Effect of Ketamine Treatment on Neuronal Oscillatory Activity in the Nucleus Accumbens and Hippocampus in an Animal Model of Treatment-Resistant Depression.

by

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ABSTRACT

EFFECT OF KETAMINE TREATMENT ON NEURONAL OSCILLATORY ACTIVITY IN THE NUCLEUS ACCUMBENS AND HIPPOCAMPUS IN AN ANIMAL MODEL OF TREATMENT-RESISTANT DEPRESSION.

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Depression is a globally prevalent disorder characterized by negative symptoms including amotivation and suicidality. Ketamine is a treatment that can provide rapid, long-lasting antidepressant effects, but also exhibits harsh psychomimetic side effects that greatly hinder its accessibility and tolerability. Macroscopic neuronal oscillations are essential for communication within the brain and are dysregulated in several neuropsychiatric disorders including depression. This study therefore used the Wistar-Kyoto (WKY) animal model of treatment resistant depression to examine the effects of ketamine on neural oscillatory activity in two brain regions implicated in the pathophysiology of depression, the nucleus accumbens (NAc) and hippocampus (HIP). At baseline, WKY males showed abnormalities in delta and theta power in the NAc and HIP, but not NAc-HIP coherence. Female WKY rats showed similar abnormalities in HIP, but not NAc, and increased NAc-HIP theta coherence. Ketamine administration normalized the dysfunction in NAc of males and theta coherence between NAc-HIP in females.
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LIST OF ABBREVIATIONS

TRD – Treatment-resistant depression
NMDA(R) – N-methyl-D-aspartate (receptor)
WKY – Wistar-Kyoto
WIS – Wistar
HPA – Hypothalamic-pituitary-adrenal
BDNF – Brain derived neurotrophic factor
NAc – Nucleus accumbens
HIP – Hippocampus
PFC – Pre-frontal cortex
EEG – Electroencephalogram
DBS – Deep-brain stimulation
LFP – Local field potential
Dnmt3a – DNA methyltransferase 3 alpha
GABA – Gamma-aminobutyric acid
1 Introduction

1.1 Depression

Depression is a severe neuropsychiatric disorder with a lifetime prevalence of approximately 20%, and which manifests with a variety of negative symptoms including a depressed mood, fatigue, and anhedonia, or the inability to experience pleasure (1). Depression’s burden to society has been well documented, costing Canada over thirty billion dollars per year (2). This includes both personal and work related costs, however the decrease in work productivity and performance is the major contributor (2). Despite its profound negative impact and a large body of existing research examining the pathogenesis of the disorder, the cellular mechanisms underlying depression are not fully understood (3). Early research has implicated monoamine neurotransmitters such as dopamine, norepinephrine, and serotonin as being crucial to depression, and this is supported by evidence showing some therapeutic effects of monoamine oxidase inhibitors and serotonin and norepinephrine re-uptake inhibitors (3, 4). However, it appears to be more complex as it has also been demonstrated that while further reducing monoamine levels in individuals with depression exacerbated symptoms, it did not alter mood in healthy individuals, implying additional mechanisms that may be independent of neurotransmitter levels (3). Furthermore, these antidepressant often take weeks for symptom relief, have undesirable side effects, and the relapse rates are quite high (3, 5, 6). A significant proportion of patients also do not respond to antidepressant treatments (7). As a result, the focus of research has slowly shifted away from monoamines in order to examine other potential neuropathological mechanisms (3).

The etiology of depression is further complicated by notable sex differences in both prevalence and symptom expression. Women are twice as likely to experience depression as men,
but the reasons are poorly understood (8). However, evidence suggests this susceptibility may be linked to biological differences within the endocrine system, particularly in sex hormones (8). In women, depression is more likely to be coupled with anxiety symptoms as well as other adverse effects such as weight gain (8). It is also likely that innate differences in stress sensitivity between men and women, with women exhibiting increased stress responsivity, lead to increased female risk (9). Unfortunately, the majority of current clinical and preclinical studies either selectively utilizes males, or in those studies that use both sexes data are pooled without evaluating sex as a biological variable. This has unfortunately resulted in a significant knowledge gap in the mechanistic understanding of enhanced female depression susceptibility.

1.1.1 Treatment-Resistant Depression

The recovery rates of persons with depression that received antidepressant treatment are low, approximately 70% of affected individuals, and thus there remains a significant portion of individuals who receive no relief from the disorder. Despite the heterogeneity between patients with depression, attempts have been made to classify and group specific subsets of patients. The general consensus is that when a patient fails to respond to at least one trial of antidepressants of normally sufficient dose and length, their disorder is termed treatment-resistant depression (TRD) (7, 10).

1.1.2 Ketamine as an Antidepressant

Ketamine is a drug that was most commonly used in veterinary medicine as an anaesthetic, but more recently it was shown that, when administered in low doses, it exhibits unique antidepressant effects (11). Unlike traditional antidepressants, ketamine is fast-acting and is highly effective in treating TRD, with antidepressant responses lasting from 1 to 3 weeks depending on
the dose and number of administrations (11). Unfortunately, the drug has significant limitations as a result of its numerous side effects, which include cognitive impairments, hemodynamic changes, psychomimetic symptoms, and the induction of schizophrenia-like behaviours (11). Importantly, these dissociative side effects last up to 2 hours after ketamine administration (11). The drug is known to act as a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist (11).

However, a meta-analysis of ketamine and a variety of glutamate receptor modulators, including other NMDA antagonists, revealed that ketamine uniquely displayed significant clinical value when treating patients with TRD (12). This indicates that ketamine exhibits other mechanistic properties, currently unknown, that contribute to its antidepressant effects. Therefore, to elucidate these mechanisms, many studies look to interpret how ketamine affects regions of the brain implicated in depression.

1.2 Neural Circuitry of Depression

Studies have been unable to find a cause-and-effect relationship between depression and a single brain region. Indeed, many brain regions have been implicated to varying degrees in the limbic and cortical areas, but the neural circuitry amongst them is still poorly understood (13). Two regions which have been shown to have important relationships to depression as well as the mediating effects of antidepressants are the nucleus accumbens (NAc), and the hippocampus (HIP) (13–15) (Figure 1.1).
**Figure 1.1:** Sagittal section through the center of the adult human brain showing the general location of the regions of interest in depression; nucleus accumbens, and hippocampus. Adapted from Duvernoy, H.M. 1999 (16).
1.2.1 Hippocampus

The HIP is a structure in the limbic region of the brain responsible for facilitating learning and memory. From a structural standpoint, the HIP has been linked to depression with depressed persons exhibiting decreased HIP size, which was shown to correlate with depressive episode duration (17). For this reason, the reduced size is hypothesized to be a consequence of the disorder’s symptoms, with size returning to normal in remitted patients, rather than a predictor of the severity of the disorder (18, 19). This is supported by the fact that one of depression’s largest effects on cognition involve impairments to encoding and retrieval of episodic memory, functions highly dependent on the HIP (20).

The HIP is also an area of the brain which takes part in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis (21). Prolonged activation of the HPA axis is seen in depression (21, 22). If the HPA axis is over stimulated, there is a consequential increase in glucocorticoid production (19, 23). In rodents, increasing glucocorticoid levels results in reduced neurogenesis and dendritic branching in the HIP (24, 25). Furthermore, chronic administration of a selection of antidepressants increases neurogenesis in the rodent HIP (26). These findings are supported by post-mortem studies of depressed individuals which revealed similar reductions in neurogenesis and dendritic branching within this region (27). Interestingly, only 50% of those with depression show an increase in glucocorticoid secretion (21) indicating that alterations in HPA function may not play a significant role in all persons that display this disorder, and a finding consistent with the known heterogeneity of depression. Nonetheless, several studies have evaluated a role for HIP in mediating ketamine effects and have shown this region may be key to its therapeutic effects (28–30). For example, there are strong positive correlations for humans between a smaller HIP size and
improved therapeutic efficacy of ketamine (30). Another study revealed significant reductions in the NR2B subunit of NMDAR, the long term potentiation, and NMDAR mediated excitatory postsynaptic currents in the HIP of depression-like mice, which were all restored as a result of ketamine treatment (28).

1.2.2 Nucleus Accumbens

The NAc is a structure of the reward pathway of the brain with a primary role being to integrate information from cortical and limbic regions to coordinate goal-directed behaviors (31). For this reason, it is thought to play a role in depression, with major symptoms of anhedonia, or the inability to feel pleasure, and amotivation suggesting disruptions to NAc function (31). A role for the NAc in depression is supported by both clinical and preclinical studies. For example, deep brain stimulation (DBS) of the NAc induced rapid therapeutic effects in TRD (32–36). Further, in rodent studies, the manipulation of biochemical targets in NAc has been shown to induce or alleviate depressive-like symptoms. Glutamate injected into the NAc, for instance, decreased swimming in rats in the forced swim test indicative of pro-depressant effects (31). It was also shown that optogenetic stimulation of the glutamatergic input to NAc from the HIP promoted susceptibility to developing depression-like behaviour (37). Conversely, the same effect was not observed when afferents from the prefrontal cortex (PFC) to the NAc were optogenetically stimulated, with those glutamatergic inputs promoting resilience to depression-like behaviour (37). Ketamine is known to act as an NMDA glutamate receptor antagonist, with preferential inhibition of GABAergic interneuron activity (12, 38). This increases excitatory neuron function and achieves, in theory, the same effect as in these previous studies (12, 38). This points to ketamine possessing additional unknown mechanisms of action as other NDMAR antagonists cannot treat
depression (12, 38). Regardless, this study identifies the network between HIP and NAc as potentially pivotal to the manifestation and symptoms of depression. This idea is further supported by evidence showing that ketamine treatment to a chronic stress mouse model induced unique transcriptional changes in the NAc that were not seen in any other region, nor were they replicated by the serotonin reuptake inhibitor imipramine (14), indicating that the NAc may be a critically important region for ketamine’s mechanism of action.

These regions play a role not only in the pathology of depression, but form part of an intricate network, that when dysregulated, manifests with negative symptoms. However, due to its complexity, one can see that the source of dysregulation may be heterogeneous and difficult to target, giving rise to TRD. This study therefore aims to provide a better understanding of the effects of ketamine versus a traditional antidepressant on activity of this network in both male and females animals.

1.3 Neuronal Oscillations as a Biomarker of Depression

Neuronal oscillations can be defined as the summed rhythmic electrical activity of groups of neurons (39). A group of neurons firing in a synchronized pattern can communicate important information to other brain regions, either spontaneously or as a result of a stimulus (39). For example, upon having a new object come into sight, information relevant to that image and related neural responses would be communicated through many forms of synchronized neuronal firing. To communicate long distances across brain regions requires large networks of low frequency oscillations (1-12Hz) while local communication tend to occur at higher frequency oscillations (12-100Hz) (39). The oscillations tend to be grouped into prescribed frequency ranges within 0.05 to 100Hz, although can go as high as 500Hz (40). The most relevant frequencies for long range
communication are: delta (1-4Hz), theta (4-8Hz) and alpha (8-12Hz), and for close range communication the frequencies are: beta (12-30Hz), low gamma (30-60Hz) and high gamma (60-100Hz) (40).

The prevailing hypothesis called “communication-through-coherence” states that coherence must occur between two groups of oscillating neurons in order for them to communicate with each other (41). Groups of neurons can only receive and send information at the peaks of excitability fluctuations (41). Therefore, in order for two groups of neurons to communicate, their oscillations must be timed in such a way that the sending group sends the information and the receiving groups peaks when the information arrives (41). When such a timed co-ordination occurs, the groups are coherent, or synchronous (Figure 1.2) (41). There is a growing body of research linking dysregulation of neuronal oscillations to neuropsychiatric disorders, such as obsessive compulsive disorder, schizophrenia, as well as depression (40, 42, 43). In humans, neuronal oscillations are commonly collected using electroencephalograms (EEG). It is important to note that while EEGs measure large areas of activity from the scalp, they cannot measure NAc or HIP activity.

1.3.1 Alpha Frequency (8-12Hz)

In depression, and other neuropsychiatric disorders, disturbances in alpha power are often observed using EEG. Specifically, asymmetries in the power of alpha waves between hemispheres of the frontal cortex have been shown to reflect a standard depressive state in humans (40, 44, 45) and are characterized by decreased alpha activity in the left lobe, and increased activity in the right lobe (40, 45). Within the right PFC, increased alpha activity correlates with increased depression severity scores (45). Unfortunately, the same pattern of frontal alpha symmetries cannot be used to distinguish TRD from healthy controls (40, 46). EEG studies suggest that this alpha asymmetry
Figure 1.2: Representation of coherence between oscillating groups of neurons with the same frequency. Red and blue are coherent, while black and blue are not (42).
may be associated with anxiety co-morbidity, rather than the symptoms of depression itself (47). In the limbic region, heightened alpha rhythm activity has been implicated in the NAc of TRD patients (40, 42). Unfortunately, the study only compared those with TRD to obsessive-compulsive patients, and did not implement a healthy control (42). Therefore, the causes and implications of the alpha asymmetry are yet to be determined (40), but it may to be indicative of a unique role for the NAc in TRD, and warrants investigation.

1.3.2 Theta Frequency (4-8Hz)

Theta oscillations have also been demonstrated to play an important role in depression (40). As mentioned previously, DBS of the NAc has been shown to be an effective treatment strategy for patients with TRD (48). While the mechanism of DBS remains unclear, studies of neuronal oscillations in brain regions following NAc DBS have been performed, and they provide some valuable insights for this research (48, 49). It was revealed that the NAc DBS enhanced subsequent theta power in the NAc, but the exact significance of the changes in theta power was not explored (48). It is possible that the increased theta power represents improvements in regulation of NAc neuronal activity associated with reward experience (anhedonia in depression) (32). A second study looked at time-dependent changes as a result of NAc DBS (49). They found that the initial stimulation increased low frequency power within the region, but that was no longer apparent after 6 hours, instead appearing as altered coherence between other regions (49). A similar type of treatment for psychotic depression is electroconvulsive therapy (50). Upon successful treatment of a cohort of patients, it was shown that increased theta power was the primary frequency change in the brain (50). Another study found the best EEG predictor of an antidepressant response in patients to be elevated theta power in the frontal region (51). It has also been shown that theta
rhythms are synchronized over long ranges in seconds, but only in healthy patients, not those with depression (52).

1.3.3 Delta (1-4Hz), Beta (12-30Hz), Gamma (30-100Hz) Frequencies

Within the delta frequency, many studies have indicated a higher delta power relating to a depressed state. Some EEG studies have reported baseline increased delta power associated with depression (53, 54), and the induction of depressive symptoms via tryptophan depletion was associated with a corresponding increase in EEG delta power (55). The beta frequency has also been implicated in depression, but may be involved in a different way than patients with alpha asymmetry (47). Heightened beta power is a clinical marker for those who have experienced recurrent (>1) bouts of depression (47). The importance of beta frequencies in the limbic regions are not well characterized, with some studies finding non-significant changes after DBS treatment (56). The relationship between gamma oscillations and depression is poorly characterized to this day. In the frontal regions, reduction in gamma synchronization has been linked to the therapeutic effect of one antidepressant, the selective serotonin reuptake inhibitor paroxetine (57). Our previous findings have highlighted the importance of gamma power in a chronic stress model of depression in rats, whereby depressive-like symptoms are induced through repeated stressors (58). In the NAc, increased low gamma power was associated with stress resilience in only female rats, while only the male rats showed increase high gamma power in the HIP region and increased high gamma coherence between the NAc and HIP (58).

1.3.4 Effects of Antidepressants and Ketamine on Neuronal Oscillations

Neural activity has been demonstrated to change in both humans and rats after the administration of many classes of antidepressants. In humans, some EEG studies have been
employed to investigate global power changes after antidepressant administration (45, 59). Saletu
et al. (66) found an interesting change as a result of both imipramine and fluvoxamine
administration, mainly surrounding alpha activity. While imipramine lowered alpha power,
fluvoxamine inversely yielded increased alpha power (59). Because fluvoxamine was associated
with more impactful improvements to concentration, psychomotor activity, and mood, the authors
suspected imipramine’s decrease in alpha power may be partly reflective of its sedative side effects
(59). Indeed, they found 80% of the imipramine-treated subjects expressed significant sedative
side effects compared to 50% in the fluvoxamine-treated subjects (59). While interesting, the study
was limited because it only captured changes that occurred shortly after a given drug
administration, changes that were both therapeutic and sedative, making them difficult to delineate
in the EEG. Another study showed that the effects of the antidepressant paroxetine on neural
activity are still apparent after 72 hours, namely reduced delta and theta and with increased beta
(60). While the researchers only gave paroxetine to healthy non-depressed volunteers, it still
indicates the potential effects of antidepressants in power and time-course in EEGs (60). This is
contrasted by more recent study that examined EEG alpha power as a predictor of therapeutic
response to fluoxetine (61). In this study, while treatment responders could be pre-identified by
increased alpha power and alpha asymmetry between hemispheres, these measures did not change
or improve after a successful therapeutic outcome (61). With regards to ketamine, many oscillatory
changes in EEG are found after administration, including reduced delta, theta and alpha, and
elevated frontal gamma (62, 63). However, these studies were performed within 2 hours following
ketamine administration, capturing and focusing on the cognitive schizophrenia-like side effects
of the drug, making it impossible to delineate between oscillatory changes that could be therapeutic
or involved with side effects, as they might overlap during that time. This lack of information is reflected in the animal literature, with the majority of local field potential (LFP) recordings being taken immediately after ketamine injection (64, 65). In this initial period, rats display similar changes, with increased gamma power being highlighted (64, 65). However, this increased gamma power is also shown by MK-801, a non-therapeutic NMDAR antagonist that also induced schizophrenia-like symptoms (64), meaning it may not be a good indicator of ketamine’s therapeutic response.

What is currently lacking in the literature is a dissociation between the negative cognitive side effects and the therapeutic effects of ketamine. The goal of this study was to examine the therapeutic aspect of ketamine on neuronal oscillations without consideration of the short-term side effects. Furthermore, these effects were characterized in the HIP and NAc, striatal regions which cannot be represented by EEGs. This information will serve as a useful biomarker of therapeutic efficacy for the preclinical development of novel drugs that aim to replicate ketamine’s success without the negative side effects. In this regard we will employ the unique Wistar-Kyoto (WKY) TRD rodent model system.

WKY rats possess good face validity (symptoms) for depression, displaying spontaneous increases in depressive-like behaviour compared to standard rat breeds in the forced swim test, a test employed to evaluate behavioural despair and used as a standard for measuring antidepressant efficacy (66, 67). These animals also exhibit heightened anxiety-like responses (67), working memory deficits (68) and disturbed sleep patterns (69), all symptoms of depression. This model has been shown to have good construct validity (molecular), exhibiting prolonged activation of the hypothalamic-pituitary-adrenal (HPA) axis (21, 22), and abnormalities in monoaminergic (70) and
brain derived neurotrophic factor (BDNF) signaling (71) commonly seen in persons with depression. With regards to predictive validity, many studies have also found that the administration of standard antidepressants such as desipramine, imipramine, and fluoxetine to these rats were ineffective in the forced swim test (10, 66). However, similar to that seen in those with TRD, administration of ketamine is effective in alleviating behavioural despair in WKY rats (10, 66). Therefore, as the WKY model system shows very good face, construct and predictive validity, it is a suitable animal model to evaluate drug treatment effects in TRD.

1.4 Research Hypothesis and Objectives

Hypotheses:

1) WKY rats will exhibit distinct oscillatory deficits in both power and coherence compared to Wistar (WIS) control rats in the NAc and HIP.

2) Ketamine, but not the traditional antidepressant fluoxetine, will normalize aberrant oscillatory activity in the regions, which will be apparent 24 hours after injection.

Objectives:

1) Establish and compare baseline oscillatory activity between WKY and WIS male and female rats.

2) Examine the effects of ketamine, fluoxetine, or saline administration to WKY rats on oscillatory activity 24 hours post-injection using WIS rats receiving saline to act as controls for normalization of oscillatory deficits.
2 Material and Methods

2.1 Animals and Housing

Adult male and female WIS (7 male, 7 female) and WKY (19 male, 19 female) rats (Charles River, QC, Canada) weighing 175-225 g at the beginning of the experiment were used. Animals were housed in a temperature-controlled colony room, maintained on a twelve-hour reverse light/dark cycle (0800h lights off; 2000h lights on) with free access to water and food. Animals were moved to single-housing and handled daily for a minimum of 7 days prior to the start of the experiment. All procedures were approved by the Animal Care Committee of the University of Guelph and were carried out in accordance with the recommendations of the Canadian Council on Animal Care (58).

2.2 Electrophysiology

Electrophysiology methods are adapted from those previously reported in our lab by Thériault et al. (58).

2.2.1 Electrode Implantation Surgery

Rats were anesthetized with isoflurane (5%), administered the analgesic carprofen (5 mg/kg, s.c.) and secured in a stereotaxic frame. Custom electrode microarrays were built using pre-fabricated Delrin templates and polyimide-insulated stainless steel wires (A-M Systems: 791600, 0.008”) and were implanted bilaterally into the NAc (AP: +1.9 mm, ML: ±1.2 mm, DV: -6.6 mm) and the CA1 region of the dorsal HIP (AP: -3.5 mm, ML: ±2.5 mm, DV: -2.6 mm). A ground/reference screw was implanted into the skull behind lambda and additional anchor screws were attached to the skull and secured with dental cement. All animals received a second carprofen
injection 24 h following surgery and recovered in their home cages for a minimum of 7 days prior to experiments being performed (58).

2.2.2 Local Field Potential Recordings

All LFP oscillatory recordings (Wireless 2100-system, Multichannel Systems) were performed in awake, freely moving animals in clear plexiglass boxes (18” x 18” x 18”). Recordings were taken for 30 min and sampled at a rate of 1000 samples/s. Routines from the Chronux software package for MATLAB (MathWorks) were used to analyze the spectral power of LFP oscillations in each region and coherence between regions. Recordings were segmented, detrended, denoised and low-pass filtered to remove frequencies greater than 100 Hz. Continuous multitaper spectral power for the normalized data (to total spectral power) and coherence was calculated for delta (1-4 Hz), theta (> 4-12 Hz), beta (> 12-30 Hz), low gamma (> 30-60 Hz), and high gamma (> 60-100 Hz) unless otherwise stated (58).

2.3 Drug Treatment

Groups of 6 to 7 WKY received treatments of either saline, fluoxetine, or ketamine. Groups of 7 WIS received saline treatment. Drug treatments were prepared fresh daily. Saline was administered as 0.9% sodium chloride in water. Fluoxetine was dissolved in saline and administered at 10mg/kg. 100mg/ml stock ketamine was diluted with saline and administered at 10mg/kg. All drugs were administered through intraperitoneal injection.

2.4 Statistical Analyses

For LFP data, routines from the Chronux software package (MATLAB, MathWorks) were used to extract individual frequency bands. Statistical analysis of baseline LFP data was performed
using an ANOVA with Sex and Strain as between subjects factors. In the case of significant main effects or interactions, individual mean differences were identified by planned independent Student’s t-test between groups. Analysis of 24-hour post-injection LFP data was performed in the WKY rats using an ANOVA with Treatment as the between subjects factor followed by Tukey's post-hoc test. To compare the saline-treated WKY and WIS rats, independent student’s t-test were performed. Computations were performed using IBM SPSS 25 software and are presented as the mean ± sem (58).

3 Results

3.1 Characterization of Baseline Oscillatory Signatures in WKY rats

Baseline neuronal oscillations were analyzed to establish sex- and strain-related differences between the male and female WIS and WKY rats. Spectral Power in the HIP and NAc were compared, as well as NAc-HIP coherence.

Normalized low and high frequency baseline power for the HIP are shown in Figure 3.1A, with quantification of each frequency band depicted in Figure 3.1B-F. Main effects of Strain were evident in the delta, theta, and beta power frequencies (Figure 3.1B-D) [delta: F(1,90)=28.353, p<0.001; theta: F(1,90)=33.455, p<0.001; beta: F(1,90)=11.883, p=0.001]. No effects of Strain were seen in low and high gamma. Main effects were also observed with Sex, with females displaying higher overall beta, low gamma, and high gamma power (Figure 3.1D-F) [beta: F(1,90)=25.632, p<0.001; low gamma: F(1,90)=12.913, p=0.001; high gamma: F(1,90)=5.125, p=0.026]. There were no main effects of Sex evident in the delta and theta frequencies. Furthermore, a Strain x Sex interaction was also revealed in the beta and low gamma frequencies.
Post-hoc tests revealed that both the male and female WKY exhibited higher delta power (male: ###p<0.001; female: ##p<0.01) and lower theta power (male: ###p<0.001; female: #p<0.05) compared to WIS rats of the same sex (Figure 3.1B,C). In the beta frequency, only WKY males showed increased beta power compared to the WIS male rats (###p<0.001). However, a sex effect in the beta frequency was evident in the WIS rats, with females showing elevated baseline beta power compared to males (beta: **p<0.01) (Figure 3.1D,E). Similarly, in low gamma, the female WIS rats exhibited significantly higher spectral power than the male WIS rats (**p<0.01), with no sex differences in the WKY rats.

Normalized baseline power and quantification for the NAc are shown in Figure 3.2. Main effects of Strain were evident in the delta, theta, and high gamma frequencies (Figure 3.2B,C,F) [delta: F(1,82)=9.102, p=0.003; theta: F(1,82)=15.232, p<0.001; high gamma: F(1,82)=11.882, p=0.001]. There were no main effects of Strain in beta and low gamma. Main effects of Sex were observed in the beta and low gamma frequencies, with females overall showing higher power in those frequencies (Figure 3.2D,E) [beta: F(1,82)=17.242, p<0.001; low gamma: F(1,82)=25.387, p<0.001]. Main effects of Sex were not observed in the delta, theta, and high gamma frequencies. Furthermore, Strain x Sex interactions were found in the delta and theta frequency bands [delta: F(1,82)=4.753, p=0.032; theta: F(1,82)=7.645, p=0.007]. Post-hoc analyses of the groups reveal that only the male WKY rats exhibited increased delta power (##p<0.01), coincident with reduced theta power (##p<0.01) compared to the WIS males, with no differences observed between the female WKY and WIS rats (Figure 3.2B,C). However, when compared to the male WKY rats, female WKY rats showed consistently altered delta (**p<0.001), theta (**p<0.001), beta
(**p<0.01), and low gamma (p<0.001) power (Figure 3.2B-E). There were no observed differences between male and female WIS rats in each region.

It was next determined whether there existed any differences in coherence between the NAc and HIP. Coherence plots and quantifications are shown in Figure 3.3. A main effect of Strain was seen in the theta frequency (Figure 3.3C) [F(1,89)=7.393, p=0.008], but not in delta, beta, or gamma. No Sex effects were prevalent, but a Strain x Sex interaction was observed in the theta frequency [F(1,89)=4.978, p=0.028]. Post-hoc analysis showed that female WKY, but not male WKY, had elevated theta coherence compared to their WIS counterparts of the same sex (#p<0.05). Furthermore, female WKY possessed higher theta coherence than male WKY rats (**p<0.01) (Figure 3.3C).

These findings demonstrate differences in the baseline oscillatory signatures between the WKY rats compared to WIS controls and point to a few key Sex differences. Specifically, the dysregulation of oscillations in the NAc of male WKY rats were not present in females. Further, although both sexes showed frequency-specific deficits in the HIP region, only females WKY show a marked increase in NAc-HIP theta coherence.

3.2 Effect of Fluoxetine or Ketamine in Normalizing WKY Oscillatory Deficits

To determine the long-lasting drug effects of the single dose of ketamine, rats were given a saline, fluoxetine, or ketamine injection and LFPs analyzed after 24 hours. Thus, there were four experimental groups. The WIS rats were used as a control group, receiving saline only, to give indications as to the directionality of drug effects in the WKY towards normalization. There were three WKY groups, receiving saline, fluoxetine (10mg/kg) or ketamine (10mg/kg). Within-Sex
Treatment effects on WKY rats were analyzed using an ANOVA, with WIS and WKY comparisons performed as done with baseline measures to evaluate group differences in oscillations.

Drug-induced changes in HIP neuronal oscillations in the male rats after 24 hours were first characterized, with results shown in Figure 3.4. Main effects of Treatment were observed in the low frequency delta and theta frequency bands (Figure 3.4B,C) [delta: F(2,35)=3.318, p=0.048; theta: F(2,35)=4.142, p=0.024]. Treatment had no main effects in the beta and gamma frequencies. Ketamine treatment increased delta power in the WKY males (*p<0.05), exacerbating the existing delta power changes evident in saline-treated WKY rats (##p<0.01). Similarly, ketamine resulted in a further suppression of theta power in these rats (*p<0.05) (##p<0.01). Drug-induced changes in spectral power in HIP of female WKY are shown in Figure 3.5. Unlike the male WKY rats, no main effects of Treatment were found. Post-hoc comparisons did not reveal any further differences between the WKY treatment groups in any frequency.

Next, drug-induced changes in the NAc of male WKY after 24 hours were characterized (Figure 3.6). Main effects of Treatment were seen in the delta, theta, and beta frequency bands (Figure 3.6B-D) [delta: F(2,25)=9.045, p=0.001; theta: F(2,25)=7.657, p=0.003; beta: F(2,25)=7.451, p=0.003], but not in gamma. Post-hoc analyses revealed that ketamine normalized existing low frequency deficits in NAc, significantly reducing delta power (*p<0.05) and increasing theta power (*p<0.05) in the WKY males. These effects were not replicated in the fluoxetine treated WKY animals which still exhibited significant differences from the WIS rats (delta: #p<0.05; theta: ##p<0.01, planned comparison). In the beta and gamma frequency bands, no significant drug effects were observed. Drug effects in NAc oscillations in the female WKY
Rats are shown in Figure 3.7. There were no Main Effects of Treatment except in the beta frequency [beta: \( F(2,30) = 5.380, p = 0.01 \)], where a significant increase in beta power by fluoxetine treatment was observed (**\( p < 0.01 \)) (Figure 3.7D).

Finally, the effects of each treatment on NAc-HIP coherence was analyzed. The male response is shown in Figure 3.8. A main effect of Treatment was shown in the theta frequency band (Figure 3.8C) \( [F(2,33) = 12.405, p < 0.001] \). No main effects of Treatment were seen in delta, beta or gamma. Consistent with the baseline data, there were no differences in theta coherence between saline-treated WIS and WKY male rats. However, both fluoxetine (*\( p < 0.05 \)) and ketamine (***\( p < 0.001 \)) significantly reduced NAc-HIP theta coherence. No other drug effects were observed in males in any of the other frequency bands. Female WKY treatment response on NAc-HIP coherence after 24 hours is shown in Figure 3.9. Similar to that observed in the male WKY rats, a main effect of Treatment in the theta frequency was evident (Figure 3.9C) \( [F(2,30) = 5.116, p = 0.012] \), but not in delta, beta or gamma. Female WKY rats exhibited an innate increase in theta coherence that was reduced by ketamine (*\( p < 0.05 \)), such that the deficit was normalized to WIS levels (#\( p < 0.05 \)). A trend towards reduced theta coherence was seen with fluoxetine (p<0.15), however it was not significant.

These results point to clear sex differences in the normalization of oscillatory responses by ketamine. Whereas male therapeutic responses to ketamine focused around delta and theta frequencies in the NAc, female responses appeared to surround the normalization of NAc-HIP theta coherence.
**Figure 3.1:** A) Baseline power spectra of neuronal oscillatory activity in the hippocampus. Untreated male WIS and WKY rats are compared in the left panels, with untreated female WIS and WKY rats compared in the right panels. Low frequency delta and theta power bands are shown in the top quadrants, and high frequency beta and gamma are shown in the bottom quadrants. Mean spectra are shown with jack-knife estimates of the sem depicted by the shaded areas. B-F) Quantification of baseline power spectra in the HIP for each frequency. Bars represent means ± sem. **p<0.01 compared to male rats of the same strain (student’s t-test); #p<0.05, ##p<0.01, ###p<0.001 compared to WIS rats of the same sex (student’s t-test).
Figure 3.2: A) Baseline power spectra of neuronal oscillatory activity in the nucleus accumbens. Untreated male WIS and WKY rats are compared in the left panels, with untreated female WIS and WKY rats compared in the right panels. Low frequency delta and theta power bands are shown in the top quadrants, and high frequency beta and gamma are shown in the bottom quadrants. Mean spectra are shown with jack-knife estimates of the sem depicted by the shaded areas. B-F) Quantification of baseline power spectra in the HIP for each frequency. Bars represent means ± sem. **p<0.01, ***p<0.001 compared to male rats of the same strain (student’s t-test); #p<0.05, ##p<0.01 compared to WIS rats of the same sex (student’s t-test).
Figure 3.3: A) Baseline coherence between the nucleus accumbens and hippocampus regions. Untreated male WIS and WKY are depicted in the upper panel, with untreated female differences shown in the lower panel. Mean coherence is shown with jack-knife estimates of the sem depicted by the shaded areas. B-F) Quantification of NAc-HIP coherence for each frequency. Bars represent means ± sem. **p<0.01, compared to male rats of the same strain (student’s t-test); #p<0.05, compared to WIS rats of the same sex (student’s t-test).
Figure 3.4: Effect of ketamine treatment on hippocampal oscillatory power in male rats. A) Power spectrum of the male HIP region 24 hours after an injection of either saline, fluoxetine, or ketamine. Low frequency delta and theta oscillations are shown in the left panel, and high frequency beta and gamma oscillations are displayed in the right panel. Mean spectra are shown with jack-knife estimates of the SEM depicted by the shaded areas. B-F) Quantification of male HIP power for each frequency after drug treatment. Bars represent means ± SEM. *p<0.01, compared to WKY saline treated rats (student’s t-test); #p<0.05, ##p<0.01, compared to WIS saline treated rats (student’s t-test).
Figure 3.5: Effect of ketamine treatment on hippocampal oscillatory power in female rats. A) Power spectrum of the female HIP region 24 hours after an injection of either saline, fluoxetine, or ketamine. Low frequency delta and theta oscillations are shown in the left panel, and high frequency beta and gamma oscillations are displayed in the right panel. Mean spectra are shown with jack-knife estimates of the sem depicted by the shaded areas. B-F) Quantification of female HIP power for each frequency after drug treatment. Bars represent means ± sem. ##p<0.01, compared to WIS saline treated rats (student’s t-test).
Figure 3.6: Effect of ketamine treatment on nucleus accumbens oscillatory power in male rats. A) Power spectrum of the male NAc region 24 hours after an injection of either saline, fluoxetine, or ketamine. Low frequency delta and theta oscillations are shown in the left panel, and high frequency beta and gamma oscillations are displayed in the right panel. Mean spectra are shown with jack-knife estimates of the sem depicted by the shaded areas. B-F) Quantification of male NAc power for each frequency after drug treatment. Bars represent means ± sem. *p<0.05, compared to WKY saline treated rats (student’s t-test); #p<0.05, ##p<0.01, compared to WIS saline treated rats (student’s t-test).
Figure 3.7: Effect of ketamine treatment on nucleus accumbens oscillatory power in female rats. A) Power spectrum of the female NAc region 24 hours after an injection of either saline, fluoxetine, or ketamine. Low frequency delta and theta oscillations are shown in the left panel, and high frequency beta and gamma oscillations are displayed in the right panel. Mean spectra are shown with jack-knife estimates of the sem depicted by the shaded areas. B-F) Quantification of female NAc power for each frequency after drug treatment. Bars represent means ± sem. **p<0.01, compared to WKY saline treated rats (student’s t-test); #p<0.05, compared to WIS saline treated rats (student’s t-test).
Figure 3.8: Effect of ketamine treatment on coherence between nucleus accumbens and hippocampus in male rats. A) Coherence between male NAc and HIP regions 24 hours after an injection of either saline, fluoxetine, or ketamine. Mean coherence is shown with jack-knife estimates of the sem depicted by the shaded areas. B-F) Quantification of NAc-HIP coherence for each frequency in males after treatment. Bars represent means ± sem. *p<0.05, ***p<0.001 compared to WKY saline treated rats (student’s t-test).
Figure 3.9: Effect of ketamine treatment on coherence between nucleus accumbens and hippocampus in female rats. A) Coherence between female NAc and HIP regions 24 hours after an injection of either saline, fluoxetine, or ketamine. Mean coherence is shown with jack-knife estimates of the sem depicted by the shaded areas. B-F) Quantification of NAc-HIP coherence in females for each frequency after treatment. Bars represent means ± sem. *p<0.05, compared to WKY saline treated rats (student’s t-test); #p<0.05, compared to WIS saline treated rats (student’s t-test).
4 Discussion

In the present study, innate and drug-induced frequency- and sex-specific differences in the neural oscillatory activity of WKY and WIS rats were observed within the HIP and NAc. In both sexes strain differences in oscillatory circuit function were restricted to the low frequency bands. Specifically, in WKY males, increased delta power and reduced theta power was evident in both regions compared to the WIS males, with no changes in NAc-HIP coherence. Female WKY rats showed similar low frequency power differences as the males in the HIP region compared to WIS rats, but not in the NAc. However, unlike the males, WKY females exhibited elevated theta band NAc-HIP coherence. Further, we showed that ketamine, but not fluoxetine, normalized the low frequency deficits in the male WKY rats selectively in the NAc, whereas in the females a ketamine-specific normalization of NAc-HIP theta coherence was observed. These findings demonstrate that ketamine induces sex-specific effects on oscillatory circuit function that may potentially reflect disparate mechanisms underlying its therapeutic efficacy in males and females with TRD.

The importance of low frequency deficits in depression have been established. For example, clinical EEG studies have demonstrated that patients with depression possess increased global delta power (54, 72), and increased temporal theta and alpha power have been linked to improved mood (73, 74). Although the present study did not evaluate the cortical regions, with these investigations currently underway, WKY rats have been well established to show impaired attributes of depression in various behavioural tests (67, 75–77). Thus, we would anticipate seeing similar low frequency oscillatory deficits in the cortical regions of WKY rats. However, as these studies are more descriptive in nature, further research using more mechanistic approaches to link deficits in oscillatory activity directly to depression-like behaviour are required.
The sex-related differences observed between male and female WKY rats are more difficult to interpret as, to our knowledge, this is one of the first studies that has employed female WKY rats in its experimental design, and there is a gross lack of pre-existing clinical and preclinical reports that incorporated both sexes. Our results demonstrated sex-specific innate differences in innate low frequency changes in the HIP and NAc, and studies are beginning to reveal an important link between these regions in depression (37, 78). A recent study by Williams et al. (78) using mice demonstrated the importance of glutamatergic inputs from the HIP to the NAc. They found that males had testosterone-dependent lower excitability than females in HIP-NAc neurons, which was associated with increased stress-resilience in a stress-depression model (78). Furthermore, they were able to use DREADD (Designer Receptors Exclusively Activated by Designer Drugs) stimulation of the HIP-NAc neurons to reduce the males’ stress-resilience, and DREADD inhibition of female HIP-NAc neurons to increase the females’ stress-resilience (78).

In this study, females, but not males, displayed an increase in NAc-HIP theta coherence providing additional evidence of sex-dependent differences in this pathway that may be reflective of a differential HIP-NAc stress-response. Upon examination of power changes within the NAc, only the male WKY rats exhibited altered delta and theta compared to the WIS parent strain. One gene that may mediate these differences is Dnmt3a (encoding DNA methyl transferase 3) (79). Expression of this gene is elevated in human patients with depression, and a recent study by Hodes et al. (2015) showed in a murine depression model system increased Dnmt3a expression to different levels between the sexes (79). Furthermore, knockout of Dnmt3a in the female mice not only eliminated development of negative behavioral stress-responses but shifted the transcriptional stress response to be more similar to males (79). It is useful to reiterate here that the WKY possess
a hyper-sensitivity to stress, as well as dysregulation of HPA axis activity (80). Therefore, the oscillatory differences seen between the male and female WKY rats in NAc may be due to alterations in transcriptional profiles in response to stress mediated, at least in part, by differing $Dnmt3a$ expression. These findings may be reflective of broad sex-related differences in neural activity and transcriptional stress responses in the WKY model of TRD.

Treatment of the WKY with ketamine provides many potential insights into its rapid, long-lasting therapeutic action. Despite the HIP being important in depression, with reduced size and glial count (18, 25), as well as the delta and theta power deficits reported herein, ketamine did not normalize neural oscillatory deficits in the region. Our previous evidence has shown the dorsal HIP plays a key role in stress-induced depression (58), and other research has shown an important role for the dorsal HIP (28, 29, 81) and ventral HIP (82, 83) in mediating in ketamine action. It is possible we did not capture oscillatory changes within the HIP due to the timing of the recordings, which may not have been consistent with the timing of ketamine actions within this region, actions that may have occurred earlier post-injection. This is supported by our previous findings showing that changes in dorsal HIP activity occur early with stress exposure, acting as a hub to induce a cascade of neuroanatomical functional changes that underlie the manifestation of depression-like behaviour in rats (58). Therefore, if ketamine effects on dorsal HIP activity occurred within a shorter time frame, these changes would not have been evident in our experimental design. In female WKY rats, ketamine did normalize NAc-HIP coherence, but as the directionality of coherence measures is unknown through this methodology, we cannot be certain which structure initiated this communication. It is therefore possible that ketamine effects in NAc are either longer
lasting than those of the HIP or are slower to develop and thus we were able to observe these changes 24 hours after ketamine exposure.

Many reports have evaluated the molecular effects of ketamine in HIP and cortical structures, with less known of its effects in NAc. Ketamine acts as a NDMAR antagonist, and in HIP and cortical regions it is thought to induce excitatory glutamatergic neurotransmission through inhibition of GABAergic inputs, thereby promoting coherent network activity with other regions in the depression network (84, 85). Through this pathway, BDNF release would be increased, leading to altered activity of downstream targets such as glycogen synthase kinase-3β and mammalian target of rapamycin complex 1, and ultimately promoting synapse formation and maturation (84, 85). This interpretation is supported by studies that have targeted GABA<sub>A</sub> receptors, known to express in the HIP and PFC (85, 86). Activation of these GABA<sub>A</sub> receptors was shown to stimulate network coherence in a similar fashion to ketamine, allowing for enhanced excitatory neurotransmission and leading to rapid antidepressant action (85, 86) presumably via inhibition of GABAergic activity. Additionally, other studies have shown that chronic administration of ketamine in an animal model of depression restored diminished levels of BDNF in the HIP, as well as in NAc (87). In NAc, ketamine has been reported to have unique effects on transcription that are not mimicked by traditional antidepressants, and which may underlie its efficacy in depression (14). The transcriptional changes may potentially lead to the expression of proteins that alter neurotransmission, resulting in the normalization of oscillatory deficits. Interestingly, in mice, ketamine administration reduces levels of DNMT3a in PFC and HIP (88), although its effects in the NAc remain unexplored.
In conclusion, these results reveal that ketamine exerts sex-dependent effects on oscillatory signatures of WKY rats. However, more research is required to determine whether these changes underlie its antidepressant actions and to establish a molecular basis for the observed oscillatory changes, such as through differences in BDNF signaling or and the relative involvement of DNMT3a. Time courses would also reveal valuable information as to which areas ketamine may initiate action with subsequent changes throughout the depressive network.
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