreased both SWS and REM duration, specifically reducing the circadian amplitude of REM. SWS was also reduced by immobilisation stress, though it has also been reported as elevated after acute social defeat stress (Meerlo, Pragt and Daan, 1997). Chronic stress also alters circadian markers, including the diurnal rhythms of general activity, temperature regulation, corticosterone and melatonin circulation (Gorka, Moryl and Papp, 1996; S. L. Christiansen *et al.*, 2016). Further investigation revealed altered patterns of expression of clock genes (S. Christiansen *et al.*, 2016).

The WKY rat, a model for genetic predisposition to depression, displays more fragmented and circadian-related changes to sleep stages, with reduced REM and SWS in the light cycle. The overall power of the ECoG was reduced in REM sleep, but not in SWS (Dugovic *et al.*, 2000). The REM deficits were later reproduced in a study which found no SWS changes, while footshock stress has been found to increase fragmentation in the WKY but not controls (Ivarsson, Paterson and Hutson, 2005; Jamie K. DaSilva *et al.*, 2011; J. K. DaSilva *et al.*, 2011). Another selectively bred model, the FSL, has been shown to exhibit increased REM sleep without SWS defi-

cits (Shiromani *et al.*, 1988; Benca *et al.*, 1996). The WKY and FSL rats exhibit different alterations in circadian temperature regulation; the FSL exhibits phase shift (altered time of circadian peak), while the WKY exhibits greater difference between diurnal temperatures (Shiromani *et al.*, 1991; Ivarsson, Paterson and Hutson, 2005).
Anti-depressant drugs show similar actions in rodents as in humans. Monoaminergic treatments including SSRIs typically suppress REM sleep, often also decreasing total sleep including SWS (Neckelmann *et al.*, 1996; Monaca *et al.*, 2003; Sánchez *et al.*, 2007). The majority of these studies have been conducted in healthy rats rather than depressive-like models; however, REM-suppressant anti-depressants also have this effect in healthy humans (Steiger and Pawlowski, 2019). In a direct comparison, TCA and SSRI drugs suppressed REM in healthy rats, but only high dose of the SSRI citalopram had an effect in the WKY; this has been used to suggest the WKY as a specific model of treatment resistance (Ivarsson, Paterson and Hutson, 2005). In general, anti-depressant effects on sleep are reflected in rodents, suggesting validity of testing the effects and mechanisms of novel experimental treatments in these models. Indeed, ketamine was shown to increase the duration of SWS and the amplitude of its SWA in rats, an effect later replicated in humans and proposed to be part of ketamine's effect on plasticity (Feinberg and Campbell, 1993; Duncan, Sarasso, *et al.*, 2013).

1.3.4 Connecting depressive pathology with sleep disturbance

The close relationship between sleep and depression is suggestive of common or overlapping regulatory mechanisms. The regulation of sleep involves diverse networks, and the functions of sleep – incompletely understood – are thought to involve local and dispersed mechanisms. Several of these mechanisms are closely linked to impaired processes in depression.

Monoamines in sleep

Arousal and sleep-wake behaviours are principally controlled by glutamate and GABA. The monoamines, in their modulatory roles, therefore play a critical role in regulation (Jones, 2020). 5-HT and NA have been long associated with arousal; they

are highly active in wake, less so during SWS and almost silent during REM sleep (Monti, 2011; Scammell, Arrigoni and Lipton, 2017). The SCN is highly serotonergic, while the DRN projects to wake promoting nuclei including the basal forebrain, thalamus, hypothalamus and cortex (Morin, 1999; Daut and Fonken, 2019). Consistent with this, activation of the DRN, associated with glutamate co-release, has been found to lead to wakefulness and sleep fragmentation (Ito *et al.*, 2013; Weissbourd *et al.*, 2014), while 5-HT inhibition is necessary for the generation of theta rhythms in REM (Pignatelli, Beyeler and Leinekugel, 2012).

Dopaminergic activity was not previously thought to play a role in sleep-wake regulation, as mean activity did not change significantly across vigilance states (Jouvet, 1972; Miller *et al.*, 1983). Fluctuations in extracellular DA across vigilance states in the NAc and mPFC have been linked to burst firing of the VTA, which in REM resembles activity during active wake, and is reduced to slow tonic activity during SWS (Léna *et al.*, 2005; Dahan *et al.*, 2007). Further evidence for dopaminergic modulation of arousal has come from manipulation of mesolimbic projections, of which activation via NAc DRD2s maintains wake, and suppression promotes nesting behaviour even in the (active) dark cycle and in the presence of salient environmental stimuli (Eban-Rothschild *et al.*, 2016; Oishi, Suzuki, *et al.*, 2017). Within sleep, DRD2s have also been implicated in the transition from SWS to REM, with antagonism reducing REM and increasing SWS (Lima *et al.*, 2008).

As well as this role in regulating arousal, activity in dopamine-regulated circuits are also thought to play functional roles during sleep including the regulation of waking behaviour. Sleep deprivation was shown to reduce glutamatergic inputs onto dopamine receptor-expressing neurons of the NAc from both the BLA and mPFC, shifting the e/i balance and increasing reward seeking behaviours (Liu *et al.*, 2016; Wang *et al.*, 2020). During SWS, DA is also thought to play a role in modulating homeostatic processes (Mayne *et al.*, 2013), with subsequent effects on mechanisms of plasticity, another strong link between sleep abnormality and depression.

Plasticity and synaptic homeostasis

Neural plasticity represents a likely common mechanism of depression and sleep disturbance, with impaired mechanisms of plasticity disrupting sleep and dysfunc-

tional sleep impairing mechanisms of plasticity (Zhang *et al.*, 2017). As proposed in the synaptic homeostasis hypothesis, synaptic downregulation as a function of sleep is linked to SWA, in particular the up-states of slow waves (Niethard *et al.*, 2018). The suppressed generation of SWA in depressed patients may therefore reflect an impairment of this important homeostatic process and related plasticity. Expression of BDNF during wake has been correlated with amplitude of the subsequent SWA response, and its microinjection causes local SWA elevation (Huber, Tononi and Cirelli, 2007; Faraguna *et al.*, 2008). The anti-depressant effect of sleep deprivation is associated with elevated expression of neurotrophic factors including BDNF, and the subsequent period of sleep is marked by increased SWA (Gorgulu and Caliyurt, 2009; Ibrahim *et al.*, 2011). Reduced expression of BDNF in depressed patients may therefore produce an impaired homeostatic SWA response. Increases in both BDNF expression and SWA have been demonstrated after acute administration of ketamine (Feinberg and Campbell, 1993; Duncan, Sarasso, *et al.*, 2013; Duncan, Selter, *et al.*, 2013); however it is possible to increase one without the other. Sertraline increases SWA but not BDNF (Jindal *et al.*, 2003; Brunoni *et al.*, 2014), while many chronic anti-depressant treatments have been shown to increase BDNF, but not SWA (Duman and Monteggia, 2006; Björkholm and Monteggia, 2016; Castrén and Kojima, 2017). Alternatively, inadequate SWA caused by other mechanisms of impaired sleep regulation may inhibit the expression of key plasticity-related genes; sleep fragmentation has been shown to inhibit NMDA-mediated LTP in the hippocampus (Tartar *et al.*, 2006). Other factors, such as DA dysregulation may also contribute. DA activity at the DRD1 has been implicated in the regulation of up-states of SWA, while DA-depleted rats exhibited reduced SWA and diminished evoked potentials, a marker of synaptic strength (Mayne *et al.*, 2013; Galati *et al.*, 2018). It is likely that a number of mechanisms interact, and contribute to both dysfunctional sleep and impaired plasticity, in turn exacerbating the other.

1.4 The anti-depressant mechanisms of mfb-DBS

Clinical trials, discussed in **section 1.1**, have demonstrated rapid and sustained anti-depressant effects after slMFB-DBS in formerly treatment-resistant patients. Pre-clinical research is vital for uncovering the mechanisms involved in this response. This chapter will examine the pre-clinical evidence for the mechanisms of DBS in depression, with specific focus on the mfb and related targets.

1.4.1 The action and mechanisms of deep brain stimulation

DBS produces complex modulation of local and distal neural tissue

Despite a long history of efficacious treatment for the symptoms of movement disorders, the precise mechanisms by which DBS achieves its therapeutic effect are still disputed (Stefani *et al.*, 2019); for the treatment of psychiatric disorders, more so.

While low frequency electrical stimulation will generally lead to an action potential and repolarisation to a normal baseline in neural tissue, the high frequency (typically >100Hz) pulses delivered by DBS induce a complex mixture of excitatory and inhibitory effects and subsequent changes at local and distal points (figure 1.14) (Jakobs *et al.*, 2019; Benazzouz and Hamani, 2020). The direct effect is dependent on features of the cell itself, including myelination and orientation of the cell relative to the electrode (Cameron C. McIntyre *et al.*, 2004; Anderson *et al.*, 2012). Cell bodies local to the stimulation site are inhibited, primarily by a “depolarisation block”, alterations to membrane properties which prevent the firing of action potentials (Beurrier *et al.*, 2001). While the depolarisation block is thought to be reversible over time, activation of GABA afferents onto the target nuclei may also contribute to inhibition. This is because, in contrast to cell bodies, neural appendages can be repeatedly depolarised by high frequency stimulation, leading to the excitation of afferent and efferent fibres of the target, in addition to nearby fibres of other projections (Kringelbach *et al.*, 2007). Anterograde and retrograde action potentials may then modulate distal targets. The decoupling of somatic and axonal effects may therefore lead to a

complex combination of excitation and inhibition; while the electrophysiological activity of the nuclei at the stimulation site is reduced, its output to distal targets may be potentiated (Dostrovsky *et al.*, 2000; Anderson, Postupna and Ruffo, 2003; Cameron C. McIntyre *et al.*, 2004). One effect of this may be to disassociate a nucleus from its afferent inputs while differentially altering its output.

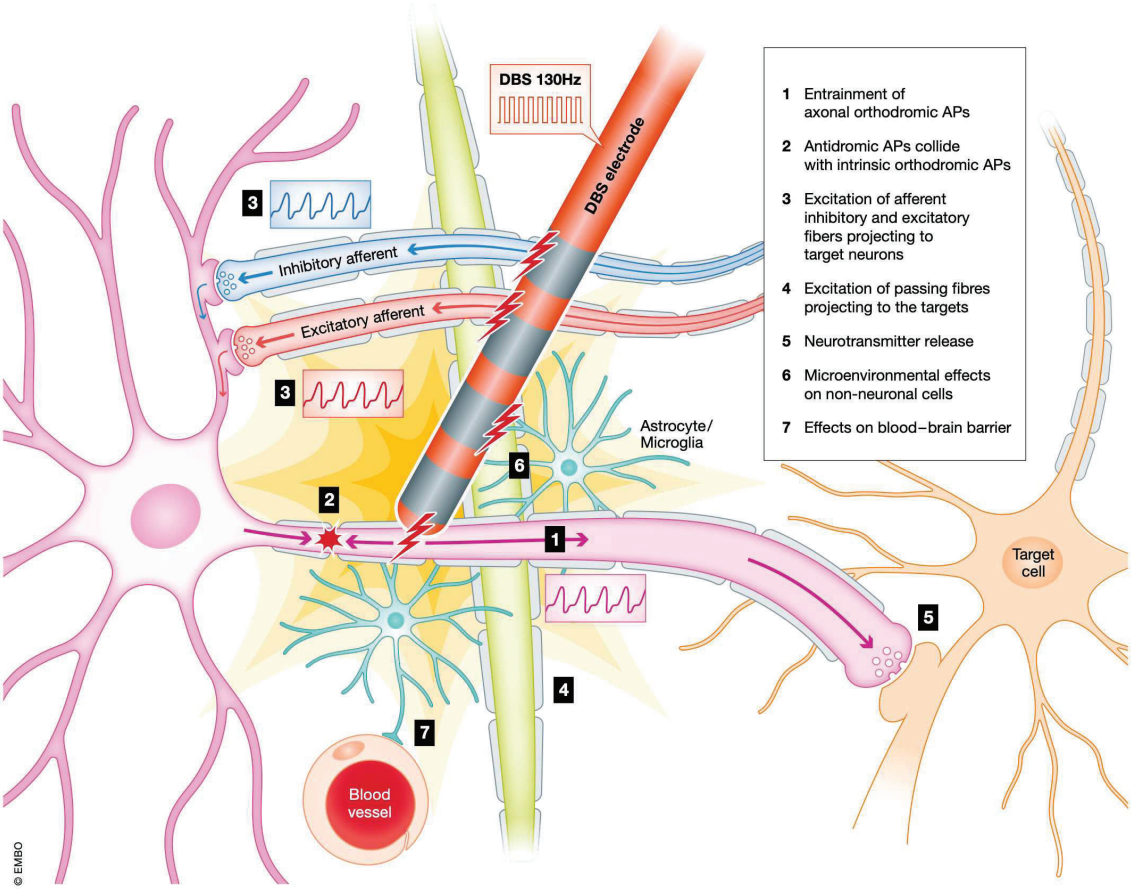

Figure 1.14. *Proposed local and distal effects of high frequency (130Hz) deep brain stimulation (DBS). The effects of DBS may act on inhibitory and excitatory afferents, efferent projections as well as other passing fibres and non-neuronal tissue, producing complex effects at local and distal points. Reproduced from Jakobs et al., 2019.*

Downstream consequences of modulation may mediate therapeutic effects

In this way, DBS can be said to create an 'information lesion' at the stimulation site, disrupting pathological activity within the local circuits it influences (Grill, Snyder and Miocinovic, 2004). However, this activity is not necessarily replaced with nor-

mal, 'healthy' firing patterns, but rather firing under the influence of the high frequency input (Cameron C McIntyre *et al.*, 2004). It is still undetermined exactly to what extent either disruption or entrained firing contributes to amelioration of symptoms. What is clear is that rather than simply overriding aberrant activity and entraining the target to its stimulation, DBS provokes numerous changes from ionic to network levels (McIntyre and Anderson, 2016).

DBS performed in pre-clinical models of depression has been shown to induce diverse neurochemical, electrophysiological, cellular and molecular changes on acute and chronic timescales. These changes may contribute directly to anti-depressant effect, or may drive cascades of further effect. In the context of depression, several paradigms modelling clinical applications of DBS have provoked changes which may form part of anti-depressant response. There is significant variation within the literature in the duration of DBS treatment. In the following sections, 'acute' stimulation will be used to refer to single applications of DBS lasting <8h; 'acute-intermittent' will refer to multiple DBS periods of <8h within one or two days (a common paradigm, modelled on drug administration before a behavioural test); 'chronic-intermittent' will refer to multiple DBS periods delivered over more than two days; 'chronic-continuous' will refer to DBS delivered for 24h per day over more than two days. In each case, unless otherwise stated, DBS refers to high frequency stimulation (100-130Hz), which is used by most investigators to reflect clinical applications (Drobisz and Damborská, 2019; Benazzouz and Hamani, 2020).

DBS alters activity of depression-related networks

DBS of nuclei within the mesolimbic and mesocortical networks produces various changes in local and distal areas. vmPFC-DBS produced neural activation, measured by immediate early gene (IEG) expression, both locally and in distal targets including other frontal areas and the HC (Veerakumar *et al.*, 2014; Jiménez-Sánchez, Linge, *et al.*, 2016). NAc-DBS produces distal electrophysiological changes, elevating beta and gamma power in the PFC, OFC and locally in the NAc, as well as altering communication between these nuclei (McCracken and Grace, 2007, 2009). DBS at the level of the VTA can also be used to entrain local circuits to a 'healthy' firing

pattern, however this involved specific patterns of low frequency, rather than the high frequency paradigms commonly and clinically used in DBS (Friedman *et al.*, 2012; Gazit *et al.*, 2015).

DBS modulates monoaminergic transmission

Monoaminergic pathways may also be modulated, with differential effects dependant on target and model. In healthy rats, acute vmPFC-DBS produced an anti-depressant behavioural response alongside 5-HT, DA, and NA release in the PFC, reduced SERT expression in the DRN and increased expression of post-synaptic 5-HT1B receptor in the PrL and DRN (Jiménez-Sánchez, Castañé, *et al.*, 2016; Volle *et al.*, 2018). In these healthy animals, behavioural response was not affected by 5-HT depletion; in stressed rats, on the other hand, the anti-depressant effects of chronic-intermittent vmPFC-DBS were dependant on an intact 5-HT system (Hamani *et al.*, 2012). Acute DBS of the NAc shell induced DA and 5-HT release locally in the NAc, but not the mPFC, of healthy rats, without changes in VTA firing (Sesia *et al.*, 2010; Sesia, Bizup and Grace, 2014); DBS of the NAc core did induce DA and 5-HT release in the PFC (van Dijk *et al.*, 2012). Contrasting with the effects in control animals, both acute-intermittent and chronic-intermittent DBS of the NAc core in the WKY rat reduced the expression of tyrosine hydroxylase (TH), a rate-limiting molecule in the synthesis of DA and NA, and the chronic paradigm resulted in reduced release of DA and NA themselves in the PFC (Falowski *et al.*, 2011).

These varied results demonstrate the common capability of DBS at various targets to modulate monoamine release. Opposing effects, including induction and inhibition of monoamine release and local and distal points have been reported, highlighting the importance of target selection. Similarly, divergent effects in control animals and depressive-like models emphasises the importance of the underlying system in the response to DBS.

DBS alters plasticity mechanisms

DBS also produces changes which suggest an influence on plasticity in local and distal areas. Chronic-intermittant vmPFC-DBS increased measures of dendritic ar-

borisation and density in the DRN, vmPFC and HC in healthy and stressed animals (Veerakumar *et al.*, 2014; Etiévant *et al.*, 2015; Chakravarty *et al.*, 2016). BDNF expression in the PFC and HC was also elevated by chronic-intermittant vmPFC-DBS in stressed but not healthy animals; the effect was blunted by 5-HT depletion (Hamani *et al.*, 2012; Bambico *et al.*, 2015). Acute vmPFC-DBS induced Glu release in the PFC of healthy animals, and in the OBX depression model, increased various markers of plasticity in the PFC including BDNF; anti-depressant effects at the behavioural level were found to be dependent on the upregulation of mechanistic target of rapamycin (mTOR), a protein associated with synaptic reorganisation (Jiménez-Sánchez, Castañé, *et al.*, 2016; Jiménez-Sánchez, Linge, *et al.*, 2016). Low frequency VTA-DBS has also been shown to induce BDNF expression in the PFC and HC in the FSL model of depression (Friedman, Frankel, *et al.*, 2008). In other investigations of DBS, stimulation of cerebellar outputs was also shown to elevate markers of synaptic density and reorganisation and increase LTP in distal motor cortex targets (Cooperrider *et al.*, 2014).

Summary

These results, showing varying modulation of monoaminergic transmission and mechanisms relating to plasticity, suggest potential substrates for anti-depressant effect following DBS applied to networks implicated in depression. Subsequent from initial effects on neural tissue, such DBS can influence the electrophysiological activity of local and distal neurons, producing altered neurotransmission in monoaminergic and glutamatergic systems. This appears to include stimulating or inhibiting release, which may then contribute to changes in receptor and transporter expression. These effects bear resemblance to conventional anti-depressant treatment, which indicate overlap between mechanisms. However, vmPFC-DBS was shown to elevate 5-HT levels to a lesser extent than SSRI treatment; this suggests that the effects of vmPFC-DBS on receptor expression are not entirely due to compensatory mechanisms, but may involve more direct mediation (Volle *et al.*, 2018). This is supported by anti-depressant effect of DBS in SERT knockout mice, in which SSRIs have a reduced effect (Bregman *et al.*, 2018). Rapid induction of plasticity – while possibly 5-HT-mediated (Hamani *et al.*, 2012) - may underlie faster response

to treatment compared to conventional anti-depressants. Reciprocal facilitation between monoaminergic and plasticity-related changes may induce an environment of positive feedback, improving network functionality. This modulation of monoaminergic and markers of plasticity appears possible via DBS at various sites in the network, and is, at least in part, dependant on the underlying physiology of the animal. Depressive-like animals showed differential changes in monoaminergic release and plasticity markers in response to DBS compared to healthy animals.

1.4.2 The effects of mfb-DBS

Despite the promise of clinical trials, a clear understanding of the anti-depressant mechanisms of mfb-DBS is yet to emerge. Given the diversity of the mfb as a tract, and the non-selective nature of electrical stimulation such as DBS, it is highly likely that the response is multi-faceted. A fundamental aspect is thought to be an interaction with a dysfunctional dopaminergic system. As discussed in the previous section, modulation of the DA system has been identified as a feature of conventional and novel anti-depressants (Friedman, Friedman, *et al.*, 2008; Moreines *et al.*, 2017; Rincón-Cortés and Grace, 2017), a role supported by experimental evidence (Chaudhury *et al.*, 2013; Tye *et al.*, 2013; Moreines, Owrutsky and Grace, 2017). The parameters of DBS used in the clinical setting are not thought to effectively recruit dopaminergic fibres directly; however, the eventual modulation of mesocortical and mesolimbic pathways, potentially via descending glutamatergic connections between the mPFC and VTA, is thought to be crucial to eventual response (Schlaepfer *et al.*, 2014; Ashouri Vajari *et al.*, 2020; Döbrössy *et al.*, 2021). Experimental manipulation of the mPFC has indeed suggested DA-mediated anti-depressant effects, thought to be related to its top-down regulation of VTA activity (Moreines, Owrutsky and Grace, 2017; Hare *et al.*, 2019). In the section, evidence from pre-clinical research for the effects of mfb-DBS will be discussed.

Non-DBS paradigms of mfb stimulation modulate behaviour, DA and plasticity

The ability of mfb manipulation to modulate frontal DA release has already been well documented, in research principally investigating reward-related behaviours. Vari-

ous frequencies of electrical stimulation, and maximally at 50-65Hz, were shown to lead to DA release in striatal areas (Kuhr *et al.*, 1984; Stamford, Kruk and Millar, 1986). Other research has utilised intracranial self-stimulation (ICSS), a form of DBS in which short trains of stimulating current are delivered to the implanted animal in response to behaviour in an operant conditioning context. Protocols of mfb-ICSS have been extensively used to investigate and manipulate reward-, learning- and memory-based processes, potentiating conditioned responses (Olds and Milner, 1954; Shizgal and Matthews, 1977). Relating to these augmented reward and memory responses, mfb-ICSS has been shown to stimulate the release of DA and 5-HT in the NAc, and increase the expression of Arc and other plasticity-related mRNAs in hippocampal regions (Nakahara *et al.*, 1989; Kádár *et al.*, 2018; Puig-Parnau *et al.*, 2020). It should be noted that these paradigms differ significantly from clinical DBS application. Early demonstrations of stimulation involved short periods (1-10s) of stimulation at frequencies lower than clinical DBS (Kuhr *et al.*, 1984; Stamford, Kruk and Millar, 1986). mfb-ICSS is often performed at high frequency, and is most effective at higher frequencies than DBS (200-500Hz (Kong *et al.*, 2019)). Although ICSS protocols are often implemented over an extended time period, the stimulation is not regular or continuous, which likely results in a different response dynamic. These findings provide valuable clues as to the capabilities of mfb stimulation, such as the modulatory effects on monoamines and interaction with mechanisms of plasticity; however, they cannot be presumed to mirror exactly the mechanisms of mfb-DBS.

mfb-DBS produces various anti-depressant-like behavioural effects

Applications of different high frequency paradigms of mfb-DBS have been shown to have antidepressant effects in rodent models. In naive rats, immobility behaviour in the FST was reduced by both acute-intermittent stimulation (Bregman *et al.*, 2015; Dandekar *et al.*, 2017). This effect, alongside reversal of anhedonic-like phenotype was shown after chronic-intermittent and chronic-continuous stimulation in CMS rats (Furlanetti *et al.*, 2015; Dandekar *et al.*, 2019), and after both acute-intermittent stimulation and chronic-continuous stimulation in the FSL (Edemann-Callesen *et al.*, 2015; Thiele *et al.*, 2020). Alongside these conventional measures of anti-depress-

ant response, chronic-continuous mfb-DBS has also been shown to improve cognitive performance in spatial memory tests (Thiele *et al.*, 2018).

mfb-DBS modulates monoaminergic transmission

The modulation of monoamines during mfb-ICSS paradigms suggest that similar mechanisms may play a role in the actions of mfb-DBS. Addressing this possibility, Bregman and colleagues (2015) investigated the extracellular release of DA and 5-HT in the NAc in anaesthetised, healthy rats. Using a microdialysis probe, samples were collected at 30-minute intervals during and after a 1h period of acute stimulation. No change was found in DA or 5-HT release. However, the static sample collection used in this method is not sensitive to the rapid dynamics of NAc DA release. Indeed, further investigation of the acute effects of mfb-DBS have suggested immediate modulation of DA release and turnover. Using fast-scanning cyclic voltammetry (FSCV), a technique with high temporal resolution, Klanker and colleagues (2017) demonstrated the acute (<1m) release of DA in the NAc of freely-moving, non-depressive-like animals (figure 1.15A). At the onset of stimulation, DA release was immediately elevated, and remained elevated for the duration of recording (40s). During the following period of continuous DBS, spontaneous transient elevations in DA (associated with burst firing (Robinson, Heien and Wightman, 2002)) were monitored, but were not different in frequency or amplitude; neither was DA release in response to unexpected food reward. Building on this work, Ashouri-Vajari and colleagues (2020) further examined the dynamic of acute DA response in the NAc of both non-depressive-like rats and in the depressive-like FSL model. Brief, 5s periods of mfb-DBS were delivered using different pulse-widths, a parameter thought to affect afferent fibre recruitment (Grace and Bunney, 1983; Bielajew, Jurgens and Fouriezos, 1987), and the subsequent DA response was monitored over 50s. At clinically relevant pulse widths, mfb-DBS differentially invoked DA release in non-depressive-like and depressive-like rats, with FSL rats showing a larger and more prolonged response to stimulation, potentially continuing beyond the measurement period (figure 1.15B). Taken together, these studies confirm that mfb-DBS induces DA release in the NAc, and are suggestive of continued elevation beyond the immediate onset of stimulation, though this must be verified. The differential

response of depressive-like animals may have implications for downstream effects of stimulation, such as changes in receptor expression. In non-depressive-like animals, upregulation of the DRD2 was observed in the PFC after acute-intermittent mfb-DBS, despite no changes being observed in tissue levels of DA and its metabolites (Dandekar *et al.*, 2017). Changes to post-synaptic receptors were also seen after chronic-continuous mfb-DBS in the FSL, with both DRD1 and DRD2 upregulated in the NAc after chronic treatment with the D2 antagonist raclopride (Thiele *et al.*, 2020).

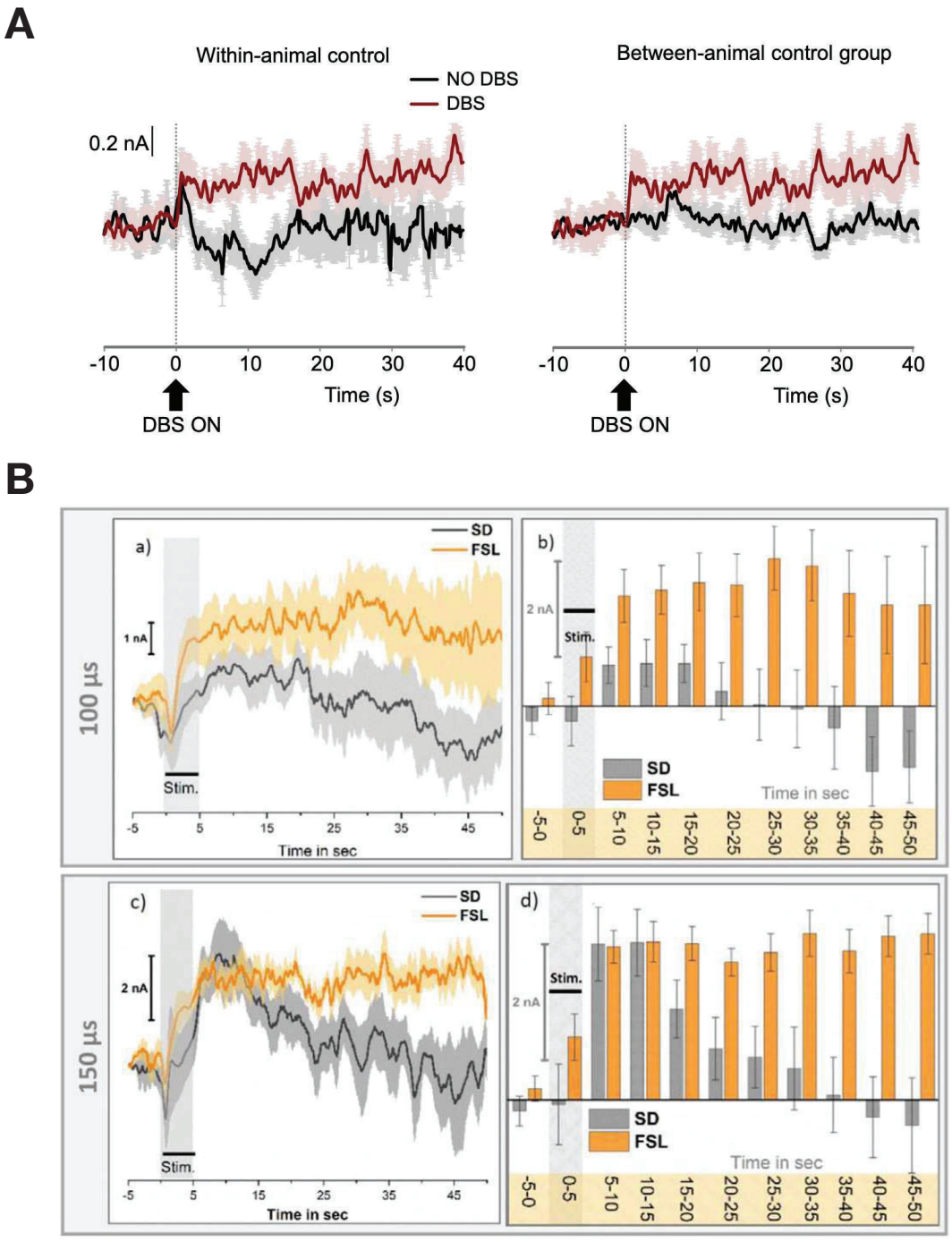

Figure 1.15 *Dopamine (DA) release in the nucleus accumbens (NAc) as measured by fast scanning cyclic voltammetry.*
mfb-DBS induced DA release in the NAc non-depressive-like animals (A; B (grey lines)), and induced sustained, higher magnitude release in the FSL (B, orange lines) at clinically relevant parameters. Adapted with permission from Klanker et al., 2017 (A) and Ashouri Vajari et al., 2020 (B).

The theoretical basis of the anti-depressant effects seen after mfb-DBS involves modulation of DA transmission (Schlaepfer *et al.*, 2013, 2014). These evidence suggest an interaction with the dopaminergic pathways contained within the mfb from the onset of stimulation and with subsequent effects on transmission. However, the dynamics and extent of these interactions, and their relationship with treatment response, remain to be explained. While evidence is strong for acute NAc release (Klanker *et al.*, 2017; Ashouri Vajari *et al.*, 2020), it is not known the extent to which this is sustained (Dandekar *et al.*, 2017). This initial elevation does not appear to affect spontaneous or reward-related burst firing from the VTA, an effect which has been previously linked to anti-depressant effect, including that of low-frequency VTA-DBS (Friedman, Frankel, *et al.*, 2008; Friedman, Friedman, *et al.*, 2008; Tye *et al.*, 2013). Rather, it could be that this change in DA release is related to interruption of pathological activity through changes in the dynamic environment of the network (Florence *et al.*, 2016). This may be the foundation of downstream changes, which may be the result of altered cell physiology rather than simply compensatory responses to elevated DA release. The adaptations of the mesolimbic and mesocortical dopaminergic systems over longer time periods must be examined, particularly in models of depression, given the differential response demonstrated by Ashouri-Vajari and colleagues (2020).

The effects of mfb-DBS on plasticity and inflammation

Little research has been carried out on the wider effects of mfb-DBS thus far. Using the CMS model of depression, Dandekar and colleagues (2019) found that, alongside anti-anhedonic behavioural effects, chronic-intermittent mfb-DBS elevated plasma, cerebrospinal fluid (CSF) and hippocampal levels of BDNF, which were depleted in untreated CMS animals. No BDNF changes were observed in the NAc; disappointingly, BDNF in the PFC was not reported. Alongside this BDNF elevation, levels of adrenocorticotropic hormone, a marker of stress, were reduced in plasma and CSF in DBS-treated animals, as were the levels of several inflammatory cytokines in the NAc and hippocampus.

The results of this study are suggestive of the potential wider effects of mfb-DBS. Taken alongside reported effects on plasticity markers of DBS paradigms in key mfb

targets including the VTA and vmPFC (Friedman, Frankel, *et al.*, 2008; Hamani *et al.*, 2012; Bambico *et al.*, 2015; Jiménez-Sánchez, Linge, *et al.*, 2016), and similar modulation resulting from mfb-ICSS (Kádár *et al.*, 2018; Puig-Parnau *et al.*, 2020), the effects of mfb-DBS on plasticity, particularly in the PFC, may be a fruitful avenue for future research.

Effects of mfb-DBS on sleep

The MFB contains pathways heavily implicated in sleep, including arousal-associated monoaminergic and glutamatergic projections from the brainstem to the forebrain and GABA-ergic fibres associated with sleep-onset (Scammell, Arrigoni and Lipton, 2017), suggesting substrate for modulation of the MFB to influence sleep. However, to the best of our knowledge, no formal investigation has been conducted into the effects of slMFB or mfb-DBS on sleep. During clinical investigations, anecdotal evidence suggests patients report subjectively better sleep (private correspondence, members of the FORSEE clinical trial study team). However, given the close relationship between sleep and depression and the importance of resolving sleep-related symptoms, the effects of MFB-DBS on sleep necessitates further study.

Summary

The effects of DBS in general, and mfb-DBS specifically, remain the subject of debate; ample evidence exists for a variety of immediate and downstream effects. In circuits relevant to depression, anti-depressant DBS has shown modulation of monoaminergic and glutamatergic signalling, leading to electrophysiological and molecular changes. Pre-clinical investigations of mfb-DBS support the hypothesis of dopaminergic modulation as a feature of anti-depressant response, without a clear picture yet emerging. mfb-DBS delivered at clinically-relevant parameters induces prolonged DA release in the NAc, and may lead to alterations of synaptic receptor expression. The longer term dynamics of release and response remain to be described.

Dopaminergic modulation is unlikely to be the only relevant effect of mfb-DBS. Although few studies to date have examined the wider effects of mfb-DBS, other

paradigms of stimulation and DBS in related structures have suggested other mechanisms which may be relevant. These include modulation of serotonergic, noradrenergic and glutamatergic transmission, plastic and inflammatory responses. The direct relationship between mfb-DBS and these responses merits further investigation. Overall, the mechanisms of mfb-DBS remain to be fully elucidated.

1.5 The Flinders Sensitive Line rat as a model of depression

The FSL is a selectively bred strain of rat, derived from the Sprague Dawley. Originally bred for their sensitivity to cholinergic agents, depressive-like behaviour was documented and became the basis of further research (Overstreet, 1986; Overstreet and Wegener, 2013). Often described as a genetic model of depression, the FSL can perhaps more accurately be described as a model of genetic predisposition or gene-environment interaction, as not all FSL rats show spontaneous depressive-like behaviours and some behaviours may be more related to the stress-sensitivity of the animals compared to controls (Wegener, Mathe and Neumann, 2012). The FSL is now a well-established and accepted model for depressive-like phenotypes, displaying a reasonable level of face, construct, aetiological and predictive validity.

1.5.1 Face validity: depressive-like phenotypes in the FSL

Face validity refers to the resemblance of phenotypes in a model to the modelled human disease. The FSL rat exhibits several behaviours considered to be analogous to features of human depression. A principal measure of depressive-like behaviour in rodents in immobility in the FST (Porsolt, Le Pichon and Jalfre, 1977). The FSL exhibits increased immobility in the FST compared to controls, as well as reduced exploratory behaviour in the open field and responsiveness in operant conditioning (Overstreet and Russell, 1982; Thiele *et al.*, 2016, 2018), suggesting motivational and psychomotor deficits.

Anhedonia, a key symptom of depression, is generally not considered spontaneously present in the FSL (Overstreet and Wegener, 2013). Measured by the SPT and intracranial self-stimulation (ICSS) paradigms, FSL rats show no difference under normal conditions, but anhedonia-like behaviour in the SPT is induced by chronic stress (Pucilowski *et al.*, 1993; Matthews *et al.*, 1996). More recently, anhedonic-like behaviour in the FSL has been suggested using a separate paradigm, the sucrose consumption test (SCT) (Rea *et al.*, 2014; Edemann-Callesen *et al.*, 2015). The SCT is purported to differentially measure the ‘wanting’ aspect of behavioural

reward compared to the SPT, and involves a mild stressor of food restriction (Enkel *et al.*, 2010; Schneider, Heise and Spanagel, 2010; Meyerolbersleben, Winter and Bernhardt, 2020). The anhedonic-like behaviour observed may therefore still be linked to a stress-response, or may show a more specific amotivational phenotype, linked to 'wanting' rather than 'liking'. The SCT will be discussed further in **chapters 3 and 5**. Although not necessarily continuously present, as the FSL appears sensitive to stress in terms of developing anhedonia, this can be considered reasonable face validity in terms of predisposition to depression.

In terms of sleep, the FSL shows partial face validity; FSL rats enter REM sleep faster and spend more time overall (Shiromani *et al.*, 1988; Benca *et al.*, 1996), and also exhibit a circadian deficit in the form of phase-shifted diurnal temperature regulation (Shiromani *et al.*, 1991). However, neither of the studies examining sleep architecture reported changes to SWS.

The FSL also exhibits cognitive disturbances which resemble those of patients with depression. These include impaired ability to learn active avoidance behaviours when faced with a shock (Overstreet, Rezvani and Janowsky, 1990) and impaired spatial, object recognition and olfactory memory performance (Gómez-Galán *et al.*, 2013; Thiele *et al.*, 2016; Cook *et al.*, 2017).

Overall, the FSL can be said to exhibit reasonable face validity as a model of depression and depressive vulnerability.

1.5.2 Construct validity: physiological abnormalities in the FSL

Construct validity refers to the biological mechanisms which underlie the phenotype of a model, and how this reflects the human disease. As the pathophysiology of depression is not well understood, construct validity can be hard to assess. The construct validity of the FSL was initially considered in relation to its sensitivity to cholinergic agents, a feature also seen in depressive patients (Janowsky *et al.*, 1972; Overstreet and Russell, 1982). However, growing research into the biology of the FSL has suggested dysfunction of several mechanisms which are theoretically associated with human depression.

Several abnormalities of the monoaminergic systems have been identified in the FSL. In the NAc, FSL rats have elevated tissue content and lower turnover of 5-HT, DA and NA (Zangen, Overstreet and Yadid, 1997, 1999). Lower synthesis of 5-HT has also been reported in limbic areas (Hasegawa *et al.*, 2006). The release of DA into the extracellular space, particularly in relation to 5-HT-stimulated release, is impaired (Zangen *et al.*, 2001; Friedman *et al.*, 2007). This deficient DA-5-HT interaction has been proposed as the result of hyperfunction of the 5-HT2C receptor (Dremencov *et al.*, 2005), while FSL rats also exhibit lower expression of the 5-HT1A and 2A and higher expression of the 5-HT1B receptors (Österlund, Overstreet and Hurd, 1999; Nishi, Kanemaru and Diksic, 2009). In the PFC and hippocampus, abnormal expression of 5-HT receptors has also been found, including reduced 5-HT2A and increased 5-HT2C expression (du Jardin, Müller, *et al.*, 2017). FSL rats exhibit increased striatal density of 5-HT transporter molecules (Hvilsom *et al.*, 2019), but lower expression of the vesicular monoamine transporter in the NAc and VTA (Schwartz *et al.*, 2003). Despite these apparent changes in 5-HT transmission, depletion of 5-HT does not alter the behaviour of FSL rats, but does blunt treatment response (du Jardin *et al.*, 2016; du Jardin, Liebenberg, *et al.*, 2017), a phenomenon similar to that observed in humans (Willner, Scheel-Krüger and Belzung, 2013).

In addition to elevated DA tissue content and impaired release in the NAc (Zangen, Overstreet and Yadid, 1999; Zangen *et al.*, 2001; Friedman *et al.*, 2007), the FSL also exhibits differential DA receptor expression, with elevated striatal DRD1s compared to controls, and reduced DRD2s when single, but not group, housed (Bjørnebekk, Mathé and Brené, 2007)**.** Friedman and colleagues (2008) demonstrated altered burst firing of VTA dopamine neurons. This altered firing contributed to an altered electrophysiological profile, a phenotype also observed in the vmPFC, NAc and STN (Friedman *et al.*, 2012; Voget *et al.*, 2015).

Observed electrophysiological changes in these areas may relate to Glu-GABA as well as DA functioning. Supporting this, FSL rats were shown to exhibit elevated baseline Glu transmission, and tissue levels in the NAc (Gómez-Galán *et al.*, 2013; Voget *et al.*, 2015) and to exhibit differential expression of AMPA and NMDA receptor subunits associated with glutamatergic transmission (Ryan *et al.*, 2009; Gómez-Galán *et al.*, 2013; Treccani *et al.*, 2016). Glutamatergic changes are theorised to

contribute to altered plasticity in depression; indeed FSL rats exhibit inhibited LTP and reduced BDNF expression in the hippocampus (Ryan *et al.*, 2009; Elfving *et al.*, 2010; Gómez-Galán *et al.*, 2013).
Overall, multiple abnormalities in systems theorised to be altered in depression mean the FSL has reasonable construct validity.

1.5.3 Predictive validity: anti-depressant response in the FSL

Predictive validity relating to depression is the ability of a model to reflect the anti-depressant response of known anti-depressant treatments, and show no response when administered non-anti-depressant treatments. In response to conventional anti-depressants, the FSL has typically shown very good predictive validity, with chronic application of all major of classes reducing behavioural phenotypes; acute treatment consistently fails to alter behaviour, as is this case in human patients (Overstreet and Wegener, 2013). This has been demonstrated in the TCAs imipramine and desipramine, the SSRIs sertraline, paroxetine, citalopram and fluoxetine, and atypical anti-depressants such as nefazodone (Pucilowski and Overstreet, 1993; Zangen *et al.*, 2001; Dremencov *et al.*, 2004; Overstreet, Keeney and Hogg, 2004). When using the same protocol, the psychostimulant amphetamine did not reduce immobility, which can produce false positives in the FST (Overstreet *et al.*, 1995). These conventional treatments have been shown to affect the physiology in the FSL, suggestive of potential mechanisms in human patients. For example, imipramine and nefadozone have been shown to correct the impaired DA-5HT interaction and DA release in the NAc (Zangen *et al.*, 2001; Dremencov *et al.*, 2004); desipramine to normalise the altered burst firing of the VTA (Friedman, Friedman, *et al.*, 2008) and to increase 5-HT synthesis (Sato, Skelin and Diksic, 2011).
Novel anti-depressant treatments have also been tested in the FSL. Ketamine has shown anti-depressant behavioural effects in the FSL (du Jardin *et al.*, 2016; du Jardin, Liebenberg, *et al.*, 2017; Silva Pereira *et al.*, 2017; Treccani *et al.*, 2019; Ardalan *et al.*, 2020), as in humans (Berman *et al.*, 2000). The mechanisms of ketamine's action have been linked to increased BDNF expression, increased hippocampal volume and dendritic arborisation in the FSL, but not in controls (Silva Pereira *et*

al., 2017; Treccani *et al.*, 2019; Ardalan *et al.*, 2020), coherent with theories of increased plasticity as a mechanism in human patients (Matveychuk *et al.*, 2020). DBS has also been investigated in the FSL, with anti-depressant effects shown after low-frequency VTA- and vmPFC-DBS (Friedman *et al.*, 2012; Gazit *et al.*, 2015; Bruchim-Samuel *et al.*, 2016); and after high frequency mfb- and vmPFC-DBS (Rea *et al.*, 2014; Edemann-Callesen *et al.*, 2015; Thiele *et al.*, 2018, 2020). These anti-depressant responses are coherent with clinical trials suggesting successful anti-depressant response in human patients after SGC- (Mayberg *et al.*, 2005; Lozano *et al.*, 2008) and slMFB-DBS (Schlaepfer *et al.*, 2013; Fenoy *et al.*, 2016, 2018; Bewernick, Kayser, Gippert, Coenen, *et al.*, 2017; Bewernick, Kayser, Gippert, Switala, *et al.*, 2017; Volker A. Coenen *et al.*, 2018; Coenen *et al.*, 2019).

While the predictive validity of anti-depressant mechanisms is not always verifiable in human patients, some, such as elevated BDNF expression are thought to correspond. Many treatment effects are not seen in behaviour or physiology in non-depressive-like controls (Overstreet *et al.*, 2005; Friedman, Friedman, *et al.*, 2008; Edemann-Callesen *et al.*, 2015; Silva Pereira *et al.*, 2017; Treccani *et al.*, 2019), which suggests an interaction between treatment and underlying pathology; therefore, in a model with reasonable construct validity, anti-depressant mechanisms can be said to provide evidence of potential mechanisms in humans.

1.5.4 Aetiological validity: the onset and course of the phenotype

Aetiological validity is not considered one of the key criteria for a model for depression as proposed by Willner (Willner, 1984). However, given the heterogenous nature of depression, it is an important factor to consider. Models induced by environmental stressors, such as CMS, can be said to have high aetiological validity given the role of stress in triggering human depression (Czéh *et al.*, 2016). Considering the wide range of proposed mechanisms underlying depressive symptoms, directly induced models (genetic knockout mice, optogenetic manipulation, for example) often lack aetiological validity due to their narrow focus. Selectively bred models, such as the FSL, lack some aetiological validity as they do not reflect the progressive onset and episodic nature of the disease. However, as the FSL shows sensitivity

to stress, as in the induction of anhedonia-like behaviours (Pucilowski *et al.*, 1993), and some symptoms have been suggested to occur in transient fashion (Thiele *et al.*, 2016), it does show some validity as a model of gene-environment interaction (Wegener, Mathe and Neumann, 2012; Czéh *et al.*, 2016).

1.5.5 Selection of the model

No animal model perfectly fulfils the criteria of validity outlined by Willner (1984) and more recently adapted (Czéh *et al.*, 2016; Czéh and Simon, 2020), reflecting the difficulty of modelling a complex mental disorder such as depression. Some models strongly replicate certain aspects of the disease, for example by experimentally manipulating specific implicated systems, providing strong but specific construct validity. Others aim to develop an aetiologically accurate model of the whole disease. Both methods have advantages, and together provide complementary insights. As a congenital model, the FSL has questionable aetiological validity; however its susceptibility to stress suggests some resemblance to the onset of human symptoms. The construct validity of the FSL was initially considered narrow, based solely on cholinergic sensitivity. However, recent evidence suggests reasonable construct validity in a wider array of mechanisms, including monoaminergic and glutamatergic signalling. The FSL exhibits reasonable face validity and excellent predictive validity in terms of anti-depressant treatments.

For the experiments presented in the following chapters, the FSL was deemed an appropriate model on the strength of these considerations. As a crucial aim was to investigate the outcomes and mechanisms of mfb-DBS, strong predictive validity of the model was important in order to adequately compare with the results of clinical trials. Construct validity was also an important consideration. Although the focus of this thesis was the dopaminergic system and its interactions, as the mechanisms of MFB-DBS are not well understood it was felt important to measure the system in the context of a broad model of depression, rather than, for example, a model of

experimentally modified dopaminergic activity. Finally, as the impact of MFB-DBS on sleep was of interest, face validity in this regard was important.

2. Summary and aims

Depression is a debilitating condition, and the problem of treatment resistance is exacerbated by the incomplete understanding of both the underlying pathophysiology and mechanisms of treatment response. Resolving sleep disturbances, a serious and common problem in depression, represents an important facet of recovery. MFB-DBS represents a promising treatment, but its full effects on depressive pathology, including sleep, and its mechanisms of action are not fully understood. Theoretical links and previous experimental work suggest a role of dopamine and mechanisms of plasticity in the effects of MFB-DBS. The broad aims of this thesis were therefore multiple:

- to investigate the effects of mfb-DBS on sleep disturbances in an appropriate rodent model, the FSL
- to investigate physiological changes resulting from mfb-DBS in the FSL, in relation to its anti-depressant effects, particularly with regard to changes in dopaminergic transmission and synaptic plasticity

Research projects

In order to fulfil the aims of the thesis as described above, three experiments were carried out.

In **experiment one (chapter 4)**, the relationship between mfb-DBS and sleep disturbances in depression was investigated. First, sleep disturbances in the FSL model were characterised beyond the published literature by conducting electrophysiological recordings in deep structures alongside traditional sleep assessment using ECoG and EMG. After baseline characterisation, the impact of 24h mfb-DBS on sleep measures was investigated.

In **experiment two (chapter 5)**, the effects of 24h mfb-DBS on behaviour, dopaminergic signalling and synaptic plasticity were investigated in the FSL. Depressive-like behaviours were assessed in the FST and SCT before and after mfb-DBS, and

the expression of dopaminergic receptors and markers of synaptic plasticity were assessed in stimulated animals.

Finally, in **experiment three (chapter 6)**, the effects of mfb-DBS of different durations on monoaminergic transmission were investigated. mb-DBS was conducted in FSL rats for either 2h or 24h, and the tissue content of DA and 5-HT assessed.

3. Materials and methods

3.1 Animals

Depressive-like FSL rats

All FSL rats used in these experiments originated from the colony maintained by the Department of Stereotaxic and Interventional Neuroscience laboratory at the Universitätsklinikum Freiburg. The colony originated from Duke University, USA, and breeding continued at the Uniklinik Neurozentrum and the University of Freiburg's ZBMZ animal facility.

Screening for depressive-phenotype

All FSL rats bred in our facility were screened for a depressive-like phenotype at an age of <12 weeks via the FST (described below). Animals exhibiting behaviour over a minimum threshold (>20% immobility) were considered for experimental use. All experiments were conducted before the rats were above 6 months of age, due to evidence that some depressive-like phenotypes may be transient (Thiele et al., 2016).

Non-depressive controls

Age-matched Sprague Dawley (Ctrl) rats, sourced from Charles River, Germany (for experiments conducted in Freiburg) or Janvier, France (for experiments conducted in Strasbourg), were selected as non-depressive-like controls.

General animal protocols

Animals were group housed, with food and water available ad libitum, under a 12h:12h light/dark cycle. Before any initial protocol, rats were handled daily for at least 7 days. After all surgical procedures, animals were housed individually. After surgery, animals were allowed a minimum of 7 days recovery before any further experimental procedures. For two weeks after surgery, animals were checked daily,

with weight monitored and pain management provided if deemed necessary (pain management via non-steroidal anti-inflammatory, Carprofen, 4mg/kg or Metacam, 1mg/kg; or opioid buprenorphine, 0.3-0.5mg/kg). Outside of recovery periods, animals were checked daily and weights monitored weekly.

Connection to stimulation and recording systems

For procedures involving DBS and/or electrophysiological recording, animals were connected via flexible cable to a rotating joint (custom built, University of Freiburg; PlasticsOne/P1 Technologies, USA, University of Strasbourg), allowing unrestricted movement in the home cage and incorporating both stimulation and recording channels. Before any procedure, animals were were habituated to the cable over 72h.

Adherence to ethical standards

All procedures were carried out in accordance with the EU Directive 2010/63/EU concerning the protection of animals used for scientific purposes. All experiments had the approval of the veterinary board for research at the University of Freiburg or Strasbourg, respective of where the experiment took place. All efforts were made to minimise the unnecessary distress, pain and suffering of the animals used.

3.2 Behavioural testing

The Forced Swim Test

Rationale

The FST is a commonly used measure of depressive-like phenotype and anti-depressant effect in rodent models of depression (Slattery and Cryan, 2012; Yankelevitch-Yahav *et al.*, 2015). Immobility in a test period is taken as a measure of a 'despair'-like phenotype.

When the test is conducted over multiple days, as in the Porsolt (Porsolt, Le Pichon and Jalfre, 1977) and chronic despair model (CDM) (Sun *et al.*, 2011) versions, the pre-test sessions act as a stressor and can be used to induce the immobile pheno-

type, and other depressive-like symptoms in the case of the CDM mouse. A single-day version of the test can be used in models of depression exhibiting spontaneous phenotypes, including the FSL (Overstreet and Wegener, 2013). In the following experiments, according to the protocols established in the laboratory (Thiele *et al.*, 2016, 2018, 2020), a two-day protocol was utilised to allow the comparison of the FSL with non-depressive-like controls in response to treatment.

Increased locomotor activity resulting from a treatment can result in false positives in the FST. This is particularly of concern in treatments thought to act on dopaminergic systems, due to the crucial role of DA in movement. At the onset of mfb-DBS, an increase in locomotor activity is seen as the animal engages in 'seeking' behaviours, and possibly related to further dopaminergic modulation. This initial response appears diminished within hours, and both unpublished monitoring and published reports from this lab (Furlanetti *et al.*, 2015) and others (Edemann-Callesen *et al.*, 2015) have indicated no motor changes after mfb-DBS. However, as a precaution in the current experiments, the forced swim test was always administered at least 24h after the end of DBS treatment (Overstreet and Wegener, 2013).

Procedure

Animals were placed in a perspex cylinder (40cm x 20cm) filled with water at 21-23°C. The water level was such that the animal could neither escape the cylinder, nor touched the bottom with their tails. The FST was performed over two days. In the first day, animals were habituated to the cylinder for 15m. On the second day, animals were placed in the cylinder for the duration of a 7m trial period. The trial session was recorded from the side, and assessed offline. Immobility was defined as three of four limbs stationary, floating with no struggling behaviour. The time spent immobile was expressed as a percentage of total time.

Sucrose Consumption Test

Rationale

In order to assess an anhedonic-like phenotype in the FSL rat, and measure the effect of mfb-DBS on this aspect, the SCT was used (Enkel *et al.*, 2010; Schneider,

Heise and Spanagel, 2010). The SCT was selected as opposed to the SPT, a commonly used measure of anhedonia in depressive models. The FSL rat reportedly does not exhibit anhedonic-like behaviour in the SPT unless exposed to chronic stressors (Pucilowski *et al.*, 1993), consistent with observations in our laboratory (Thiele *et al.*, 2016). Sucrose consumption, rather than preference, is proposed to measure the 'wanting' to a greater degree than to the 'liking' aspect of reward-related behaviour; the activity of the DA system is associated with 'wanting', and its disruption has been shown to modulate sucrose consumption but not preference (Meyerolbersleben, Winter and Bernhardt, 2020). The high calorific value of the solution, coupled with mild food restriction, adds motivational salience to the reward (Grønli *et al.*, 2005; Scheggi, De Montis and Gambarana, 2018). The element of choice between two bottles in the SPT may also add the dimension of behavioural reinforcement and reward learning (Enkel *et al.*, 2010; Schneider, Heise and Spanagel, 2010). To specifically measure the purported dopaminergic, 'wanting' aspect of reward, a single-bottle SCT was used.

Procedure

24h before testing, animals were habituated for 2h to a solution of sweetened condensed milk (SCM, Nestlé, Milchmädchen gezuckerte Kondensmilch and water at a 1:3 ratio). Rats were food (but not water) deprived for 18h before being given free access to the SCM solution for 15m. Bottles were weighed before and after the test period, the amount of solution normalised to the body weight of the animal and expressed at mg solution consumed/kg body weight. All tests were performed at the same time (relative to the room's light/dark cycle), due to the potential circadian effect on sucrose intake (Scheggi, De Montis and Gambarana, 2018).

3.3 Electrodes

All electrodes used in the following experiments were constructed by hand, adapted from previous protocols used in the laboratories. After construction, the passage of current between the distal tips of each electrode was tested with an ohmmeter. Before surgical implantation, electrodes were immersed in 70% ethanol.

Bipolar electrodes for deep brain stimulation

Teflon-coated Platinum-Iridium wire (90-10%) with an inner diameter of 125µm (World Precision Instruments, USA) was cut to lengths of approximately 25mm. Approximately 2mm of insulation was stripped from one end, and a gold-coated steel tip appropriate for the connection piece fixed with solder to the exposed wire. Pairs of single electrodes were twisted together from the midway point to form a single bipolar electrode, which was then straightened. The ends of the two electrodes were cut to create a distance of approximately 0.5mm between tips. The bipolar electrode was inserted into a stainless steel cannula (length approximately 3-5mm), created from a cut needle, with approximately 9mm exposed below the cannula for insertion into the brain. The cannula was secured to the electrode with superglue (Loctite).

Monopolar electrode pairs for recording of local field potentials

Teflon-coated Tungsten wire with an inner diameter of 76µm (World Precision Instruments, USA) was cut to lengths of 25mm. Approximately 2mm of insulation was stripped from one end, and a gold-coated steel tip appropriate for the connection piece fixed with solder to the exposed wire. Pairs of such electrodes were inserted into a stainless steel cannula cut from a needle (approximately 3-5mm). The length of electrode extending below the cannula was dependant on the target structure, adjusted to a length of approximately 1mm greater than the depth of the target co-ordinate. Electrode pairs were arranged with the tips adjacent to each other and fixed to the cannula with superglue (Loctite).

Screw electrodes for ECoG recording, referencing and ground

Lengths of teflon-coated Tungsten wire with an inner diameter of 76µm (World Precision Instruments, USA) were stripped of 2mm insulation at either end. At one end, a gold-coated steel tip appropriate for the connection piece was fixed with solder to the exposed wire. At the other, the wire was soldered to a 1.2mm stainless steel flat-bottomed screw.

EMG recording electrode

Lengths of teflon-coated Tungsten wire with an inner diameter of 76µm (World Precision Instruments, USA) were stripped of approximately 10mm insulation at one end, and a spherical tip of solder attached to the tip. At the other end, approximately 2mm of insulation was stripped and a gold-coated steel tip fixed with solder to the exposed wire.

3.4 Surgery

Surgery was performed under isoflurane anaesthesia delivered in O_2, induced at 4% and maintained at 1.5-2.5% for the duration of surgery. Temperature was managed via electronic heat pad (University of Freiburg) or thermal blanket (University of Strasbourg). Before incision, animals received subcutaneous lidocaine (Lurocaïne, Xylovet 1%, 2 mg/kg) at the incision site, and the eyes were covered with ophthalmic gel (Lubrithal) to prevent drying of the cornea. Anti-septic was applied to the top of the head, and an incision made along the midline of the skull. Tissue was cleared to expose the landmarks of bregma and lambda. The dorsal-ventral (DV) coordinates of the skull at the two landmarks were used to ensure the flat plane of the skull, and the tooth bar adjusted accordingly. The anterior-posterior (AP) distance between bregma and lambda was measured, and used to calculate an AP correction factor to account for the size of the animal (AP correction = bregma AP co-ordinate - lambda AP co-ordinate / 9). AP co-ordinates of all target regions were multiplied by this correction factor. Stereotactic coordinates of each target were calculated from bregma, and burr holes drilled at these positions. Additional burr holes were drilled for the insertion of 1.2mm stainless screws to provide stability. In the case of surgery for electrophysiological recording, additional holes were drilled over the frontal and cerebellar regions for ECoG and ground electrodes. In all surgeries, a total of 4 screws were inserted to ensure stability of the headpiece.

Deep brain electrodes were mounted in the arm of the stereotaxic frame, and target coordinates were taken once more from bregma. Before insertion, the tip of each electrode was coated with a fluorescent dye (DiI, Molecular Probes, Inc.) to facilitate

verification of electrode placement. Each electrode was lowered to the level of the dura, and the DV coordinate taken from this point. The electrode was then lowered slowly to the target depth and secured in position with glue (Superbond, C&B).

For electrophysiological recordings, screw electrodes for ECoG and electrophysiological reference were inserted to the level of the dura above the frontal and cerebellar regions. A screw electrode acting as electrophysiological ground was inserted into the skull. Finally, an EMG electrode was inserted at the rear of the incision site into the nuchal muscle. Electrodes were inserted into to a connection pedestal (PlasticsOne/P1 Technologies, USA, in experiments at the University of Strasbourg; custom built, in experiments at the university of Freiburg) and adhered to the skull with acrylic dental cement (Paladur).

Post-operatively, all animals received analgesia (Metacam, 1 mg/kg, University of Strasbourg; buprenorphine, 0.5mg/kg, University of Freiburg) and were allowed seven days of recovery time before further experimental procedures.

3.5 Deep brain stimulation

In all experiments, deep brain stimulation was delivered continuously using a biphasic, square wave signal with a pulse width of 90µs and a frequency of 130Hz, via a pulse generator (custom built, University of Freiburg; A-M Systems, USA, University of Strasbourg). These parameters were selected primarily in order to replicate clinical application of slMFB-DBS (Schlaepfer *et al.*, 2013; Bewernick, Kayser, Gippert, Coenen, *et al.*, 2017), and in relation to previous pre-clinical work (Edemann-Callesen *et al.*, 2015; Thiele *et al.*, 2018, 2020; Ashouri Vajari *et al.*, 2020). While low frequency mfb-DBS (5-75Hz) has been shown to induce NAc DA release in acute applications (Kuhr *et al.*, 1984; Stamford, Kruk and Millar, 1986; Gonon, 1988), 20Hz mfb-DBS was unable to produce anti-depressant response in the rodent (Bregman *et al.*, 2015), and frequency below the typical high-frequency (>100Hz) application of DBS (Benazzouz and Hamani, 2020) is unlikely to be mechanistically relevant to the clinical application of 130Hz. The pulse width used is shorter than optimal for the recruitment of unmyelinated dopaminergic neurons

(Ashouri Vajari *et al.*, 2020), but again reflects the parameters used in DBS applications in clinical and pre-clinical applications.

Current was delivered between 50-300μA, a range selected according to previous work in the Freiburg laboratory establishing safe and effective chronic application of mfb-DBS (Furlanetti *et al.*, 2015; Furlanetti, Coenen and Döbrössy, 2016). Current was titrated for individual each electrode, using behavioural response previously established in the laboratory as a marker for successful stimulation of the MFB (Furlanetti *et al.*, 2015; Thiele *et al.*, 2018). Titration – starting at 50μA increasing stepwise by 50μA to a maximum current of 300μA – was initiated with the animal in a state of quiet rest, and current increased until a distinct change towards an alert state characterised by explorative, "seeking" behaviour was observed (Coenen *et al.*, 2011; Panksepp and Watt, 2011), in the absence of side effects such as dystonic or erratic movements, or rotational behaviour. The titrated current was maintained for the duration of the DBS period.

mfb-DBS was applied in each experiment for a duration of 24h. The mechanistic response of mfb-DBS appears to be dynamic, and it is unclear when anti-depressant effect begins, while clinical application is continuous and chronic. Previous work has investigated immediate acute response (seconds-minutes) (Klanker *et al.*, 2017; Ashouri Vajari *et al.*, 2020), but this is likely different from anti-depressant mechanisms after chronic stimulation. Both intermittent mfb-DBS within 24h and chronic stimulation over several days have produced anti-depressant behavioural responses (Edemann-Callesen *et al.*, 2015; Thiele *et al.*, 2018; Dandekar *et al.*, 2019). 24h was therefore selected as a stimulation duration likely sufficient to induce anti-depressant response, but producing a mechanistic reaction beyond the acute response. In experiment three, an additional stimulation period of 2h was applied to one group, in order to model a point between the immediate acute response and response after 24h.

3.6 Electrophysiology

Rationale

Oscillatory activity describes the electrical signal generated endogenously by the brain's neural circuits. Brain oscillations, such as those recorded by EEG or electrodes implanted in tissue to record local field potentials (LFPs), represent the summed electrical activity produced by fluctuations in excitability across large populations of neurons (Buzsáki, Anastassiou and Koch, 2012). Oscillatory activity can be defined in terms of three dimensions: frequency (the speed of the oscillation); power (the amplitude of the signal); and phase (the position of the wave at a given point in time). In analysis of neural signals, frequency and power are the most commonly assessed features. An individual waveform derived from neural signal contains activity at multiple oscillating frequencies, which can be separated and the amplitude of each assessed. These frequencies are typically separated into 'bands', including delta (0.5-4Hz), theta (4-8Hz), alpha (8-12Hz), beta (15-30Hz) and gamma (30-100Hz) (Cohen, 2014). While the exact boundaries of these groupings are not fixed, and thus may vary between reports, they are based upon observed functional groupings, thought to be derived from different neurophysiological events and architecture (Buzsáki, 2006; Cohen, 2014). Different frequency bands are therefore associated with different behavioural and physiological functions, and variations in amplitude can reflect functional and physical changes. For instance, activity in the delta band (0.5-4Hz) corresponds to the SWA of SWS, with putative role in synaptic homeostasis; theta activity (4-8Hz) is strongly associated with active behaviour in wake and with REM sleep, thought to be related to mechanisms of learning and memory; gamma activity (30-100Hz) is associated with the activity of populations of excitatory and inhibitory neurons, and is thus theoretically related to e/i balance, several cognitive functions and mechanisms of plasticity (Buzsáki, 2004, 2006; Buzsáki and Wang, 2012).

The principle measure of sleep, the EEG, and its equivalent in the rodent, the ECoG, are derived from these principles. The summed oscillatory activity of large neuronal

populations can be recorded from electrodes placed on the scalp (EEG) or on the surface of the brain (ECoG). The characteristic oscillations of different vigilance stages (as discussed in **section 1.3**) can then be used to determine the timing of sleep stages, and be assessed themselves for their oscillatory content. From electrodes implanted into deep structures, LFP recordings can provide information on the oscillatory activity of populations of neurons in sleep and in other vigilance states.

Data collection

Electrophysiological recording sessions were conducted in the home cage, with animals filmed throughout. Electrophysiological signals were referenced against the universal reference electrode (at the level of the cerebellum), amplified x1000 and sampled at 1kHz. Signal was then digitised via LabChart acquisition software (AD Instruments, New Zealand), with data stored for offline analysis.

Sleep scoring

Analysis of time spent in vigilance states was performed using ProFusion software (Compumedics Ltd, Australia). ECoG and EMG signal were divided into 10s epochs, and each epoch manually scored as “wake”, “SWS” or “REM”. “Wake” state was identified by typically desynchronised ECoG activity of high frequency, with concurrent EMG activity; “SWS” by characteristic slow waves of low-frequency, high-amplitude ECoG activity and low EMG; and “REM” sleep by the presence of characteristic theta waves and minimal EMG activity. Video recordings were used to verify any case of doubt. Sleep architecture measurements of each vigilance state’s duration, episode number and mean length were averaged over 3h periods and delineated according to Zeitgeber time (ZT) period (24h clock adjusted to the light-dark cycle, with ZT 0 defined as the beginning of the light cycle and ZT 12 being beginning of the dark cycle). For each vigilance state, circadian amplitude was defined as its total duration during the light cycle – during the dark cycle.

Spectral analysis

Signal from ECoG and deep electrodes were imported into MATLAB (Mathworks, USA) for spectral analysis. Original scripts for analysis were written specifically for the datasets, utilising the Chronux (Bokil *et al.*, 2010) and MATLAB Signal Processing (Mathworks, USA) toolboxes. Upon importation, signal from pairs of deep electrodes was locally referenced via the first derivation method (in which the signal of one electrode in the pair is subtracted from the other) in order to remove elements of distal signal. 50Hz line noise was removed using a dedicated function (Eiber, 2021). Artefacts were identified as timepoints with an amplitude ±3 standard deviations from the mean, and were removed from the signal along with 100ms from either side. Periods of signal were divided according to vigilance state according to the criteria above (in the section, 'sleep scoring'). Episodes of each vigilance state during 3h-long periods were concatenated, bandpass filtered between 0.5 and 200Hz and transformed using the multitaper method, using five Slepian tapers, implemented via Chronux (Purpura and Bokil, 2008). Spectrograms were inspected to verify the presence of spectral peaks within relative frequency bands (figure 3.1). Spectral power was averaged over classical pre-defined frequency bands (delta 0.5-4Hz, theta 5-10Hz, beta 10-30Hz, gamma 30-90Hz), log-transformed and normalised to total recorded power (0.5-200Hz). This data was presented according to vigilance state and ZT period.

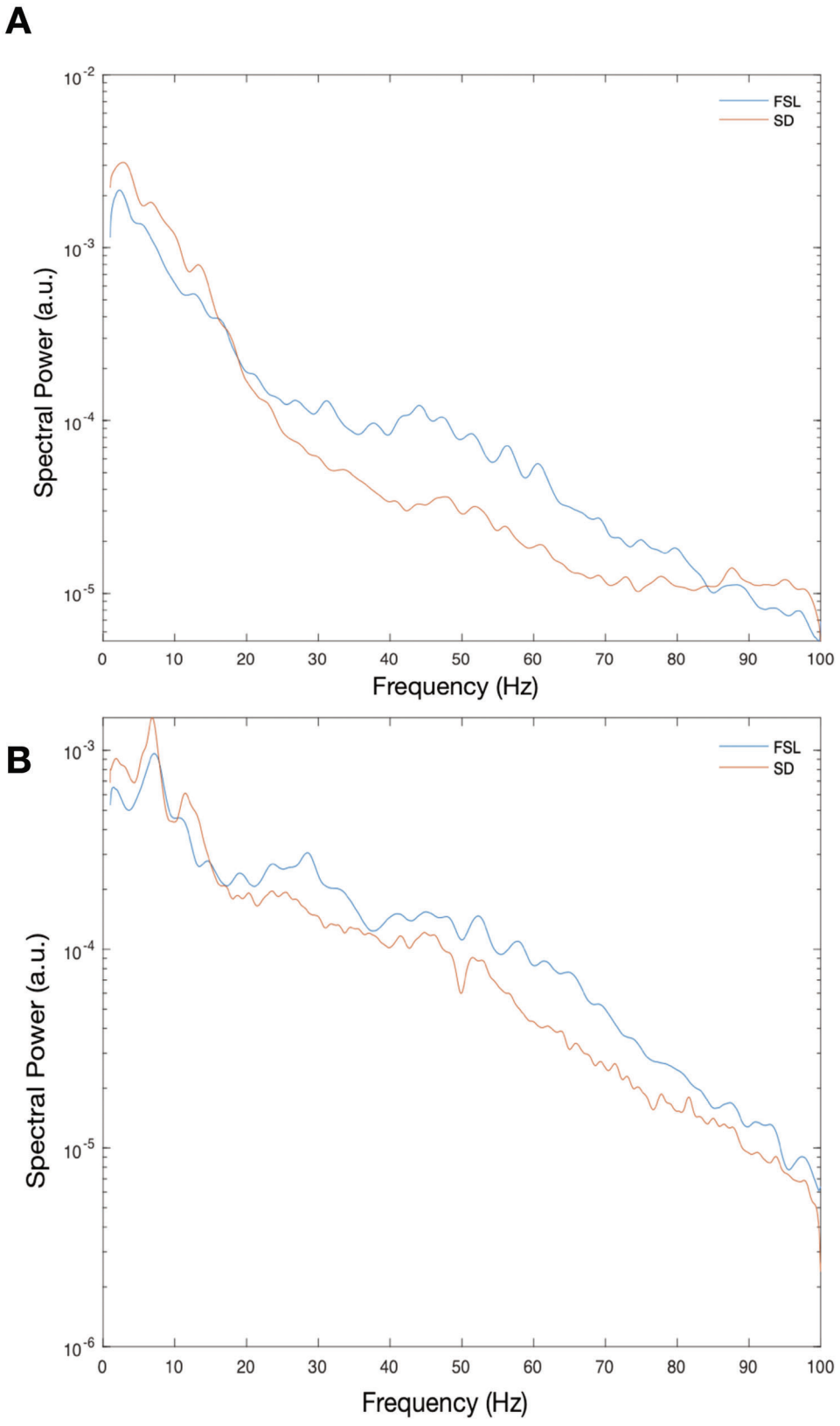

Figure 3.1. *Example spectrograms taken from ECoG recordings. Individual episodes during the light cycle of SWS (A) and REM sleep (B), in the Sprague Dawley (red) and FSL (blue).*

3.7 Tissue collection

Histological assessment

At the end of experimental procedures, animals were terminally anaesthetised with the administration of sodium pentobarbital (Dolethal, 100 mg/kg, i.p., University of Strasbourg) or ketamine (10%)/xylazine (2%) solution (University of Freiburg), and transcardially perfused with 4% PFA solution. Following a 2h period of post-fixation immersion within 4% PFA, brains were suspended in a 20% sucrose solution for 48h before being frozen. 40µm sections were cut using a cryostat and mounted on slides in the presence of DAPI for fluorescent imaging. Electrode placement was verified visually under a fluorescence microscope. Electrodes judged to be placed outside of the target structure were excluded from relevant analyses.

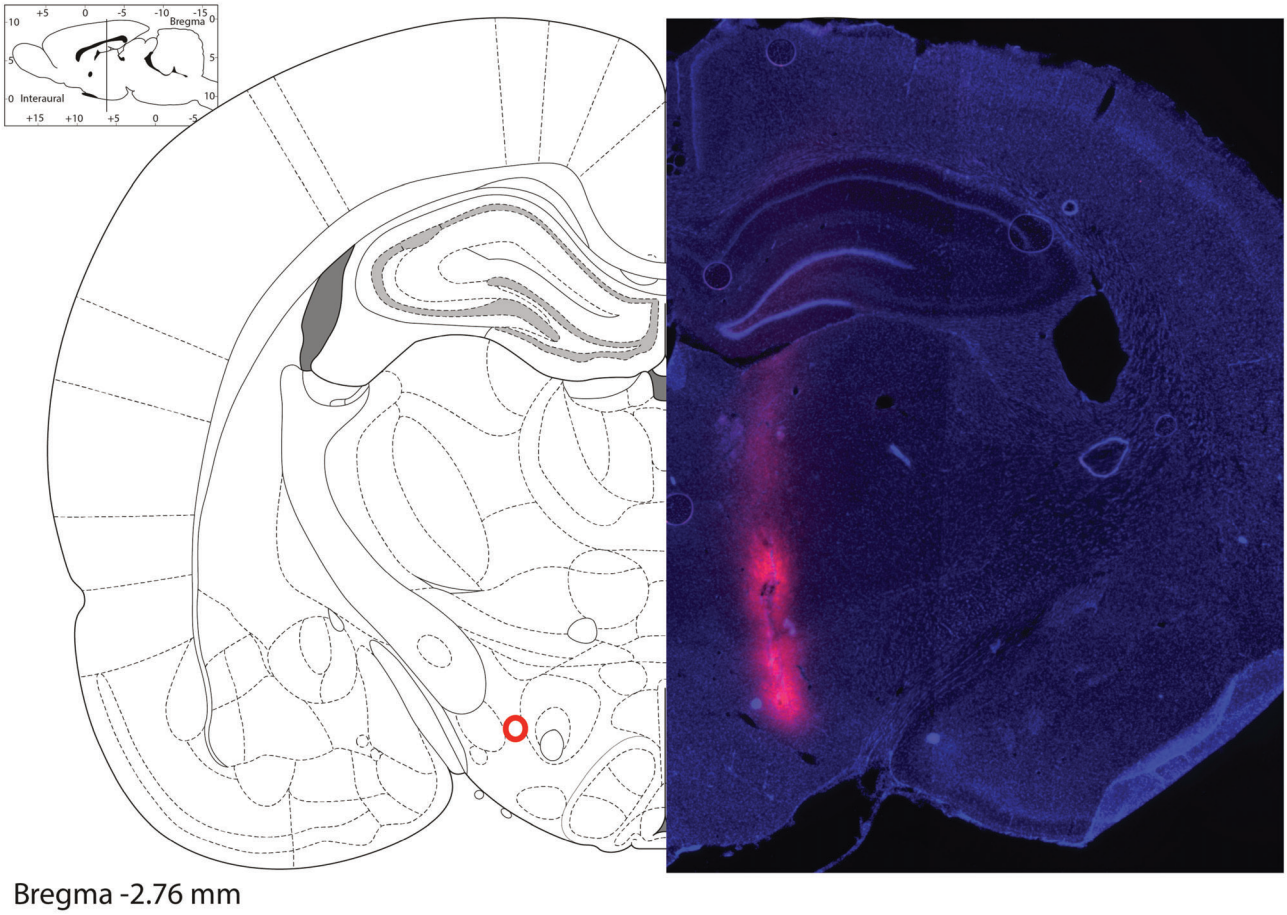

Figure 3.2. *Estimation of electrode placement in the mfb.*
Before implantation, the electrode is lightly coated in fluorescent DiI. Electrodes imaged with fluorescent microscopy (right) were compared with target co-ordinates in the Paxinos and Watson Rat Brain Atlas 7th Edition (2007, left).

Micro dissection

For tissue required for further neurochemical or molecular analysis, immediately at the cessation of mfb-DBS, animals were disconnected from the stimulation apparatus and euthanised by decapitation. The brain was rapidly removed, and the frontal portion dissected on a glass plate frozen on dry ice to remove the mPFC and NAc in coronal sections. Samples were stored at -80°C until prepared for analysis.

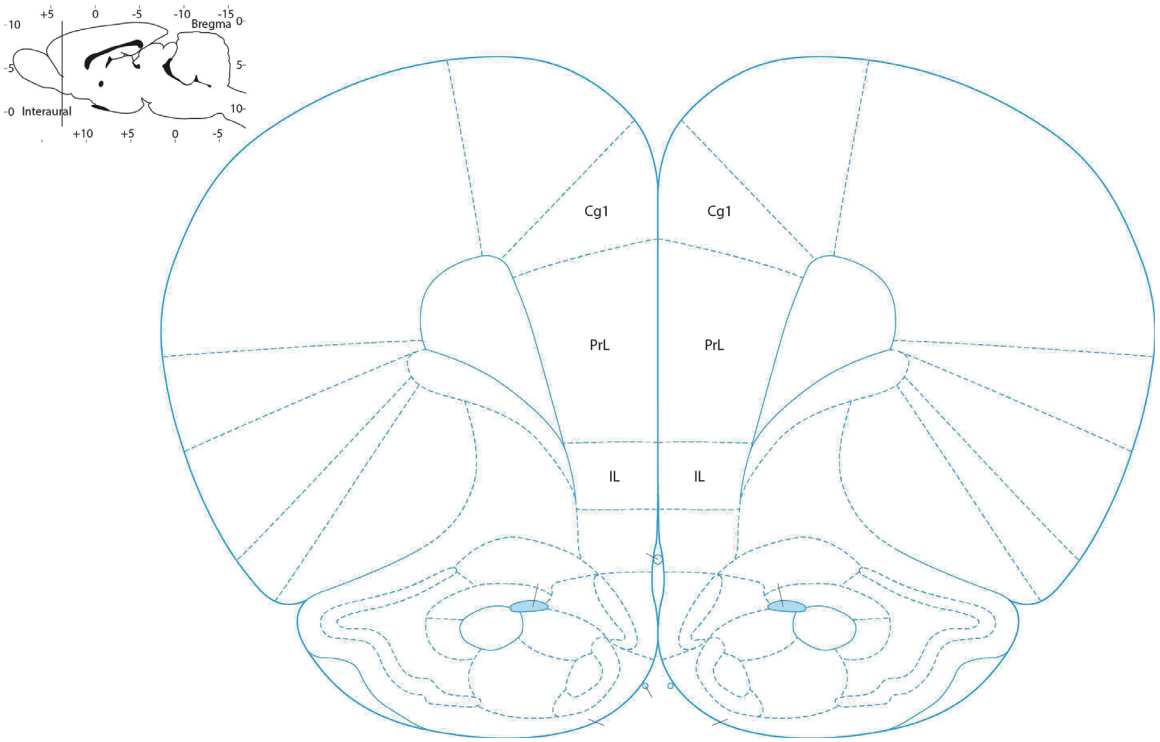

Figure 3.3. *Target tissue of the mPFC for collection after micro dissection. Adapted from the Paxinos and Watson Rat Brain Atlas 7th Edition (2007). Cg1 = anterior cingulate cortex (ACC); PrL = pre-limbic cortex; IL = infralimbic cortex.*

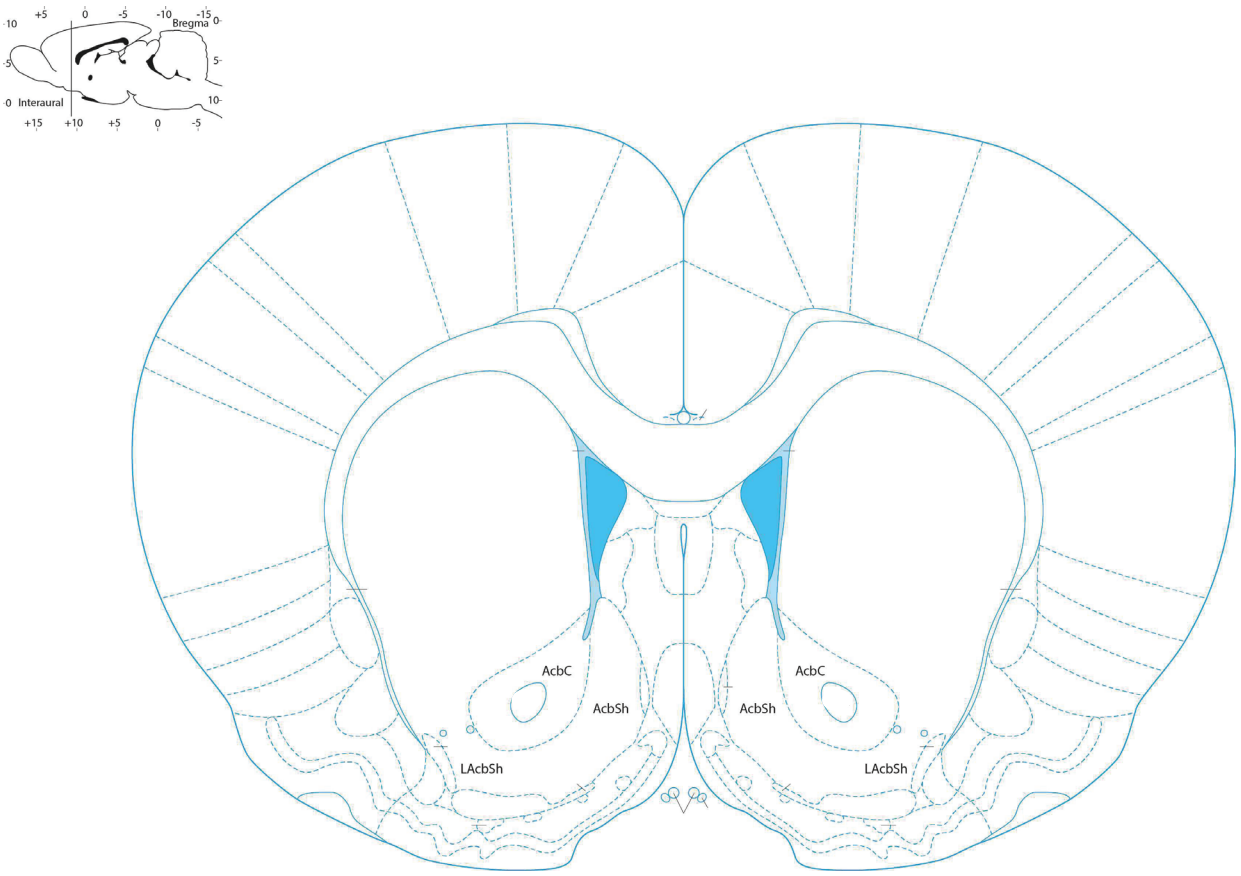

Figure 3.4. *Target tissue of the NAc for collection after micro dissection. Adapted from the Paxinos and Watson Rat Brain Atlas 7th Edition (2007). AcbC = nucleus accumbens core; AcbSh = nucleus accumbens shell; LAcbSh = lateral nucleus accumbens shell.*

3.8 Quantitative real-time PCR

Rationale

Quantitative real-time polymerase chain reaction (qPCR) is a widely used technique for the quantitative assessment of the expression of messenger RNA (mRNA). In qPCR, specific strands of genetic material are targeted with complementary oligonucleotides (primers) and amplified by repeated thermal cycles. By the addition of a fluorescent probe, which fluoresces at a level corresponding to the quantity of ge-

netic material amplified, a measure of the target genetic material can be made by recording the cycle at which the signal exceeds the background level (cycle threshold, Ct). Relative expression of the target gene can then be estimated by measuring this Ct value against that of reference genes, selected for their stable expression in the given tissue (Nolan, Hands and Bustin, 2006; Vos *et al.*, 2016).

RNA isolation and cDNA synthesis

Isolation of RNA from sample tissue was carried out using a variation of the method described by Chomczynski and Sacchi (2006). Working on dry ice, tissue samples were first mechanically homogenised using a pestle, before addition of 500µL GTC denaturing buffer (4M Guanidinium Thiocyanat, 25mM sodium citrate, 0,1M 2-Mercaptoethanol) and further manual homogenisation with a pipette tip. 50ML sodium acetate, 500µL phenol and 100µL chloroform-isoamylalcohol were added, samples vortexed, incubated on ice for 15m and centrifuged at 12000RPM for 15m. Samples were then precipitated in 400µl isopropanol, incubated over night, centrifuged once more for 20m, washed twice with 70% ethanol and centrifuged for 15m. Pellets were dried, dissolved in RNase-free Tris-HCl buffer (pH 7.0), and RNA concentrations determined using a Nanodrop (ThermoFisher, USA). Synthesis of complementary DNA (cDNA) was performed using 1 µg of total RNA with an oligo(dT) primer (ThermoFisher), and Superscript III reverse transcriptase (ThermoFisher). Synthesised cDNA was stored at -20°C until use.

mRNA expression analysis

The quantitative real-time PCR was performed using a Light Cycler 480 RT-PCR System (Roche, Switzerland) using Fast SYBR Green Mastermix (ThermoFisher). A reaction solution of 15µl was used (3µl cDNA, 7.5µl SYBR Green, 0.225µl each forward and reverse primer, 4.05µl ultrapure water) with a 40 cycle quantification programme. Samples were processed in duplicate. Target gene mRNA levels were automatically normalised to the reference genes glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and acidic ribosomal phosphoprotein P0 (Arbp). Melting curve analysis was performed automatically and checked visually at the end of each run. Expression of mRNA was presented proportionally, with data normalised to the

expression of the first animal of the non-stimulated group of the specific cycle. The primer sequences and annealing temperatures used were as follows:

Homer1a: 5`-CAAACACTGTTTATGGACTG-3`, 5`-TGCTGAATTGAATGTGTACC-3`, annealing temperature 65°C;
Arc: 5`-AGTCTTCAGAGCCAGGTGAATGAC-3', 5`-TCTGTGCAGGCAGCTTCAAGA-3', annealing temperature 65°C;
NPAS4: 5`-CATCAACTCCAGAGCCAAGTTCA-3`, 5`-TCCCCTCCACTTCCATCTTCAT-3`, annealing temperature 65°C;
DRD1: 5`-GCCTTTGGGTCCCTTTTGTA-3`, 5`-TCATACTGGAAAGGGCTGGA-3`, annealing temperature 58°C;
DRD2: 5`-ATGGCTGTATCCCGAGAGAA-3`, 5`-AATTTCCACTCACCCACCAC-3` annealing temperature 58°C;
TH: 5`-CTTTGACCCAGACACAGCA-3`, 5`-TGGATACGAGAGGCATAGTTC-3`, annealing temperature 58°C;
GAPDH: 5`- TGTCCGTCGTGGATCTGAC-3`, 5`-CCTGCTTCACCACCTTCTTG-3`, annealing temperature 62°C;
Arbp: 5`-CCTGCACACTCGCTTCCTAGAG-3`, 5`-CAACAGTCGGGTAGCCAATCTG-3`, annealing temperature 62°C.

3.9 High Performance Liquid Chromatography

Rationale

High performance liquid chromatography (HPLC) is an established technique for quantitative analysis of the chemical content of tissue, in which molecules in a solvent (the 'mobile phase') are separated according to polar interaction with the constituent 'stationary phase' of the column they are passed through (Hamilton and Sewell, 1982). The time required for molecules to pass through the column (retention time) is determined by the polarity of the mobile phase, standard phase and molecule, and is detected by the electrochemical charge produced at a detection plate as molecules leave the column. By comparing the voltage and retention times

against curves prepared with standards of known concentration, HPLC can provide quantitative analysis of the concentration of certain molecules in a given sample. In application of this method, HPLC with electrochemical detection has been validated for the detection of monoamines in rodent neural tissue (Kempf and Mandel, 1981; Viljoen *et al.*, 2018).

Procedure

DA, its metabolites 3,4- dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were detected via HPLC with amperometric electrochemical detection (Decade, Antec, the Netherlands). An electrochemical flowcell (VT-03, Antec, the Netherlands) with a glassy carbon working electrode and Ag/AgCl reference electrode was used, with a detection potential set at 0.8V. Temperature was maintained at 30°C. Tissue from the mPFC and NAc from ipsi- and contralateral hemispheres to mfb-DBS were separately analysed. Samples, maintained at 4°C, were individually homogenized in 150 uL of a mobile phase (50mM citric acid, 40mM Na2HPO4, 0.8mM EDTA, 0.3mM octanesulfonic acid and 1% Methanol in ultrapure water) by sonication before sampling via autosampler (Triathlon Spark, the Netherlands). 20µl samples were injected through a C18 column (750-mm length, 4.6-mm internal diameter, 3-µm particle size, Ultrasphere ODS, Beckman, USA) at a 0.5-ml/min flow rate. Total analysis duration was 60m. Standard solutions of each target (Sigma Aldrich) were prepared in 10 mM HCl, and appropriate dilutions in the mobile phase used to produce a 3-point curve for each. Retention times under the used conditions conditions were 6.0m (DOPAC), 7.4m (DA), 9.4m (5-HIAA), 14.6m (HVA) and 16.4m (5-HT). Concentration was calculated using Azur acquisition software v4.5 (Datalys, France), normalised against sample weight and presented as ng/mg of tissue. Concentrations of DA, DOPAC, HVA, 5-HT and 5HIAA were presented alongside ratios of metabolites to their respective precursor (DOPAC/DA, HVA/DA and 5HIAA/5-HT), as measures of turnover.

3.10 Statistical analysis

Statistical analyses were performed in Graphpad Prism v9.0.1. As appropriate, differences between groups were assessed using unpaired t-tests, one- or two-way ANOVAs, followed by post-hoc comparisons in the event of significance reported by ANOVA. In the cases where the same animals were tested over time, or prior to and post-mfb-DBS, repeated measures designs were used. The threshold for significance for all experiments was set at 0.05, and data expressed as mean +SEM.

4. Slow wave sleep deficits in the FSL model of depression: modulation by medial forebrain bundle deep brain stimulation[1]

4.1 Introduction

Sleep disturbances are a complex feature of depression, present in the vast majority of patients (Steiger and Pawlowski, 2019). Insomnia and hypersomnia are accompanied by physiological abnormalities, including an increased amount of time spent in REM sleep, and reduced power of SWA during SWS (Pillai, Kalmbach and Ciesla, 2011; Baglioni *et al.*, 2016). As well as being a significantly disruptive presence in the lives of patients (Mayers, van Hooff and Baldwin, 2003), the deeper relationship between sleep and depression makes the effect on sleep an important consideration for any novel treatment. As commonly prodromal and residual symptoms which can be predictive of future episodes (McClintock *et al.*, 2011; Alvaro, Roberts and Harris, 2013), sleep may be linked to recurrent episodes and treatment response (Ehlers, Havstad and Kupfer, 1996; Mendlewicz, 2009; Baglioni *et al.*, 2011). However, the effects of anti-depressants on sleep is varied. Given the typical endotype of increased REM sleep, a REM-suppressant effect has often been considered an

[1] The work presented in this chapter was submitted to the journal *Sleep* as: Slow wave sleep deficits in the FSL model of depression: effects of medial forebrain bundle deep brain stimulation, Gardner W, Fuchs F, Durieux, L, Bourgin, P, Coenen VA, Döbrössy M, Lecourtier L. 2021.

anti-depressant mechanism. This is indeed the effect of several anti-depressants, but others have no effect or elevate REM (Asarnow, 2020; Pandi-Perumal *et al.*, 2020). Remitted depressed patients often report improved sleep even without changes to the architecture (Mayers and Baldwin, 2005), which suggests there may be more to treating sleep perception than architecture.

The MFB network contains monoaminergic, glutamatergic and GABAergic systems implicated in sleep regulation and mechanisms of plasticity underlying SWA (Oishi and Lazarus, 2017; Galati *et al.*, 2018). While there is some anecdotal evidence of improved perception of sleep quality after slMFB-DBS (private correspondence, FORSEE study team), no specific investigation into the effects of mfb-DBS/MFB-DBS on sleep has been conducted.

In the current experiment, the effects of 24h mfb-DBS on sleep were examined in the FSL rat. In early characterisations of the model, the FSL was found to exhibit decreased latency to REM sleep, and increased time spent in REM overall (Shiromani *et al.*, 1988; Benca *et al.*, 1996), alongside phase-shift of circadian-controlled temperature changes (Shiromani *et al.*, 1991), suggesting the FSL is a suitable model for sleep-related symptoms in depression. In the FSL, mfb-DBS has been shown to have anti-depressant effects on behaviour (Edemann-Callesen *et al.*, 2015; Thiele *et al.*, 2018), while other pre-clinical work has demonstrated effects on physiology, including altered dopamine release in the nucleus accumbens (Dandekar *et al.*, 2017; Klanker *et al.*, 2017; Ashouri Vajari *et al.*, 2020), effects which could influence sleep regulation (Oishi and Lazarus, 2017). The current experiments aimed to further characterise the baseline sleep phenotype of the FSL, and investigate the effects of mfb-DBS upon sleep-related parameters. Electrophysiological recordings of the NAc, PrL and dorsal CA1 HC (CA1) were made alongside standard ECoG/EMG recordings during sleep, before and after 24-hours of mfb-DBS. Behavioural phenotype was measured via the FST, in order to confirm the anti-depressant action of mfb-DBS.

4.2 Materials and methods

Experimental design

FSL rats (*n* = 8) underwent sleep assessment before and after mfb-DBS, with a further FSL cohort selected for naive (non-DBS) behavioural testing in the FST (*n* = 6). Sprague Dawley (Ctrl) rats (*n* = 6) were used as non-depressive-like controls for sleep assessment without DBS. The experimental timeline is summarised in figure 4.1. Animals underwent electrode implantation surgery before 48h of pre-DBS baseline recording, immediately followed by 24h mfb-DBS, or sham-DBS in the case of control animals. Two further 24h recording periods were conducted at 1 and 7 days post-DBS. After the final recording period, a behavioural phenotype was measured in the FST. Animals were then euthanised and brain tissue collected for the verification of electrode placement.

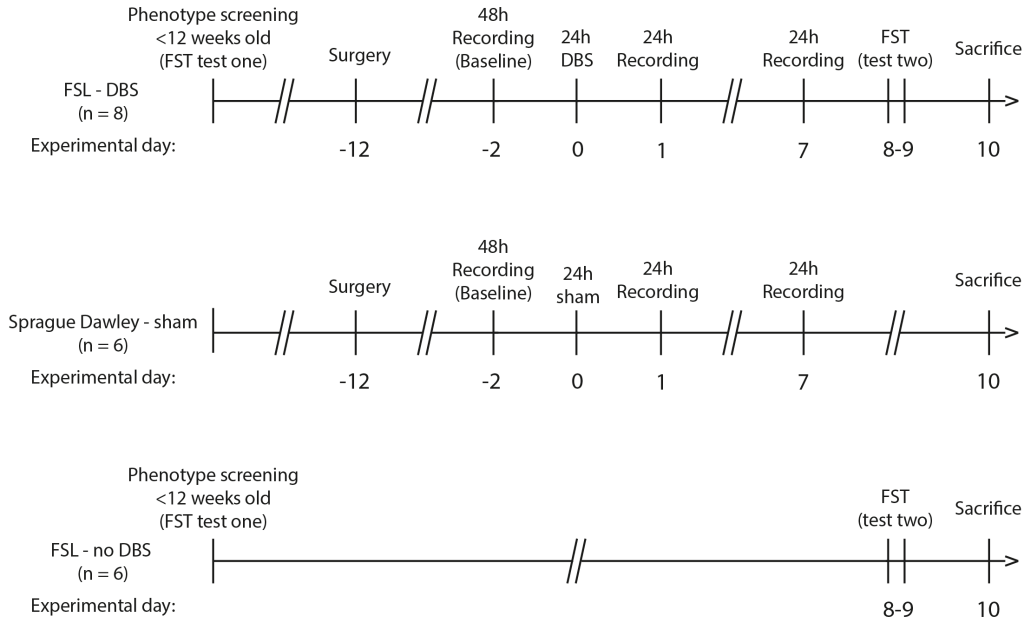

Figure 4.1. *Experimental timeline.*
FSL = Flinders Sensitive Line. FST = Forced swim test. DBS = Deep brain stimulation. For 'sham' sessions, animals remained connected to the recording/stimulation apparatus for the period of the session.

Experimental procedures

Protocols and analysis were carried out as described in chapter 3: the FST (**section 3.2**), DBS (**section 3.5**), electrophysiological recording and assessment (**section 3.6**) and tissue collection for histological assessment (**section 3.7**).

Surgery

Surgery was performed as described in **section 3.4**. Electrodes for mfb-DBS were bilaterally inserted into the medial forebrain bundle (AP -2.7, ML ±1.7, DV -8.0). Pairs of monopolar electrodes for the recording of LFP signal were inserted into the pre-limbic cortex (PrL, AP +2.8, ML ±0.7, DV -3.0), nucleus accumbens (NAc, AP +1.0, ML ±1.4, DV -7.1) and dorsal CA1 region of the hippocampus (CA1, AP -3.8, ML ±3.0, DV -2.0) (figure 4.2). Additionally implanted were an ECoG electrode at the level of the dura, and an EMG electrode in the nuchal muscle.

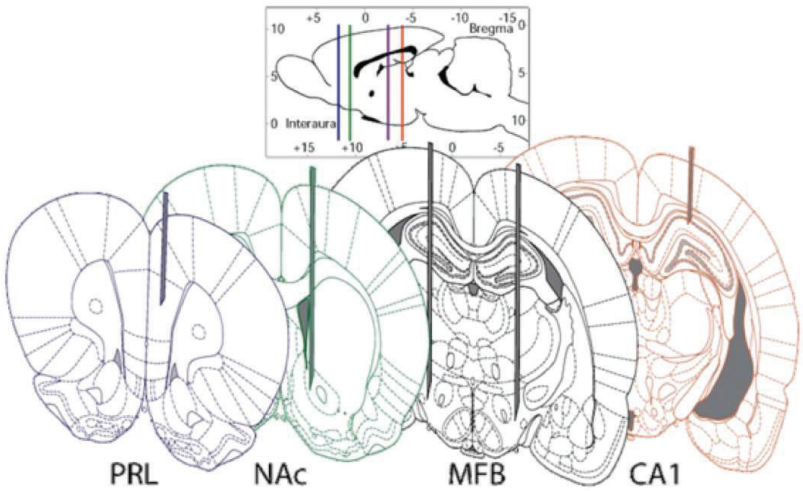

Figure 4.2. *Deep electrode implantation for rats in the sleep groups. Unilateral electrodes for recording LFP signals were implanted in the pre-limbic cortex (PrL), NAc (nucleus accumbens) and dorsal CA1 of the hippocampus. Electrodes for deep brain stimulation were implanted bilaterally into the medial forebrain bundle (MFB). Precise coordinates are presented in section 4.3. Brain atlas images adapted from Paxinos and Watson (2007).*

Statistics

Statistical analyses were performed as described in **section 3.10**. Specifically, repeated measures two-way ANOVA was used to evaluate measurements over different ZT periods, between groups or within groups at different experimental time points. Comparisons between specific ZT periods were performed using Fisher's post-hoc test when appropriate after significant interactions were reported by ANOVA. Unpaired t-tests or repeated measures one-way ANOVA were used to compare groups in measures where ZT period was not a factor. Greenhouse-Geisser correction was applied where appropriate to control for the effects of repeated measures in ANOVA and post-hoc comparisons. The threshold for significance was set at 0.05, and data expressed as mean +SEM.

4.3 Results

Two implanted FSL and one control rat were excluded from the sleep and spectral analysis due to poor quality signal. After histological assessment, one FSL was excluded from all post-DBS analysis due to improper placement of the MFB electrode. The CA1 data from the same FSL and one control were excluded at all time points due to poor placement of this electrode. A summary of the positions of electrodes included in analysis is shown in figure 4.3.

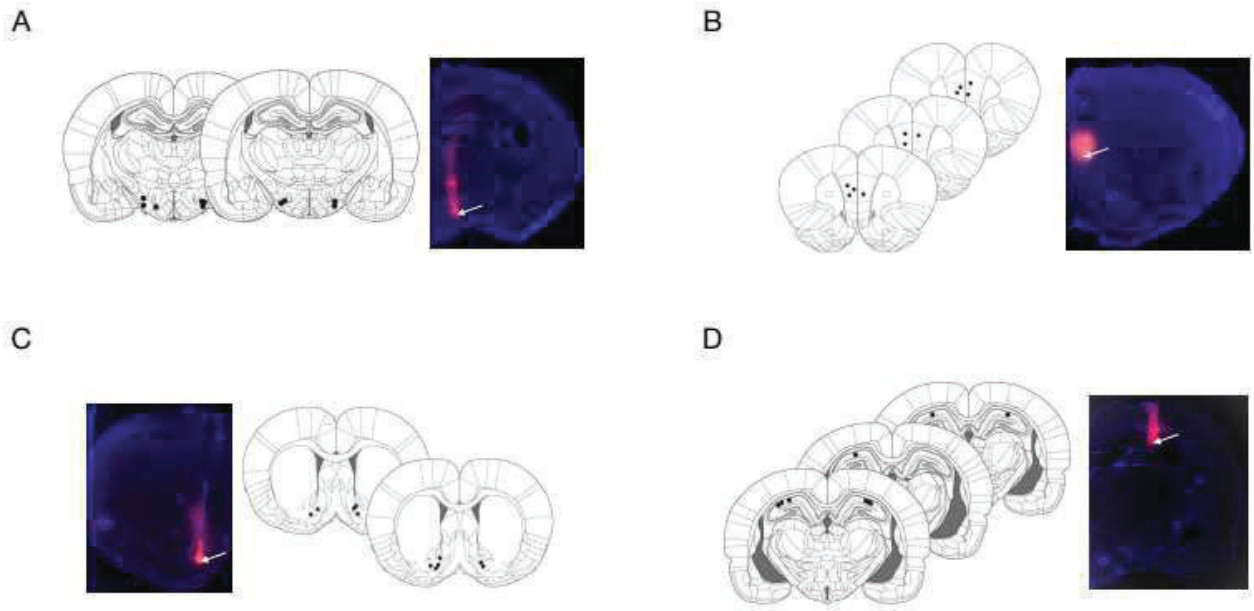

Figure 4.3. *Positions of electrodes included in the study.*
Electrodes were implanted in the (A) MFB, (B) PrL, (C) NAc and (D) CA1. White arrow indicates approximate location of electrode tip. Images adapted from Paxinos and Watson 7th edition, 2007.

Sleep in the FSL at baseline

Sleep architecture

ECoG and EMG recordings were used to assess sleep in controls and the FSL over 48h baseline. In these measurements, FSL rats exhibited various abnormalities in sleep architecture compared to non-depressive-like controls.

As predicted by earlier studies, FSL rats showed clear changes to REM sleep, consistently spending more time overall in REM over 24h (two-way ANOVA, group factor $F(1, 9) = 10.85$, $p = 0.0093$, Fig. 4.4C). Episodes of REM in the FSL were more numerous (group factor $F(1, 9) = 22.70$, $p = 0.001$, Fig. 4.4I), but of shorter average duration (group factor $F(1, 9) = 22.87$, $p = 0.001$, Fig. 4.4F), describing a pattern of more fragmented REM sleep. The circadian amplitude of REM sleep, a measure of how a vigilance state is influenced by circadian rhythm, was not significantly different between groups ($t = 1.063$, $p = 0.32$, Fig. 4.4L).

Changes to the architecture of SWS were also present in the FSL, and were highly ZT-phase dependant: time spent in SWS during the wake-dominated dark cycle was slightly higher in FSL rats, whereas it was clearly reduced during the sleep-dominated light cycle (group-ZT period interaction, $F(7, 63) = 2.83$, $p = 0.012$, with significant post-hoc interactions at ZT0-2, $p = 0.017$ and ZT6-8, $p = 0.0073$, Fig. 4.4B). These light-dark cycle effects are further described by a 24.5% (±7.6%) mean reduction of circadian amplitude of time spent in SWS compared to controls (unpaired t-test, $t = 3.21$, $p = 0.011$, Fig. 4.4K). Average length of SWS episodes also showed ZT-related differences, with significantly longer episodes during the final three hours of the dark cycle (group-ZT period interaction, $F(7, 63) = 2.52$, $p = 0.024$, post-hoc interaction at ZT21-23, $p = 0.049$ Fig. 4.4E), and borderline-significantly shorter episodes during the onset of the light cycle (post-hoc interaction at ZT0-2, $p = 0.059$, Fig. 4.4E).

Architecture relating to wake was not different between groups, with FSL rats and controls spending similar total time awake (group factor $F(1, 9) = 0.62$, $p = 0.45$, Fig. 4.4A), with similar mean episode duration (group factor $F(1, 9) = 0.15$, $p = 0.71$, Fig. 4.4D), and number of episodes (group factor, $F(1, 9) = 0.08$, $p = 0.78$, Fig. 4.4G); the circadian amplitude of time awake was not different between groups ($t = 1.70$, $p = 0.12$, Fig. 4.4J).

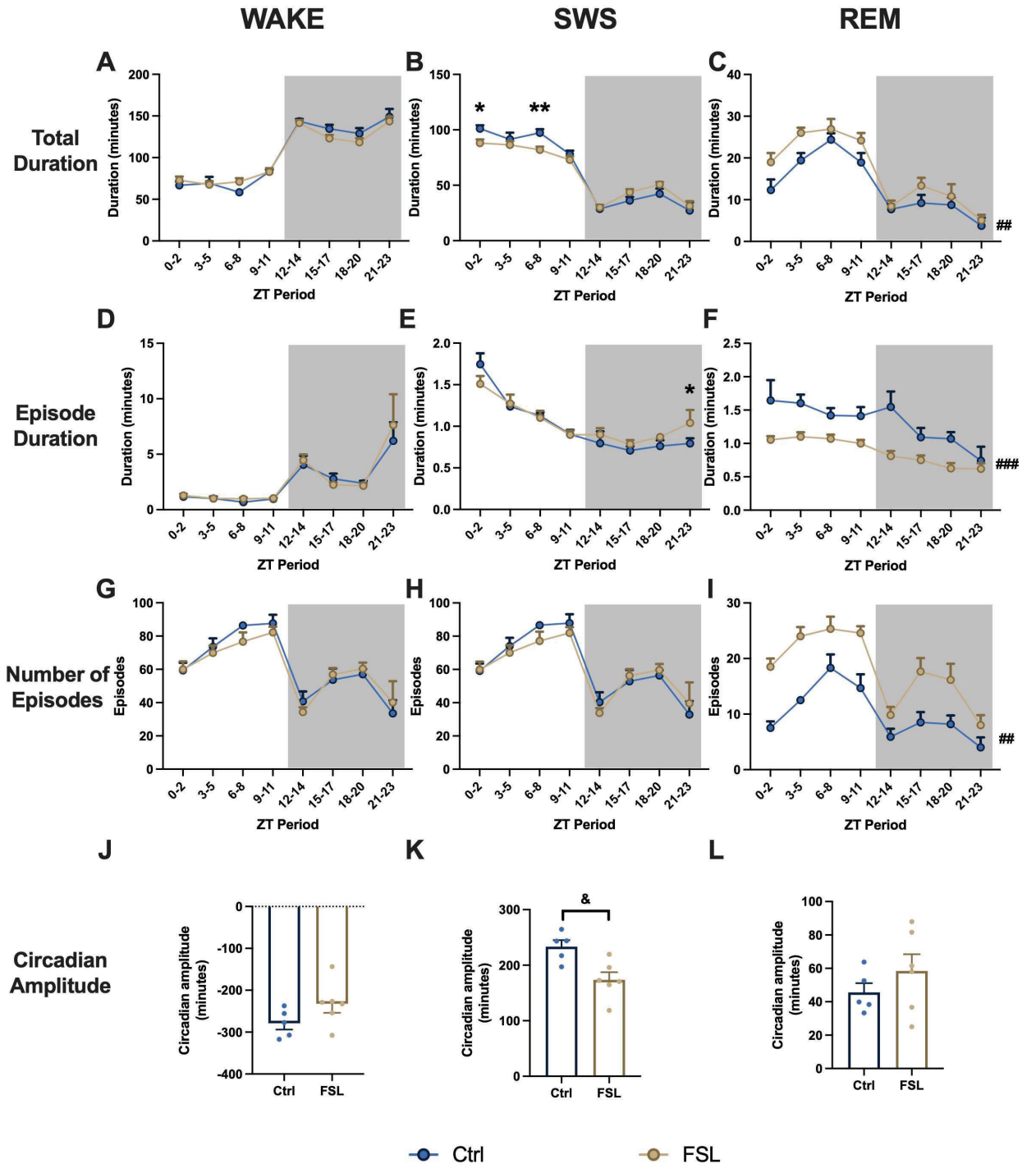

Figure 4.4. *Baseline sleep architecture in controls and the FSL.*
*Sleep architecture measures in control and FSL rats over 24h at baseline, in 3h bins. Grey background indicates dark period. Total duration of (A) wake, (B) SWS and (C) REM sleep; mean episode duration of (D) wake, (E) SWS and (F) REM sleep; number of episodes of (G) wake, (H) SWS and (I) REM sleep; circadian amplitude of (J) wake, (K) SWS and (L) REM sleep. # = significant difference between control and FSL according to two-way ANOVA; * = significance according to ZT-specific post-hoc comparison; & = significance according to unpaired t-test. For all, n = 5 (control) and 6 (FSL).*

Spectral analysis

SWS

FSL rats exhibited various abnormalities in oscillatory activity during SWS. Firstly, they exhibited significant reductions in SWS delta power in ECoG during specific ZT points (group-ZT period interaction, $F(7,63) = 6.45$, $p < 0.0001$, Fig. 4.5A). As delta power rose in control animals in the dark cycle in response to mounting sleep pressure, in the FSL delta remained low (post hoc interactions: ZT15-17, $p = 0.032$; ZT18-20, $p = 0.0066$; ZT21-23, $p = 0.038$). Delta power peaked in both groups in the first portion of the light period, but this peak was significantly lower in FSL rats (post-hoc comparison, ZT0-2, $p = 0.04$). In the NAc, the circadian pattern of delta was more closely matched between the two groups, but power was significantly lower in FSL rats overall (group factor, $F(1,9) = 12.07$, $p = 0.007$, Fig. 4.5E). In the PrL and CA1, FSL rats showed a tendency towards elevated and reduced delta power respectively, but neither of these differences reached significance (PrL group factor, $F(1, 8) = 2.970$, $p = 0.12$, Fig. 4.5C; CA1 group factor, $F(1, 7) = 1.60$, $p = 0.25$, Fig. 4.5G).

Alongside changes to delta activity, FSL rats also exhibited elevated gamma oscillations during SWS. In global ECoG signal, gamma power was significantly higher in FSL rats than controls (group factor, $F(1, 9) = 13.17$, $p = 0.0055$, Fig. 4.5B), and rose prominently across the dark cycle. Gamma was also significantly elevated in the FSL in the NAc (group factor, $F(1, 9) = 6.89$, $p = 0.028$, Fig. 4.5F), while activity in the PrL and CA1 was not significantly different between groups (PrL, group factor, $F(1, 9) = 0.53$, $p = 0.49$, Fig. 4.5B; CA1, $F(1, 7) = 2.31$, $p = 0.17$, Fig. 4.5H).

REM

Despite architectural changes, FSL rats showed no changes in spectral activity during REM sleep. Theta oscillations were of particular interest as potential markers of REM sleep quality; however measurements of theta were not significantly different

between groups in ECoG signal (group factor, $F(1, 9) = 0.17$), $p = 0.69$, Fig. 4.6A), in the PrL (group factor, $F(1, 9) = 2.40$, $p = 0.16$, Fig. 4.6B), NAc (group factor, $F(1, 9) = 0.49$, $p = 0.50$, Fig. 4.6C) or in the CA1 (group factor, $F(1, 7) = 0.45$, $p = 0.53$, Fig. 4.6D). No differences in any structure were found in other frequency bands investigated (data not shown).

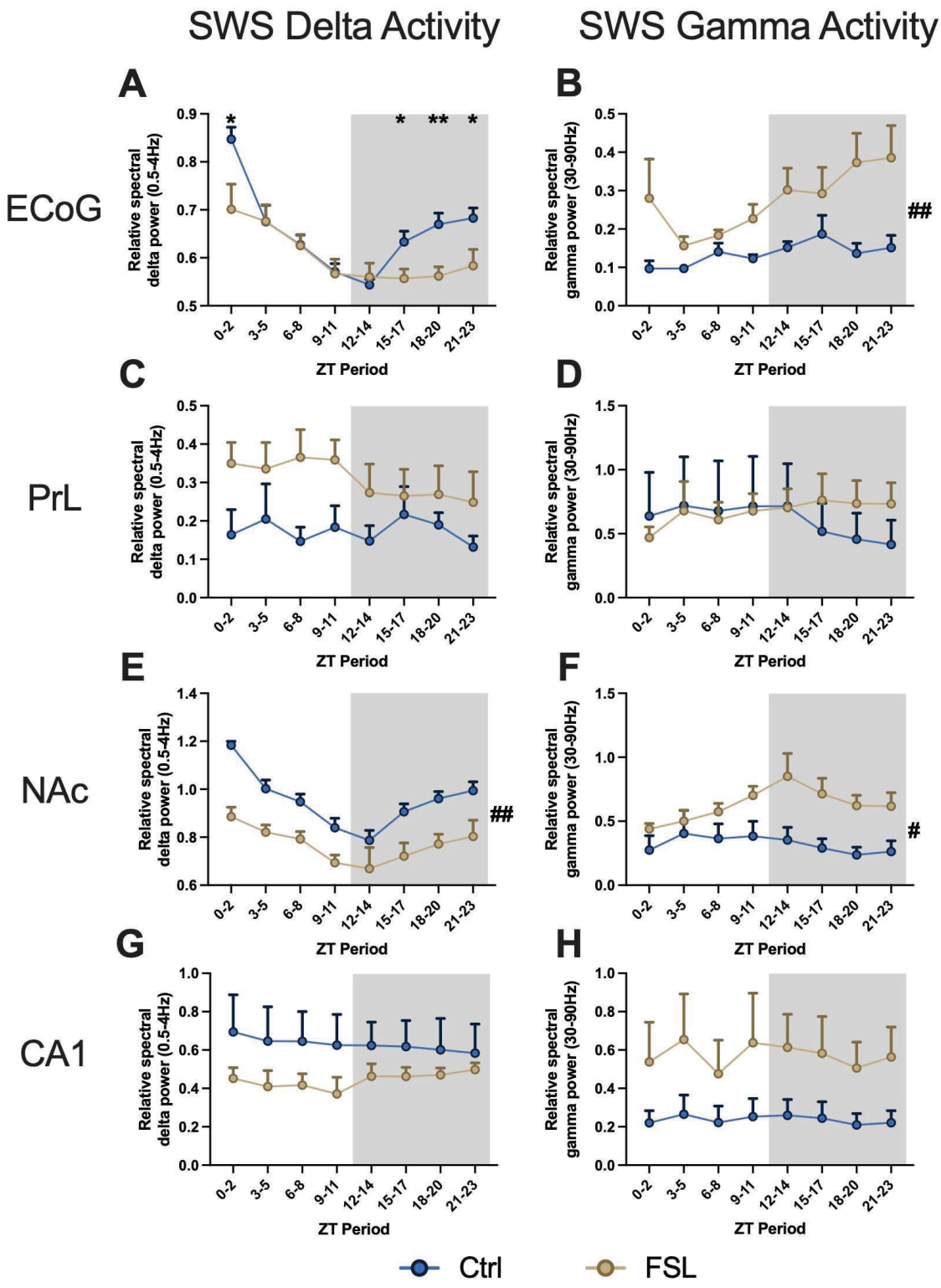

Figure 4.5. *Baseline SWS spectral activity in controls and the FSL. Spectral activity during SWS and control and FSL rats over 24h at baseline, in 3h bins, relative to total power. Grey background indicates dark period. ECoG delta (A) and gamma (B) activity; PrL delta (C) and gamma (D) activity; NAc delta (E) and gamma (F) activity; CA1 delta (G) and gamma (H) activity. For ECoG, PrL and NAc, n = 5 (control) and 6 (FSL); for CA1, n = 4 (control) and 5 (FSL).*

(continued from previous page) *# = significant difference between control and FSL according to two-way ANOVA; * = significance according to ZT-specific post-hoc comparison.*

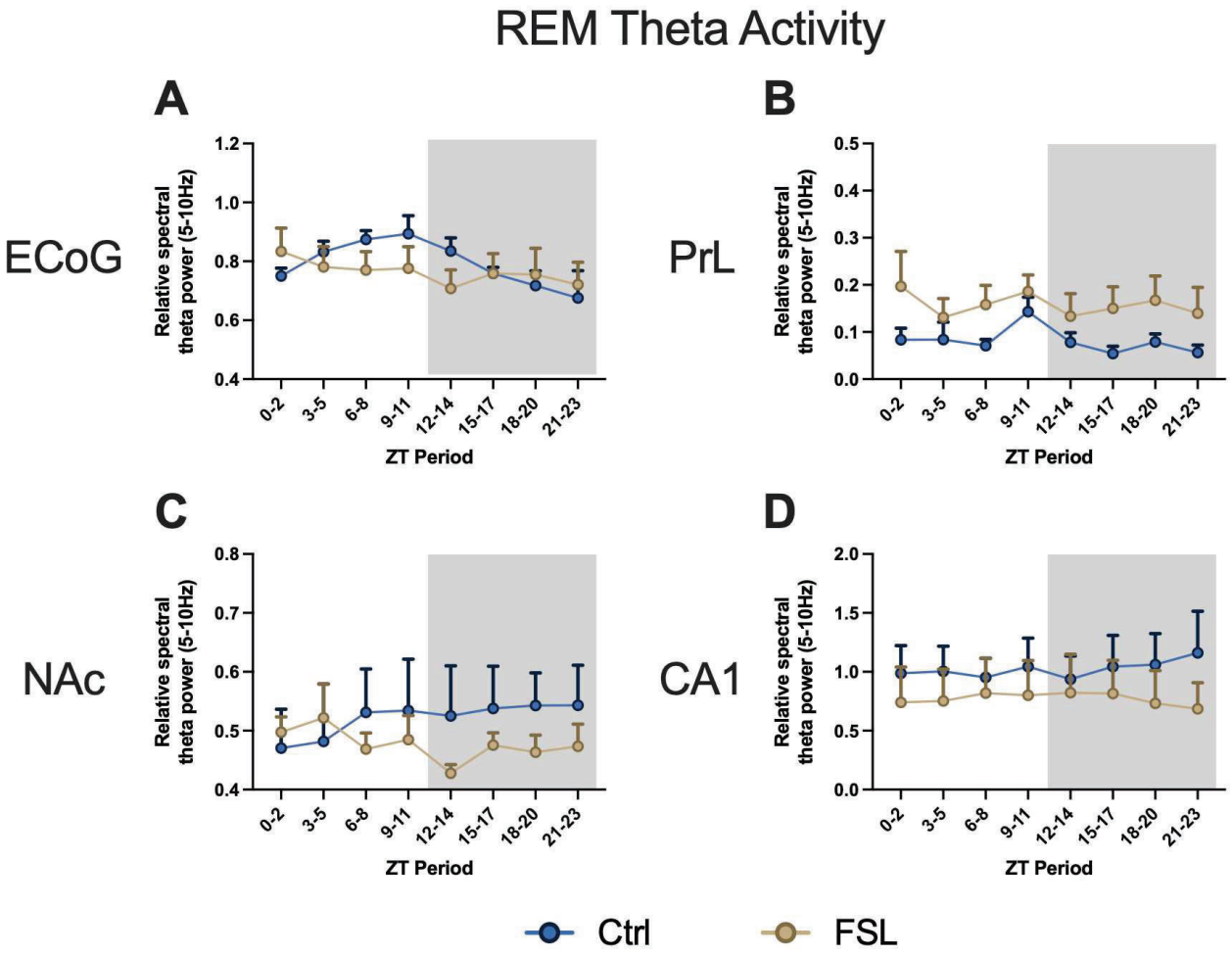

Figure 4.6. *Baseline REM spectral activity in controls and the FSL. Spectral activity during REM sleep in control and FSL rats over 24h at baseline, in 3h bins, relative to total power. Grey background indicates dark period. Theta activity in the (A) ECoG, (B) PrL, (C) NAc and (D) CA1. n = 5 (control) and 6 (FSL); for CA1, n = 4 (control) and 5 (FSL).*

Effects of mfb-DBS

Behaviour

When tested 9 days after mfb-DBS, FSL rats showed a significant reversal of depressive-like phenotype reflected in the reduction of immobility in the FST, compared to an unimplanted cohort which showed no significant change in behaviour (group-treatment interaction, $F(1, 11) = 12.91$ $p = 0.0042$; test 1 vs test 2 comparison, DBS group $t = 6.51$, $p < 0.0001$; naive group, $t = 1.16$ $p = 0.27$; Fig. 4.7).

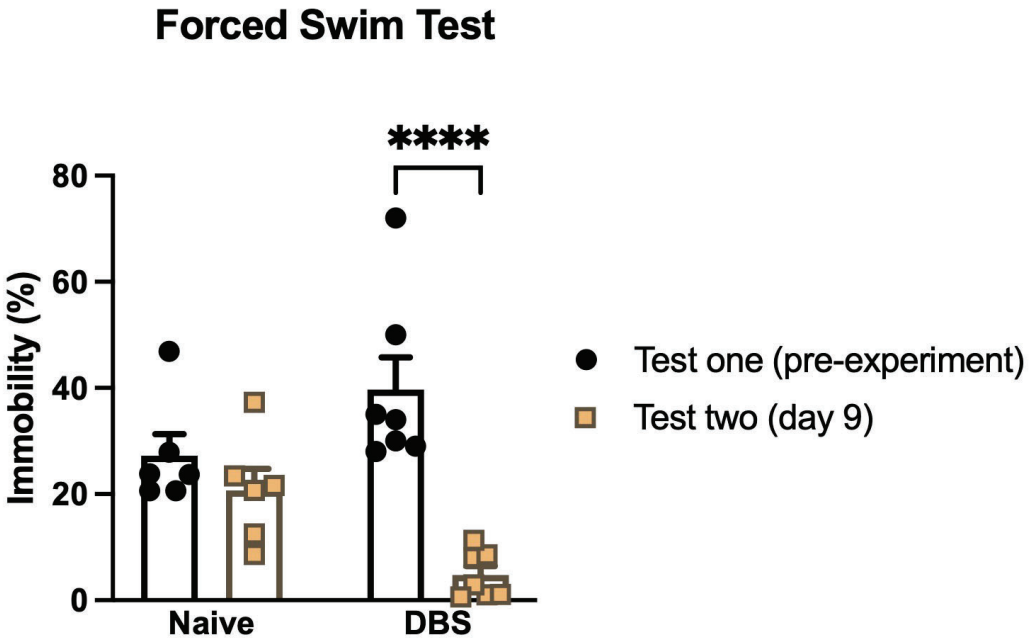

***Figure 4.7**: Immobility as measured in the forced swim test. Test one (pre-experiment phenotype screening) and test two (experimental day 9), in unstimulated FSL rats and FSL rats with mfb-DBS. * = significance indicated according to two-way ANOVA with Fisher's post-hoc comparisons. For unstimulated FSL, n = 6; for FSL with mfb-DBS, n = 7.*

Sleep architecture

Sleep architecture showed little change after mfb-DBS. Within-group assessments showed no change in FSL rats after mfb-DBS in the overall duration or mean duration of episodes of any vigilance states (experimental timepoint factor: FSL wake total duration, $F(1.63, 6.53) = 1.34$, $p = 0.32$, Fig. 4.8A; FSL wake mean episode duration, $F(1.91, 7.64) = 2.93$, $p = 0.12$ Fig. 4.8B; SWS total duration, $F(1.42, 5.69) = 2.20$, $p = 0.20$, Fig. 4.8C; SWS mean episode duration, $F(1.36, 5.43) = 1.20$, $p = 0.34$, Fig. 4.8D; REM total duration, $F(1.81, 7.23) = 0.37$, $p = 0.69$, Fig. 4.8E; $F(1.92, 7.68) = 1.57$, $p = 0.27$, Fig. 4.8F). The number of episodes of wake and SWS showed a difference in the FSL after DBS, with more transitions between the two states during the dark cycle and fewer during the light cycle (experimental time point factor: number of wake episodes, $F(1.36, 5.42) = 9.69$, $p = 0.020$, Fig. 4.8G; number of SWS episodes, $F(1.36, 5.42) = 10.73$, $p = 0.016$, Fig. 4.8I). The number of REM episodes was not different (experimental time point factor, $F(1.37, 5.48) = 0.50$, $p = 0.57$, Fig. 4.8K). Circadian amplitude was not different after DBS in any vigilance state (one-way ANOVA: wake, $F(1.94, 7.74) = 0.43$, $p = 0.66$, Fig. 4.8H; SWS, $F(1.32, 5.29) = -0.012$, $p > 0.99$, Fig. 4.8J; REM, $F(1.37, 5.49) = 0.20$, $p = 0.75$, Fig. 4.8L). Overall, while these data provide some evidence of small changes to SWS, the effect of mfb-DBS on sleep architecture appears to be minimal, with no apparent influence on the architecture of REM sleep.

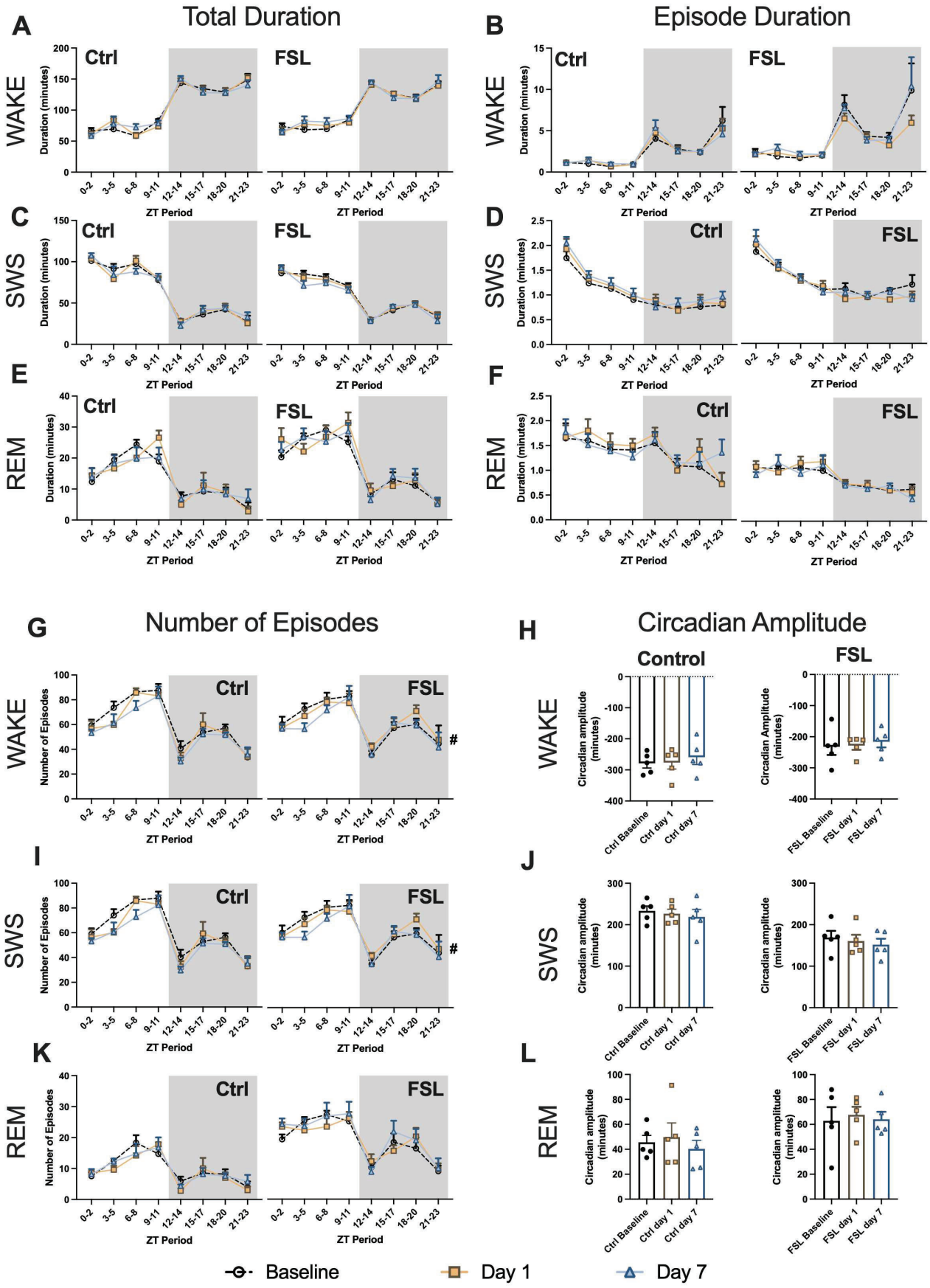
A
Total Duration
B
Episode Duration
WAKE
SWS
REM
Ctrl
FSL
Duration (minutes)
ZT Period
C
D
E
F
G
Number of Episodes
H
Circadian Amplitude
Control
FSL
Circadian amplitude (minutes)
Ctrl Baseline
Ctrl day 1
Ctrl day 7
FSL Baseline
FSL day 1
FSL day 7
I
J
K
L
#
Baseline
Day 1
Day 7

Figure 4.8 *Sleep architecture in control and FSL rats at baseline, experimental days 1 and 7* ***(previous page).*** *Represented over 24h expressed in 3h bins. Grey background indicates dark period. Dashed line represents baseline data. Total duration of wake (A), SWS (C) and REM sleep (E); mean episode duration of wake (B), SWS (D) and REM sleep (F); number of episodes of wake (G), SWS (I) and REM sleep (K); circadian amplitude of wake (H), SWS (J) and REM sleep (L). # = significant differences between each experimental time point according to two-way ANOVA. For all, n = 5 (control) and 5 (FSL).*

Spectral analysis

SWS

Analysis of post-stimulation global ECoG signal showed small changes to SWS delta power (Fig. 4.9A). In comparisons between baseline and post-DBS timepoints within the FSL, a significant interaction suggested potential changes at certain ZT periods (experimental timepoint-ZT period interaction, $F(3.09, 11.93) = 3.47$, $p = 0.05$); however post-hoc comparisons between groups did not reach significance for any ZT period.

Comparing control and FSL groups, baseline differences at ZT0-2, ZT15-17, and ZT21-23 disappeared at day 1 (ZT period-group interaction, $F(7, 56) = 2.97$, $p = 0.01$; post-hoc ZT0-2 $p = 0.65$; post-hoc ZT15-17 $p = 0.14$; post-hoc ZT21-23 $p = 0.80$) and day 7 (ZT period-group interaction, $F(7, 56) = 2.22$, $p = 0.046$; post-hoc ZT0-2 $p = 0.33$; post-hoc ZT15-17 $p = 0.35$; post-hoc ZT21-23 $p = 0.099$). This ablation of difference is difficult to interpret and is perhaps in part due to variation in the control group, however at day 1 appears to be explained by increased FSL delta power at ZT0-2 and ZT21-23. This may suggest that post-DBS, FSL rats are be able to reach higher levels of SWS delta power, but not in a sustained fashion.

In deep structures, delta power was not statistically significant in the FSL between baseline and post-DBS time points; however comparisons between control and FSL may suggest changes from baseline in the PrL. In the PrL, delta power was elevated in the FSL compared to controls at post-stimulation day 1 (group factor, $F(1, 8) = 10.80$, $p = 0.011$, Fig. 4.9C), and was reduced enough at 7 days post-DBS in FSL rats to remove these significant group differences (group factor, $F(1, 8) = 2.40$, $p = 0.16$, Fig. 4.9C). In the NAc, control-FSL group differences apparent at baseline remained at day 1 (group factor, $F(1, 8) = 14.64$, $p = 0.0050$, Fig. 4.9E) and day 7

(group factor, F (1, 8) = 12.12, p = 0.0083, Fig. 4.9E). In CA1, no changes after stimulation or between groups were apparent (Fig. 4.9G). Overall, while not showing a definitive effect, these results provide some suggestion that mfb-DBS can influence SWS delta oscillations, potentially allow higher delta power to be attained at the start of the light cycle in global ECoG, and elevating PrL delta immediately after stimulation.

Analysis of other frequency bands during SWS revealed specific effects of mfb-DBS on gamma oscillations. In global ECoG signal, DBS resulted in suppression of elevated gamma observed at baseline during the dark cycle (experimental time point-ZT period interaction, F (2.55, 10.19) = 4.27, p = 0.038; post-hoc differences between baseline and day 1 significant at ZT12-14 (p = 0.045) and ZT18-20 (p = 0.046), near significance at ZT21-23 (p = 0.064), reducing the pattern of rising gamma power through the dark cycle seen at baseline. At day 7, the difference remained significant at ZT18-20 (p = 0.041) but not ZT12-14 (p = 0.11), and the pattern of rising gamma throughout the dark cycle appeared to remain diminished.

In the PrL, FSL rats also showed changes following mfb-DBS (experimental time point factor, F (0.714, 3.57) = 8.94, p = 0.041, Fig. 4.9D), with lower gamma 1 day after stimulation and an apparent rebound at 7 days. In the NAc, while gamma power did not significantly change after DBS within the FSL group (experimental time point factor, F (1.56, 7.78) = 1.05, p = 0.38, Fig. 4.9F), the difference observed at baseline between groups was not significant 1 day post-DBS (group factor, F (1, 8) = 0.18, p = 0.68, Fig. 4.9F), however variation in the control group as well as reduction in the FSL appeared to contribute to this convergence. The difference between groups was present again at day 7 (group factor, F (1, 8) = 5.90, p = 0.041, Fig. 4.9F). In the CA1, SWS gamma was not different between time points in the FSL (F (1.42, 5.68) = 1.22, p = 0.34, Fig. 4.9H). Overall, suppression of SWS gamma was apparent in global ECoG signal and in the PrL one day after mfb-DBS, with some evidence of effect in the NAc. After seven days, a rebound of gamma activity occurred in the PrL but was not seen in ECoG.

REM

No changes were observed in FSL rats after mfb-DBS in REM sleep theta oscillations in either global ECoG (experimental time point factor, $F(1.12, 4.46) = 0.72$, $p = 0.45$, Fig. 4.10A) or hippocampal signal ($F(1.67, 6.67) = 1.21$, $p = 0.35$, Fig. 4.10D), the two main areas of interest, nor in the PrL ($F(1.24, 4.95) = 1.31$), $p = 0.32$, Fig. 4.10B) or NAc ($F(1.75, 7.02) = 0.38$, $p = 0.68$, Fig. 4.10C). No other changes in any structure were observed in other frequency bands during REM after mfb-DBS (data not shown).

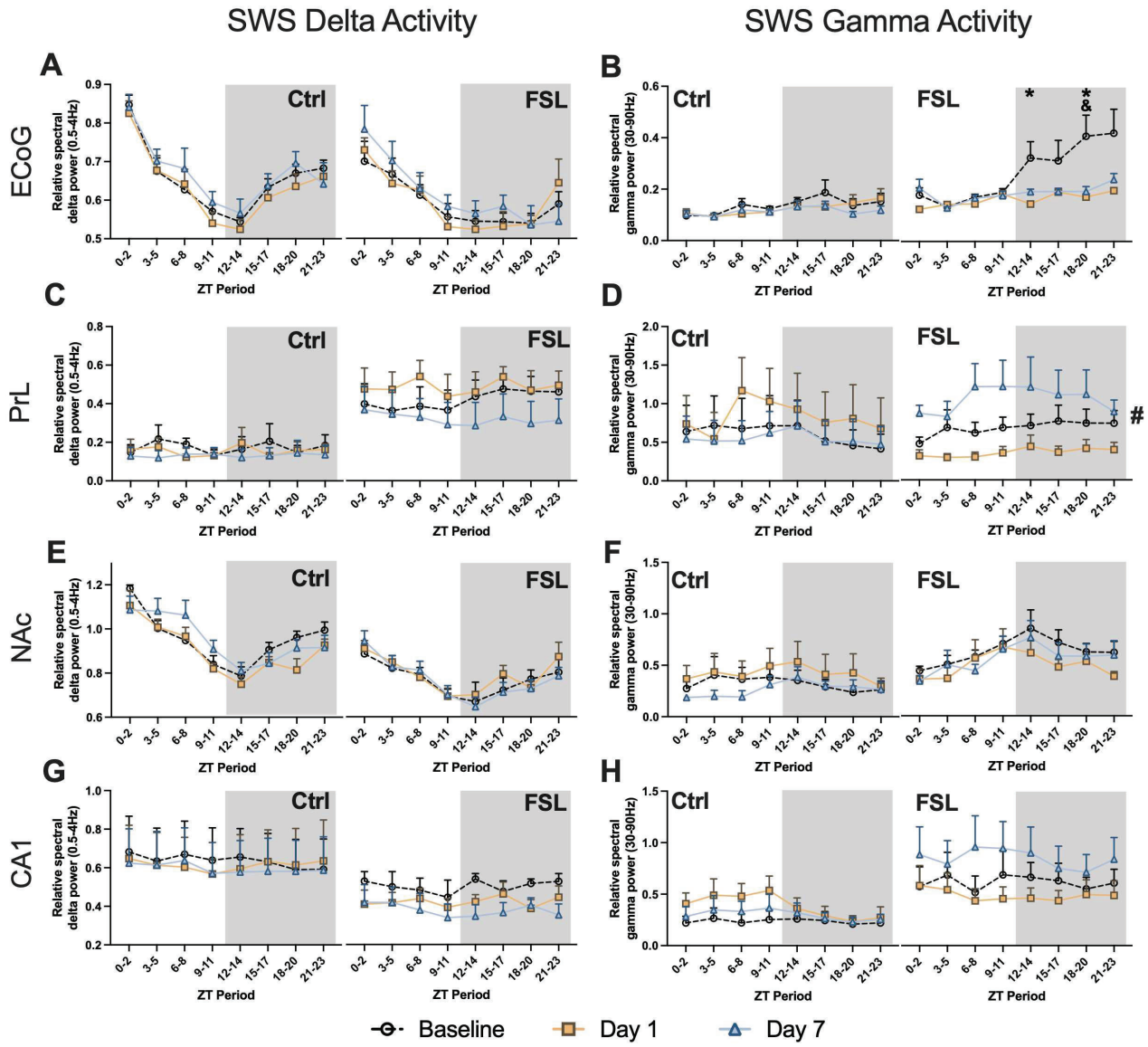

***Figure 4.9**: Time course of SWS spectral activity before and after mfb-DBS. Spectral activity during SWS in control and FSL rats over 24h at baseline, day 1 and day 7. Data in 3h bins, relative to total power. Grey background indicates dark period. Dashed line represents baseline data. ECoG delta (A) and gamma (B) activity; PrL delta (C) and gamma (D) activity; NAc delta (E) and gamma (F) activity; CA1 delta (G) and gamma (H) activity. For ECoG, PrL and NAc, n = 5 (control) and 5 (FSL); for CA1, n = 4 (control) and 5 (FSL). * = significant difference at specific ZT period between baseline and day 1, according to post-hoc comparison. & = significant difference at specific ZT period between baseline and day 7, according to post-hoc comparison. # = significant differences between each experimental time point according to two-way ANOVA.*

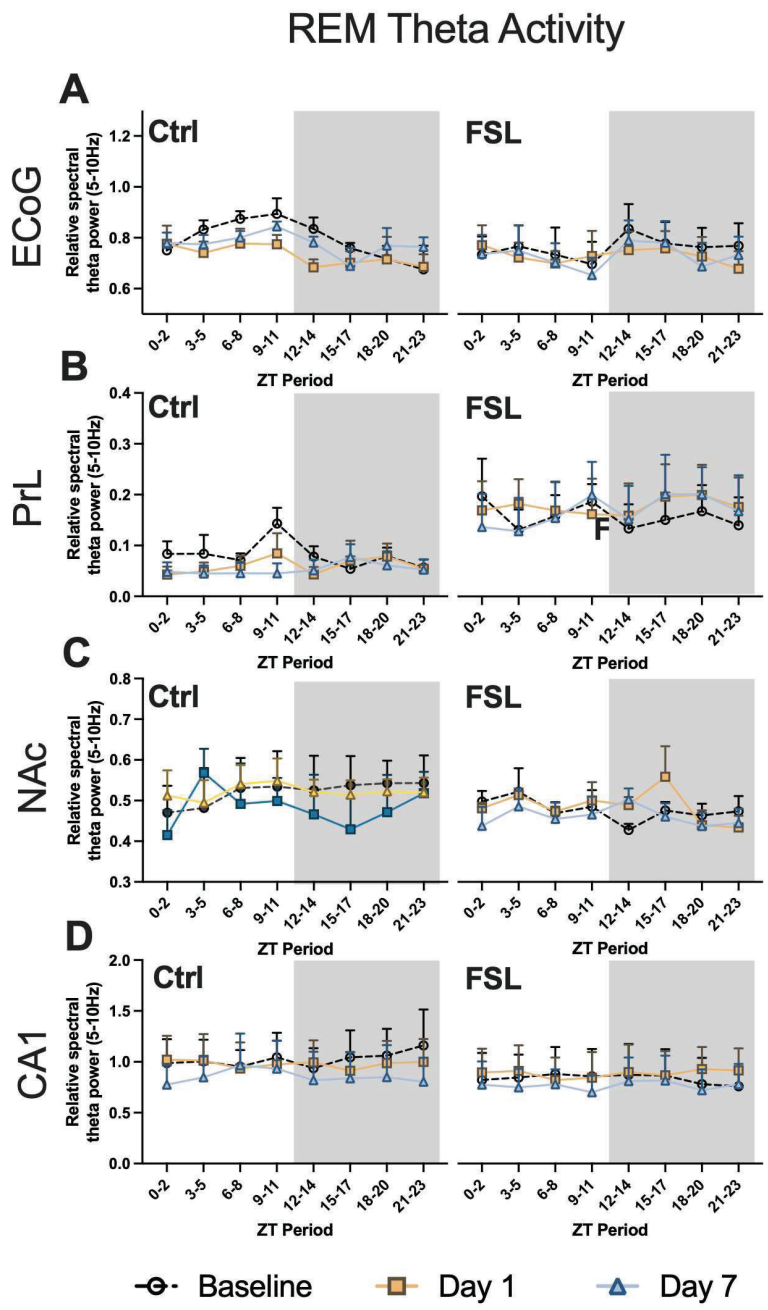

***Figure 4.10**: Time course of REM spectral activity before and after mfb-DBS. Spectral activity during REM sleep in control and FSL rats over 24h at baseline, day 1 and day 7. Represented in 3h bins, relative to total power. Grey background indicates dark period. Dashed line represents baseline data. Theta activity in the (A) ECoG, (B) PrL, (C) NAc and (D) CA1. n = 5 (control) and 5 (FSL); for CA1, n = 4 (control) and 5 (FSL).*

4.4 Discussion

Sleep anomalies are frequently associated with MDD, nevertheless it is not known how the experimental treatment of slMFB-DBS effects this symptom. Clinical trials of slMFB-DBS have shown rapid and lasting anti-depressant effects on diagnostic inventory scales (Schlaepfer *et al.*, 2013; Fenoy *et al.*, 2016, 2018; Bewernick, Kayser, Gippert, Coenen, *et al.*, 2017; Bewernick, Kayser, Gippert, Switala, *et al.*, 2017; Volker A. Coenen *et al.*, 2018; Coenen *et al.*, 2019), and pre-clinical studies have suggested effects on diverse depressive-like behavioural phenotypes (Bregman *et al.*, 2015; Edemann-Callesen *et al.*, 2015; Furlanetti *et al.*, 2015; Dandekar *et al.*, 2017; Thiele *et al.*, 2018) and physiology (Dandekar *et al.*, 2017; Klanker *et al.*, 2017; Ashouri Vajari *et al.*, 2020; Thiele *et al.*, 2020). Despite some anecdotal reporting by clinical trial patients of improvements in sleep quality, no in-depth clinical or pre-clinical investigation has been conducted to date into the effects of slMFB-DBS or mfb-DBS on sleep. Using the FSL model of depression, the current study confirmed REM sleep deficits in the model, while demonstrating changes to the architecture and physiology of SWS. FSL rats exhibited a reduced circadian effect on time spent in SWS, reduced SWA and elevated high frequency gamma oscillations, abnormalities also present locally in the NAc. 24h mfb-DBS produced a behavioural anti-depressant effect and suppressed SWS gamma oscillations without influencing sleep architecture.

Distribution and quality of SWS are abnormal in the FSL

Further characterisation and refinements of sleep disturbances in the FSL suggest a stronger face validity for this model of depression than previously described, while highlighting the importance of SWS in depression. Changes to the architecture of REM sleep have been noted in the FSL (Shiromani *et al.*, 1988; Benca *et al.*, 1996), and are replicated here. Benca and colleagues (1996) reported neither quantitative nor qualitative SWS abnormalities, but this discrepancy with our results may be due to methodological differences, including their use of longer sleep scoring epochs (30s) and overall EEG amplitude to assess SWS quality. Our results suggest the FSL

does in fact show a range of sleep abnormalities. Quantitative changes to REM sleep are prominent, but our data suggest the underlying physiology is not qualitatively different. SWS, on the other hand, shows changes to both quantity and quality. Normal sleep is regulated by two processes: *S*, the homeostatic drive for sleep; and *C*, circadian regulation (Borbély *et al.*, 2016). Reduced delta power across the dark cycle suggests a deficiency in the homeostatic drive for sleep, reduced sleep depth and quality (Borbély *et al.*, 1981, 2016; Dijk, 2009), while a flattening of the circadian amplitude is suggestive of reduced circadian influence on SWS. As a vital regulatory process, dysfunction of process S may be a driver for wider sleep disruption in depression. Deficits in SWA may also be the result of, or contribute to, reduced plastic functioning, as a marker of reduced synaptic downscaling during SWS (Tononi and Cirelli, 2003, 2006). Diurnal temperature variation, as a marker of circadian rhythm, has been previously shown to be irregular in the FSL (Shiromani *et al.*, 1991), and our results add further evidence that altered circadian control is also part of the wider depressive phenotype.

Abnormal gamma oscillations as a feature of dysfunctional SWS

While reduced SWA has been previously observed in depressed human patients (Argyropoulos and Wilson, 2005), elevated gamma oscillations in the FSL further point to SWS as a state of dysfunction in depression. High frequency oscillations have been suggested as a marker of disrupted sleep in insomniacs, negatively correlated with perceived sleep quality (Perlis *et al.*, 2001). Here, we add to this picture by describing the elevation of gamma power with rising sleep pressure, with SWA remaining diminished when it would be expected to rise. Whether the generation of these gamma rhythms plays a role in the inhibition of SWA, or is a by-product of disrupted sleep is unclear. Gamma oscillations are prominent both in wake, during which they are associated with processing of sensory information, coherent patterns of neural activity and cognitive processes (Nir *et al.*, 2007; Fitzgerald and Watson, 2018), and in REM sleep (Cantero *et al.*, 2004; Osorio *et al.*, 2020). During SWS they are associated with the active ‘up’ state of slow waves (Valderrama *et al.*, 2012). Altered balance between arousal and de-arousal has been proposed as a fundamental dimension in several mental disorders (Baglioni *et al.*, 2016), and the

combination of reduced SWA and elevated gamma would result in a more metabolically active brain (Bergel *et al.*, 2018) which may interfere with restorative aspects of SWS (Schmidt, 2014).

It has been suggested that residual high frequency oscillations during SWS may represent a failure to suppress ongoing cognitive activity (Perlis *et al.*, 1997). However, the fact that gamma appears to rise across the dark cycle, rather than immediately increasing when the animals spend more time awake, may be more suggestive of a relationship with sleep pressure rather than ongoing cognition. On the other hand, local abnormalities may support the idea of ongoing activity. These patterns – reduced SWA, elevated gamma — were observed locally in the NAc, a structure strongly implicated in MDD due to its various roles in encoding reward and aversion, novelty and motivation. During sleep, the NAc is thought to play a role in emotional and reward-based memory processing (Perogamvros and Schwartz, 2012), and may have a role in sleep regulation (Qiu *et al.*, 2012; Oishi, Xu, *et al.*, 2017). The NAc is reactive to changes in sleep (Sardi *et al.*, 2018) and NAc gamma oscillations are sensitive to stress (Iturra-Mena *et al.*, 2019). While the link between gamma in wake and sleep is unclear, aberrant gamma oscillations are a growing area of interest in depression, proposed as a potential marker for the disease and subsequent suicide risk (Fitzgerald and Watson, 2018; Arikan *et al.*, 2019). Abnormalities are reported in humans during various behavioural states (Pizzagalli *et al.*, 2006; Strelets, Garakh and Novototskii-Vlasov, 2007; Lee *et al.*, 2010; Liu *et al.*, 2014), and in the anaesthetised FSL (Voget *et al.*, 2015), suggesting impairments of underlying processes fundamental to several brain states (Fitzgerald and Watson, 2018). For example, impaired excitatory/inhibition (e/i) balance, mediated by gamma-generating interneuron circuits is implicated in depression and may contribute to the problem of residual arousal (Thompson *et al.*, 2015; Page and Coutellier, 2019). With the range of pathophysiology thought to contribute to depressive phenotypes, it is likely that homeostatic dysfunction and other contributors to gamma abnormalities exist simultaneously. These dysfunctions may exacerbate one another, with impaired response to sleep pressure preventing the suppression of gamma-generating activity, in turn contributing to a hyper-aroused brain impeding deeper sleep, for example.

mfb-DBS alters behaviour without affecting sleep architecture

The anti-depressant effects of MFB-DBS are thought to be mediated, in part, via modulation of ascending monoaminergic projections to frontal and striatal nuclei (Schlaepfer *et al.*, 2013), which also have roles in sleep (Monti and Monti, 2007; Lima *et al.*, 2008; Monti, 2011; Qiu *et al.*, 2012; Chang *et al.*, 2014; Oishi, Xu, *et al.*, 2017). Despite the shared substrate, and the common effects of anti-depressants on both mood and sleep, mfb-DBS produced anti-depressant changes in behaviour (as previously demonstrated (Bregman *et al.*, 2015; Edemann-Callesen *et al.*, 2015)) without altering sleep architecture. This effect on one without the other may suggest more selective modulation of implicated circuits than systemic pharmacological anti-depressants.

mfb-DBS suppression of SWS gamma may represent improved sleep

mfb-DBS did alter sleep physiology, in the suppression of SWS gamma as sleep pressure increased. Although no significant concurrent rise in SWA was observed, reduction in gamma may represent an improvement in quality of SWS. We propose elevated gamma as a disruptive, energy-consuming feature of dysfunctional SWS in depression, suppression of which may therefore represent a marker or mechanism of anti-depressant response. mfb-DBS affecting SWS physiology without affecting architecture again highlights the apparent independent modulation of the various regulatory processes connecting mood and sleep. Differences in ECoG and PrL gamma response – lack of ZT specificity and the rebound at 7 days in the PrL, dark cycle specific with less obvious rebound in ECoG – may also be suggestive of varying, localised effects. One aspect affected aspect of sleep may relate to perception, which is negatively correlated with high frequency activity during SWS (Perlis *et al.*, 2001). Subjective sleep perception has been reported to change independently of polysomnographic changes (Perlis *et al.*, 1997): depressed patients perceive lower sleep quality than control subjects despite suffering similar objective levels of disturbance (Mayers, van Hooff and Baldwin, 2003), and report improved sleep after treatment even when sleep architecture contradicts this (Mayers and Baldwin, 2005). Suppression of gamma oscillations may be a biological substrate for

changes in sleep perception without major changes in architecture. “Perception” is not possible to measure in an animal model, but this proposal would concur with anecdotal data of patients’ self reporting during trials of slMFB-DBS. This must be verified in formal investigations of sleep following slMFB-DBS in clinical trials. Perception change in the absence of architecture change has been reported with serotonergic modulators (Mayers and Baldwin, 2005), which have separately been found capable of suppressing gamma activity (Puig *et al.*, 2010). However, the majority of serotonergic anti-depressants do suppress REM (Wang *et al.*, 2015), while DBS has many other potential mechanisms, including interaction with inhibitory circuits which can also modulate gamma and may also be candidate mechanisms (Sun *et al.*, 2015; Jakobs *et al.*, 2019).

Overall, links between sleep quantity, quality and circadian factors in depression remain incompletely described. Future studies should investigate the presence of elevated SWS gamma alongside reduced SWA and sleep architecture changes in other models of depression. Manipulations involving sleep deprivation could give insight into the relationship between elevated gamma and sleep pressure. Finally, studies on the impact of other anti-depressant treatments, both those which do and do not affect architecture, on SWS gamma may give important clues as to mechanisms regulating sleep quantity and quality.

Limitations

mfb-DBS was applied for 24h in this study, whereas clinically the treatment is chronic and continuous. Previous studies have illustrated the dynamic nature of response to mfb-DBS, with dopaminergic response potentially evolving over minutes to hours (Bregman *et al.*, 2015; Klanker *et al.*, 2017; Ashouri Vajari *et al.*, 2020). A lack of definitive understanding on when DBS response becomes ‘chronic’, alongside technical limitations preventing recording during continuous stimulation represent weaknesses of the current study. 24h was sufficient to produce anti-depressant and physiological effects, providing evidence for potential mechanisms, but effects on sleep may evolve with continuous, chronic stimulation. Whether SWS gamma is continually suppressed after chronic mfb-DBS, and whether chronic stimulation

may eventually affect sleep architecture are key questions in determining the effects of the treatment.

Conclusion

The presented data emphasise the fundamental importance of SWS deficits in affective disorders, demonstrating their presence in a validated model and illustrating abnormalities in the quantity, circadian rhythm, and physiology of SWS. Elevated gamma oscillations alongside diminished SWA form part of this pathophysiology. Gamma activity, as a marker or mechanism, may be of particular relevance due to its connection with other aspects of depressive pathophysiology. 24h mfb-DBS was able to produce an anti-depressant behavioural effect and suppress SWS gamma without altering sleep architecture, suggesting specific, independent modulation of circuits believed to share many biological substrates. This raises further questions about the mechanisms of mfb-DBS as a treatment, while modulation of gamma oscillations may represent an anti-depressant mechanism common with other treatments.

5. Modulation of dopaminergic receptor expression and induction of plastic mechanisms by medial forebrain bundle deep brain stimulation

5.1 Introduction

The complexity of the MFB suggests that the anti-depressant action of MFB-DBS is almost certainly multifactorial (Döbrössy *et al.*, 2021). A prominent theory regarding its effects relate to the modulation of ascending dopaminergic projections to the NAc and mPFC, which are implicated in anhedonic and amotivational symptoms of depression (Russo and Nestler, 2013; Schlaepfer *et al.*, 2013, 2014). Pre-clinical experimental work provides evidence for this supposition. Induced dopamine release in the NAc after stimulation of the MFB is well established (Olds and Milner, 1954; Shizgal and Matthews, 1977; Kuhr *et al.*, 1984; Stamford, Kruk and Millar, 1986). More recently, it was shown that acute mfb-DBS induces DA release in the NAc differentially in depressive-like and non-depressive-like rats, with the response in the FSL of greater magnitude and sustained for longer than in controls (Klanker *et al.*, 2017; Ashouri Vajari *et al.*, 2020), and changes in DA receptor expression have also been suggested in healthy rats, and in the FSL when challenged with DA receptor blockade (Dandekar *et al.*, 2017; Thiele *et al.*, 2020). These reports provide evidence for modulation of the ascending DA pathways as a consequence of mfb-DBS, but how this manifests as anti-depressant effect is unclear.

The action of rapid-onset antidepressants, such as ketamine and forms of DBS, are theorised to be related to the induction of Glu-dependant plasticity (Duman,

Deyama and Fogaça, 2021). Dopaminergic modulation by MFB-DBS is thought to arise via interaction with glutamatergic fibres, rather than via direct recruitment, as the unmyelinated DA projections present in the tract are not preferentially stimulated at the parameters of DBS used clinically (Ikemoto, 2010; Schlaepfer *et al.*, 2013, 2014). Given that both the glutamatergic and dopaminergic systems are involved in regulating plastic mechanisms (Belujon and Grace, 2014; Rincón-Cortés and Grace, 2020), a role of plasticity alongside DA modulation is suggested in MFB-DBS. Indeed, it has been recently shown that mfb-DBS can induce BDNF expression in the HC (Dandekar *et al.*, 2019).

In the current experiment, we investigated in the FSL the expression of DA DRD1 and DRD2 receptors and the catecholamine synthesising enzyme tyrosine hydroxylase (TH) in the NAc and PFC, alongside three markers of synaptic plasticity, namely the expression of the IEGs Arc, H1a and NPAS4. Dopamine transmission is, to an extent, self-regulating, with the expression of receptors sensitive to changes in dopaminergic tone, with changes in agonism able to induce up- or downregulation of receptors (Elhwuegi, 2004). High affinity DRD2s are sensitive to tonic changes in extracellular DA, while DRD1s are more readily activated by the phasic release associated with burst firing, and are less sensitive to concentration changes (Dunlop and Nemeroff, 2007; Goto, Otani and Grace, 2007). As well as altering as part of compensatory mechanisms to maintain steady functioning, the expression of receptors may also be independently modulated by the environment, including stress and the action of drugs including anti-depressants, which may effect the functionality of dopaminergic transmission (Scheler, 2004). TH is a critical enzyme in the synthesis of DA and NA, considered a rate limiting factor and marker of anabolism (Elhwuegi, 2004). Its expression and activity are regulated in a number of ways, including by the activity of DRD2 autoreceptors (Pothos *et al.*, 1998), changes to transcription rates and the stability of the mRNA (Kumer and Vrana, 2002). Levels of TH expression can be modulated by stress and different classes of anti-depressants, and may represent direct changes to cellular mechanisms or feedback responses to changes in catecholamine activity (Nestler *et al.*, 1990; Angulo *et al.*, 1991).

The markers of synaptic plasticity investigated were three IEGs: the transcription factor NPAS4 and two effector proteins, H1a and Arc. NPAS4 is a rapidly expressed

IEG found only in neural tissue, which directly regulates the expression of BDNF (Sun and Lin, 2016). Its expression regulates excitatory synaptic inputs onto inhibitory circuits, and inhibitory inputs onto excitatory neurons, therefore functioning as a circuit-wide homeostatic controller of functional response, crucial for maintaining e/i balance (Bloodgood *et al.*, 2013; Spiegel *et al.*, 2014). NPAS4 regulates a number of effector proteins via its induction of BDNF, including Arc. Arc is an important molecule in regulating synaptic strength, dendritic spine density and morphology (Korb and Finkbeiner, 2011; Li, Pehrson, *et al.*, 2015); it is also implicated in mechanisms of long-term Glu-dependant plasticity, as it is reciprocally controlled by and modulates Glu receptors including AMPARs (Bramham *et al.*, 2008). Furthermore, Arc appears to be suppressed in chronic stress models of depression, and is elevated in the frontal cortices and hippocampus after SSRI and MAOI treatment (Pei *et al.*, 2004; Li, Pehrson, *et al.*, 2015). Another regulator of Glu transmission via post-synaptic remodelling of metabotropic receptors, H1a induction is also implicated in activity-dependant synaptic plasticity (Clifton *et al.*, 2019). Induction of H1a has been demonstrated as critical to the anti-depressant action of imipramine, ketamine and sleep deprivation, leading to the suggestion that it may be a final common mechanism of various treatment (Serchov *et al.*, 2015).

The expression of these molecules was investigated alongside behaviour in the FSL. The FSL exhibits spontaneous depressive-like behaviour in the FST, and anhedonia-like phenotype after chronic stress (Pucilowski and Overstreet, 1993; Overstreet and Wegener, 2013). However, recent reports have suggested a more spontaneous anhedonic phenotype may be present, linked to the measurement of 'wanting' behaviour in the SCT rather than 'liking' behaviour in the SPT (Rea *et al.*, 2014; Edemann-Callesen *et al.*, 2015). Here we aimed to reproduce these reports, and the anti-depressant effect of mfb-DBS on behaviour as reported by Edemann-Callesen and colleagues (2015).

5.2 Materials and methods

Experimental design

The experimental timeline is summarised in Figure 5.1. Animals were divided into cohorts for behavioural testing (Ctrl, *n* = 10; FSL, *n* = 12) or qPCR analysis (Ctrl, *n* = 8; FSL, *n* = 8). These cohorts were further divided into sham (Ctrl- and FSL-) and DBS (Ctrl+ and FSL+) groups (*n* = 5 and 6 per group for controls and FSL respectively in the behavioural cohort; *n* = 4 for all groups in the qPCR cohort).

In the behavioural cohort, animals underwent baseline tests of the FST and SCT before surgery. All animals were then implanted with bilateral DBS electrodes in the mfb. After recovery and habituation to a cable allowing free movement in the home cage, rats underwent 24h mfb-DBS. Rats in the qPCR cohort were euthanised immediately after mfb-DBS, and those in the behavioural cohort after further testing, with tissue collected as described in **section 3.7**.

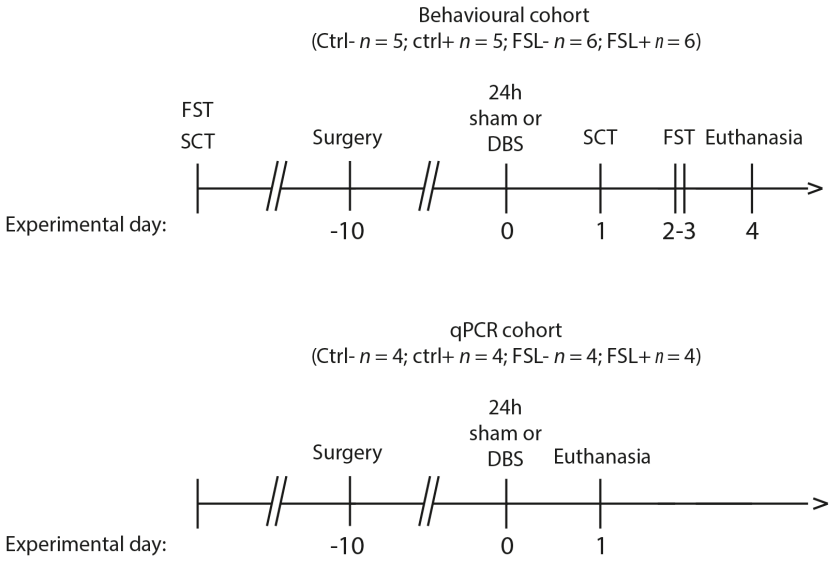

Figure 5.1. *Experimental timeline.*
Ctrl- = control with sham-DBS; Ctrl+ = control with mfb-DBS; FSL- = FSL with sham-DBS; FSL+ = FSL with mfb-DBS; FSL = Flinders Sensitive Line; FST = forced swim test; SCT = sucrose consumption test; DBS = deep brain stimulation.

Experimental procedures

Protocols and analysis were carried out as described in chapter 3: the FST and SCT (**section 3.2**), DBS (**section 3.5**), tissue collection (**section 3.7**) and qPCR analysis (**section 3.8**).

Surgery

Surgery was performed as described in **section 3.4**. Electrodes for mfb-DBS were bilaterally inserted into the medial forebrain bundle (AP -2.7, ML ±1.7, DV -8.0).

Statistics

Statistical analyses were performed as described in **section 3.10**. For baseline behavioural data, sham and DBS group data was pooled and assessed using unpaired t-tests. Changes within sham and DBS groups for control and FSL animals were evaluated using repeated measures two-way ANOVA. Significant interactions were assessed using the Holm-Šidak method. qPCR data between groups was assessed using unpaired t-tests. Data were expressed as mean +SEM, with a significance level set at $p = 0.05$.

5.3 Results

Due to electrode faults, the final groups numbers for control animals in the qPCR cohort was *n* = 3 for both Ctrl- and Ctrl+ groups.

Behavioural testing

FSL rats exhibit depressive-like phenotypes In the FST and SCT

In baseline measurements of depressive-like phenotypes, FSL rats exhibited increased mobility in the FST (unpaired t-test, t = 3.69, *p* = 0.0015, Fig. 5.2A) and decreased sucrose consumption in the SCT (t = 3.67, *p* = 0.0015, Fig 5.2B) compared to controls, indicating both a “despair-like” and anhedonic-like phenotype.

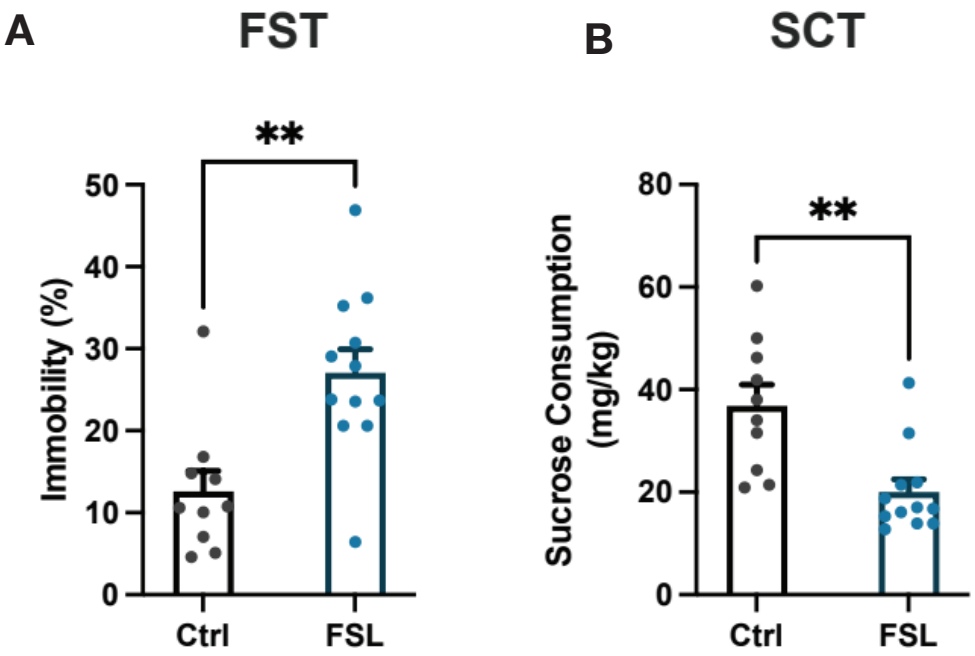

Figure 5.2. *Behavioural measures of control and FSL animals at baseline. As measured in the FST (A) and SCT (B). Significance reported according to unpaired t-test; n = 10 (Ctrl); n= 12 (FSL).*

mfb-DBS reduces immobility in the FST in both the FSL and control animals

In the FSL rat, immobility in the FST was reduced in animals receiving mfb-DBS, with no change seen in sham animals (two-way ANOVA, test time x treatment group interaction, $F(1, 10) = 11.80$, $p = 0.0064$; post-hoc comparison between tests, DBS group, $p = 0.0028$; sham group, $p = 0.64$, Fig. 5.3). Similarly, mfb-DBS reduced immobility in stimulated control animals, while sham stimulation had no effect (test time x treatment group interaction, $F(1, 8) = 5.49$, $p = 0.047$; post-hoc comparison between test time, DBS group, $p = 0.0066$; sham group, $p = 0.33$, Fig. 5.3).

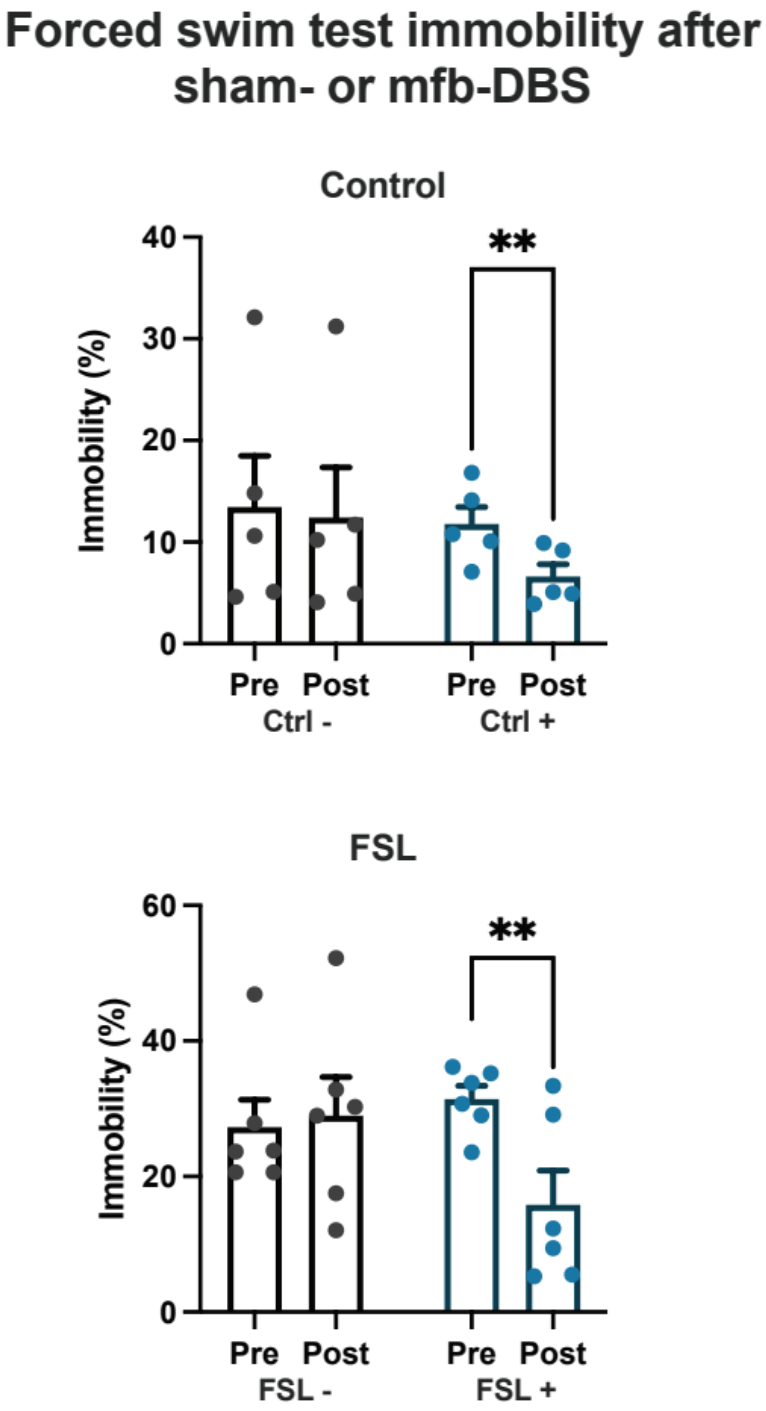

Figure 5.3. *Immobility in control and FSL animals as measured in the FST, pre- or post- 24h sham- or mfb-DBS.*
Significance according to post-hoc assessment after two-way repeated measures ANOVA. n = 5 (control groups) and 6 (FSL groups).

mfb-DBS increases sucrose consumption in the FSL but not control animals

In the FSL rat, sucrose consumption increased in the SCT in DBS but not sham animals (test time x treatment group interaction, $F(1, 10) = 5.39$, $p = 0.043$; post-hoc comparison between test time, DBS group, $p = 0.05$; sham group, $p = 0.52$, Fig. 5.4). In non-depressive control animals, no differences were found in the sham or DBS groups between baseline and post-DBS tests (test time x treatment group interaction, $F(1, 8) = 0.0044$, $p = 0.95$, Fig. 5.4).

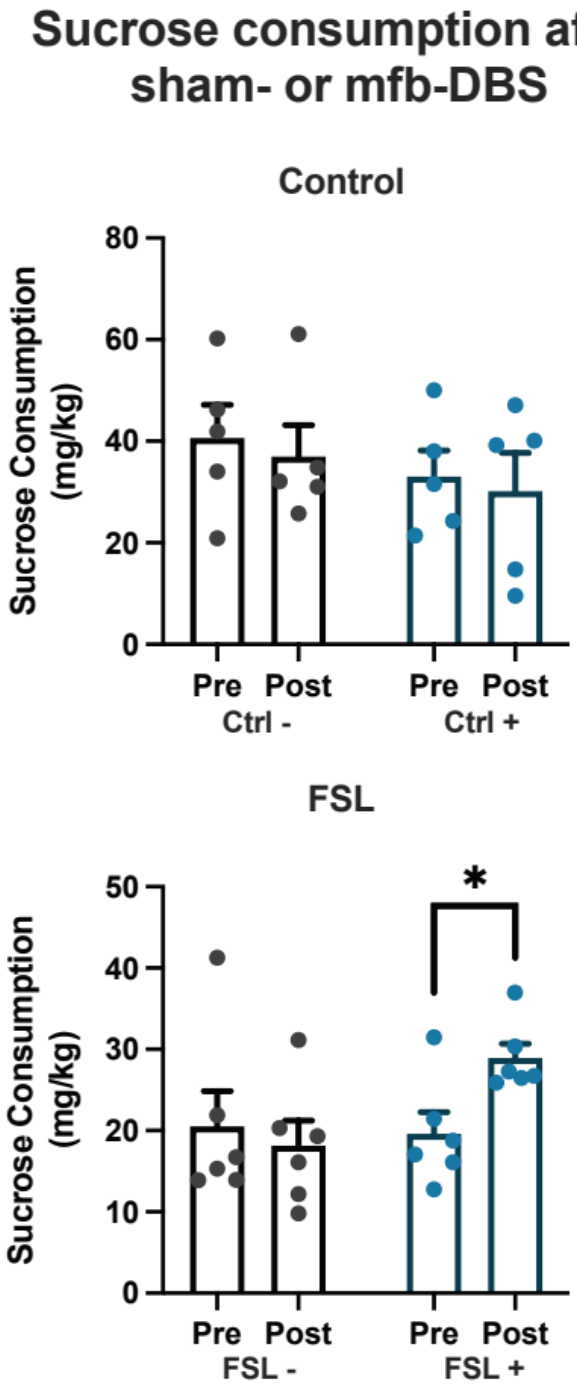

Figure 5.4. *Sucrose consumption in control and FSL animals, pre- or post- 24h sham- or mfb-DBS. Significance according to post-hoc assessment after two-way repeated measures ANOVA. n = 5 (control groups) and 6 (FSL groups).*

mfb-DBS differentially alters DA receptor expression in non-depressive and FSL rats

24h mfb-DBS modulated the expression of DA receptors in both non-depressive-like controls and FSL rats, with changes dependant on the model.

In the FSL rat, 24h mfb-DBS altered D1 and D2 receptor expression in a region-specific manner (Fig. 5.5). In the PFC, mfb-DBS resulted in a 1.52±0.21-fold increase in DRD1 receptor expression ($t = 2.49$, $p = 0.047$, Fig. 5.5A) compared to sham-DBS FSL rats, without producing a significant change in expression of DRD2 ($t = 1.06$, $p = 0.33$, Fig. 5.5B), or TH ($t = 1.42$, $p = 0.21$, Fig. 5.5C). In the NAc, DRD1 levels were not affected ($t = 0.89$, $p = 0.41$, Fig. 5.5A), but rather DRD2 expression was significantly reduced, by a factor of 0.46 (±0.16; $t = 2.96$, $p = 0.025$, Fig. 5.5B). TH expression appeared reduced after DBS with a trend towards significance in the NAc ($t = 2.22$, $p = 0.069$, Fig. 5.5C).

In control rats, 24h mfb-DBS resulted in significant reductions in the expression of both D1 and D2 receptors (Fig. 5.6). In the PFC, D1 receptors were reduced by a factor of 0.67±0.18 compared to sham-stimulated controls ($t = 3.83$, $p = 0.019$, Fig. 5.6A); D2 receptors were reduced by 0.72±0.11 ($t = 6.44$, $p = 0.003$, Fig. 5.6B). This was accompanied by a 2.08±0.25-fold increase in TH expression ($t = 4.39$, $p = 0.012$, Fig. 5.6C). D1 and D2 receptor expression were also reduced in the NAc: DRD1 by a factor of -0.41±0.13 ($t = 3.06$, $p = 0.038$, Fig. 5.6A), and DRD2 by a factor of 0.43±0.13 ($t = 3.40$, $p = 0.027$, Fig. 5.6B). TH expression was not different between sham- and mfb-DBS groups in the NAc ($t = 0.71$, $p = 0.52$, Fig. 5.6C).

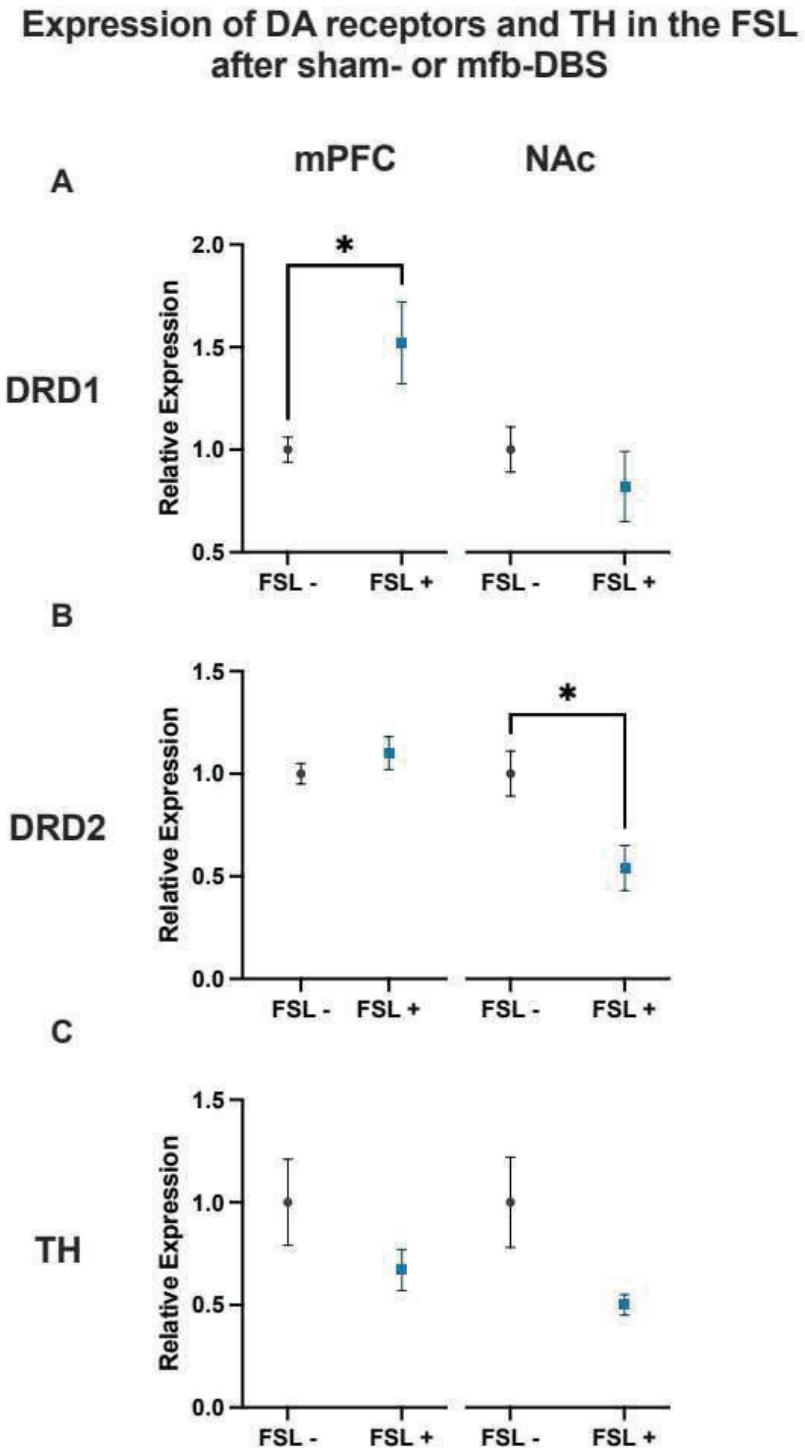

Figure 5.5. *Expression of dopamine receptors and in response to mfb-DBS in the FSL. Expression of DRD1 (A), DRD2 (B) and TH (C) in the mPFC and NAc of FSL animals after 24h sham- (FSL-) or mfb-DBS (FSL+). Significance indicated according to unpaired t-test. For all groups, n = 4.*

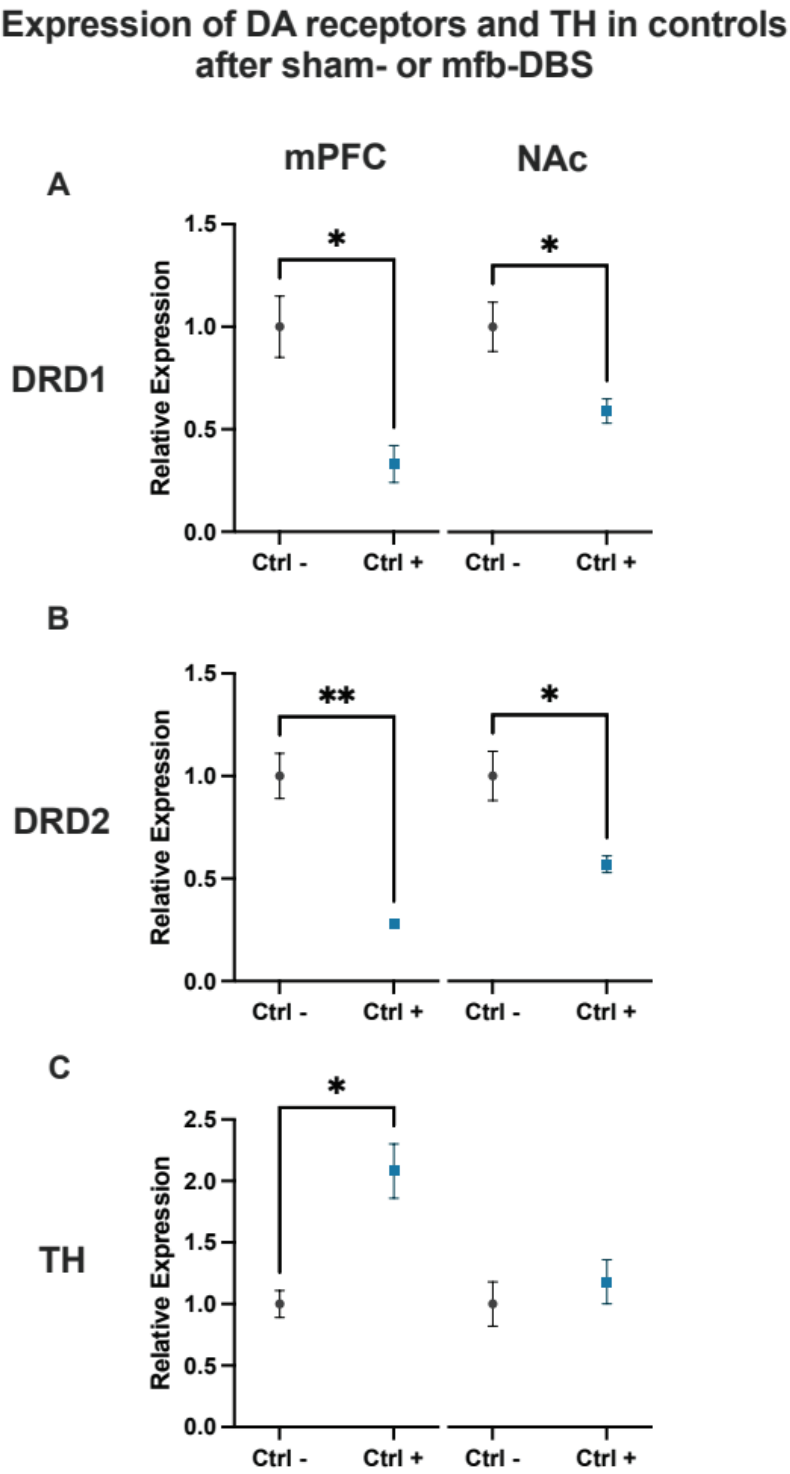

Figure 5.6. *Expression of dopamine receptors and in response to mfb-DBS in controls. Expression of DRD1 (A), DRD2 (B) and TH (C) in the mPFC and NAc of control animals after 24h sham- (Ctrl-) or mfb-DBS (Ctrl+). Significance indicated according to unpaired t-test. For all groups, n = 3.*

mfb-DBS induces changes in PFC IEG-like molecule expression in the FSL

In the PFC of the FSL, 24h mfb-DBS induced a significant, 1.58±0.22-fold increase in Arc expression (t = 2.79, *p* = 0.032, Fig. 5.7A) compared to sham-stimulated FSL rats, and an increase of similar magnitude in H1a (1.55±0.18-fold increase, t = 2.62, *p* = 0.039, Fig. 5.7B). PFC NPAS4 expression was not significantly altered (t = 1.51, *p* = 0.18, Fig. 5.7C). In the NAc, no changes were observed in Arc (t = 0.026, *p* = 0.98, Fig. 5.7A), in H1a (t = 0.18, *p* = 0.87, Fig. 5.7B) or in NPAS4 (t = 0.81, *p* = 0.45, Fig. 5.7C) expression.

In contrast to these effects in the FSL, mfb-DBS did not induce changes in the PFC of control animals compared to sham-DBS controls (Fig. 5.8). No change was observed in expression of H1a (t = 0.38, *p* = 0.72, Fig. 5.8A), Arc (t = 0.84, *p* = 0.45, Fig. 5.8B), or NPAS4 (t = 0.48, *p* = 0.66, Fig. 5.8C). However, unlike in the FSL, a change was observed in the NAc, with Arc expression reduced (-0.41±0.10) in stimulated controls compared to sham controls (t = 0.41, *p* = 0.015, Fig. 5.8A). Expression of H1a (t = 1.48, *p* = 0.21, Fig. 5.8B) and NPAS4 (t = 1.17, *p* = 0.31, Fig. 5.8C) remained unchanged.

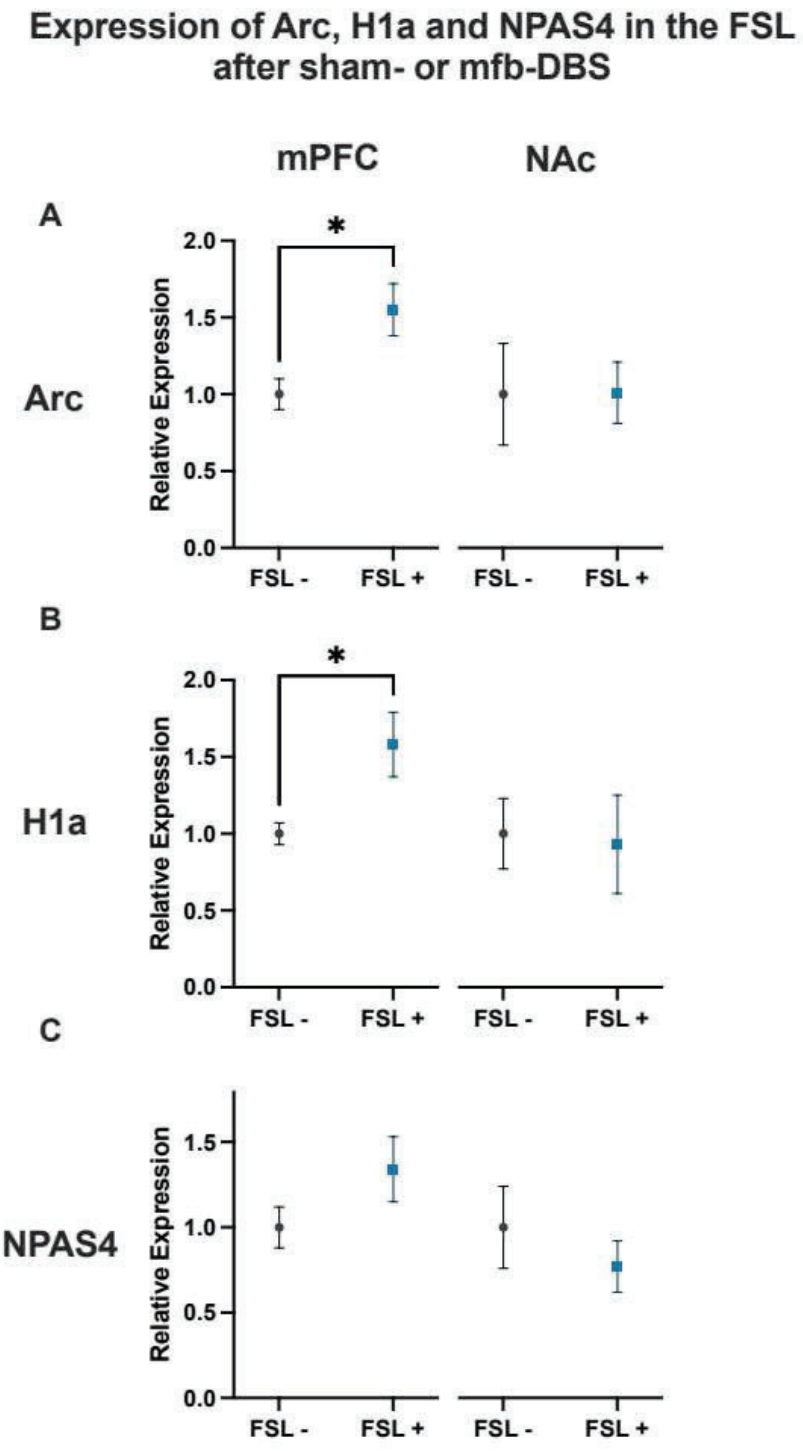

Figure 5.7. *Expression of plasticity markers in response to mfb-DBS in the FSL. Expression of Arc (A), H1a (B) and NPAS4 (C) in the mPFC and NAc of FSL animals after 24h sham- (FSL-) or mfb-DBS (FSL+). Significance indicated according to unpaired t-test. For all groups, n = 4.*

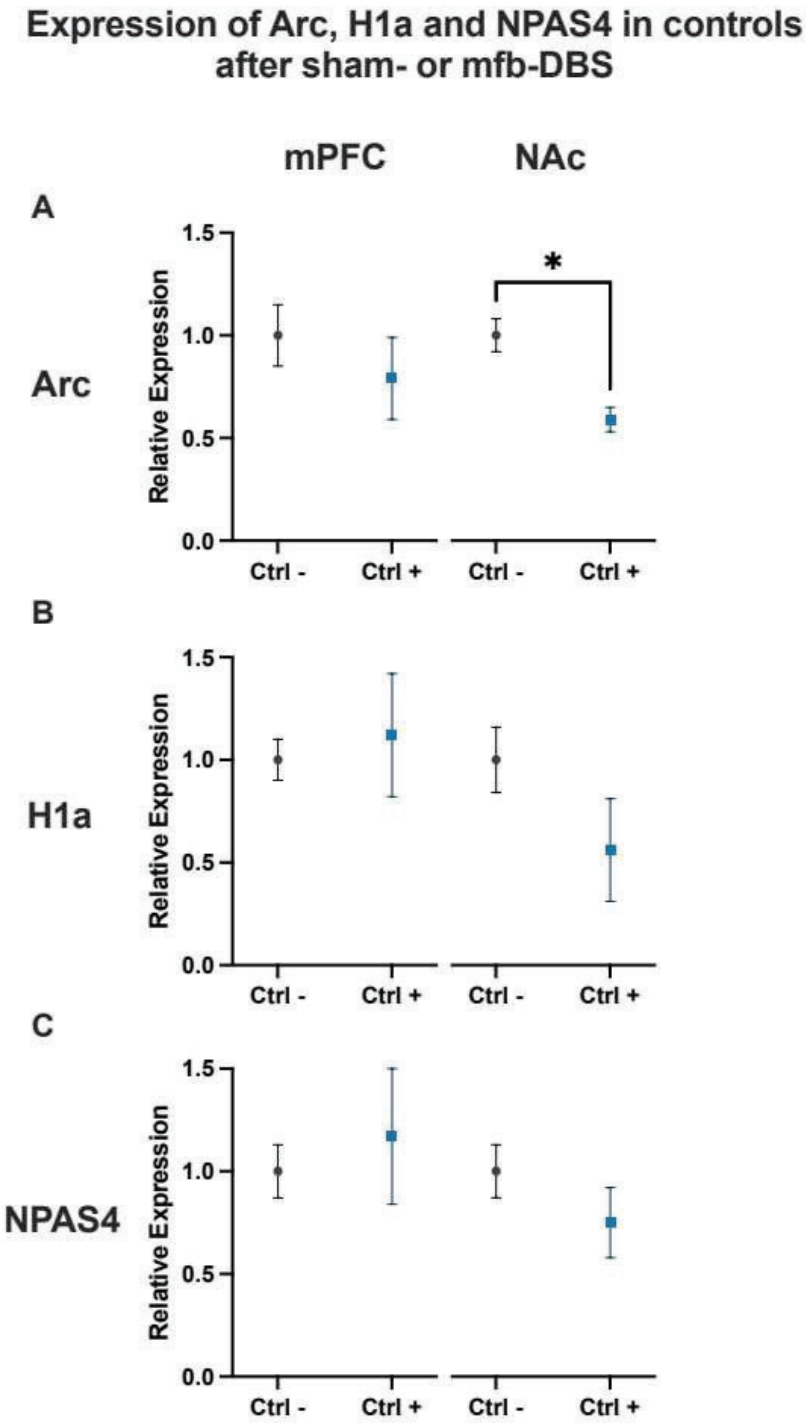

Figure 5.8. *Expression of plasticity markers in response to mfb-DBS in controls. Expression of Arc (A), H1a (B) and NPAS4 (C) in the mPFC and NAc of control animals after 24h sham- (Ctrl-) or mfb-DBS (Ctrl+). Significance indicated according to unpaired t-test. For all groups, n = 3.*

5.4 Discussion

FSL rats exhibited depressive-like behaviours, including anhedonic-like behaviours, which were reversed by 24h of mfb-DBS. These behavioural effects were accompanied by an increase of DRD1, H1a and Arc expression in the mPFC, and a reduction in the expression of NAc DRD2s. In control animals, DBS decreased immobility in the FST, but did not increase sucrose consumption. In these animals, expression of DRD1 and DRD2s was reduced in both the mPFC and NAc, while TH was increased in the mPFC.

FSL rats display an anhedonic-like phenotype without chronic stress

The FSL rat has long been held to not exhibit anhedonic-like phenotype in the SPT, except after exposure to chronic stress (Pucilowski *et al.*, 1993; Overstreet and Wegener, 2013; Thiele *et al.*, 2016). As CMS can induce such anhedonic behaviours in non-depressive-like strains of rats (Willner, Muscat and Papp, 1992), and as a core symptom of depression, this represents a significant weakness in the face validity of the FSL. However, two recent studies of the FSL have suggested reduced sucrose consumption in the SCT without chronic stress (Rea *et al.*, 2014; Edemann-Callesen *et al.*, 2015). Compared to the SPT, the direct measures of sucrose consumption are thought to more narrowly measure aspects of 'wanting', relating to motivational salience, associated with dopaminergic functioning in reward systems (Berridge and Kringelbach, 2015; Meyerolbersleben, Winter and Bernhardt, 2020). The replication of these previous reports suggests that an anhedonic-like phenotype separate from that inducible in healthy rats by chronic stress is indeed present, adding significantly improving the face validity of the FSL. However, given the reported sensitivity of sucrose preference to a variety of stressors and methodological differences (Scheggi, De Montis and Gambarana, 2018), further replication of these results in the SCT would be beneficial to confirm the phenotype. As the 'wanting' aspect of reward is more specifically representative of dopaminergic response, this phenotype may reflect previously reported dysfunction of the mesolimbic dopaminergic system in the FSL, including altered VTA firing (Friedman, Friedman, *et al.*, 2008; Friedman

et al., 2012) and DA release in the NAc (Zangen *et al.*, 2001). However, given the complexity of substrates underlying rewards and debatable distinction between them in these behavioural tests (Der-Avakian and Markou, 2012), the prospective role of dysfunctional DA in the SCT must be specifically experimentally investigated.

mfb-DBS alleviates anhedonic-like symptoms in the FSL but has no effect on sucrose consumption in healthy animals

After 24h mfb-DBS, the anhedonic-like phenotype of the FSL rat was alleviated, but there was no effect on sucrose consumption of non-depressive-like animals. This difference between strains in the SCT is consistent with results reported after short duration, acute-intermittent mfb-DBS (Edemann-Callesen *et al.*, 2015) and suggests an interaction with underlying depressive pathophysiology relating to anhedonic symptoms, rather than a potentiation of normally functioning hedonic circuits.

In both the FSL and in non-depressive-like animals, mfb-DBS has been shown to induce DA release in the NAc in the acute period after stimulation onset (Klanker *et al.*, 2017; Ashouri Vajari *et al.*, 2020), but for how long this release is sustained is not clear (Bregman *et al.*, 2015).

In the FSL, TH showed a non-significant trend for reduced expression, which may suggest reduced DA synthesis, possibly in response to increased DA activity (Kumer and Vrana, 2002). The expression of DRD2s was also reduced, which may also be indicative of increased agonism and compensatory down-regulation (Elhwuegi, 2004; Scheler, 2004). Together, this is coherent with sustained increase in extracellular DA release, following the acute elevation previously reported (Ashouri Vajari *et al.*, 2020). Furthermore, DRD2 activation in the NAc has been associated with increased motivational drive and anti-depressant behaviour (Tye *et al.*, 2013; Gallo *et al.*, 2018), suggesting a possible role in the behavioural response seen in the FSL.

In control animals, both DRD1 and DRD2s were downregulated, without a change in TH expression. This differential response to stimulation may be representative of continued divergent changes in DA release following stimulation, or may be the result of differential direct modulation of receptor and TH expression in the two strains.

Markers of plasticity and DRD1 expression are elevated in the FSL mPFC after mfb-DBS

In the mPFC of the FSL, 24h mfb-DBS resulted in an upregulation of the DRD1. Absence of changes to TH or DRD2 expression may suggest that DA synthesis and tonic release are largely unchanged (Dunlop and Nemeroff, 2007; Goto, Otani and Grace, 2007). An upregulation of DRD1s, independent of changes in extracellular DA, may therefore facilitate increased activation, which has been linked to anti-depressant effect in previous reports (D'Aquila *et al.*, 1994; Scornaiencki *et al.*, 2009; Hare *et al.*, 2019).

A possible mechanism for DRD1 regulation is modulation by glutamate signalling and glutamate-dependant plasticity, which appears to have a bi-directional modulatory relationship with DA via DRD1s (Wolf, Mangiavacchi and Sun, 2003; Pei *et al.*, 2004; Do, Kerr and Kuzhikandathil, 2007). Concurrently, potentiation of plastic mechanisms may also contribute to both DRD1 upregulation and ultimate response, as stress is thought to weaken pre-frontal networks via the reduction of DRD1 expressing cells (Arnsten, 2015). Supporting this hypothesis, FSL rats – but not controls – showed increased markers of Glu-dependant synaptic plasticity in the form of H1a and Arc expression after mfb-DBS. Both H1a and Arc are implicated in synaptic reorganisation, with H1a crucial in regulating post-synaptic densities and Arc in synaptic strength (Inoue *et al.*, 2007; Hu *et al.*, 2010; Korb and Finkbeiner, 2011); H1a induction has been implicated in the anti-depressant response to SSRIs, sleep deprivation, ketamine and ECT (Kato, 2009; Serchov *et al.*, 2015). Facilitation of plastic processes is thought to be crucial as the final outcome of various anti-depressant treatments. Our results suggest mfb-DBS facilitates mPFC plasticity after 24h in the FSL, which may be linked to the observed behavioural effects.

In the mPFC of control animals, DRD1 and DRD2 expression was reduced, while TH expression was elevated. Together, this may suggest an elevated capacity for DA synthesis and release, resulting in a downregulation of receptor expression as a result of increased agonism. This activity may be linked to the response of control animals in the FST.

Differential responses may represent divergent secondary responses

The presented results highlight the distinct effects of mfb-DBS on healthy and depressive-like physiology. The divergence in response is coherent with evidence from other studies of mfb- and vmPFC-DBS, which have also shown differential interaction between controls and depressive-like animals in monoaminergic (Hamani *et al.*, 2012; Jiménez-Sánchez, Linge, *et al.*, 2016; Ashouri Vajari *et al.*, 2020) and plastic modulation (Hamani *et al.*, 2012; Bambico *et al.*, 2015). These altered responses may have their foundation in the initial response to stimulation (for example, in the greater magnitude DA release induced in the NAc of the FSL (Ashouri Vajari *et al.*, 2020), which may then provoke stronger compensatory mechanisms); they may arise from differences in the efficacy or form of the compensatory mechanisms themselves; or they may represent alternate direct modulation of other cellular processes, which then lead to divergent expression.

In the mPFC, the data suggests that mfb-DBS may sustain increased dopaminergic synthesis, and perhaps release, in control animals, while in the FSL no change to synthesis or increased DA tone is suggested. Schlaepfer and colleagues (2013) suggest that DA modulation by mfb-DBS is the result of activation of descending glutamatergic fibres to the VTA, possibly resulting in increased dopaminergic tone in the ascending mesolimbic and mesocortical pathways. This may underlie the effect seen in the NAc of both control and FSL animals, and in the mPFC of controls. In the FSL, however, it appears the end response is different in the mPFC. This may be due to differential initial recruitment of VTA afferents, or physiological or functional differences in the mesocortical projections from the VTA. Earlier work has shown abnormal burst firing in the VTA of the FSL (Friedman, Friedman, *et al.*, 2008), which may therefore respond differently to altered afferent influence. Although this abnormal firing can be normalised by VTA-DBS, this was by direct, low-frequency rather than high frequency stimulation delivered to distal fibres(Friedman *et al.*, 2012; Gazit *et al.*, 2015). Investigation of the activity of the VTA in the FSL in response to mfb-DBS is a necessary further step in characterising the anti-depressant response.

However, given the wide dysregulation of network activity implicated in depression, the influence of other abnormalities is likely. The variability in dysfunction of the dopaminergic system in depressive-like states also necessitates research in to the effects of mfb-DBS in other models of depression, in order to truly understand the substrates of modulatory effect.

Limitations

The physiological response of mfb-DBS is dynamic, as previously demonstrated (Bregman *et al.*, 2015; Klanker *et al.*, 2017; Ashouri Vajari *et al.*, 2020). The distinction between acute and chronic response, and when this becomes anti-depressant, is not clear. The results presented provide a single snapshot of this response, and are limited in the information they provide about the progression of response. In terms of effects on extracellular DA release, our speculations as to the dynamics underlying the response of receptor and TH expression must be verified by extracellular recording techniques, such as microdialysis or fast-scanning cyclic voltammetry (FSCV). Unfortunately, these techniques are limited by the required balance between acute temporal resolution and feasibility of long-term application. Solutions must likely be found in experimental design to examine long-term changes to DA dynamics after mfb-DBS.

mfb-DBS was applied for 24h, as previous reports have indicated that intermittent stimulation within this timeframe is sufficient for anti-depressant response (Edemann-Callesen *et al.*, 2015; Dandekar *et al.*, 2017). Adaptive changes to dopamine receptor and TH expression, and induction of the selected plasticity markers are relatively fast events, so 24h should be representative of changes beyond the acute effects. Although further adaptive changes cannot be ruled out, behavioural effects indicate that changes seen may be part of anti-depressant effect.

Conclusions

Our results suggest DRD1 upregulation and facilitation of plasticity in the mPFC, in particular through H1a induction, as mechanisms of anti-depressant response after mfb-DBS. These two mechanisms may be interconnected by reciprocal modulation. A lack of evidence for changes to DA release in the mPFC potentially suggests

modulation by other systems; glutamate and serotonin are candidates. In the NAc, changes in receptor expression may provide evidence for continued extracellular DA release after 24h mfb-DBS; this must be confirmed by long-term monitoring of extracellular concentrations.

The current study was able to replicate recently reported anhedonic-like behaviour in the FSL model of depression, upon which 24h mfb-DBS had an anti-depressant effect. The data also provide further evidence of dopaminergic modulation involved in the anti-depressant response of mfb-DBS, while suggesting differential modulation of healthy and pathological function. In depressive-like animals, evidence of plastic changes in the mPFC were found alongside changes to dopamine transmission. This highlights the importance of the interaction between DBS and the pathology of DBS, and the alteration, rather than normalisation, of dopaminergic function as potential anti-depressant effect.

6. Modulation of frontal monoamine concentration by medial forebrain bundle deep brain stimulation in the FSL model of depression

6.1 Introduction

From the very outset of pharmacological treatment of depression, the augmentation of the activity of monoamines has been regarded as a principle mechanism of anti-depressant action (Pereira and Hiroaki-Sato, 2018). Despite the monoamine deficiency hypothesis of depressive pathology proving inadequate to describe the disorder itself, strong evidence still associates monoamines as part of anti-depressant effect (Willner, Scheel-Krüger and Belzung, 2013). The action of monoaminergic anti-depressants is not thought to be the direct result of monoaminergic elevation, rather downstream mechanisms are implicated. However, these final (common or alternate) pathways to depressant response appear to rely on initial monoaminergic modulation – depletion and antagonism of NA, 5-HT or DA can prevent or reverse the actions of anti-depressants (Delgado *et al.*, 1993, 1999; Miller *et al.*, 1996; Willner, Hale and Argyropoulos, 2005; du Jardin *et al.*, 2016). Novel anti-depressants, whose action may not be primarily monoaminergic, may also depend on such effects. Ketamine, primarily an NMDA receptor antagonist, alters the activity of VTA DA neurons (Belujon and Grace, 2014) and stimulates 5-HT and NA release in the PFC (López-Gil *et al.*, 2019); its behavioural effects can be blunted or blocked by 5-HT depletion (du Jardin *et al.*, 2016; du Jardin, Liebenberg, *et al.*, 2017), and by both DRD1 or DRD2 antagonism (Li, Zhu, *et al.*, 2015a; Hare *et al.*, 2019). Similarly, vmPFC-DBS increases 5-HT release in the PFC, and 5-HT depletion blocks its effects in

depressive-like but not control rats (Hamani *et al.*, 2012; Jiménez-Sánchez, Linge, *et al.*, 2016). Monoamines and their modulation therefore appear to play a prominent role in both conventional and novel anti-depressants.

mfb-DBS, interacting with the same circuitry as vmPFC-DBS, is theorised to effect its anti-depressant response in part via dopaminergic modulation in mesolimbic and mesocortical pathways (Schlaepfer *et al.*, 2013, 2014; Döbrössy *et al.*, 2021). Acute applications of mfb-DBS in rats induce DA release in the NAc on a timescale of seconds to minutes in both depressive-like and control animals (Klanker *et al.*, 2017; Ashouri Vajari *et al.*, 2020). However, the dynamic beyond this is uncertain. The results of both Klanker and colleagues (2017) and Ashouri Vajari and colleagues (2020) suggest that DA release extends beyond the recorded time frame; however Bregman and colleagues (2015), taking measurements at 30m intervals for 2h in non-depressive-like animals, found no change in DA release.

FSL rats exhibit elevated tissue content of NA, DA and 5HT in the NAc compared to controls (Zangen, Overstreet and Yadid, 1997, 1999). However, DA release into the extracellular space is diminished, due in part to abnormal interaction between the DA and 5-HT: local release of 5-HT in the NAc typically stimulates DA release, but this response is not elicited in the FSL (Zangen *et al.*, 2001; Dremencov *et al.*, 2004). This has been theorised as the consequence of hyper-functionality of the 5-HT2C receptor, which inhibits DA release (Dremencov *et al.*, 2005, 2011). Anti-depressant response to desipramine in the FSL has been associated with reduced tissue content and greater extracellular release of DA through improved responsiveness to local 5-HT (Zangen *et al.*, 2001; Dremencov *et al.*, 2004; Friedman *et al.*, 2007).

In the current study, we compared tissue content of DA and 5HT in the FSL after two protocols of mfb-DBS: 2h and 24h of stimulation. These durations were chosen to consider a state beyond the previously reported acute response, and also a more extended time point, where acute response may have given way to more long-term effects. As unilateral mfb-DBS has been reported as sufficient to induce an anti-depressant behavioural response (Dandekar *et al.*, 2017, 2019), here we used such a protocol to separately examine the effects on the ipsi- and contralateral hemispheres to DBS.

6.2 Materials and methods

Experimental design

The experimental timeline is summarised in figure 6.1. FSL rats were divided into three groups to receive 0h (*n* = 8), 2h (*n* = 8) or 24h (*n* = 8) of unilateral mfb-DBS. Within each group, implantation was alternated between the left and right hemisphere for each rat, so that each group consisted of an equal number of left- and right-implanted DBS electrodes. After surgery, animals were allowed at least 7 days of recovery time, before being habituated to the stimulation apparatus for 72h. After habituation, each animal received DBS according to their group. Immediately upon the cessation of DBS, the animal was sacrificed and tissue collected for analysis of dopamine and serotonin tissue content.

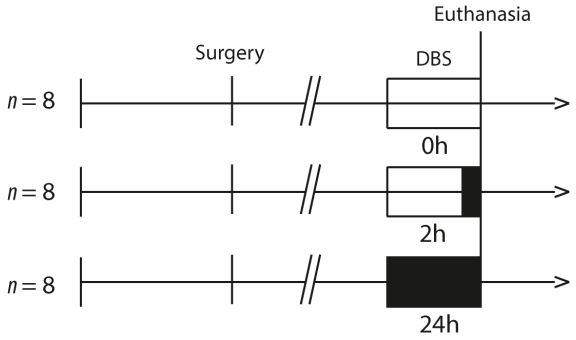

Figure 6.1. *Experimental timeline.*
FSL rats were divided into three groups. After surgery and recovery, mfb-DBS was applied for 0h, 2h or 24h. At the cessation of stimulation, animals were immediately euthanised and tissue collected.

Experimental procedures

Protocols and analysis were carried out as described in chapter 3: DBS (**section 3.5**), tissue collection (**section 3.7**) and HPLC analysis (**section 3.9**).

Surgery

Surgery was performed as described in **section 3.4**. Electrodes for mfb-DBS were unilaterally inserted into the medial forebrain bundle (AP -2.7, ML ±1.7, DV -8.0).

Statistics

Statistical analyses were performed as described in **section 3.10**. Data from un-stimulated rats were pooled between both hemispheres to use as a baseline. Group means from ipsilateral and contralateral hemispheres were compared to the baseline group using unpaired t-tests. The threshold for significance was set at 0.05, and data expressed as mean +SEM.

6.3 Results

Due to technical problems arising during the processing of tissue for HPLC analysis, the number of samples included in NAc analysis was reduced to $n = 4$ for 2h- and 24h-DBS groups in both hemispheres.

Modulation of NAc DA content by mfb-DBS

In the NAc, DA tissue content showed a trend towards increase in the ipsilateral hemisphere to DBS after 2h (unpaired t-test, $t = 2.05$, $p = 0.068$), and was significantly reduced after 24h in both the ipsilateral ($t = 4.23$, $p = 0.0017$) and contralateral ($t = 2.89$, $p = 0.0016$) hemispheres (Fig. 6.2A).

The levels of DA metabolites followed similar patterns: DOPAC concentration was significantly reduced in both hemispheres at 24h (ipsilateral: $t = 5.47$, $p = 0.0003$; contralateral: $t = 6.08$, $p = 0.0001$, Fig. 6.2B); as was HVA (at 24h, ipsilateral: $t = 4.59$, $p = 0.001$; contralateral: $t = 5.85$, $p = 0.0002$, Fig. 6.2C).

Modulation of NAc DA turnover by mfb-DBS

The similar patterns and extent of changes to DA and DOPAC concentrations resulted in no changes in DOPAC/DA ratio from baseline, except in the contralateral hemisphere at 24h, which saw a significant reduction in DOPAC/DA ($t = 3.02$, $p = 0.013$, Fig. 6.2D). In contrast, HVA was differentially depleted, and the ratio of HVA/DA was lower than baseline after 2h in both the ipsilateral ($t = 3.51$, $p = 0.0056$, Fig 6.2E) and contralateral hemispheres ($t = 2.94$, $p = 0.015$); after 24h this difference was only significant in the contralateral hemisphere ($t = 4.30$, $p = 0.0016$).

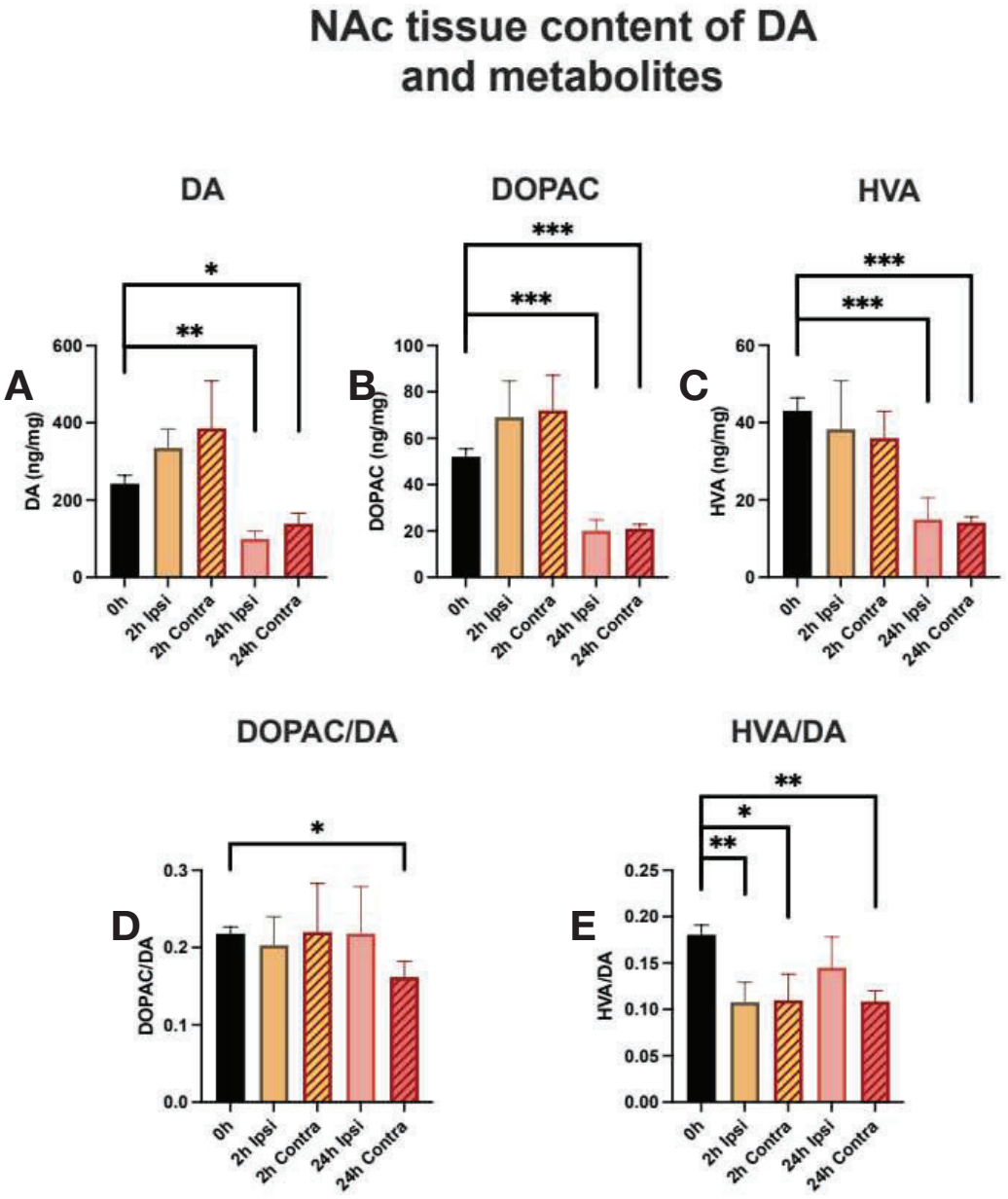

Figure 6.2. *Tissue concentration of dopamine and its metabolites in the nucleus accumbens. Concentration of dopamine (A) and its metabolites DOPAC (B) and HVA (C), and the ratios of DOPAC/DA (D) and HVA (E) in the NAc of the FSL, after 0h, 2h and 24h mfb-DBS. Striped bars indicate hemisphere contralateral to DBS. Significance indicated according to unpaired t-test. For 0h, n = 8; for all other groups, n = 4.*

Modulation of NAc 5-HT content by mfb-DBS

5-HT content in the NAc was also modulated by mfb-DBS. After 2h mfb-DBS, 5-HT concentration was elevated in the contralateral hemisphere (t = 2.74, p = 0.021, Fig 6.3A) but not in the ipsilateral (t = 0.85, p = 0.42). After 24h, 5-HT was significantly reduced from baseline in the ipsilateral hemisphere (t = 3.42, p = 0.0065), but not the contralateral (t = 1.66, p = 0.13).

Modulation of NAc 5-HT turnover by mfb-DBS

Concentration of 5-HT metabolite 5HIAA showed a similar pattern to that of 5-HT after mfb-DBS. Contralateral 5HIAA was elevated after 2h mfb-DBS (t = 2.38, p = 0.038, Fig. 6.3B), while after 24h concentration in both hemispheres was significantly reduced (ipsilateral: t = 3.25, p = 0.0087; contralateral: t = 3.25, p = 0.0087). The similarity in changes between 5-HT and 5HIAA after mfb-DBS resulted in no significant difference in 5HIAA/5-HT ratio from baseline in any group (Fig. 6.3C).

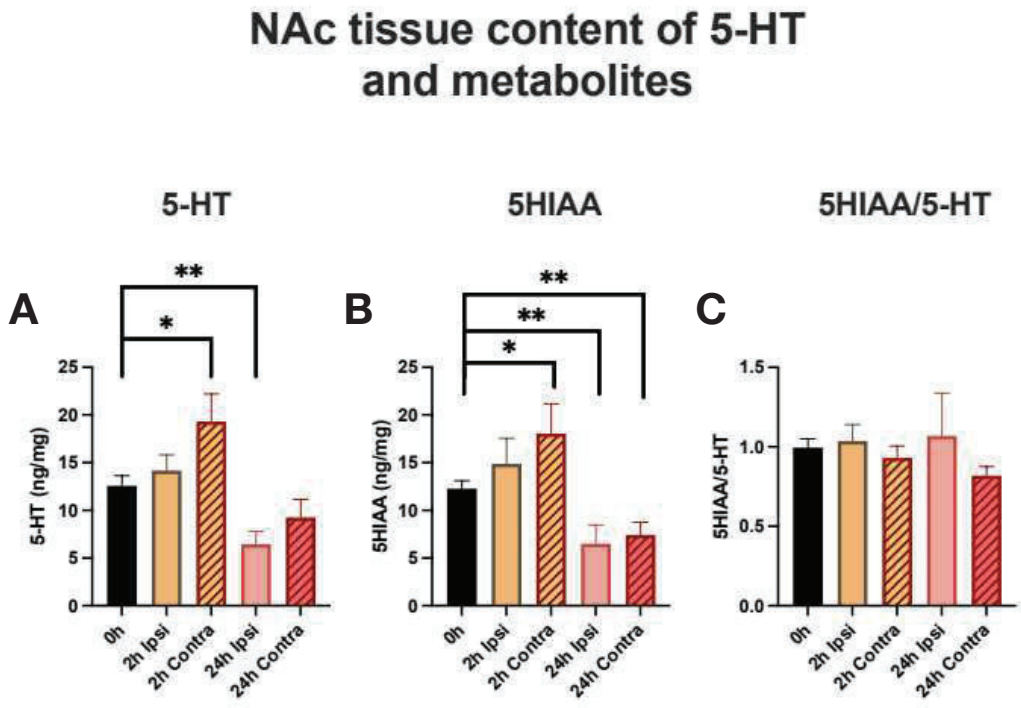

Figure 6.3. *Tissue concentration of serotonin and its metabolites in the nucleus accumbens. Tissue concentration of 5-HT (A) and its metabolite 5HIAA (B), and the ratio of 5HIAA/5-HT (C) in the NAc of the FSL, after 0h, 2h and 24h mfb-DBS. Striped bars indicate hemisphere contralateral to DBS. Significance indicated according to unpaired t-test. For 0h, n = 8; for all other groups, n = 4.*

Modulation of mPFC DA content by mfb-DBS

In the PFC, 2h of mfb-DBS caused a significant increase in the tissue content of DA in the hemisphere receiving stimulation ($t = 3.03$, $p = 0.0097$, Fig. 6.4A), but no influence was seen in any other group.

Modulation of mPFC DA turnover by mfb-DBS

The absolute concentration of the metabolite DOPAC was not significantly different in any group after mfb-DBS (Fig. 6.4B). However, the increase in DA and trend for decrease in DOPAC in the ipsilateral hemisphere after 2h led to a significant decrease in DOPAC/DA ratio in this group ($t = 3.04$, $p = 0.0095$, Fig. 6.4D).

HVA was significantly elevated in both hemispheres after 2h mfb-DBS (ipsilateral: $t = 2.21$, $p = 0.044$; contralateral: $t = 3.09$, $p = 0.008$, Fig. 6.4C); after 24h, HVA remained significantly elevated in the contralateral hemisphere ($t = 2.93$, $p = 0.011$), while in the ipsilateral hemisphere the increased concentration was borderline-significant ($t = 2.07$, $p = 0.058$). The ratio of HVA/DA was significantly elevated at 24h in the contralateral hemisphere ($t = 2.21$, $p = 0.045$, Fig. 6.4E), with a trend towards elevation also seen in the contralateral hemisphere at 2h ($t = 2.04$, $p = 0.06$).

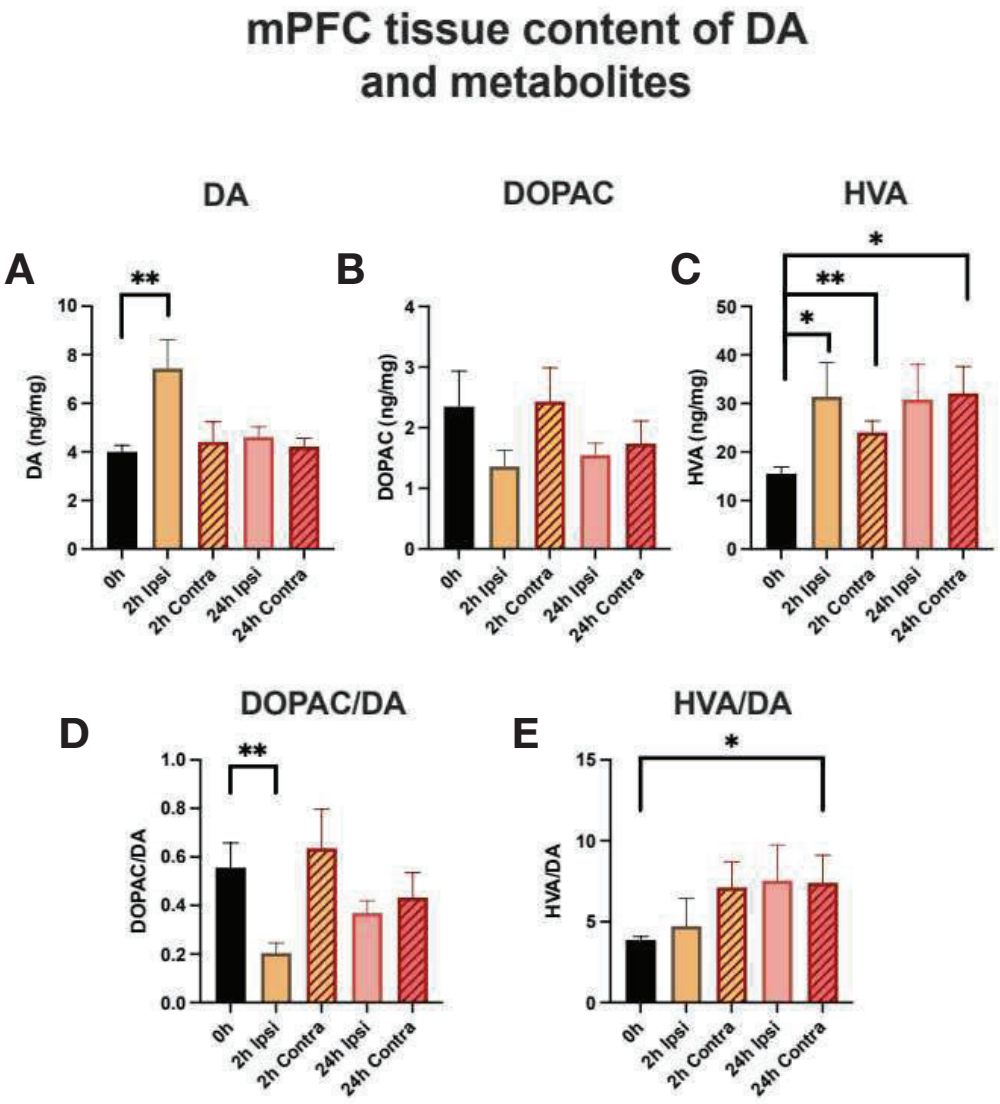

Figure 6.4. *Tissue concentration of dopamine and its metabolites in the medial pre-frontal cortex. Concentration of dopamine (A) and its metabolites DOPAC (B) and HVA (C), and the ratios of DOPAC/DA (D) and HVA (E) in the mPFC of the FSL, after 0h, 2h and 24h mfb-DBS. Striped bars indicate hemisphere contralateral to DBS. Significance indicated according to unpaired t-test. For all groups, n = 8.*

Elevation of 5-HT content after 2h but not 24h mfb-DBS

Similar to the effect seen in DA, 5-HT concentration was elevated in the hemisphere receiving mfb-DBS (t = 2.16, p = 0.049, Fig. 6.5A), but not in any other group compared to baseline.

Modulation of PFC 5-HT turnover by mfb-DBS

The concentration of 5-HT metabolite 5HIAA was elevated after 2h DBS in the both the ipsilateral (t = 2.65, p = 0.019, Fig. 6.5B) and contralateral (t = 3.15, p = 0.007) hemispheres, and after 24h in the contralateral hemisphere (t = 2.2, p = 0.045). The ratio of 5HIAA/5-HT was significantly elevated in the contralateral hemisphere to stimulation after both 2h (t = 3.08, p = 0.0082, Fig. 6.5C) and 24h (t = 2.56, p = 0.023).

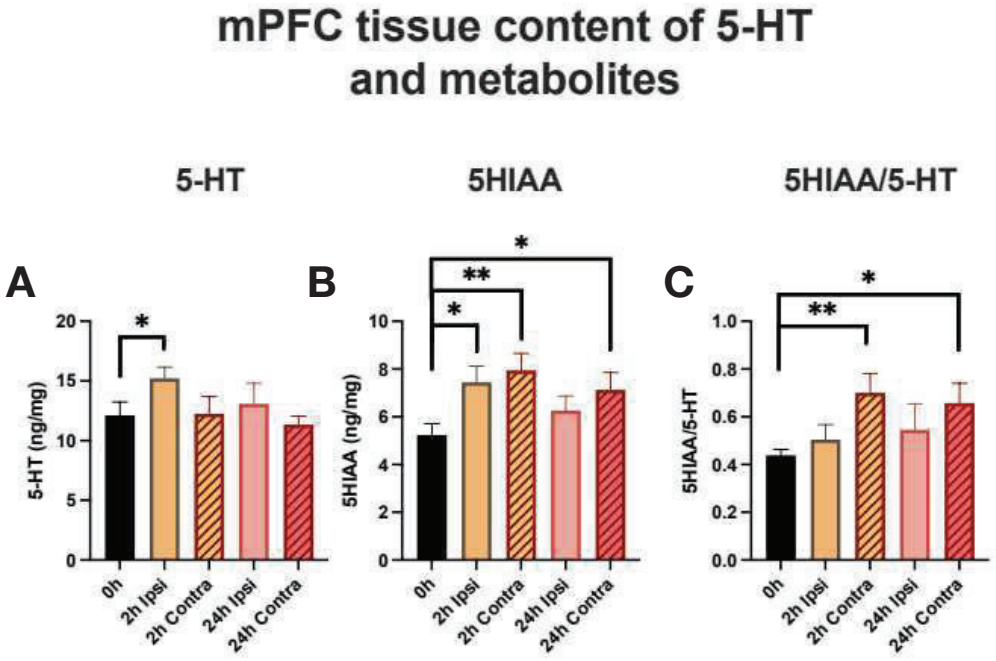

Figure 6.5. *Tissue concentration of serotonin and its metabolite in the medial pre-frontal cortex. Tissue concentration of 5-HT (A) and its metabolite 5HIAA (B), and the ratio of 5HIAA/5-HT (C) in the mPFC of the FSL, after 0h, 2h and 24h mfb-DBS. Striped bars indicate hemisphere contralateral to DBS. Significance indicated according to unpaired t-test. For 0h, n = 8; for all other groups, n = 4.*

6.4 Discussion

mfb-DBS produced changes in the dopaminergic and serotonergic content of tissue in the NAc and mPFC, dependant on region and duration of stimulation. Modulation in the NAc was marked by a decrease in DA, 5-HT and their metabolites after 24h mfb-DBS. In the mPFC, the concentrations of DA and 5-HT were elevated in the stimulated hemisphere after 2h of mfb-DBS, with changes in metabolism evident at 2h and 24h..

Reduced synthesis of DA and 5-HT after 24h mfb-DBS in the NAc

The response of both DA and 5-HT in the NAc to mfb-DBS suggests a response that develops over time. After 2h mfb-DBS, concentrations of DA and its metabolites were largely unchanged, although reduced HVA/DA ratio may be suggestive of reduced extracellular metabolism. HVA is largely derived from the metabolic actions of catechol-O-methyltransferase (COMT) in the synaptic space, while DOPAC is largely formed by the breakdown of newly synthesised or re-uptaken DA within the dopaminergic neuron (Sharp, Zetterström and Ungerstedt, 1986; Zetterström *et al.*, 1988; Soares-Da-Silva and Garrett, 1990; Elhwuegi, 2004). A reduction in the HVA/DA ratio therefore may indicate a reduction in DA being metabolised outside of the cell. With no change in overall DA concentration of DOPAC-related metabolism, this may suggest reduced release or increased reuptake, but with no significant change to intracellular metabolism.

After 24h, the concentration of DA and both of its metabolites were reduced in both hemispheres. The concurrent reductions in transmitter and metabolite are indicative of decreased synthesis of DA, with no significant change in the overall rate of metabolism in the ipsilateral hemisphere, indicated by the constant transmitter/metabolite ratios. In the contralateral hemisphere, a decreased turnover of DA is indicated by reduced metabolite ratios, but the DA concentration remained low, again suggestive of a low rate of synthesis.

Concentration of 5-HT and its metabolite 5HIAA were elevated only in the contralateral hemisphere after 2h of stimulation, indicating an increase in synthesis rather than a reduction in metabolism. After 24h, 5-HT was reduced in the ipsilateral hemisphere, and 5HIAA reduced in both hemispheres. As with DA, the concurrent reduction of both transmitter and metabolite, resulting in a consistent ratio between the two, suggests that these changes are due to reduced anabolism of the transmitter, rather than significant changes to metabolic processes.

Changes to DA concentrations in the mPFC may indicate elevated release

In the mPFC, DA concentration was significantly elevated in the stimulated hemisphere after 2h mfb-DBS. The concurrent reduction in DOPAC/DA ratio indicates this was the result of reduced intracellular turnover; this is also suggested by elevated levels of HVA. The reduction in intraneuronal metabolism appears to be greater than the increase in extracellular metabolism, as suggested by the overall rise in tissue DA. Together, this may suggest increased release or reduced re-uptake of extracellular DA, with COMT unable to metabolise all of the transmitter, leading to accumulation.

In the contralateral hemisphere after 2h, HVA concentration is also elevated. However, concentrations of DA, DOPAC and metabolite ratios are unchanged. This may indicate more available DA in the extracellular space, which may be the result of induced release, be reuptake and intracellular metabolism remain unchanged, as does the overall concentration of DA.

After 24h, HVA and its ratio to DA were elevated in the contralateral hemisphere and appeared to be elevated in the stimulated hemisphere, suggesting more extracellular DA available for metabolism. The unchanged concentrations of DOPAC and DA suggest the overall metabolism of DA is not notably changed. Together, this may be suggestive of increased release of DA into the extracellular space, leading to a shift to COMT metabolism and HVA production.

Changes to 5-HT in the mPFC may involve altered metabolism

The concentration of 5-HT was also elevated in the mPFC after 2h mfb-DBS in the ipsilateral side to stimulation. This was mirrored by an increase in 5HIAA, indicating

increased synthesis of 5-HT in this hemisphere. At 24h mfb-DBS, this effect had disappeared, and concentrations of 5-HT and its metabolite were at baseline levels. Interestingly, in the contralateral hemisphere, 5-HT concentration was consistent, but elevation in the concentration of 5HIAA and the metabolite/transmitter ratio indicated an increased rate of metabolism, which may be indicative of consistent synthesis but increased release.

Reduced DA synthesis in the NAc may reflect anti-depressant response

It has been previously demonstrated that the FSL exhibits elevated levels of DA, 5-HT and NA in NAc tissue (Zangen, Overstreet and Yadid, 1997, 1999). This elevated tissue level is accompanied by reduced release into the extracellular space (Zangen *et al.*, 2001; Dremencov *et al.*, 2004; Friedman *et al.*, 2007). Two mechanisms have been proposed to explain inhibited release: electrophysiological recordings suggest that the FSL is less capable of long bursts of phasic VTA activity required to induce sufficient DA release (Friedman, Friedman, *et al.*, 2008); the DA released by shorter bursting may be rapidly cleared before interacting with postsynaptic receptors. Dremencov and colleagues (2005) demonstrated hyperfunctionality of the 5-HT2C receptor in the FSL, which has an inhibitory effect on DA release in the NAc. Impaired DA release and subsequent hypo-agonism of both autoreceptors and postsynaptic receptors may disinhibit DA synthesis, leading to elevated tissue content without inducing release (Floresco *et al.*, 2003).

After chronic treatment with desipramine, anti-depressant response was associated with reduced DA and 5-HT tissue content (Zangen, Overstreet and Yadid, 1997, 1999). Friedman and colleagues (2007) showed extracellular release elevated after this treatment, while others have shown that baseline extracellular levels were unchanged, but DA release inducible by 5-HT local release was potentiated, possibly via reduced action of the 5HT2C receptor (Zangen *et al.*, 2001; Dremencov *et al.*, 2005). Longer burst firing of the VTA was also facilitated by desipramine, suggesting another possible mechanism for increased extracellular release (Friedman, Friedman, *et al.*, 2008).

Reduced tissue content of DA in the NAc of the FSL may therefore reflect a reduction in synthesis brought about by normalisation of dopaminergic transmission dynamics, after both chronic TCA treatment, and after 24h of mfb-DBS as demonstrated here. This may be mediated through increased efficiency of phasic DA firing, or through ameliorated interaction with other transmitter systems including 5-HT. This may be mediated through reduced function of the 5HT2C receptor, which is also an inhibitor of serotonergic activity (Dremencov *et al.*, 2011), suggesting a similar mechanism may lead to the reduced synthesis of 5-HT also observed alongside that of DA after mfb-DBS.

mfb-DBS modulation of mPFC monoamines may be mechanistically different

The modulation of monoamines in the mPFC suggested different mechanisms of action from that in the NAc. In the mPFC, changes in concentration were consistent with alterations in metabolism and release, rather than synthesis. Elevations in DA after 2h may be representative of mechanisms of transmitter reuptake and removal being overwhelmed, a proposed action of DBS (Hamani and Temel, 2012). This response was not sustained after 24h, which may suggest adaptive changes coming into play, but the evidence presented here suggests more extracellular DA available after 24h mfb-DBS. Whether this is linked to altered local synaptic or modulatory mechanisms, or changes to the behaviour of the VTA neurons projecting to the mPFC is unclear.

Changes to 5-HT in the mPFC were different still; while an increase in synthesis was suggested in the stimulated hemisphere at 2h, this effect was absent at 24h. Apparent changes in metabolism were present only in the contralateral hemisphere to stimulation at both 2h and 24h, suggesting differential influences of stimulation between the hemispheres, presumably mediated via the subsequent network effects of stimulation.

The continued dynamic response of mfb-DBS

Given the apparent dynamic effects of mfb-DBS, it is likely that the response to stimulation is polyphasic. Whether the state observed after 24h mfb-DBS is therefore reflective of a sustained state requires verification. Similarly, further investiga-

tions into the responses of various mechanisms to mfb-DBS are required to confirm the extent and substrate of changes suggested by our results. However, the reduced NAc monoamine content after chronic desipramine treatment (Zangen, Overstreet and Yadid, 1997, 1999), and the anti-depressant effects demonstrated after 24h or less of mfb-DBS (experiments one and two of this thesis, Edeman-Callesen et al., 2015; Dandekar et al., 2017) suggest that this may reflect a common anti-depressant modulation of the FSL, achieved more rapidly by mfb-DBS.

Limitations

The techniques utilised in the current study involved the assessment of monoamine concentrations whole tissue; that is to say both the intracellular and extracellular content. While previous studies have suggested the informative value in whole tissue content relating to monoamines in depression (Zangen, Overstreet and Yadid, 1997, 1999), other techniques differentiating extracellular release may be more informative. A second limitation, as discussed in the experiment of chapter 5, is the static nature of tissue collection post-mortem. The response of monoamines to mfb-DBS is dynamic, and techniques with good temporal resolution are beneficial. However, techniques with excellent dynamic sensitivity such as FSCV tend not to be suitable for measurements over long time periods. Therefore, for a broad examination of gross modulation of monoamines, post-mortem tissue was deemed appropriate in order to provide further insight into longer-term changes induced by mfb-DBS. These techniques must be used in complementary fashion across studies to build a picture of the dynamic response to mfb-DBS.

Conclusions

The results of this study reveal features of monoaminergic modulation by unilateral mfb-DBS, extending the growing image of the dynamic response and suggesting potential aspects of anti-depressant mechanism. Differential modulation of DA and 5-HT were observed in the mPFC and NAc, in turn suggestive of different potential mechanisms of action at these sites.

7. General Discussion

Despite promising clinical trials, the full effects and mechanisms of MFB-DBS are not well understood. Building on the theoretical basis and clinical exploration of DBS of the SGC, a regulator of corticolimbic networks, MFB-DBS is proposed to allow modulation of several previous targets of DBS in this network (Schlaepfer *et al.*, 2014). Projections of the VTA to the NAc and mPFC are thought to be significant in the response to MFB-DBS, associated as they are with emotional processing, reward-related and motivational behaviours, and adequate stress response, processes variously implicated as dysfunctional in depression (Russo and Nestler, 2013; Douma and de Kloet, 2020). While the dopaminergic components of the mesolimbic and mesocortical pathways are associated with these functions, the MFB is thought to be a highly diverse tract, and the wider corticolimbic network is modulated by the activity of a variety of neurotransmitters also implicated in depressive pathology, including serotonergic, noradrenergic, glutamatergic and GABAergic transmission. A broad aim of this thesis was therefore to provide insight into some of the mechanisms by which mfb-DBS influences these systems, with a focus on interactions of the dopaminergic system and its projections to the mPFC and NAc, and the effects of these mechanisms on depressive-like behaviours and sleep disturbances in the FSL model of depression.

7.1 The FSL as a model for depressive-like symptoms: implications for depression

The FSL was selected as a suitable model for investigating the effects of mfb-DBS, due to its reasonable face, predictive and construct validity (Belzung and Lemoine, 2011), including the reported presence of REM sleep disturbances (Shiromani *et al.*, 1988; Benca *et al.*, 1996). In investigating the effects of mfb-DBS, attention was paid to the baseline phenotype of the FSL, in order to fully characterise the model and its response to treatment. In doing so, we describe previously unreported SWS

deficits in the model, and provide supporting evidence for a recently reported anhedonic-like phenotype, previously thought to be absent in the FSL.

Slow wave sleep deficits in the FSL

REM sleep disturbances – decreased latency to and greater time spent in REM sleep – are the most strongly associated sleep disturbance with depression (Baglioni *et al.*, 2016), while the consistency of alterations to the time spent in SWS has been debated (Benca *et al.*, 1992; Pillai, Kalmbach and Ciesla, 2011; Baglioni *et al.*, 2016). SWA during SWS, a marker of sleep homeostasis, has been shown to be typically decreased during early sleep in depression (Borbély *et al.*, 1984, 2016). Increased REM, and its suppression by anti-depressant drugs, have therefore been the principle focus of much research into sleep and depression. SWS abnormalities have received less attention, despite having been proposed at times as a potential marker for genetic predisposition, symptom severity and treatment response (Ehlers, Havstad and Kupfer, 1996; Nissen *et al.*, 2001; Pillai, Kalmbach and Ciesla, 2011). Our selection of the FSL as a suitable model to measure the sleep response to mfb-DBS was based on the presence of REM-related abnormalities, with no SWS deficits previously reported in the model (Shiromani *et al.*, 1988; Benca *et al.*, 1992). Replicating these elevated REM sleep reports, we also observed circadian-dependant changes to the architecture of SWS and a reduced pattern of SWA, with a shifted power spectrum to high frequency gamma range oscillations at points of high sleep pressure. These observations, in a model also displaying the more commonly-reported REM deficits, suggest a significance of SWS in depression which may be more widely overlooked. Recently, the action of ketamine on SWA has been reported as part of the anti-depressant mechanism (Duncan, Selter, *et al.*, 2013), building on previous work demonstrating the critical role of SWA in the rapid anti-depressant action of sleep deprivation (Nissen *et al.*, 2001; Landsness *et al.*, 2011). Demonstrating SWA deficits in the FSL, resembling the altered distribution across sleep in human patients, not only supports the face validity of the model, but suggests these deficits as a potential indicator of certain forms of endogenous depression, broadly supporting earlier suggestions that SWS abnormalities may be useful prodromal markers, perhaps indicative of treatment response and symptom severity (Ehlers,

Havstad and Kupfer, 1996; Pillai, Kalmbach and Ciesla, 2011; Duncan, Selter, *et al.*, 2013; Goldschmied *et al.*, 2019). As far as we are aware, SWA reduction has not been demonstrated in another rodent model. This may be due to lack of reporting, as several studies of sleep in the CMS model, for example, appear not to have investigated amplitude of ECoG activity (Cheeta *et al.*, 1997; Grønli *et al.*, 2004); in the WKY, on the other hand, SWA has been specifically reported as not different from controls (Dugovic *et al.*, 2000). This may suggest an aspect of the physiology of the FSL is directly related to SWA deficits, making it a prospective model for patients who specifically display SWS abnormalities.

The significance of SWS gamma oscillations

The SWS deficits observed in the FSL suggest underlying deficiencies relating to sleep homeostasis, potentially revealing disrupted mechanisms of plasticity. High amplitude early night SWA and its dissipation through the night is thought to reflect the normal function of necessary synaptic downscaling, with slow waves directly implicated in this process (Tononi and Cirelli, 2003, 2006). This process allows for normal synaptic adaptivity during wake. The relationship between SWA and plasticity is reciprocal; high SWA leads to more LTP in subsequent wake, and activity during wake leads to local increases in SWA (Huber, Tononi and Cirelli, 2007; Hanlon *et al.*, 2009). Reduced SWA activity in depression could therefore be the result of either a deficiency of synaptic formation and strengthening during wake, requiring a lower SWA response in sleep; or an impairment of the downscaling process in sleep, resulting in impaired plastic processes. Our data suggest that SWA reductions in the FSL are accompanied by the presence of irregularly powerful gamma oscillations, which increase with mounting sleep pressure in a manner expected of SWA. Gamma oscillations have been linked to the activity of GABAergic interneurons, particularly somatostatin-expressing (SST) populations (Fee, Banasr and Sibille, 2017). SST neurons have been linked to dendritic plasticity, and are crucial in maintaining the balance of inhibition which creates the up-state environment of slow waves, fundamental to SWS synaptic regulation (Niethard *et al.*, 2018).

High frequency oscillations (including the gamma range) have been previously identified as a disruptive element in insomniac sleep with and without depression (Perlis

et al., 2001), and suggested as an underlying substrate of poor sleep perception (Perlis *et al.*, 1997). We suggest that, further to this, these oscillations are an active factor in the dysregulation of homeostatic processes mediated during SWS, and linked to deficient SWA. This may be linked to aberrant activity of interneuron populations, connecting gamma abnormalities in sleep to those observed in other states via shared underlying substrate (Fitzgerald and Watson, 2018). Although a correlation with homeostatic drive is suggested by gamma oscillations rising with sleep pressure in our data, further investigations could examine the relationship of local activity during wake and subsequent gamma power; the relationship between this wake activity and SWA is a demonstrable feature of the synaptic homeostasis hypothesis, which we suggest may be subverted by aberrant gamma. The relationship between markers of plasticity at baseline and SWA/gamma may also prove interesting: elevated frontal SWA has been reported in young, female subjects with depression (Frey *et al.*, 2012; Plante *et al.*, 2013); in female FSL rats, BDNF expression was unexpectedly found to be elevated in the PFC, while male rats has comparable expression to controls (Angelucci *et al.*, 2000). In our data, SWA deficits and gamma elevation were not found in the PrL of our male FSL rats, despite these deficits being recorded in the frontal ECoG electrode and NAc. These instances of higher SWA may be related to hyperactivity during wake and sleep which have been observed in humans in prefrontal areas (Nofzinger *et al.*, 2005). This relationship between activity during wake, plasticity markers and gender relating to abnormal SWA/gamma may be worth exploring further, and again may provide useful insight into delineating different forms of depression. In particular the issue of sex is an aspect requiring further research, given the disparity in prevalence between men and women (World Health Organisation, 2017), and the bias towards using male animals in research (unfortunately continued in the current work) (Dalla *et al.*, 2010).

As a proposed marker of depression across various behavioural states, abnormal gamma during sleep may represent a further reflection of connected underlying dysfunction (Fitzgerald and Watson, 2018). The further exploration of the presence of these abnormalities in other depressive models and in human patients is an important next step in establishing their relevance to depression, their link with dysfunctional sleep homeostasis and other underlying mechanisms.

Anhedonic-like behaviour in the FSL

The second phenotype of note we observed in the FSL before treatment was anhedonic-like behaviour, as measured in the SCT. Although a core feature of depression, anhedonia is conceptually difficult to assess. The various aspects of reward-related behaviour which may be deficient, and thus lead to the diagnostic symptom of anhedonia, are not typically (or easily) assessed in human patients (Treadway and Zald, 2011). Anhedonia may therefore be the result of varying underlying causes in different patients, including aspects of appetite, hedonic and emotional impact, learning and memory (Der-Avakian and Markou, 2012). Anhedonia-like behaviours are measured in animal models using various paradigms which also reflect differing aspects of reward. The specific aspects of reward modelled by standard tests are debated, creating further complications in translating results to humans (Rizvi *et al.*, 2016; Scheggi, De Montis and Gambarana, 2018). The translation of pre-clinical elements of anhedonia to humans is therefore difficult, but categorising exactly what is measured is important for examining the different elements of these behaviours and their neural underpinnings (Treadway and Zald, 2011).

Sucrose consumption test as a measure of 'wanting'-related anhedonia

The most common measure of anhedonia in depression models is the SPT, in which animals are presented with a choice between water and a sweetened solution. Preference for the sweet solution as a percentage of total liquid intake is taken as a measure of hedonic behaviour, normally considered a measure of the 'liking' element of reward (Der-Avakian and Markou, 2012), and it has been suggested that the element of choice in such a test introduces decision making and behavioural reinforcement, and thus represents broader aspects of reward (Schneider, Heise and Spanagel, 2010). In terms of dopaminergic function, the most pertinent aspect of reward is motivational/incentive salience, or 'wanting' (Berridge and Robinson, 1998; Berridge, 2007; Berridge and Kringelbach, 2015). While preference between two solutions indicates a pleasurable association ('liking'), it has been argued that argues that the amount consumed is more related to motivational drive ('wanting') (Meyerolbersleben, Winter and Bernhardt, 2020): increased extracellular DA in the NAc has been linked to active consumption of food reward and to consumption

beyond physiological need (Hajnal and Norgren, 2001; McCutcheon and Roitman, 2019), but is not associated with a hedonic aspect (Berridge and Kringelbach, 2015). Investigating this difference, Meyerolbersleben and colleagues (2020) showed that moderate reduction in mesolimbic DA led to decreased consumption but no change in preference of sweet solution. The SCT, used here, is therefore proposed as more sensitive to the 'wanting' aspect of reward than the SPT. Motivational salience in this respect is increased by a short period of food restriction, while the acute test duration and single-bottle paradigm reduce aspects of behavioural reinforcement, reward learning and decision making (Enkel *et al.*, 2010; Schneider, Heise and Spanagel, 2010).

The role of DA in anhedonic-like behaviour in the FSL

A reduced 'wanting' behaviour, which can be influenced by diminished DA transmission (Meyerolbersleben, Winter and Bernhardt, 2020), is coherent with reported mesolimbic dopaminergic abnormalities in the FSL (Zangen, Overstreet and Yadid, 1999; Friedman *et al.*, 2007, 2012; Friedman, Friedman, *et al.*, 2008). 'Liking' behaviour and strictly hedonic aspects of reward are not thought to be mediated by DA, and are more likely related to μ-opioid receptor and endocannabinoid-mediated transmission (Der-Avakian and Markou, 2012; Berridge and Kringelbach, 2013). CMS rats have previously been shown to exhibit similar mesolimbic dopaminergic deficits to the FSL (Chang and Grace, 2014; Moreines, Owrutsky and Grace, 2017); a protocol similar to SCT, of food restriction followed by limited-time calorific-food availability, the Froot Loops® consumption assay, recently demonstrated anhedonic-like phenotype after CMS in healthy rats (Dandekar *et al.*, 2019). Taken together, this suggests anhedonic-like behaviour related to motivational salience is present in the FSL and can be induced by CMS in non-depressive-like rats. The work of Meyerolbersleben and colleagues (2020) suggests that these deficits are mediated in part by dopaminergic dysfunction. However, these behaviours are also modulated by various other systems implicated in depression, including GABAergic transmission, glutamatergic projections from the mPFC to NAc, the activity of the ACC and amygdala amongst others (Der-Avakian and Markou, 2012; Knowland and Lim, 2018). While a clear delineation between motivational salience and consummatory

reward processing in the SCT and SPT in a matter of debate, there does appear to be a difference in the behaviour of the FSL between the two tests. Alterations to 'liking' behaviour are not normally present in the FSL or control rats, but can be induced in both by CMS (Willner, Muscat and Papp, 1992; Pucilowski *et al.*, 1993; Dandekar *et al.*, 2019). This may suggest differences in anhedonic states – and their underlying substrates – between endogenous or prodromal depressive symptoms, and those more directly linked to stress. The variation in these states may also provide nuance in predicting treatment response, as has been suggested for anhedonic behaviours in patients (Treadway and Zald, 2011; Vrieze *et al.*, 2013). Given the reported interaction and crossover between systems modulating reward (Goldstein Ferber *et al.*, 2021), further investigations into the relationship between the development of the various aspects of anhedonia in different models is required to give insight into differential presentations of depression.

Face validity of the anhedonic-like phenotype in the FSL

As noted by Rea and colleagues (2014), the mild stressors of laboratory handling (shown to affect sucrose intake in the SPT (Clarkson *et al.*, 2018)) and mild food restriction may be enough to induce the phenotype in the stress-sensitive FSL, rather than the phenotype being truly 'spontaneous'. The same study saw anhedonic-like behaviour in control animals, the related Flinders Resistant Line (FRL) strain (Rea *et al.*, 2014). Although this may suggest stress-induced anhedonia, the phenotype was not seen in controls when the Sprague Dawley rat was used (Edemann-Callesen *et al.*, 2015). This may suggest that, while diverging from the FSL in some phenotypes, the FRL shares some susceptibility to mild stress. Our data replicates the difference seen by Edemann-Callesen and colleagues between the FSL and Sprague Dawley controls. Whether this difference is an inherent behaviour in the FSL, or related to exaggerated stress vulnerability, remains uncertain. Our animals were single housed, a mild stressor which has shown to produce reduced NAc DRD2 expression in the FSL (Bjørnebekk, Mathé and Brené, 2007). However, the possibility of mild stress-induction would not necessarily suggest equivalence with the phenotype observed in the SPT after chronic stress (Pucilowski *et al.*, 1993), due to the large difference in magnitude of the stress protocol, and the presence of various

mild lab stressors, such as handling, in studies which produced null findings in non-CMS FSL rats (Thiele *et al.*, 2016). Neither would this take away from the face validity of this anhedonic-like phenotype, as human depression is episodic and frequently precipitated by stress; the vulnerability of the FSL to mild stress-induced behaviour compared to controls would suggest validity as a model of depressive predisposition (Overstreet and Wegener, 2013).

Our replication of previous results suggests reasonable validity of the SCT paradigm. In the context of these recent works, the results presented here suggest an anhedonic phenotype in the FSL different (at least in part) from that induced by chronic stress measured in the SPT. We suggest that the phenotype present without chronic stress is related to motivational salience, and to some degree to dopaminergic dysfunction reported to be present in the model. This phenotype may be spontaneously occurring or induced by mild stress, reflecting either the inherent hypo-functionality or stress-sensitive nature of the mesolimbic dopaminergic system in the FSL.

Summary

Our results suggest the presence of two important features of depression in the FSL model. We have demonstrated circadian-related SWS architecture changes and reduced SWA, abnormalities which reflect observations in human patients and may reflect deficient mechanisms of plasticity. These abnormalities add to growing evidence suggesting the particular relevance of SWS in depression. Highlighting elevated gamma oscillations during SWS – which may be a marker or causative agent in disturbed sleep, as well as a marker of wider dysfunction in depression – our results highlight a relatively unremarked-upon feature of SWS dysfunction.

Further, we support recent reports suggesting the presence of an anhedonia-like phenotype in the FSL in the absence of chronic stress. This phenotype may represent a distinct mechanism to the established, chronic stress-induced phenotype previously reported, and may be related to dopaminergic dysfunction suggested in the model. Together, these results suggest a stronger face validity of the FSL than previously appreciated, and provide signposts for further research, to investigate these phenotypes and their relevance to human depression.

7.2 Effects and potential mechanisms of mfb-DBS

Relating to previously established and experimentally demonstrated phenotypes of the FSL, we investigated the anti-depressant effects of mfb-DBS on behaviour and sleep, and measured physiological changes utilising methods of electrophysiology, neurochemical and molecular assessment. In terms of behaviour, we found 24h mfb-DBS capable of alleviating a depressive-like phenotype in the FST and an anhedonic-like phenotype in the SCT. mfb-DBS altered aspects of physiology within SWS, without having an effect on the overall architecture of sleep. Finally, we demonstrated several modulations of monoaminergic transmission after mfb-DBS in the FSL, which may provide insight into the treatments mechanisms of action. In control animals, mfb-DBS had differential effects, highlighting the specific interaction with depressive dysfunction after mfb-DBS.

The anti-depressant effects of mfb-DBS on behaviour

24h mfb-DBS showed an anti-depressant-like effect in the FST and anhedonic-like effect in the SCT. In the FST, this effect has been previously demonstrated after acute-intermittent applications, in which DBS is applied the day before and then the day of behavioural testing for various durations (8h followed by 4h before testing (Dandekar *et al.*, 2017); 4h followed by 2h (Bregman *et al.*, 2015); 2x 30m followed by 15m, with DBS continuing into behavioural tests (Edemann-Callesen *et al.*, 2015)), as well as after chronic-intermittent (8h per day for 7 days (Dandekar *et al.*, 2019)) and chronic-continuous (10 and 21 days (Furlanetti, Coenen and Döbrössy, 2016; Thiele *et al.*, 2018, 2020). While these results clearly demonstrated the anti-depressant effects of mfb-DBS, acute-intermittent and even chronic-intermittent applications are not necessarily representative of clinical applications. Our results demonstrate, as predicted by these previous works, the effectiveness of continuous application of mfb-DBS after 24h. This may be a reasonable reflection of the rapid response (within days) after onset of chronic stimulation in humans (Schlaepfer *et al.*, 2013).

The use of the FSL and the question of treatment resistance

As in previous pre-clinical studies of mfb-DBS (aside from Edemann-Callesen et al., 2015), behavioural response was observed after cessation of stimulation. In chapter 4, the FST was conducted at day 9 after stimulation, and in chapter 5 conducted at day 3 after stimulation; the SCT was conducted at one day post-DBS. This suggests the effects of mfb-DBS are sustained at least over several days in the FSL. This may contrast with the clinical situation; after discontinuation of slMFB-DBS, symptoms are reported to reappear in patients within hours or days (Kilian *et al.*, 2019). This raises some questions about the predictive validity of anti-depressant effects of mfb-DBS observed in the FSL. This sustained effect may be linked to physiological differences between rodents and humans, or may be linked to the severity and resistant nature of the underlying pathology. Human patients involved in trials of slMFB-DBS necessarily suffer from severe, treatment-resistant forms of the disease (Schlaepfer *et al.*, 2013; Coenen *et al.*, 2019; Kilian *et al.*, 2019). The FSL as a model of depressive symptoms is generally responsive to treatment (Overstreet and Wegener, 2013), and severity of the condition in individual animals can only be measured via the flawed proxy of behavioural tests; how this relates to severity of human depression is uncertain. Investigating whether this discrepancy is related to treatment resistance thus requires a model of TRD. The question of a treatment resistant model can be considered at odds with most established animal models of depression, as responsiveness to treatment represents one of the key criteria of validity as proposed by Willner (1984). Recently addressing this issue, Willner and Belzung (2015) proposed that a model of TRD would require a validated model of depression, which did not respond to conventional anti-depressant drugs, but was responsive to novel anti-depressants such as ketamine and DBS, which have shown efficacy in human patients with TRD. The combination of the CMS procedure in the WKY rat has recently been demonstrated as a potential candidate, resistant to the effects of citalopram, imipramine and venlafaxine but responding to ketamine and vmPFC-DBS (Willner *et al.*, 2019). Some questions remain about this model, such as its apparent lack of anhedonic-like phenotype, which may represent an important factor in TRD (Nestler and Carlezon, 2006; Alloy *et al.*, 2016); furthermore, a lack of clear clinical definition of TRD and understanding of how it may be

mechanistically separate from treatable depression make construct validity hard to assess (Bartova *et al.*, 2019). Despite this, the CMS-WKY may represent a useful tool in terms of further investigating the effects of mfb-DBS. In terms of the presented work, in the absence of a fully validated model of treatment resistance, we believe the FSL to represent an adequate model to assess mechanisms of mfb-DBS due to its reasonable validity as a model of vulnerability and endogenous depression. Willner and Belzung (2015), in discussing how best to model TRD, argue in favour of moving away from stress-based models towards models of risk factors and vulnerability. The FSL, while clearly imperfect due to its responsiveness to conventional treatment, may therefore represent a reasonably valid model in terms of predisposition, in the absence of a validated model of TRD. However, as we have demonstrated, the physiological response of non-depressive-like and depressive-like rats can significantly vary; it would be prudent to compare the responses to mfb-DBS of the FSL and other standard models of depression with the CMS-WKY and other putative models of TRD in order to explore potential responsive differences. This would serve to test the usefulness of standard models in examining mfb-DBS mechanisms, while also potentially providing insight into mechanisms of treatment resistance.

Modulation of DA by mfb-DBS

A key theoretical substrate of mfb-DBS effect is its modulation of dopaminergic systems, specifically the ascending pathways from the VTA which form a component of the MFB. These pathways are implicated in depression, in particular symptoms of anhedonia and amotivation (Russo and Nestler, 2013; Fox and Lobo, 2019), in the response to conventional anti-depressants (Muscat, Sampson and Willner, 1990; Muscat, Papp and Willner, 1992; Willner, Hale and Argyropoulos, 2005) and to novel, fast-acting treatments such as ketamine (Belujon and Grace, 2014; Kokkinou, Ashok and Howes, 2018). Previous pre-clinical work has supported a role of DA modulation in the response to mfb-DBS, presenting an early picture of acute and longer-term response. The acute response (<1m) to mfb-DBS has been demonstrated to include the release of DA into the extracellular space in the NAc (Klanker *et al.*, 2017); in response to a 5s stimulation period, this response was of greater

magnitude and duration in the FSL than in control animals (Ashouri Vajari *et al.*, 2020). At an increased timescale, measuring at 30m intervals over 2h of mfb-DBS, Bregman and colleagues (2015) recorded no release of DA or 5-HT in the NAc. Investigations of endpoint effects have suggested long-term modifications to dopaminergic systems, including the upregulation of DRD2s in the PFC of non-depressive-like rats after acute-intermittent mfb-DBS (Dandekar *et al.*, 2017), and upregulation of NAc DRD1 and DRD2s in FSL rats under the challenge of DRD2 antagonist raclopride after 10 day chronic-continuous mfb-DBS (Thiele *et al.*, 2020). These studies describe both direct modulation of DA release and changes to receptor profiles which may be a direct result of stimulation or a compensatory response, suggestive of considerable interaction between mfb-DBS and dopaminergic transmission. Building on these works, our results add to the developing picture of dopaminergic response to mfb-DBS in the FSL, showing differential modulation in the NAc and mPFC, including changes in tissue concentration and site-specific alterations to receptor profiles.

Modulation of the NAc

After 24h, our data suggest an inhibition of synthesis of DA in the NAc, indicated by lower tissue concentration and possibly lower TH expression, accompanied by a reduction in the expression of DRD2. Inhibition of DA synthesis may seem counter-intuitive as a feature of anti-depressant response, given the hypothesised hypofunction of the dopaminergic systems; however, the FSL has been shown to exhibit elevated tissue concentration in the NAc, possibly from disinhibited synthesis caused by lack of DA release into extracellular space (Zangen, Overstreet and Yadid, 1999; Zangen *et al.*, 2001; Floresco *et al.*, 2003; Friedman *et al.*, 2007). DA synthesis is not necessarily followed by release (Berry *et al.*, 2018), with the movement of DA into the extracellular space regulated not only by the activity of pre-synaptic DRD2s, but also the activity of post-synaptic DRD2s, likely mediated through Glu, GABA or endocannabinoid signalling (Anzalone *et al.*, 2012), and 5-HT via the 5-HT2C receptor (Dremencov *et al.*, 2011). Previous work has suggested reduction in overall tissue content may be part of a response which concurrently involves increased extracellular release (Zangen, Overstreet and Yadid, 1999; Zangen *et al.*,

2001; Friedman *et al.*, 2007). Our data is also coherent with increased extracellular release and DRD2 agonism, with subsequent inhibition of synthesis and down-regulation of the DRD2 (Dunlop and Nemeroff, 2007). Activation of DRD2s has been linked to increased motivational drive (Trifilieff *et al.*, 2013; Gallo *et al.*, 2018) and anti-depressant response (Tye *et al.*, 2013), suggesting increased agonism may play a part in the behavioural response seen in our animals. Alternatively, a reduction in DRD2 expression may be a more direct consequence of mfb-DBS. DBS of the STN has been shown capable of modulating NAc DA receptor expression in DA depleted animals (Carcenac *et al.*, 2015), while mfb-DBS affected expression after DRD2 blockade (Thiele *et al.*, 2020); these results suggest mechanisms of receptor regulation independent of DA activity. A downregulation of NAc DRD2s would be contrary to the demonstrated effects of chronic treatment with some conventional anti-depressants, which have been shown to increase NAc DRD2 expression (Dziedzicka-Wasylewska, Willner and Papp, 1997; Ainsworth *et al.*, 1998). This may suggest alternate mechanisms of achieving anti-depressant response. One such mechanism may be suggested by the proposed action of anti-psychotic medications, typically DRD2 antagonists which can augment anti-depressant response; it has been suggested that their action may lead to homeostatic upregulation of dopaminergic function via compensatory mechanisms (Moreines *et al.*, 2017). The DRD2 downregulation seen after 24h mfb-DBS may therefore be part of a wider dynamic response, perhaps inducing changes in activity across the mesocorticolimbic network. For instance, decreasing DRD2 stimulation in the NAc is thought to potentiate the response to inputs from the mPFC (Goto and Grace, 2005; Goto, Otani and Grace, 2007). Direct mfb-DBS downregulation of NAc DRD2 activity may therefore facilitate the top-down regulation of the NAc by the mPFC.

Modulation of the mPFC

Changes to dopaminergic function are also apparent in the mPFC itself. Here, 24h mfb-DBS resulted in elevated expression of the DRD1, while changes in metabolism suggest an increase in extracellular DA availability. Together, these combined effects may lead to increased activation of DRD1s, which has been previously associated with anti-depressant effect and stress resilience (Scornaiencki *et al.*, 2009; Shino-

hara *et al.*, 2018; Hare *et al.*, 2019). DRD1s can be differentially activated by weak and strong agonism, leading to the suggestion of an 'optimal' DRD1 tone (Seamans and Yang, 2004; Floresco, 2013); it is possible in this regard that mfb-DBS modulation helps establish an environment for efficient activation of mPFC DRD1s. The DRD1 has been shown to regulate the monoaminergic stress response of the NAc, mediated via DRD2-expressing neurons of the NAc (Doherty and Gratton, 1996; Stevenson and Gratton, 2003; Goto, Otani and Grace, 2007); this may suggest a functional link between changes in DRD1 activity in the mPFC and DRD2 expression in the NAc after mfb-DBS. The activity at DRD1s is also strongly linked regulatory mechanisms of plasticity (Wolf, Mangiavacchi and Sun, 2003; Huang *et al.*, 2004; Iasevoli *et al.*, 2014); the anti-depressant effects have been linked to increased expression of synaptogenesis-related proteins, which may also be linked to stress resilience (B. Zhang *et al.*, 2017; Shinohara *et al.*, 2018). Alongside DRD1 upregulation, mfb-DBS resulted in increased expression of the mRNA encoding H1a and Arc proteins in the mPFC. Increased expression of these proteins is linked to improved regulation of Glu-dependant plasticity. Arc is critical for regulating synaptic strength, dendritic remodelling and controlling multiple mechanisms of adaptive plasticity, in part by influencing the activity of Glu AMPARs (Shepherd *et al.*, 2006; Bramham *et al.*, 2008; Nikolaienko *et al.*, 2018); H1a mediates synaptic reorganisation of Glu NMDARs and metabotropic classes of receptors (Szumlinski, Kalivas and Worley, 2006). Induction of these proteins is a likely route to improved regulation of plasticity-related functions, and both have been identified as part of anti-depressant response (Pei *et al.*, 2003; Serchov *et al.*, 2015). Reciprocal regulation between DRD1s and plastic mechanisms including the expression of H1a (Huang *et al.*, 2004; Ghasemzadeh *et al.*, 2009; Datko *et al.*, 2017) suggest the upregulation of DRD1s and the enhanced regulation of plasticity may be concurrent, although it is possible one specifically leads the other after mfb-DBS. It has been suggested that the final common action of all anti-depressants is the stabilisation of the plastic environment in vulnerable areas such as the mPFC, restoring inadequate mechanisms and preventing aberrant adaptive responses (Rincón-Cortés and Grace, 2020). In this context, it may be speculated that dopaminergic modulation is the foundation of subsequent plastic changes. However, as dopaminergic modula-

tion after mfb-DBS is theorised to be indirect, secondary to the activation of glutamatergic projections between frontal areas and the VTA (Ikemoto, 2010; Schlaepfer *et al.*, 2013), it is also feasible that changes to glutamatergic transmission directly influence these activity-dependant mechanisms of plasticity. Even if this is the case, dopaminergic systems may still play a critical role: it has been demonstrated that the anti-depressant effects of ketamine, which directly modulates Glu transmission via NMDARs, can be blocked by the antagonism of either DRD1 or DRD2s (Li, Zhu, *et al.*, 2015b; Hare *et al.*, 2019). Again, the state observed after 24h of mfb-DBS is a snapshot of a presumably dynamic response which may evolve further with continued stimulation. Arc and H1a are both short-lived proteins behaving as immediate early genes (de Bartolomeis and Iasevoli, 2003; Rao *et al.*, 2006), which suggests continuous induction in response to stimulation. Investigation into the expression of these proteins after cessation of stimulation (and the correlation with behavioural response) could provide insight into the regulation as part of anti-depressant response. If H1a and Arc are continually upregulated after cessation of DBS, it may suggest that these plastic changes are secondary to modulation of other systems; if their upregulation is a primary effect of mfb-DBS, it may be expected that their expression returns to a baseline level in the absence of stimulation.

Sleep and plasticity after mfb-DBS

The upregulation of Arc and H1a molecules after 24h mfb-DBS suggests possible substrate for increased SWA during SWS. Both Arc and Homer genes are specifically upregulated during wake, and are thought to be related to the synaptic activity which is dependant on – and subsequently influences – SWA (Cirelli, Gutierrez and Tononi, 2004). The critical role of Arc in regulating synaptic strength provides a direct theoretical link, suggesting increased Arc-mediated synaptic potentiation could lead to increased SWA during subsequent sleep. Indeed, reduction of LTP-related molecules including Arc has been shown to blunt SWA (Cirelli and Tononi, 2000; Cirelli, Gutierrez and Tononi, 2004; Cirelli *et al.*, 2005). However, despite this upregulation of Arc and H1a, we did not observe potentiation of SWA during subsequent sleep. The reduction of SWS gamma – which was abnormally elevated in the FSL at baseline, following a distribution that would normally be associated with SWA -

after mfb-DBS may be indicative of changes to the activity of interneuron populations, affecting the e/i balance (Fee, Banasr and Sibille, 2017). This balance is crucial for the slow wave up-state environment during which synaptic downscaling is thought to take place (Niethard *et al.*, 2018); the normalisation of gamma activity may therefore represent a beneficial change, tuning the environment to allow these homeostatic processes. However, this would be expected to be accompanied by a concurrent increase in SWA.

Ketamine has been shown to concurrently increase SWA and BDNF expression (Duncan, Sarasso, *et al.*, 2013), while sleep deprivation's anti-depressant effects are associated with the level of SWA rebound in subsequent sleep (Ehlers, Havstad and Kupfer, 1996; Nissen *et al.*, 2001; Duncan, Selter, *et al.*, 2013). Ketamine is also known to potentiate gamma oscillations in rodents (Hunt, Raynaud and Garcia, 2006; Hakami *et al.*, 2009) and humans (Muthukumaraswamy *et al.*, 2015; Shaw *et al.*, 2015). However, these investigations have been conducted during wake, and gamma oscillations, including aberrant activity reported in depression, are highly state-dependant (Fitzgerald and Watson, 2018). As far as we are aware, although elevated SWS gamma has been previously identified as a marker of disrupted SWS in depressed insomniacs (Perlis *et al.*, 2001), gamma has not been investigated during SWS in relation to ketamine or sleep deprivation response. Therefore, whether a reduction in gamma alone contributes, or indeed is sufficient to improve the efficiency of synaptic downscaling during sleep and thus enhance plasticity remains to be established. The increased expression of Arc and H1a may suggest a role in the absence of SWA changes, but the relationship will require deeper investigation. Investigations on the effects of ketamine and sleep deprivation in the FSL could provide comparisons to the effects of mfb-DBS on SWA and the expression of plasticity-related molecules. It is once again pertinent to consider the potential evolution of response to mfb-DBS beyond 24h of stimulation. Although Arc and H1a were shown to be upregulated after 24h, it is unknown whether this effect is potentiated further with continuing stimulation. Theoretically, if response continues to elevate with more chronic stimulation – as is applied in the clinical setting – an effect on SWA may be expected to emerge. Pursuing this line of investigation would provide greater insight into the relationship between the plasticity mediated by mfb-DBS

and its effects sleep, in particular the role of SWS gamma changes observed here. Further investigation should further explore whether sleep architecture remains unchanged after extended continuous stimulation, or if this is similarly a consequence of the relatively short duration of mfb-DBS applied here.

Finally, a missing piece of this jigsaw is an investigation into the effects of clinical slMFB-DBS on sleep. Anecdotal reports suggest a subjective improvement in sleep in patients receiving slMFB-DBS (private correspondence, FORSEE trial team); as subjective perception of sleep has been linked to high frequency oscillations during SWS (Perlis *et al.*, 1997, 2001), it would be of interest to examine if the reduction in gamma power observed in the FSL is replicated in patients and corresponds with changes in sleep perception. As EEG studies into sleep are one of relatively few opportunities to non-invasively monitor markers of depressive response, the comparison of responses between human patients and the results reported here would provide a valuable measure of the validity of pre-clinical work into the actions of mfb-DBS.

Summary

Our results provide further insight into dopaminergic modulation after mfb-DBS, supporting the theory that such changes may play an influential role in the response to treatment. However, the precise dynamics of this response remain uncertain. Whether modulations seen at 24h are fundamental to anti-depressant response, or whether they represent a step during polyphasic response which develops further after extended stimulation is unknown. It should be noted that anti-depressant behavioural effects were seen at this point, which suggests the physiological changes observed may be representative of an anti-depressant effect, though this does not discount the further evolution of the response. It is possible that the crucial facet of anti-depressant response to mfb-DBS is the disruption of pathological activity; forcing changes in network activity that interrupt or 'reset' dysfunctional processes. Florence et al. (2016) describe tissue affected by DBS as typically reaching a 'new dynamic equilibrium'. This is suggested in our data by changes to receptor profiles, tissue concentration, electrophysiological activity and markers of synaptic plasticity. The correspondence of these changes to anti-depressant-like behavioural response

suggests that whether or not these changes are representative of the final equilibrium reached after mfb-DBS, they are likely involved in the observed anti-depressant response, and may be indicative of mechanisms more generally.

Limitations

The principal limitations of the work conducted in this study are the length of treatment and the relevance of the model used. As discussed, pre-clinical investigations of mfb-DBS have utilised various paradigms, including intermittent and chronic applications. This variation in the literature, alongside limitations of various techniques in terms of the ability to monitor a dynamic response across a significant period of time, present a challenge in truly representing the clinical application of slMFB-DBS in a pre-clinical setting. Compounding this, animal models of depressive disorders, in particular the treatment resistant elements, are imperfect. The difference observed in the physiological response of non-depressive-like animals and our model, the FSL, highlight the importance of using a biologically relevant system when considering the effects of mfb-DBS. These caveats must be borne in mind when interpreting the results presented in this thesis. However, when interpreted with caution, we believe the results still hold value in providing insights in to potential mechanisms and pathways of treatment response in depression.

7.3 Conclusions and outlook

In relation to the aims of this work, the results presented in this thesis provide insight into the two proposed areas of investigation. We have presented here evidence of the effects of mfb-DBS on sleep symptoms in a model of depressive-like symptoms. After demonstrating physiological abnormalities during SWS, we observed that mfb-DBS modulated this aspect of sleep in a manner we propose may be beneficial to regular sleep. We found that mfb-DBS appears to have no effect on the architecture of sleep, a detail which may be pertinent to managing the treatment of patients. Further, we provide supporting evidence relating to two main theories of the mechanisms of mfb-DBS. Firstly, we have shown modulation of dopaminergic systems, expanding on previous work suggesting interaction with mesolimbic and

mesocortical circuitry as a medium for anti-depressant response. Secondly, we have demonstrated an influence of mfb-DBS on markers of the regulation of synaptic plasticity. We propose that the interaction between these two mechanisms may be the foundation of anti-depressant response after mfb-DBS, and is likely relevant to the clinical application of slMFB-DBS.

The complex mechanisms and outcomes of mfb-DBS and its clinical counterpart remain to be fully elucidated. The presented work has provided insights into behavioural response and potential physiological mechanisms relating to this promising but incompletely understood treatment; it provides a framework relating to sleep, plasticity and dopaminergic function, on which future investigations can build.

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Medial forebrain bundle deep brain stimulation in a rodent model of depression: modulation of sleep abnormalities and mechanisms of anti-depressant effects

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Résumé

La dépression est un état complexe dont les symptômes variés comprennent des troubles du sommeil, pour lequel les traitements conventionnels sont inadéquats pour une minorité importante de patients. La stimulation cérébrale profonde (DBS) du faisceau médian du cerveau antérieur (MFB) représente un traitement prometteur pour ces patients. L'objectif de cette thèse était d'étudier les effets de la MFB-DBS sur le sommeil et la physiologie, en utilisant des méthodes comportementales, électrophysiologiques, moléculaires et neurochimiques chez le FSL rat modèle de dépression. Après avoir identifié des déficits du sommeil lent dans ce modèle, nos résultats suggèrent des modulations des oscillations gamma pendant le sommeil, la transmission dopaminergique et la plasticité neuronale comme mécanismes thérapeutiques potentiels de la MFB-DBS. En explorant ces effets, ce travail fournit un aperçu de ce traitement prometteur et élargit notre compréhension générale des mécanismes antidépresseurs.

Mots clés : Dépression ; faisceau médian du cerveau antérieur ; stimulation cérébrale profonde ; dopamine ; sommeil ; oscillations gamma

Résumé en anglais

Depression is an incompletely understood mental health condition with a range of symptoms including sleep disturbances, for which conventional treatments are inadequate for a substantial minority of patients. Deep brain stimulation (DBS) of the medial forebrain bundle (MFB) represents a promising new treatment modality for these patients. The aim of this thesis was to investigate the effects of 24h MFB-DBS in terms of sleep and physiology, utilising behavioural, electrophysiological, molecular and neurochemical methods in the Flinders Sensitive Line rat model of depression. After identifying previously unreported deficits in slow wave sleep in the model, our results suggested changes to gamma oscillations during sleep, modulation of dopaminergic transmission and plastic mechanisms as potential therapeutic mechanisms of MFB-DBS. By exploring these effects, this work provides insight into this promising treatment and broadens our general understanding of anti-depressant mechanisms.

Keywords: Depression; medial forebrain bundle; deep brain stimulation; dopamine; sleep; gamma oscillations.