KETAMINE’S EFFECT ON SINGLE-UNIT ACTIVITY IN THE STRIATUM

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1. ABSTRACT
Ketamine is a commonly used anesthetic; however, when administered at low-dose sub-anesthetic levels, ketamine has been shown to relieve chronic pain and treatment-resistant depression. Recent studies have shown that sub-anesthetic doses of ketamine reduce L-DOPA-induced dyskinesia (LID), an impairment of voluntary movement, in rodent models of Parkinson’s disease (PD) and in human patients. As an N-methyl-D-aspartate (NDMA) receptor antagonist, we can predict ketamine’s effects on the brain by already knowing how NMDA receptors function. Recent data from our previous experiments show how ketamine alters brain oscillations, inducing sustained high-frequency oscillations in regions such as the dorsolateral striatum. Knowing that ketamine alters oscillatory brain activity we further investigated how it affected single-unit activity in these regions. Single-unit recording measures activity of a single neuron in a desired area of the brain by measuring the rate of change in voltage during an action potential. Here we investigated how repeated exposure to ketamine alters the single-unit activity in the striatum. Results show that the isolated cells respond in a variety of ways to ketamine, some being inhibited while others were excited. Further analysis looked at variance, spiking patterns, and waveform shape to further understand ketamine’s effect on neural activity.
2. INTRODUCTION

2.1 Parkinson’s Disease

   Parkinson’s disease is a prevalent disease that affects nearly one million people in the United States alone, making it the second most common neurodegenerative disease [25]. It is characterized by motor symptoms such as tremors, bradykinesia, rigidity and impaired balance and coordination. However, PD also includes non-motor symptoms, such as cognitive deficiencies and sleep disturbances [25].

   Parkinson’s has been categorized as a sporadic disease, the cause of the disease is still unknown, even after extensive research has been done since it’s discover in 1817 [17]. However pathological and genetic clues have helped scientists better understand the disease in recent years. Similar to other neurodegenerative disorders, aging is the most important risk factor, with people over the age of 60 having a higher risk than the younger population. Furthermore, the disease is not directly genetically linked, with only 15% of people with PD reporting having family members having the disease [25].

   Even though it is categorized as a sporadic disease, very few environmental factors have been found to attribute to the causation of PD, mostly related to toxins or injuries. Genetic factors, sporadic or inherited, are more likely to cause PD.

2.1.1 Etiology

   Parkinson’s disease is primarily caused by a progressive degeneration of dopaminergic neurons in the brain region known as the substantia nigra pars compacta. This is the hallmark explanation for the causation of PD, however when the first symptoms of PD become present, roughly 30% of the neurons in the substantia nigra have already been lost [14].
The process in which the dopaminergic cells die is yet unknown; however several gene mutations have been discovered that play a role in dopamine cell function. These mutations have been found in both sporadic and familial PD and have a variety of point mutations or polymorphisms that cause PD. One of these identified genes is a gene that encodes for α-synuclein. A-synuclein protein aggregates are a primary component of Lewy bodies in the substantia nigra of patients with sporadic and inherited PD [14]. These Lewy bodies have appeared to be a major factor for neuronal dysfunction and cell death [14]. The most common gene associated with PD is the autosomal dominant LRKK2 gene mutation. Its function is not fully known; however, Corti et.al state that LRRK2 mutant variants are toxic to neurons, causing neuronal death [8].

There are other mutations and pathogenic mechanisms that contribute to PD, these are just a few well known variants. However, it is important to note that other factors can contribute to dopaminergic cell death, including oxidative damage and abnormal cellular calcium, to name a few [14].

2.1.2 The Basal Ganglia and Dopamine

Dopamine is an important neurotransmitter, affecting multiple pathways involving reinforcement, reward, cognitive functions, and motor controls. Dopamine is produced in the pars compacta of the substantia nigra. The substantia nigra forms part of the basal ganglia, which is comprised of four major sections: the striatum, globus pallidus, substantia nigra, and subthalamic nucleus [14]. The substantia nigra projects its dopaminergic neurons to the striatum. The neurons in the striatum are responsible for coordinating movement by integrating input from the cortex and substantia nigra [29]. The cortex provides the striatum with sensory information as well as plans for future movement, and the substantia nigra provides dopamine, which is
needed to coordinate the inputs the striatum has received [29]. Dopamine provides inhibition as well as excitation to the striatum. In order to coordinate movement, the neurons in the striatum project to other nuclei of the basal ganglia, where information is processed and then sent back to the cortex, including the motor cortex.

From the striatum, neurons can project to the internal globus pallidus and thalamus, forming the direct pathway. Striatal neurons can also project to the external globus pallidus to the subthalamic nucleus to the thalamus, forming the indirect pathway. The direct pathway is a dis-inhibitory pathway, disinhibiting the motor cortex, allowing for movement. In the direct pathway, dopaminergic neurons from the substantia nigra provide excitatory input on D1 type dopaminergic receptors. The cells with the D1 type receptors project to the internal globus pallidus inhibiting this region. The inhibition of the internal globus pallidus in turn disinhibits the thalamus. Less inhibition in the thalamus allows for excitatory projections to the motor cortex, allowing for an increase in movement. The direct pathway is summarized in Figure 1.

The indirect pathway, on the other hand, is a “dis-dis-inhibitory” pathway, whose role is to modulate the dis-inhibition of the direct pathway [24]. In the indirect pathway, the dopaminergic neurons from the substantia nigra provide inhibitory input to the striatum. This inhibitory input is mediated by D2 type dopaminergic receptors that project to the external globus pallidus. The inhibition of the external globus pallidus leads to less inhibition on the subthalamic nucleus. The release of inhibition on the subthalamic nucleus sends excitatory input to the internal globus pallidus. When neurons in the internal globus pallidus are excited, the thalamus becomes inhibited. Inhibition of the thalamus means less excitation to the motor cortex, decreasing movement. The indirect pathway is also shown in Figure 1.
A balance between the direct and indirect pathway is crucial in order to obtain regular movement, inhibiting involuntary movement and allowing for voluntary movement. In Parkinson’s, the production of dopamine in the substantia nigra is reduced, diminishing the dopaminergic projections to the striatum, causing an imbalance between the direct and indirect pathways. The reduction of input in the striatum causes an increase in the dominance of the indirect pathway (the no-go pathway). The excess inhibition put on the thalamus makes it less likely to activate motor neurons [24]. This imbalance produces slowness, or even absence of movement, characteristic of PD.

In summary, the loss of dopaminergic neurons in the substantia nigra is what produces the imbalance of excitation and inhibition in the basal ganglia, affecting movement. This neurodegeneration in the substantia nigra has been linked with α-synuclein. A-synuclein has been shown to regulate the production of dopamine by interacting with tyrosine hydroxylase (TH), the rate limiting enzyme that converts TH to L-3,4-dihydroxyphenylalanine (L-DOPA), the precursor of dopamine [31]. In PD α-synuclein is overexpressed; overexpression of α-synuclein
reduces activity of TH, reducing the overall production of dopamine. This finding is important; however, a reduction in dopamine production is also a natural result of aging [31]. Nonetheless, α-synuclein also plays a more important role in synaptic vesicle function and dopamine release. Recent evidence in dopaminergic systems has shown that an absence of α-synuclein led to elevated dopamine release, while over-expression of α-synuclein led to a decreased dopamine release. It was shown that accumulation of α-synuclein impairs not only neurotransmitter release, but also impairs vesicle recycling and the stability of t-SNARE complex stability. Dopamine’s actions in the striatum are also regulated by reuptake back into the pre-synaptic terminal by the dopamine transporter (DAT), terminating dopamine signaling [31]. In vivo studies have shown that α-synuclein is a regulator of DAT function, controlling the trafficking of DAT to the cell membrane, accelerating dopamine reuptake [31].

2.1.3 Treatments

Parkinson’s is still an incurable degenerative disease; however, there are a variety of medications, or mixture of medications, that can be taken at any stage of the disease to manage the symptoms. Dopaminergic medications are the most common therapeutic options for dealing with the motor symptoms of PD. Dopaminergic medications include monoamine oxidase (MAO-B) inhibitors, dopamine agonists, and levodopa (L-DOPA). MAO-B inhibitors reduce dopamine metabolism at the synapse, slowing the process of dopamine breakdown [25]. They are effective for early-stages of PD or in conjunction with other therapies in more advanced stages; however, they are not as effective as dopamine agonists or levodopa. Dopamine agonists activate pre-and post-synaptic dopamine receptors directly [25]. The agonists stimulate parts of the brain that are usually influenced by dopamine, tricking the brain into thinking that it is receiving the dopamine it needs [23]. Agonists can also be used alone or in conjunction with other medications;
however, they still are not as effective as levodopa. With the variety of dopaminergic therapies, levodopa remains the most effective oral treatment.

As mentioned in section 2.1.2, L-DOPA is the precursor of dopamine in dopamine synthesis. This dopaminergic medicine is given orally as the active dopamine precursor, L-DOPA, because dopamine cannot cross the blood-brain barrier. L-DOPA can cross the blood-brain barrier, and once inside a dopaminergic neuron it can be converted to dopamine [25]. To further enhance its effects, L-DOPA is given with the addition of a catechol-o-methyl transferase (COMT) inhibitor. COMT inhibitors reduce the metabolism of L-DOPA by decreasing its breakdown in the stomach, increasing the L-DOPA half-life making it last longer. Even with the help of COMT inhibitors, L-DOPA effects tend to wear off usually resulting in increased dosage of L-DOPA. Increasing dosage only will work up to a certain point before considerable side effects occur.

The most common and debilitating side effect from L-DOPA treatment is levodopa-induced dyskinesia (LID). The dyskinesia (spontaneous, involuntary movement) occurs after increased dosing and long-term use of L-DOPA. It is not well known how L-DOPA causes LID; however, dyskinesia only becomes present after the dopaminergic therapy has been in use for a prolonged period of time [28]. Knowing the pathogenesis of LID can help create a treatment for an incapacitating side effect to the most effective PD treatment. One proposed mechanism of LID is based off of recent observations that show an overactivity of glutamatergic systems that use N-methyl-D-aspartate (NMDA) receptors in the basal ganglia [28]. Attempts to treat LID based off of this proposed mechanism suggests using NMDA receptor antagonists to decrease the hyperactivity of the receptors.
2.2 Ketamine

Ketamine is a commonly used anesthetic, that when administered at a sub-anesthetic level, can be used to treat chronic pain as well as treatment-resistant depression [11,32]. Knowing that it can hold more functions than being just an anesthetic, studies have been performed to find novel ways this preexisting drug can be used in other contexts. Discovering new uses for already approved drugs is beneficial for the medical and scientific community, saving time and money. One of these new treatments for ketamine is for the reduction of LID in Parkinson’s patients. Ketamine acts on the brain in many different ways, binding to multiple receptors, leaving its mechanism of action largely unknown.

2.2.1 Molecular Mechanisms

Among its many mechanisms of actions, ketamine is most commonly known for being a non-competitive NMDA receptor antagonist. Inhibiting the NMDA receptor produces ketamine’s well-known dissociative effects. The glutamatergic NMDA receptor is an important receptor in post-synaptic terminals, critical for memory and cognitive function [14]. Long-term potentiation is also inhibited via the NMDA receptor blockade produced by ketamine. This blockade also increases release of dopamine by down-regulating the inhibitory pathway produced when the NMDA receptor is activated via GABA interneurons [27].

Not only does antagonizing the NMDA receptor increase dopamine release, but ketamine also has a high binding affinity for the dopamine D2 receptor [15]. Out of the five dopamine receptors present in the brain, ketamine is a partial agonist on the D2 autoreceptor, a g-coupled protein receptor located in high density in the striatum, particularly in the nucleus accumbens. When activated, D2 receptors decrease the neural activity of dopaminergic neurons [14]. There are several D2 agonists that are used as treatment for PD. From our knowledge of PD, this
appears to be contradictory to how we know PD works. The D2 receptor’s inhibition on movement could be used as a treatment for decreasing involuntary movement. As mentioned earlier, the stability between the inhibition and dis-inhibition of movement is altered in PD. D2 agonists could regulate these two pathways by re-establishing balance. In addition, ketamine’s up-regulation of dopamine through NMDA hypofunction can be counterbalanced with the activation of D2 receptors.

Furthermore, ketamine also binds to opioid receptors when administered in high doses, causing antagonistic effects on the Mu opioid receptors (MOP) and Kappa opioid receptors (KOP)[11]. MOP receptors are typically found in the ventral tegmental area (VTA), another region of the brain that produces dopamine whose projections send to the nucleus accumbens, a region in the ventral striatum. Opiates that bind to the MOP receptors inhibit GABA interneurons, neurons that typically inhibit dopaminergic projections to the nucleus accumbens in the VTA, allowing for dopamine release [14].

2.3 Measuring Brain Activity

Apart from understanding ketamine’s molecular mechanisms, it is important to know how brain activity is affected when under the influence of this drug. Neurons are excitable cells that encode information with electrical signals, transmitting this information via synapses with other neurons [9]. These electrical signals can be recorded in a variety of ways, using both intracellular and extracellular electrodes. Intracellular electrodes are placed directly into the soma of a neuron, measuring the varying potentials of one cell while extracellular electrodes sense action potentials from nearby neurons.
2.3.1 Local Field Potentials and Single-Unit activity

Typically, neurons respond to stimuli in ensembles, forming synchronized action potentials, more commonly referred to as field potentials, and extracellular electrodes can detect this summed activity [14]. The synchronized activity between the ensemble of neurons is interpreted as oscillations. Electroencephalography (EEG) and local field potentials (LFP) are common forms of recording methods that detect neuronal oscillations. EEG recordings detect electrical activity by placing electrodes on the skull, measuring oscillations from the outermost part of the cortex. LFPs use intracranial electrodes, allowing for better resolution of oscillations, as well as a more precise recording of desired regions, including those deep in the brain.

Oscillations are believed to underlie cognitive processing, and have been divided into well-defined frequency bands. The frequency bands defined as follows:

- Delta (<4Hz)
- Theta (4-8 Hz)
- Alpha (8-13Hz)
- Beta (15-30 Hz)
- Gamma (30-80Hz)
- High frequency (130-180 Hz)

The frequency of neuronal oscillations is influenced by factors such as number, size and type of neuron involved in the generation of the oscillation, as well as the degree of synchronization between neurons [4,5]. The different frequency bands of oscillations do not act independently, instead they interact with each other. Interactions between oscillations, cross-frequency coupling, has been linked to cognitive functions such as reward-signaling, decision-making, working
memory, attention and learning [13]. The coupling of oscillations varies across brain regions and change in response to sensory, motor and cognitive events [6,7].

Abnormal oscillatory activity can also be a determinant for neurological and psychiatric diseases. For example, in PD there is an exaggerated synchronization in the beta frequency band between the motor cortex and the basal ganglia, as well as changes in the gamma range after dopaminergic intake [18,22]. Recording oscillations provides useful input at the level of neural populations; however, this is also a drawback. Measuring the summation of activity from a large group of neurons is helpful in understanding the average activity; however, this also means it is capturing many neural processes.

Recording single-unit activity, the activity of an individual neuron, provides better resolution of how a particular neuron responds. The action potentials of individual neurons are recorded with the same electrodes used for local-field measurement. These extracellular electrodes record the currents surrounding the cell body of an active neuron. Action-potentials are characterized by brief (~1 msec) changes (“spikes”) in voltage measured at the tip of the electrode. Single-units assist in understanding circuits and possible connections between these circuits as well as among different regions in the brain; while LFPs can relate to the bigger picture of the functions of large neural ensembles.

2.3.2 Ketamine’s effects on brain activity

We have reviewed ketamine’s molecular mechanisms, and saw that its main function is as an antagonist to the NMDA receptor. The NMDA receptor is important in synaptic plasticity and brain development, so it should be assumed that blocking it would have implications on
brain activity. Since brain oscillations and cross-frequency coupling vary between brain regions, ketamine’s effects also vary depending on the recorded area.

In an experiment performed by Nicolas et al. in the motor circuit of the basal ganglia, ketamine was shown to induce an increase in low gamma band oscillations (~50 Hz) as well as high gamma band oscillations (~80 Hz). The high gamma band were constrained in the basal ganglia nuclei and directly related to motor behavior, showing high gamma oscillations during hyperactivity [22]. In PD patients, an increase in high gamma activity in the basal ganglia has also been linked to dopaminergic stimulation [3,22]. Lastly, ketamine caused an overall increase in high frequency oscillations (~150 Hz) throughout the entire motor circuit of the basal ganglia with a high correlation with motor behavior, meaning an increase in movement was highly correlated with the increase in high frequency oscillations [22].

In the hippocampus, Caixeta et al. revealed that ketamine also increased gamma and high frequency oscillations, as well as altered cross-frequency coupling in various layers of the hippocampus. In particular, low frequency theta oscillations were coupled with high frequency oscillations during peak locomotion [6]. Interestingly, theta and gamma oscillations were coupled at low doses, yet disrupted at higher doses of ketamine. This further proves that oscillations vary not only in different regions, but are also dose-dependent.

Focusing on our experimental aims, Alonso-Frech et. al present a study focusing directly on neural oscillations and single-unit activity in LID. They found that theta band oscillations, particularly in the subthalamic nucleus, are associated with LID expression in PD. This further suggests that specific motor states have a particular oscillatory activity. In single-unit activity too, there is decreased firing rate as well as abnormal firing patterns in the same regions with the low frequency oscillations [1].
Overall, neuronal synchrony is important for selecting and routing information across brain regions as well as within particular regions. Depending on the dosage and the brain region, ketamine can increase or decrease oscillatory activity, possibly regulating the abnormal activity found in PD as well as LID, making it a potential treatment.

3. MATERIALS AND METHODS

3.1 Animals

Male Sprague-Dawley rats (n=3) were used and housed in a temperature and humidity controlled room with 12 h reversed light/dark cycles. They had free access to food and water at all times. All animals were treated as approved by the Institutional Animal Care and Use Committee, University of Arizona and in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

3.2 Surgical Procedures

3.2.1 Electrode Arrays

Each animal was implanted with two 32-channel chronic electrode arrays for neurophysiological recordings. Each array was hand-made, using manufactured EIB boards. Each array contains 18 stereotrodes loaded into glass silica. The stereotrodes were cut to specific depths in order to reach target regions. The study targeted multiple regions, including the striatum, hippocampus, nucleus accumbens, motor cortex and somatosensory cortex. Figure 2 shows the two arrays used, along with the target regions for each array.
Figure 2. Electrode arrays used to record neurophysiological data. A: Array 1, targeting the primary motor cortex, DLS, DMS and NAc. B: Array 2, targeting the hippocampus and primary somatosensory cortex.

3.2.2 Surgery

The aforementioned arrays were implanted in anesthetized rats during surgery. The arrays were implanted bilaterally following the coordinates from Paxinos Rat Atlas:

Motor cortex: AP: 1.7, ML: +/-2.8, DV: 1.4 mm
DS: AP: 1.5, ML: +/-2.8, DV: 4.2 mm
STN: AP: -3.8, ML: +/-2.5, DV: 7.7 mm
HC: AP: -3.8, ML: +/-2.5, DV: 2.7 mm

Parietal Cortex: The electrodes in the parietal cortex were used to test and control for the volume condition of the LFP signal on the motor cortex probes. AP: -3.8, ML: +/-3.2, DV: 1.2 mm

A screw was implanted above the cerebellum as a reference for the LFP signals.

Animals were given a week for recovery post-implantation, and supplied with pain medications.

3.3 Experiment

Experiment sessions occurred once a week per rat. Recordings were conducted in the rats’ home cage and unconstrained movement was monitored with motion tracking via an
overhead camera. The sessions began with a 1-hour baseline before the first injection. Injections followed every two hours, totaling 5 injections over 11-hour recording sessions (Figure 3).

Injections were introduced intraperitoneally (i.p.), each rat receiving three ketamine sessions (Ketamine hydrochloride, 20 mg/kg) (Tocris Bioscience, Bristol, UK), one saline session (physiological saline, SAL), as well as one baseline session.

![Experiment 1 Timeline]

*Figure 3.* Outline of the experiment, denoting length of the recording session as well as the introduction of each injection.

### 3.4 Data Analysis

Neurophysiological data was recorded with the AmpliRec system and post-processed through MatLab. LFP and single-units were analyzed with the Spike2 software and MatLab. Single-units were characterized by waveform shape and principal components. Interspike intervals were used to help determine spiking characteristics.

### 4. RESULTS

Here we focused on the single-unit activity in the dorsal and ventral striatum. The behavioral data (see Figure 4) from the rats observed supported previous studies indicating that ketamine increases locomotor activity in rats [6, 22]. From the single-unit data analyzed, eight cells were extracted from the dorsolateral striatum (DLS) and five from the nucleus accumbens (NAc). *Figure 5* shows a cluster summary of one of the neurons isolated. This summary was generated for each cell in order to analyze its characteristics, such as waveform, shape, principal
components and interspike interval. Overall, it appears that ketamine has a heterogeneous effect in both regions.

![Graph showing locomotor activity after each injection](image1)

**Figure 4:** Locomotor activity after each injection, denoted by the red line. Locomotion measured as pixel change over time (speed denotes z-score)

![Neuron cluster summary](image2)

**Figure 5.** Cluster summary of one neuron in the DLS. This summary sheet summarizes features of a neuron (e.g., firing rate, oscillatory activity, isolation from other neurons) and helps identify characteristics of what we determined as a good cell. The waveform shape contains a negative amplitude, lasting roughly 1ms. PC1 and PC2 show the principal components of the neuron (neuron of interest in blue). The principal component plots (PC1-PC3 in upper right) indicate the degree to which this neuron was separately clustered from other neurons. The ISI (interspike interval) informs us of the neuronal variability, in a good cell the first couple ms of the ISI should be empty (or very low) due to the nature of a spiking cell. The summary also includes drift detection (bottom plot) to see if voltage levels varied throughout the recording (an indication of an unstable electrode).
4.1 Nucleus Accumbens

After spike sorting single-unit activity in the NAc, five cells were identified. Various tests were performed to determine the characteristics differing these cells in order to understand the type of cells they were and if an overall trend to could be determined.

When comparing baselines (Figure 6), there was no significant difference between the cells that were up regulated versus the ones that were down regulated ($p=0.8826$). Furthermore, comparing the waveforms from two cells they appear similar, making it difficult to make any judgments (Figure 10). The variance of the single-unit activity, Figure 7, shows that the cells that were up regulated in the presence of ketamine decreased their variance, meaning their firing patterns became more predictable and tonic after injection, while those that were down regulated increased their variance, meaning more sporadic firing patterns.

![Baseline Firing Rate in NAC](image)

**Figure 6.** Baseline firing rates of up-regulated (n=3) and down-regulated (n=2) neurons. Performing a T-Test concluded that $p=0.8826$. Errors bars indicated SEM.
Figure 7. Overall change in variance before and after ketamine injection in the NAc. Up-regulated cells (n=3) decreased in variance while those that were down regulated (n=2) increased in variance. Performing a T-Test concluded that p=0.2214, error bars indicate difference in SEM.

The autocorrelation of the down-regulated cells (Figure 8) indicated bursting-like behavior due to its shape, denoted by a sharp peak. The up-regulated cells also changed shape post-injection, indicating bursting behavior as well.

Figure 8. Autocorrelation of cells before (right) ketamine and after (left) ketamine in the NAc. The down-regulated neurons showed bursting activity both before and after ketamine, while the up-regulated neurons showed an increase in bursting-like activity after ketamine. Error bars indicate SEM.
Figure 9 and Figure 10 show two peri-event time histograms (PETH) of two individual cells in the NAc that show opposing effects to ketamine. It is interesting to note that the effects of ketamine, decreased over time. By the 5th injection, the inhibition/excitation had a smaller duration and was also not as strong of a response.

Figure 9. Individual up-regulated neuron from the NAc. A: PETH shows excitation after each ketamine injection (denoted by the red line). The excitation response decreased over time, showing lower frequency excitation by the 5th injection. B: Corresponding waveform of the neuron represented in 9A (Green).
4.2 Dorsolateral Striatum

Cells that responded differentially to ketamine were also found in the DLS. Unlike in the NAc, the baseline firing rate before ketamine for the cells in the DLS (n=8) were noticeably different. The cells that were up regulated had a lower baseline than those that were down regulated (Figure 11). Just like in the NAc, the cells that were excited decreased in variance while those that were inhibited increased their variance, although not much, possibly indicating that the inhibited cells were not affected much by ketamine in the sense that their firing rate was maintained fairly tonic (Figure 12).
Figure 11. Overall baseline firing rates of all neurons that were either up regulated or down regulated in the DLS in response to ketamine. Up-regulated neurons (n=6) had a lower baseline firing rate than those that were down-regulated (n=2). Performing a T-Test concluded that p=0.0124, indicating there is a significant difference between the baseline firing rate of the up-regulated neurons compared to the down-regulated neurons. Error bars indicate SEM.

Figure 12. Overall change in variance before and after ketamine injection in the DLS. The cells that were up-regulated (n=6) showed a decrease in variance in effect to ketamine administration. The cells that were down-regulated (n=2) increased in variance slightly, showing almost no change in variance. Performing a T-Test concluded p=0.3350, showing that these differences between the two different types of cells were not significant. Error bars indicate difference in SEM.
The autocorrelation of the excited cells demonstrated that ketamine increased their firing rate, the shape of the curve did not change, the frequency just increased (Figure 13). The autocorrelation of those that were down regulated also maintained the same shape; however, this presence of the sharp peak also indicates that the down-regulated cells fired in a bursting pattern. The peak did decrease, indicating the frequency at which the cells fired decrease, but the time between each cell firing was not constant, both before and after injection. Figure 14 and Figure 15 show the PETH and waveform of two individual neurons in the DLS with opposing effects in response to ketamine.

Figure 13. Autocorrelation of cells before ketamine (right) and after ketamine (left) in the DLS. The up-regulated neurons showed an increase in firing after ketamine. The down-regulated neurons show bursting activity, denoted by the sharp peak, as well as a decrease in firing rate after ketamine. The error bars indicate SEM.
Figure 14. Individual up-regulated neuron from the DLS. **A:** PETH shows an increase in activity after each injection (denoted by the red line). **B:** Corresponding waveform of the neuron represented in **A.**
5. Discussion

With the small sample size of single-units, it is difficult to make definite claims on how ketamine affects single-unit activity in the brain, especially in relation to motor circuits in the basal ganglia. Nonetheless, these initial results provide insight on single-units and their unique responses to ketamine.

From the two responses found, ketamine could be acting both on medium spiny neurons as well as the GABA interneurons. Spiny neurons are typically inhibitory neurons, which
explains the inhibitory feedback found in the striatum. These spiny neurons are mediated by inhibitory GABA-ergic interneurons. As mentioned in the introduction, NMDA antagonists decrease the activity of these interneurons, allowing for an increase in dopamine release as well as increased locomotor activity (Figure 5). However, NMDA receptor hypofunction does not solely affect these interneurons, another underlying mechanism could be at play. The cells that we saw that were inhibited after ketamine could be these interneurons, while those that were excited by ketamine could be the dis-inhibited medium spiny neurons. This can be determined usually by looking at waveform shapes because interneurons are known to be smaller in size; however, the waveform shapes presented did not help in determining which cell had been identified.

There was an interesting response to ketamine in the NAc, where the effect of ketamine decreased in duration over time after each injection. This could implicate that these neurons become de-sensitized to the effect of ketamine, meaning that over time, they will not have as dramatic of a response than when first introduced. This decreased response could show ketamine’s affect on plasticity in these neuronal ensembles, possibly regulating cell firing patterns over time, recreating a balance that was lost during the progression of PD and LID treatment [1,3].

Single-unit activity alone, even with a large sample size, cannot be the only way to analyze ketamine’s effects. The next step would be to combine the oscillatory activity with the single-unit activity to tie everything together. Previous experiments performed in our lab analyze the LFPs in the same regions where single-unit activity was recorded. Preliminary results show that repeated doses of ketamine induce sustained gamma and HFO coupling in the DLS and MI. This suggest that there could be an additive effect to repeated ketamine injections. In addition,
we found that HFP and beta-band oscillations are anti-correlated. This could suggest a competitive role for ketamine-induced changed in oscillatory activity, which could be seen as a potential mechanism in reducing LID. Comparing the single-unit activity being collected along with the LFP data could assist in understanding the mechanism ketamine uses to increase motion in order to be a potential treatment. This has already been proven beneficial because we are studying the effects over a prolonged period of time with repeated exposure to ketamine. If it is used as a treatment, it will be used over a prolonged period of time therefore understanding the long-term effects of sub-anesthetic doses is crucial.
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