The effect of antipsychotic medication on parvalbumin interneurons in the primate prefrontal cortex: Implications for cognitive functioning in schizophrenia

Comprehensive Proposal submitted by:

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I. SUMMARY

BACKGROUND. GABAergic interneuron dysfunction contributes to the development of schizophrenia (Nakazawa, 2012). In particular, dysfunction of parvalbumin (PV)-expressing interneurons is associated with the emergence of cognitive symptoms of schizophrenia and these symptoms predict long term functional disease outcomes (Lewis, 2012; Glausier, 2017; Kaar, 2019; Bowie, 2006; Lepage, 2014). There is substantial evidence that PV interneurons within cognitive prefrontal circuits are involved in working memory and attention processing via the regulation of excitatory pyramidal cells (Wang, 2004; Homayoun, 2007; Murray, 2015). However, little is known about how typical and atypical antipsychotic medications affect PV interneurons and their role in regulating cognitive circuits in the primate prefrontal cortex.

To explore the effect of antipsychotic drugs on primate prefrontal PV interneurons, I propose to examine intrinsic and network properties of macaque monkey prefrontal PV interneurons after antipsychotic drug treatment, as well as how drugs may provide resistance to schizophrenia-like symptom formation. We will compare the effects of typical (haloperidol) and atypical drugs (clozapine). I hypothesize that each class of drugs will exert differential effects on intrinsic interneuron properties and that changes induced by clozapine will lead to greater resilience to cognitive symptom formation elicited by NMDAR drug challenge.

RESEARCH AIMS. **In Aim 1, we will use patch clamp electrophysiology to investigate changes in the intrinsic properties of PV interneurons after exposure to haloperidol or clozapine**. Changes are expected in membrane properties including action potential initiation and subthreshold properties. In order to make these results comparable to in vivo neural recordings, neural response to 'noise' stimuli resembling biological signals will be tested.

In order to examine drug effects within active cognitive circuits, **in Aim 2, we will investigate the resiliency effects of long term administration of haloperidol or clozapine on neurons in vivo under NMDAR drug challenge during a working memory task**. We will use multichannel electrode arrays to record neural activity before and after the administration of ketamine, an NMDAR antagonist that mimics symptom formation and pathophysiology in schizophrenia (Morgan, 2004; Lewis, 2012; Weickert, 2013; Starc, 2017; Frohlich, 2014). I predict that clozapine will have the greatest effect on reducing ketamine induced cognitive dysfunction and that performance will be correlated with regulatory inhibition provided by putative PV interneurons.

In Aim 3, we will examine the resiliency effect of long term administration of haloperidol or clozapine on NMDAR drug challenged PV neurons using patch clamp electrophysiology. We will record response properties of the cells before and after administering ketamine into the holding solution. NMDAR specific contributions will be measured.

SIGNIFICANCE. Findings will improve our understanding as to how current and novel treatments for schizophrenia affect PV interneurons, an important component of prefrontal circuits for cognitive computation. Critically, using a macaque monkey model facilitates our understanding of complex circuits that are unique to the expanded prefrontal cortex seen in primates, expanding on foundational research conducted in rodents and increasing the translation to patients with schizophrenia.

II. ABSTRACT

Cognitive dysfunction is a core symptom of schizophrenia and closely predicts long-term functional disease outcomes. Complex cognitive functioning relies on the neural circuits within the prefrontal cortex consisting of diverse cell types. In particular, GABAergic parvalbumin expressing interneurons maintain normal circuit functioning through providing inhibition that both stabilizes and refines circuit activity. Indeed, parvalbumin interneuron dysfunction is evidenced to play a large role in symptom formation in schizophrenia. However, the effect of common first and second generation antipsychotic drugs on parvalbumin interneurons as well as the resulting effect on primate prefrontal circuits activity is currently unknown. In this proposal, I outline a series of experiments designed to: 1) Assess the effects of common antipsychotic drugs on intrinsic properties of primate prefrontal parvalbumin interneurons, 2) Assess the effects of common antipsychotic drugs on cognitive performance and on neurons within an activated prefrontal circuit as well as resiliency to schizophrenia-like symptom formation, and 3) Identify receptor response properties underlying the resiliency effect of common antipsychotic drugs on schizophrenia-like symptom formation.

III. BACKGROUND

Schizophrenia is a debilitating and chronic mental disorder that affects approximately 20 million people worldwide (GBD, 2018). Symptoms are most often classified into three categories: positive, negative, and cognitive (NIMH, 2016). Positive symptoms include hallucinations and delusions, negative symptoms are the absence of typical emotion or behaviour such as asociality and flattened affect, and cognitive symptoms include deficits in attention, working memory, and general executive functioning (NIMH, 2016). Cognitive dysfunction has recently gained recognition as a core symptom of schizophrenia and closely predicts functional disease outcomes as deficits contribute to impaired social and vocational functioning (Bowie, 2006; Lepage, 2014). Moreover, these symptoms are challenging to treat using currently available therapies including treatment with typical and atypical antipsychotic medication (Gold, 2004). Our current lack of knowledge regarding how antipsychotic treatment affects neural activity underlying cognitive processing in humans, in particular prefrontal neuron activity, hampers the development of effective pharmaceutical treatment of cognitive symptoms.

Two commonly prescribed antipsychotic medications are haloperidol and clozapine. Haloperidol is a typical antipsychotic or first-generation antipsychotic drug (FGA) and is widely used as a reference standard for treatment of schizophrenia symptoms (Davies, 1998; Apiquian, 2003). It competitively blocks postsynaptic dopamine (D2) receptors (NIH, 2020). Despite significant side effects, haloperidol is highly effective at treating positive symptoms of schizophrenia (Apiquian, 2003). Although its ability to treat cognitive symptoms has been challenged, there is evidence that haloperidol does increase overall cognitive performance in patients with schizophrenia (Woodward, 2007).

Clozapine is a synthetic dibenzo-diazepine derivative and is recognized as a highly effective atypical antipsychotic or second-generation antipsychotic drug (SGA) (NIH, 2020b; Warnez, 2014). Clozapine acts as a receptor antagonist with high affinity for serotonin Type 2 (5HT2), dopamine Type 2 (D2), and acetylcholine muscarinic (M1) receptors. It's antagonism of dopamine type 2 receptors is weaker than haloperidol (NIH, 2020b). Clozapine is often endorsed as a gold standard of therapy and is evidenced to perform better than FGA drugs at treating cognitive deficits (Huhn, 2019; Lee, 1999).

Elucidating the primate prefrontal circuits that underlie cognitive functioning is challenging since they consist of intricate interactions between diverse cell types (Wang, 2004). Methodological limitations further hinder our ability to probe the contribution of neuronal types in cortical computations that underlie cognitive functioning in primates. Fortunately, an assortment of methods including optogenetics and genetic modification in rodents allows for more precise measurements of neuron contributions in neural circuits. Considering accumulating evidence in rodents and computational modeling, one type of neuron, parvalbumin (PV) expressing interneurons, play a key role of in the functionality of prefrontal circuits (Wang, 2004; Homayoun, 2007; Murray, 2015). PV interneurons are fast spiking GABAergic neurons that strategically innervate the soma and proximal dendrites of excitatory pyramidal cells. (Kawaguchi, 1997). This allows them to provide flexibility within cognitive circuits through dynamic inhibition of pyramidal neurons, which can serve to stabilize and refine circuit activity (Wang, 2004; Homayoun, 2007; Murray, 2015).

PV interneurons in the prefrontal cortex are largely vulnerable across many psychiatric diseases, including schizophrenia (Lewis, 2012). For example, a review of post mortem concentrations of PV interneurons concluded that the density of PV interneurons is reduced in the frontal cortex of patients with schizophrenia (Kaar, 2019). Indeed, dysfunction in parvalbumin interneurons is associated with the emergence of cognitive symptoms of schizophrenia (Kaar, 2019; Lewis, 2012). Despite the

accumulating evidence for PV interneuron dysfunction in schizophrenia, little is known about how FGA and SGA medications affect PV interneurons and how PV neuron regulation of cognitive circuits in the primate prefrontal cortex. Therefore, understanding the effect of common antipsychotic medications on PV interneurons has important implications on the treatment of cognitive dysfunction.

IV. PROJECT OVERVIEW

To explore the effect of antipsychotic drugs on primate prefrontal PV interneurons, I propose to examine intrinsic and network properties of macaque monkey prefrontal PV interneurons after antipsychotic drug treatment, as well as how drugs may provide resistance to schizophrenia-like symptom formation. In this proposal, **I outline a series of experiments designed to**: **1)** Assess the effects of common antipsychotic drugs on intrinsic firing properties of PV interneurons, **2)** Assess the effects of common antipsychotic drugs on cognitive performance and on neurons within an activated prefrontal circuit after ketamine drug challenge which mimics symptom formation of schizophrenia, and **3)** Identify receptor response properties underlying the resiliency effect of common antipsychotic drugs after ketamine drug challenge.

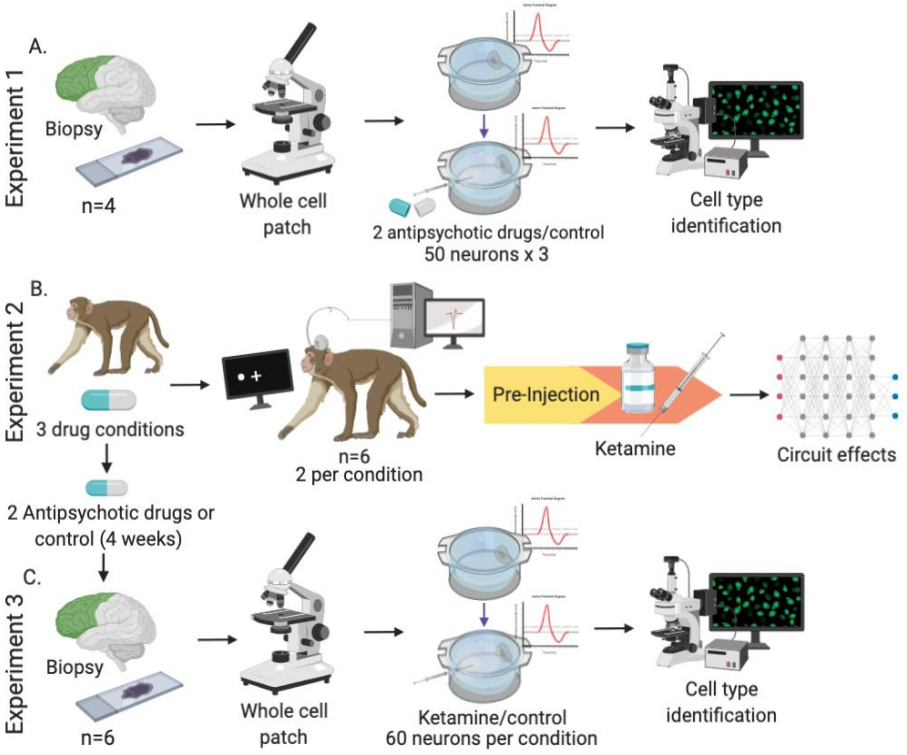


Figure. 1 **A**. Graphical depiction of general approach for experiment 1 using patch clamp electrophysiology to investigate drug induced changes in intrinsic cell properties. **B**. General approach for experiment 2 using in vivo extracellular recording paired with a working memory task.

I hypothesize that FGA and SGA medications will exert differential effects on intrinsic interneuron properties and that changes induced by clozapine will lead to greater resilience to cognitive dysfunction elicited by ketamine drug challenge. I will use a non-human primate model in experiments addressing the three aims.

To address Aim 1, I will use whole cell patch clamp electrophysiology to test the hypothesis that FGA (haloperidol) and SGA (clozapine) drugs diversely alter intrinsic firing properties of PV interneurons from control. **To address Aim 2**, I will test the hypothesis that long term administration of FGA and SGA drugs will mitigate behavioural and underlying neural effects of ketamine drug challenge. In vivo electrophysiology will be conducted using multi-electrode arrays to simultaneously record the activity of a population of neurons in order to study local circuit dynamics during performance of a prefrontal mediated task. **To address Aim 3**, I will use whole cell patch clamp electrophysiology to test the hypothesis that long term administration of FGA and SGA drugs

mitigates ketamine induced changes in PV interneuron receptor properties. Future directions for this project will also be discussed.

V. AIM 1

Rationale

The functioning of a neural circuit depends on the unique properties of the neurons that comprise it. In order to understand the specific roles that each class of neuron fulfill within a given circuit, it is important to understand the intrinsic properties of each neuron class. Neurons differ in their morphology, receptor expression, and intracellular signaling, all of which contribute to response properties of the cell (i.e. how a neuron responds to input) (Llinás, 2001). Whole cell patch clamp electrophysiology is one of the best ways to record basic information about these intrinsic properties by observing response properties through continuous and accurate recording of membrane potentials. Therefore, whole-cell patch clamp electrophysiology in current clamp mode will be used in the first aim to measure the effects of antipsychotic drugs on response properties of PV interneurons.

Using rodent tissue slices and cell culture, previous patch clamp experiments have shown that antipsychotic drugs increased excitability of neurons (Arvanov, 1997; Gemperle, 2003; Dzyubenko, 2017). Indeed, there is evidence that antipsychotic drugs influence excitatory neurotransmission, which impacts intrinsic response properties of cells (Daly, 1993; Dzyubenko, 2017). For example, one study demonstrates that haloperidol and an SGA drug that acts similar to clozapine increased excitatory synaptic input to hippocampal interneurons resulting in increased firing (Dzyubenko, 2017). Despite these findings, few patch clamp experiments are conducted in interneurons from the lateral prefrontal cortex which is unique to primates and crucial for cognition (Uylings, 2003; Seamans, 2008; Petrides, 2001; Funahashi, 2017). Therefore, we do not understand how clozapine or haloperidol influence the intrinsic firing properties of primate prefrontal PV expressing interneurons.
Hypothesis. I hypothesize that administering haloperidol and clozapine will increase the excitability of PV neurons compared to placebo control. I further hypothesize that this effect will be greater after clozapine administration than haloperidol.

Methods

Four macaque monkeys will be used for this aim. Following tissue retrieval and preparation, patch clamp recordings will be conducted in small cells with round or oval somas. Recordings will be conducted using a selection of stimulus protocols listed below. These stimuli are meant to investigate membrane properties of cells that contribute to the input/output function of the cell and to understand aspects of neural response properties that occur in vivo.

Recording protocols:

-Resting membrane potential will be measured in a 0 current configuration (I=0).
-**Ramp**: current injection of increasing intensity (20 pA per 1 second).
-**Short square pulse**: To determine rheobase. 3 ms injections starting at 80 pA and increasing in increments of 10 pA until action potential generation.
-**Long square pulse**: Duration allows neuron to reach steady-state (1000 ms starting from -80 pA to 160 pA, 20 pA increment).
-**Naturalistic Noise** (resembles in vivo inputs). Pink noise on top of square pulses (1000 ms, starting from 80 pA).

Baseline firing properties evoked by the various stimulation protocols will be recorded including action potential (AP) threshold, AP amplitude, AP frequency, and AP peak to trough width. The temporal distribution of AP trains will also be calculated as an index: (1 – (LISI/FISI)) where LISI is the average frequency of the last two inter-spike-intervals and FISI is the average frequency of the

first two inter-spike-intervals. Zero value indicates consistent firing and values close to 1 indicate firing adaptation. PV neurons tend to be non-adapting so this will be an important parameter for cell type identification (Markram, 2015). Cells will be split into three drug conditions: haloperidol, clozapine, and control (sham). After recording protocols are run at baseline, drugs will be applied to the bath in a concentration range that approximate brain concentrations that are reached after administration of therapeutic drug doses. Recording protocols will be run again and changes in firing properties will be compared in individual cells between baseline and after drug administration or sham. Changes in electrophysiological parameters from baseline will be compared between experimental conditions.

Anticipated Outcome

I anticipate that both clozapine and haloperidol will increase activity of PV interneurons, resulting in increased AP frequency and reduced AP threshold. Based on experiments conducted by Arvanov, (1997) in rodent cells, I expect clozapine to have a greater excitatory effect than haloperidol. Waveform shape and firing adaptation are not expected to change significantly.

Electrophysiological Parameters	Baseline (n=~50)	Control (n=~50)	Haloperidol (n=~50)	Clozapine (n=~50)
Resting membrane potential (mV)	−68 ± 8	No change	More +	More +
AP threshold (mV)	−41 ± 5	No change	More -	More -
AP amplitude (mV)	55 ± 11	No change	No change	No change
AP frequency (20 pA above threshold)	80 ± 9	No change	Large increase	Larger increase

Table 1. Anticipated overall differences in basic electrophysiological parameters between experimental conditions and baseline. Baseline measures are from recordings of monkey PV cells conducted by Povysheva, 2008.

Potential Challenges & Limitations

Since we will be recording from tissue slices, neurons will not be involved in active computations related to processing of cognitive tasks. Moreover, neurons will be isolated from inputs from computationally relevant cortical networks. With the addition of stimuli resembling biological signals (naturalistic noise), we hope to approach naturalistic responses as closely as possible. However, assuming a neuron's function within a given circuit (in this case a prefrontal circuit for cognitive computation) exclusively through investigating its intrinsic properties is not ideal since neurons with similar intrinsic properties may play different roles in different functional circuits (i.e.

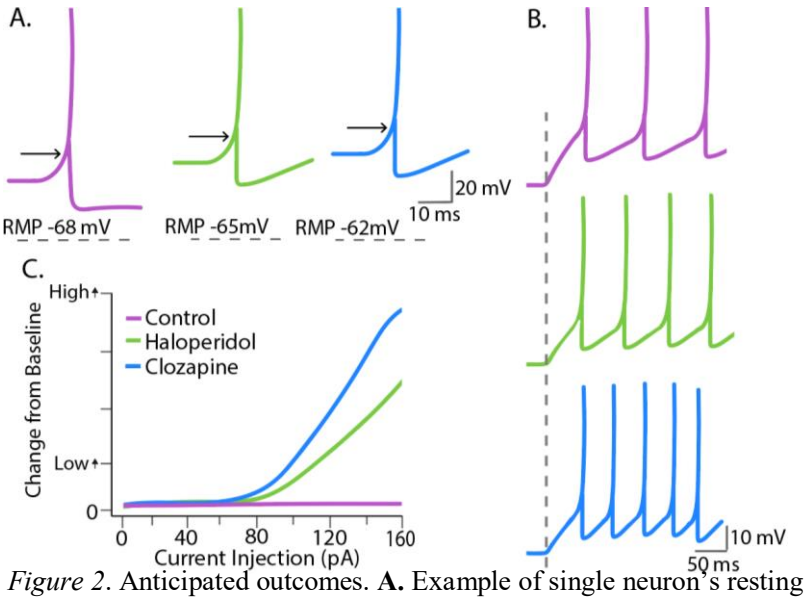


Figure 2. Anticipated outcomes. **A.** Example of single neuron's resting membrane potential during baseline recording (pink), or after administering haloperidol (green) or clozapine (blue). **B**. Example of a single neuron's firing pattern during baseline recording (pink), or after administering haloperidol (green) or clozapine (blue). **C**. Expected change of AP frequency from baseline after drug administration or

circuits for working memory or emotional processing). Neural activity during a specific function (task) must be taken into account to determine which properties of PV neurons may be appropriate for implementing the necessary computations. This is why we plan to conduct high density in vivo recordings during a working memory task in experiment 2.

VI. AIM 2

Rationale

Intrinsic firing properties allude to how a neuron may contribute to cognitive computations, but a neuron's functional role can be best understood during a behavioural task that activates a heavily researched circuit. Therefore, to understand how the activity of a PV neuron changes after exposure to clozapine or haloperidol, we plan to record PV neuron activity during a working memory task. Working memory circuits are well defined in the primate prefrontal cortex and working memory deficits are prevalent in schizophrenia (Funahashi, 1989; Constantinidis, 2018; Forbes, 2009; Starc, 2017).

Working memory (WM) is the cognitive process that allows us to briefly maintain and manipulate information perceived in our environment (Baddeley, 1986). The prefrontal circuit underlying working memory is well studied. It is hypothesized that working memory representations are enabled by persistent firing through recurrent pyramidal cell connections (Funahashi, 1989; Constantinidis, 2018). Lateral inhibition of pyramidal neurons from PV interneurons refine these representations (Rao, 2000; Wang, 2004). Glutamatergic N-methyl-D-aspartate receptors (NMDARs) are critically involved in balancing interactions between pyramidal cells and inhibitory interneurons, regulating computations that underlie working memory (Wang, 2013). Indeed, ketamine (an NMDAR antagonist) consistently elicits working memory deficits in humans and animals (Frohlich, 2014; Morgan, 2004; Malhotra, 1997; Wang, 2013). Moreover, ketamine exacerbates cognitive impairment in patients with schizophrenia (Malhotra, 1997).

Ketamine is found to have differential effects on cells types such that ketamine reduces activity of interneurons and increases activity of pyramidal cells in rodents (Homayoun, 2007). Indeed, NMDAR antagonists decrease the expression of PV in the rodent prefrontal cortex relating NMDAR hypoactivity to PV interneuron dysfunction (Cochran, 2003). This effect can also be observed in humans using brain imagining through increased prefrontal neural excitation after ketamine administration (Breier, 1997). Therefore, in order to counter the effects of ketamine, a drug would have to increase PV interneuron activity.

There is evidence that some antipsychotic drugs may impact NMDAR activity. Previous research supports the role of clozapine and, to a lesser extent, haloperidol in reversing cognitive deficits caused by ketamine as well as similar NMDAR blockers (Malhotra, 1997b; Jentsch, 1997). However, how these drugs counteract underlying neural effects of ketamine is unknown.
Hypothesis. Therefore, I hypothesize that pre-treatment with haloperidol and clozapine will reduce ketamine induced working memory deficits through increasing the activity of putative PV interneurons.

Methods

Six macaque monkeys will be used for this aim. These animals will be grouped into three drug conditions (two per condition). The first group will receive a four-week regimen of haloperidol, the second, clozapine, and the third, a placebo pill. Animals will be trained to perform a computerized spatial working memory task in which a cue will then be presented on screen in 1 of 8 possible locations and will disappear during a 1500-2000 ms delay period. A test target will then be presented

either in the same location as the cue (match) or a different location (non-match). The animals will be required to release a button if the location matches the cue location. Experimental sessions will begin after 30 minutes of the last drug dose then either ketamine (low dose; 0.4 mg/ kg) or saline will be injected intramuscularly. At least thirty correct trials per target location will be collected after injection. Seven saline control sessions and seven ketamine sessions will be recorded for each animal over a four-week span with two days between sessions to allow for drug washout. Saline and ketamine sessions will be randomly interleaved.

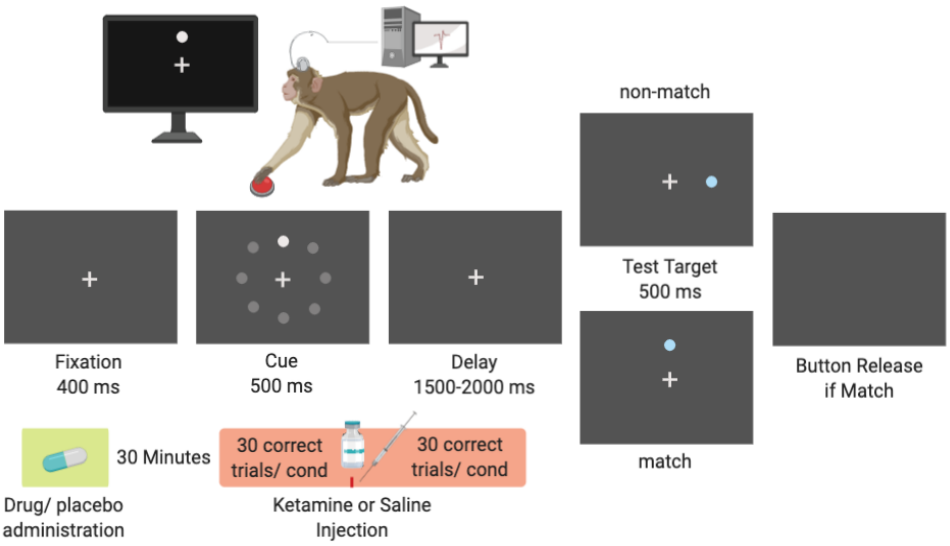
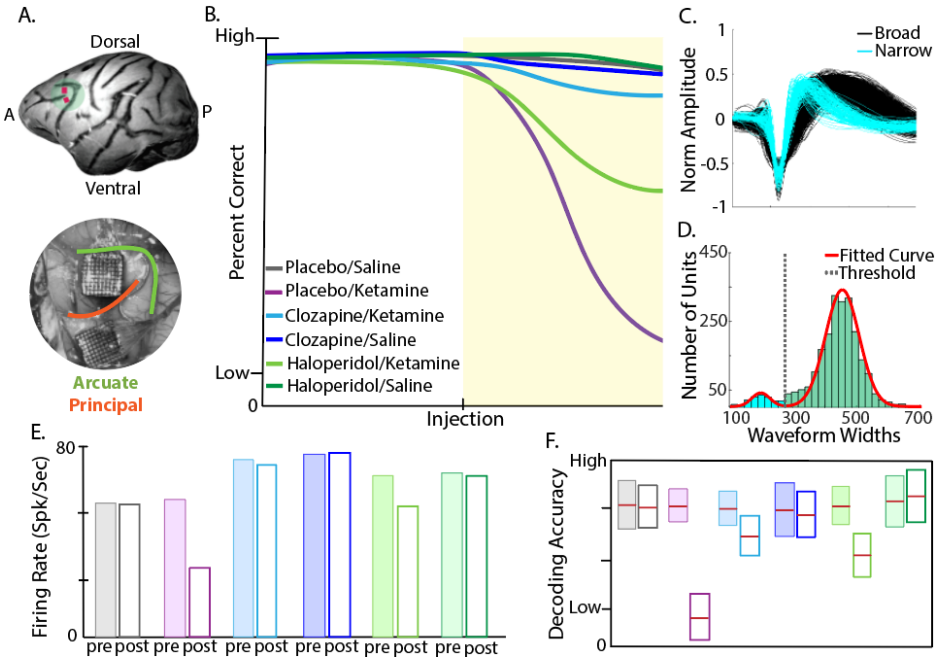


Figure 3. Depiction of behavioural task design and experimental session timeline.

Two 10x10 Utah arrays will be implanted in the prefrontal cortex (Brodmann areas 46/9) (Petrides, 2001) of all animals to record neural activity during the working memory task. Eye movement will also be recorded throughout experimental sessions.



A. Suggested site of electrode implantation in lateral prefrontal cortex. **B.** Predicted task performance (percent correct) for different drug conditions. **C**. Example of division between broad and narrow waveforms. **D**. Example of distribution of broad and narrow spiking neurons. **E.** Predicted firing rates of narrow spiking neurons before and after ketamine or saline injection. **F.** Predicted decoding accuracy before and after ketamine injection

Anticipated Outcome

Based on previous experiments, I expect a yield of ~150 neurons per session and ~10% of those neurons to be PV interneurons (~15 neurons per session). Classification of neurons will be conducted based on waveform width from peak to trough where fast, narrow spiking neurons are considered putative PV interneurons (McCormick, 1985; Gomez-Torres, 2020). After ketamine, but not saline injection, I

predict that task performance will decrease significantly from baseline levels. I predict that this reduction will be significantly less for animals pretreated with clozapine and to a lesser extent, animals pretreated with haloperidol. We predict that neurons classified as narrow spiking will increase their activity from baseline in both clozapine and haloperidol conditions. After ketamine injection, but not after saline injection, I predict that narrow spiking neurons in the placebo drug condition will decrease their firing rate. However, I predict that the firing rate of narrow spiking neurons in animals pre-treated with clozapine will not significantly change from baseline. Neurons pretreated with haloperidol are also predicted to be less affected by ketamine injection, but the compensatory effect is predicted to be weaker than clozapine.

By studying many simultaneously active neurons, we can approximate how successfully ensembles of neurons involved in the same computation represent information about a task or behaviour. The level of information content can then be correlated to performance of the task. For example, we can use machine learning to predict target location during the delay period from neural activity which tells us how strongly memory representations are encoded by neurons. I predict that after ketamine injection, we will see a decrease in the amount of information encoded in a population of neurons which can be correlated to decreased task performance. I predict that clozapine and haloperidol will counteract this effect leading to superior decoding of task related variables from neural activity. This essentially shows us how well populations of neurons within a circuit can perform task related neural computations.

Potential Challenges & Limitations.

Systemic administration of ketamine likely has global effects on the brain including regions with direct and indirect connections to prefrontal circuits of interest. An alternative approach would be to use local administration of ketamine in the prefrontal cortex (via microinjections or iontophoresis) to elicit localized effects. However, due to the volume of the macaque prefrontal cortex, it would be unlikely that local administration would elicit behavioral changes that would allow us to correlate neural activity to task related behaviour. In order to investigate local effects of ketamine on prefrontal PV neurons, we plan to use patch clamp electrophysiology in aim 3.

VII. AIM 3

Rationale

Reduced excitatory input to PV interneurons contributes to the pathophysiology of schizophrenia (Nakazawa, 2012). This is evidenced through reduced density of excitatory synapses on PV expressing interneurons in patients with schizophrenia (Chung, 2016). There is also evidence that antipsychotic drugs facilitate excitatory transmission, particularly, NMDAR neurotransmission after acute and prolonged exposure (Arvanov, 1999; Gemperle, 2003). For example, clozapine increases extracellular glutamate levels (Daly, 1993) and reverses NMDAR antagonist induced social withdrawal in rats (Corbett, 1995).

Several studies have investigated the effects of clozapine and/ or haloperidol on rodent cells in various brain regions and in cell culture using patch clamp electrophysiology. One of these studies found that clozapine and haloperidol increased NMDAR mediated responses in rodent medial prefrontal pyramidal cells (Arvanov, 1997). A further study shows that clozapine increases NMDAR mediated excitatory postsynaptic currents in rodent prefrontal pyramidal neurons (Gemperle, 2003). Clozapine is even evidenced to act as a NMDAR agonist through increasing glycine (co-agonist) binding (Schwieler, 2004). Long term use of antipsychotic drugs may also increase NMDAR expression evidenced by a study conducted by Dzyubenko, 2017 using hippocampal cell culture that

demonstrated that haloperidol and an SGA drug that acts similarly to clozapine increased synaptic density, leading to increased excitatory synaptic input to interneurons. Importantly, this increased input resulted in increased PV interneuron firing (Dzyubenko, 2017).

Despite evidence that clozapine and haloperidol influence NMDAR transmission, no studies have investigated how their effect may mitigate the NMDAR blocking effects of ketamine that lead to schizophrenia-like symptom formation. Using patch clamp electrophysiology in voltage clamp mode, we can record receptor specific activity by recording currents passing through open ion channels that cause changes in membrane potential. Therefore, in this aim, we will compare the effect of haloperidol and clozapine on NMDAR current responses in prefrontal PV interneurons using whole cell patch clamp techniques.

Hypothesis. I hypothesize that the facilitation of NMDAR responses produced by clozapine and to a lesser extent, haloperidol, will compensate for NMDAR blocking by ketamine.

Methods

We will use the same six macaque monkeys from experiment 2. Animals will be grouped based on previous antipsychotic drug exposure and put on a semi-chronic oral regimen of either haloperidol, clozapine, or a placebo control. After 4 weeks, the animals will be sacrificed and biopsies will be taken from the hemisphere opposite the implanted electrode arrays.

First, cells will be held at their resting potential and current responses will be measured to identify whether there are any overall differences present at baseline in the different drug conditions. Picrotoxin (GABA antagonist) will then be added to the bath and excitatory post synaptic currents (EPSCs) will then be recorded at +40 mV holding potential in the presence of NMDA, glycine, and physiologically relevant levels of Mg^{2+} in bath. AMPAR antagonist, NBQX, will then be added to better isolate NMDAR mediated responses. Cells will be returned to resting potential and ketamine will then be puffed in proximity to recorded cells. Cells will again be held at +40 to measure NMDAR-mediated currents. Current amplitudes will be compared before and after ketamine administration. Changes from pre-ketamine baseline will be compared between drug conditions.

Anticipated Outcome

Due to previous studies showing increased activity of neurons after exposure to clozapine and haloperidol, I predict that NMDAR currents will be increased by both drugs at baseline compared to the placebo condition. After ketamine administration, I anticipate that cells in the placebo condition will show reduced NMDAR-mediated EPSC amplitudes. I anticipate that cells in both the clozapine and haloperidol conditions will show reduced NMDAR-mediated EPSC amplitudes but will be less effected than cells in the placebo condition.

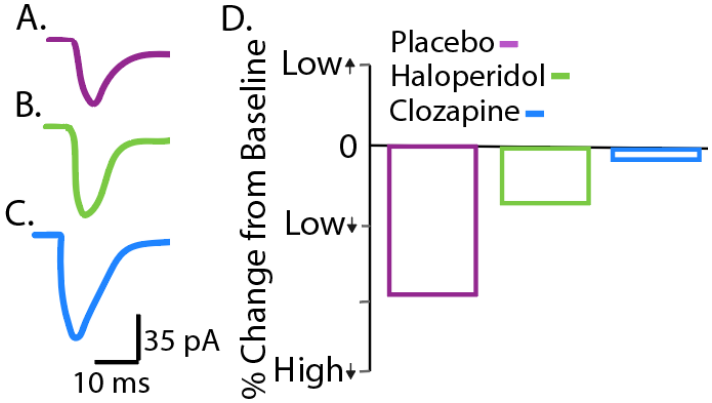


Figure 5. Anticipated results of individual neuron EPSC response. **A**. Example EPSC at baseline in placebo condition (black) **B.** Example EPSC at baseline in haloperidol condition (green). **C.** Example EPSC at baseline in clozapine condition (blue). **D.** Predicted change in overall EPSC amplitude from pre-ketamine to post-ketamine measurements.

Potential Challenges & Limitations

The current experiment does not take into account the effect of drugs on AMPA receptors that also contribute to excitatory neurotransmission or on the potential facilitation of NMDARs through AMPA receptor activation. The effect of clozapine and haloperidol on AMPA receptor response

could be investigated by holding cells at -80 mV (a potential in which NMDAR will be blocked by Mg^{2+}) and recording EPSCs. Future experiments will be conducted to investigate the role of antipsychotic drugs on AMPA receptors.

VIII. GENERAL METHODS

Animal Model

The effect of antipsychotic drugs on neurons has been primarily researched in rodents. The ability to genetically modify mice and use precise behaviour modifying techniques like optogenetics, which is challenging in non-human primates, makes rodent research indispensable. However, the agranular rodent frontal cortex has no structural/ functional equivalent of the lateral PFC (LPFC), a region in primates linked to complex cognitive computation like the maintenance of mental representations that underlie working memory (Uylings, 2003; Seamans, 2008; Funahashi, 2017). Differences in physiological properties of PV expressing interneurons have also been identified in which primate PV interneurons are more excitable then rodent cells (Povysheva, 2008). Therefore, to increase translation to patients with schizophrenia as well as create a bridge between rodent research and humans, I chose to use a non-human primate model for the current experiments.

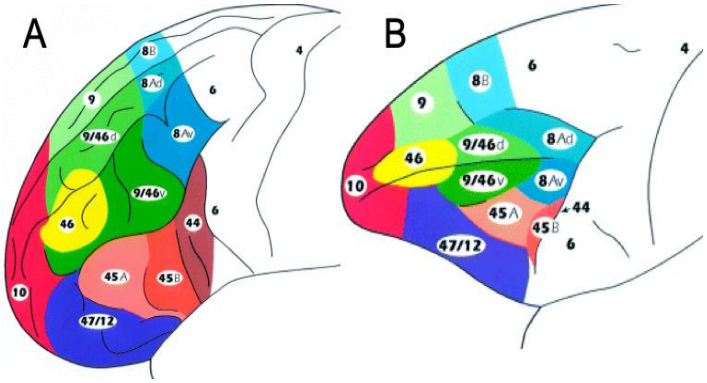


Fig. **A.** Human frontal cortex cytoarchitecture. **B.** Macaque frontal cortex cytoarchitecture. Figure adapted from Petrides, 2001

Laboratory-bred adult rhesus macaques (*Macaca mulatta*) will be used in all experiments. The anatomical and functional organization of the macaque lateral prefrontal cortex is similar to humans, including a notable expansion of lateral prefrontal cortex layer 3. This expansion includes an increase in neuron density and complexity (Petrides, 2001; Zikopoulos, 2018; Gilman, 2017). These animals are also capable of performing complex cognitive and behavioural tasks that are comparable to tasks used in human research.

Tissue Retrieval and Preparation

For experiment 1, prefrontal biopsies will be performed in four macaque monkeys. Surgery will be conducted under general anesthesia (ventilated isoflurane). After craniotomy and durectomy, ice-cold artificial cerebrospinal fluid (aCSF) will be used for irrigation of the biopsy region to restrict blood flow in order to reduce initial bleeding. The extracted tissue will then be rinsed in cold aCSF to remove blood and then transferred to a jar containing icy slicing solution saturated with carboxygen (95% O2/ 5% CO2). Tissue will be sliced to 300 um thickness and recovered at 37 degrees for 10 minutes in choline slicing solution (kept at room temperature for rest of experiment). For experiment 3, biopsies will be taken from hemispheres not implanted with electrode arrays.

Pharmaceutical Agents

Pharmaceutical application. In experiment 1, clozapine and haloperidol will be applied to bath solution. Applying these drugs to bath solution allows for high levels of control over drug concentration in order to match physiologically/ clinically relevant levels.

In experiment 2, animals will be trained to orally ingest drugs dissolved in preferred fruit juice/ pudding through a syringe twice daily in order to match clinical administration. Masking drug taste will be important for dosing and experimental control. The concentration of haloperidol will be

approximately 2.0-5.0 mg. Drug dose will be adjusted to achieve and maintain plasma concentrations that approximate therapeutic doses in patients (Zhang-Wong, 1999). Clozapine will be administered at approximately 400 to 600 mg/day a dose to maintain clozapine blood levels at therapeutic levels (Simpson, 1999; Kronig, 1995). Video monitoring will be conducted in home enclosures to assess drug induced changes in behaviour (i.e. bradykinesia, sedation, stereotyped behaviour). In experiment 3, ketamine will be puffed in proximity to a patched cell using a pipette. Puffing ketamine will reduce desensitization of membrane receptors (Feng, 2017).

Neuron Type Identification:

Neuron types are typically classified by electrophysiological properties, morphology, and/ or protein expression (Markram, 2015). For example, PV interneurons can be classified based on their fast-spiking phenotype and non-adapting firing patterns (Markram, 2015; Petilla Group, 2008) as well as their unique multipolar morphology with widely branching axons and round or oval soma shape (Tremblay, 2016). Basket cells are the most common form of PV expressing neuron and since these cells innervate the soma and proximal dendrites of pyramidal cells, they play a large role in regulating excitatory transmission. (Tremblay, 2016; Kawaguchi, 1997; Petilla Group, 2008).

After patch clamp recording is finished, cells will be filled with fluorescent dye for morphological reconstruction and cell type classification. Neurobiotin will be used for dye-filling since it spreads through cells quickly which increases structural detail (Kanjhan, 2008). Streptavidin Cy3 will be used as a fluorescent label since it binds to biotin with high affinity (BioLegend, 2020). Basket cells should be easily identifiable based on their unique morphology (Povysheva, 2008; Tremblay, 2016).

IX. FUTURE DIRECTIONS

Haloperidol and clozapine are both dopamine (D2) antagonists and positron emission tomography conducted in patients with schizophrenia shows that clozapine uniquely has an equivalent occupancy of dopamine D(1) and D(2) receptors (Tauscher, 2004). Dopamine D(1) receptors are prominent in primate prefrontal PV expressing interneurons and are implicated in prefrontal regulated cognition including working memory (Muly, 1998; Cools, 2011). Clozapine is also a 5-HT2A receptor antagonist and evidence shows that this affinity may also contribute to mitigating effects of clozapine on NMDAR drug challenge (Schwieler, 2004). Moreover, it is likely that glutamate/ dopamine interactions mediate the response of clozapine (Chen, 2002). Therefore, the independent and interaction effects of dopamine and serotonin on interneuron activity will be investigated in future studies.

X. SIGNIFICANCE & IMPACT

This proposal describes basic research using a primate animal model that aims to advance our understanding of the effect of commonly prescribed antipsychotic drugs. The primary outcome of this work will be a better understanding of drug influence on prefrontal PV interneurons and their role in cognitive processing. Since ketamine produces many of the same symptoms as schizophrenia and likely share a similar pathophysiology, these findings have important implications for explaining the underlying mechanisms that lead to cognitive deficits in patients with schizophrenia and how currently available treatment may counteract this mechanism. Findings may further elucidate the unique role of NMDAR in symptom formation and treatment. These findings provide a possible framework for treatment development aimed at mitigating cognitive dysfunction in schizophrenia.

XI. REFERENCES

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