Perturbing Neural Feedback Loops to
Understand the Relationships of Their Parts

by

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ABSTRACT

The basal ganglia are four sub-cortical nuclei associated with motor control and reward learning. They are part of numerous larger mostly segregated loops where the basal ganglia receive inputs from specific regions of cortex. Converging on these inputs are dopaminergic neurons that alter their firing based on received and/or predicted rewarding outcomes of a behavior. The basal ganglia's output feeds through the thalamus back to the areas of the cortex where the loop originated. Understanding the dynamic interactions between the various parts of these loops is critical to understanding the basal ganglia's role in motor control and reward based learning. This work developed several experimental techniques that can be applied to further study basal ganglia function.

The first technique used micro-volume injections of low concentration muscimol to decrease the firing rates of recorded neurons in a limited area of cortex in rats. Afterwards, an artificial cerebrospinal fluid flush was injected to rapidly eliminate the muscimol's effects. This technique was able to contain the effects of muscimol to approximately a 1 mm radius volume and limited the duration of the drug effect to less than one hour. This technique could be used to temporarily perturb a small portion of the loops involving the basal ganglia and then observe how these effects propagate in other connected regions.

The second part applies self-organizing maps (SOM) to find temporal patterns in neural firing rate that are independent of behavior. The distribution of detected patterns frequency on these maps can then be used to determine if changes in neural activity are occurring over time.
The final technique focuses on the role of the basal ganglia in reward learning. A new conditioning technique was created to increase the occurrence of selected patterns of neural activity without utilizing any external reward or behavior. A pattern of neural activity in the cortex of rats was selected using an SOM. The pattern was then reinforced by being paired with electrical stimulation of the medial forebrain bundle triggering dopamine release in the basal ganglia. Ultimately, this technique proved unsuccessful possibly due to poor selection of the patterns being reinforced.
To my family especially my mom and dad who have always encouraged me when I have been discouraged, loved me unconditionally, and provided me with opportunities and the space to grow

To my friend, Luke, who has always been willing to listen and keeps encouraging me to push onward
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CHAPTER 1

INTRODUCTION

The genesis for this work was an interest in understanding the role of the basal ganglia in motor control and learning. The basal ganglia is a set of four subcortical nuclei – the striatum, globus pallidus, subthalamic nucleus (STN), and the substantia nigra. Three of the nuclei can be further divided - the striatum into caudate nucleus and the putamen, the globus pallidus into the external (GPe) and internal globus pallidus (GPi), and the substantia nigra into the substantia nigra pars compacta (SNc) and the substantia nigra pars reticulata (SNr). These nuclei have complex interconnections with each other and other parts of the brain. The techniques in this work were designed to help probe interactions in hopes of gaining greater understanding of their function.

Striatal Architecture

The striatum is the main input structure to the basal ganglia and it is also the largest nuclei. The majority of its inputs are from the cortex and they come from a variety areas including motor, somatosensory, prefrontal, temporal and limbic systems (Rosell & Giménez-Amaya, 1999). The cortical inputs appear to remain segregated by their function during their progression through the basal ganglia nuclei, and they return via the thalamus to the same areas of the cortex they originated from (Alexander, DeLong, & Strick, 1986). In the case of sensorimotor inputs, they are arranged in somatotopic organization throughout the basal ganglia (Parent & Hazrati, 1995a; Parent & Hazrati, 1995b; Romanelli, Esposito, Schaal, & Heit, 2005). In addition to cortical inputs, the striatum receives inputs from the thalamus and inputs from the cerebellum via the thalamus (Bostan & Strick, 2010; Haber & Calzavara, 2009). The inputs from the
cortex and the thalamus are excitatory in nature. The striatum also receives inputs from dopaminergic neurons originating from the substantia nigra compacta and the ventral tegmental area (VTA). The activity of these dopaminergic neurons correspond to reward and the prediction of reward (Romo & Schultz, 1990; Schultz, Dayan, & Montague, 1997).

The striatum is made of three main categories of neurons. The first is the medium spiny projection neurons (SPN) which make up 90-95% of the neurons in the striatum. These neurons have GABAergic outputs that inhibit the neurons they are connected to and are the output neurons of the striatum.

The SPN’s dendritic spines have cortical synapse on their heads and dopaminergic synapses on the shaft of the spines (Freund, Powell, & Smith, 1984). When dopamine neurons are chemically destroyed, there is a significant reduction in the number of dendritic spines on SPNs which indicates dopamine’s important role in synaptic plasticity (Ingham, Hood, Taggart, & Arbuthnott, 1998). Each SPN receives about 5000 corticostriatal synapses per a neuron and the number of synapses that each cortical axon makes insures each SPN receives a unique set of cortical inputs (Kincaid, Zheng, & Wilson, 1998). These unique sets of input may make the SPN an ideal pattern recognizer. The SPNs also have a bistable membrane potential. The higher membrane potential is called the up state and the lower membrane potential is the down state with action potentials occurring mostly in the up state (Plenz & Kitai, 1998; Stern, Kincaid, & Wilson, 1997). Up and down states also determine whether the effect of dopamine is one of long-term potentiation or long-term depression.
The second type of striatal neurons are tonically active neurons (TAN). These interneurons have excitatory cholinergic outputs to SPNs. They have also been shown to be associated with the timing of reward briefly ceasing their tonic activity shortly after receiving a reward or detecting a cue associated with reward (Apicella, Legallet, & Trouche, 1997; Joshua, Adler, Mitelman, Vaadia, & Bergman, 2008; Kimura, Rajkowsi, & Evarts, 1984). Unlike dopaminergic neurons which increase firing with reward and decrease when the expected reward is missing, TAN firing decreases in both events, although the decrease in firing rate is less with a missing reward than an unexpected reward (Morris, Arkadir, Nevet, Vaadia, & Bergman, 2004). Morris et al (2004) also show that the time delay between the reward and the DA and TAN response are the same which may indicate that TAN signals the window to expect information from dopaminergic neurons about the reward value.

The third type of neuron in the striatum is the GABAergic interneuron. Like SPNs, they produce inhibitory outputs. They appear to have a stronger inhibitory effect on SPN than SPN collaterals have on other SPNs (Tepper, Koós, & Wilson, 2004). English et al (2012) conducted experiments where TANs were modified with channel rhodopsin and their results indicate that TAN activate GABAergic interneurons. By simulating the pause caused by reward and then return to tonic activity typically found in TANs, they noticed that firing activity in SPN’s briefly stopped indicating inhibitory inputs were triggered by the TANS. This may be further confirmation that TANs role in opening and closing windows for synaptic plasticity by dopaminergic inputs.

The architecture of the striatum seems to make a good candidate for the leaning of behaviors (represented as cortical patterns of activity) that are associated with reward.
The individual SPNs each have a unique set of cortical inputs that when activated together cause the SPN to go into an upstate increasing its probability of firing. The upstate when with paired dopaminergic inputs reinforces those synapses that created the upstate. The window for the dopaminergic effect is signaled by TANs and the closing of the window cause inhibition of the SPN making plasticity less likely to occur ensuring that the proper neurons are reinforced. Thus, the SPNs may act to monitor cortical activity seeking patterns of activity that were previously associated with reward.

The Basal Ganglia’s Role in the Selection of Behavior

The predominant model for understanding the function of the basal ganglia is the direct and indirect pathway model first proposed by Albin, Young and Penney (1989). When discussing this model, it important to realize that output neurons of the striatum, GPe, GPi and SNr are GABAergic and inhibitory whereas the outputs from the cortex, STN and thalamus are made up of excitatory neurons. This model divides the SPNs of the striatum into two distinct populations. The first is the direct pathway SPNs. For the direct pathway, SPNs project from the striatum to either the GPi or the SNr and inhibits the activity of neurons in those structures. Because the GPi and SNr are inhibiting the outputs from the thalamus, the direct pathway has the effect of disinhibiting the thalamus increasing its input to the cortex. This increased input from the thalamus makes the cortical behavior associated with original input in the cortex more likely to occur. For the indirect pathway, SPNs project to the GPe, then the STN and finally either GPi or SNr. Activation of this pathway results in the disinhibition of the STN. Because the STN is excitatory, there will be an increase in activity in the GPi and SNr. The inhibitory
outputs of these two nuclei will then depress the activity of the thalamus making it less likely the particular activity will occur.

The direct and indirect pathway can be distinguished by the type of dopaminergic receptors they have. The direct pathway has D1 receptors which excite the neurons when dopamine is released and the indirect pathway has D2 receptors which inhibit the neurons in the presence of dopamine (Gerfen & Surmeier, 2011). These effects indicate that dopamine encourages behaviors that are occurring at the same time as rewarding events by increasing the direct pathway activation and decreasing the direct pathway activation.

Overview of This Document

The basal ganglia like other neural structures has complex set of interactions. This work discusses a series of techniques that have been designed to help probe neural function in circuits like those of the basal ganglia. In the Chapter 2 of this work, a method of targeted short-term injections is described. The goal behind the design of this injection technique was to have a way of targeting small segments neural tissue altering its function multiple times in one experimental session. This technique would be especially useful in studying the loops that form between the cortex, basal ganglia, thalamus and then back to the cortex (Middleton & Strick, 2000; Parent & Hazrati, 1995a). Targeted injections could performed in one part of the loop and then neural activity on the other areas could be measured as the drug effects propagate through the system. It could also be used to target inputs from certain neurons such as the dopaminergic neurons or the TAN in the striatum.

The third chapter describes a possible method for detecting these drug induced perturbations in other parts of the brain. By training a self-organizing map (SOM), a
distribution of typical patterns and their rate occurrence can be created. Changes in this distribution can then be used to detect when neuron behavior has changed. This technique will hopefully be useful in detecting changes in neural activity during the learning of behaviors in places such as the cortex or striatum.

In the final chapter, a method is created that uses internal reinforcement of cortical patterns via medial forebrain bundle (MFB) stimulation is described. This method was designed to test the explanation of basal ganglia function proposed in the modeling work by Hunn (2008). This modeling work suggested that striatum learns to recognize patterns of cortical activity associated with reward and alters the activity of the thalamus through the structures of the basal ganglia. Then the thalamus influences cortical activity by reducing the signal to noise ratio of the pattern making it more likely that the rewarded patterns will occur again. This chapter will test this explanation of basal ganglia function by using MFB stimulation to release dopamine immediately after the occurrence of a selected pattern of cortical activity. According to our theoretical work, this should increase the occurrence of this selected pattern.
CHAPTER 2
LOCALIZED SHORT DURATION INHIBITION OF CORTICAL NEURONS USING MUSCIMOL MICROINJECTIONS

Introduction

The brain is a complex structure with many interconnected parts that interact with one another to process low level sensory inputs in a hierarchical way to create high level interpretations and actions that are then translated back into low level motor commands. The dynamics of these interactions are critical to understanding how the brain processes information and make decisions.

A method often used to study these interactions is to alter the functioning of one of the connected regions and then see how this perturbation affects the observed external behavior of the subject and/or the internal neural activity of connected brain regions. One common way to do this is to create permanent lesion either by destroying a region of the brain or severing the connections between connected regions. There are several drawbacks with permanent lesions. First, the same subject comparisons between normal and altered function cannot be done repeatedly over the course of the study. Second, the brain is highly adaptive over time and is able to compensate either through neural plasticity at the cellular level or through behavioral adaptation. Unless the study is focused on these adaptations, these changes over time can confound the results of these studies.

A second method creates temporary lesions using drugs injections that alter the behavior. This method’s advantage is that it allows multiple same subject comparisons between normal behavior and the drug altered behavior over the course of a study. In addition, the behavior of a neural structure can be manipulated in a variety of ways depending on the type of drug injected. The challenge is that many injections into the brain
spread far beyond the region being targeted and the effects can last for hours (Arikan et al., 2002; Edeline, Hars, Hennevin, & Cotillon, 2002; J. H. Martin, 1991; Parks, 2006).

Kliem and Wichmann (2004) demonstrated a technique that might be capable of creating localized and short-term injection. In their experiment, an injection cannula was bonded to a recording electrode and inserted into the globus pallidus pars interna allowing them to measure neural activity at the injection site. They demonstrated that neurons deactivated by injecting the GABA agonist muscimol could be rapidly switched from being deactivated to normal activity. They did this by following the muscimol injection with an artificial cerebral spinal fluid (aCSF) flush. Unfortunately, this study’s focus was on proving that their novel electrode design worked and not further quantifying the effects of this technique of injection. The experiment never addressed the effects of this injection method on neurons further away from the injection site.

The Kliem and Wichmann (2004) results became the basis of a later computational model attempting to quantify the spread of the drug at distances further away from the immediate injection location (Stukel, Parks, Caplan, & Tillery, 2008). The drug injection and aCSF flush was described using a convective-diffusion model that factored in the typical properties of the neural tissue. The model showed that small volume injections of low concentration drug when followed by an aCSF flush could be engineered to affect a well-defined and limited area of the brain. The combination of the volume and the concentration of the injected drug determined the extent and duration of the drug effect on neural tissue. For the most limited volume of neurons affected, the ideal concentration for the drug should be set just above the minimal concentration where the drug will still affect neuron activity. During a low concentration drug injection and flush, the drug is pushed further from the injection site by the remaining drug and the aCSF flush that follows spreading the drug over
an ever increasing volume of tissue and rapidly drops below the effective concentration of the
drug. The model results were strictly theoretical and were not verified experimentally.

In this chapter, a series of injection experiments were performed with the purpose of
verifying the results by Stukel et al. A solution of the drug muscimol was injected in the
cortex of anesthetized rats while the surrounding neural activity was recorded. The drug
injection was then followed by an aCSF flush for the duration of the experiment. Muscimol
was chosen because as a GABA<sub>A</sub> agonist, it inhibits neuron firing making its effects easy to
observe in neural recordings. GABA<sub>A</sub> agonists open GABA<sub>A</sub> receptors which results in an
influx of chloride ions into the neuron which effectively clamps the membrane potential
below the threshold level for an action potential (Macdonald & Olsen, 1994). Muscimol has
been shown to have effects on the scale of hours in traditional neural drug injections making
it an ideal drug to test the effectiveness of this proposed injection technique (Edeline et al.,
2002; Hikosaka & Wurtz, 1985). Finally, GABA<sub>A</sub> receptors are found throughout the brain
including two areas of interest, the cortex and nuclei of the basal ganglia (Young & Chu,
1990; Zezula, Cortés, Probst, & Palacios, 1988).
Methods

Design of cannula electrode arrays. An array combining recording electrodes with a drug injection cannula was built to allow the injection cannula and electrodes to be driven as unit in order to ensure that the injection site remained in close approximation to the recording site. A plasticine clay mold was created to hold four single site electrodes (FHC tungsten microelectrode) and 26 gauge guide cannula (Plastics One) in place at the desired distances of approximately 1 mm between electrodes. Four recording sites were chosen because the Tucker Davis Technologies (TDT) acute headstage used in this experiment was limited to four inputs. The Plastics One guide cannula was designed for 33 gauge internal cannula to be inserted into it and then for the injection tubing screw on to the guide cannula to hold the whole assembly in place. The small gauge of the internal cannula reduces the risk of injection backflow. The electrodes were positioned using a dissecting microscope and a jig to maintain the desired spacing and then pressed gently into the clay. The guide cannula was carefully placed on top of the third recording electrode in the array so the tip of the inserted injection cannula would align with the tip of the third recording electrode and be within approximately 0.5 mm (the thickness of the sidewall of the guide cannula). Epoxy was then poured into the mold and allowed to harden to form a single array. Crimp connectors were added to the electrode in order to connect to the TDT acute headstage.
**Array implantation.** All animal experiments conducted in this study were done with approval of Arizona State University IACUC. These experiments were conducted using Sprague Dawley rats in an acute preparation. Both the surgeries and experiments were performed using KX rat cocktail (100 mg/ml ketamine and 5mg/ml xylazine) or just ketamine (50 mg/ml ketamine) as anesthesia. A craniotomy was made for inserting the array into the brain. The craniotomy was centered at 3.0 mm anterior and 2.00 mm medial from Bregma and then enlarged to fit the electrode array. A second hole was drilled into the skull on the contralateral side to the craniotomy. In this hole, a ground wire was inserted between the skull and the dura and held in place by a screw.

**The experimental procedure.** After the surgical prep was completed, the rat was maintained under anesthesia for the duration the experiment. The electrode array was inserted into the brain using an implant arm attached to the stereotax. Before the array was lowered, the electrodes and the ground wire were attached to a headstage designed for acute recordings (TDT RA4AC1) which was connected to the Tucker Davis Technology (TDT) RX5 recording system via optically isolated pre-amplifier/analog to digital convertor.

The recording system was used to listen and display the cortical activity on the recording array while the array was being driven into the brain. Using the feedback from the system, the array was positioned in a location in the cortex where there was clear and distinguishable neural activity on several of the recording sites on the array. One of these channels with neural activity had to be channel 3 which was the channel closest to the injection site. The single and multi-unit recordings collected throughout this experiment
using the TDT RX5 system. The signals were sampled at a rate of 24.414 kHz and band pass filtered to pass through frequencies within the range of 300 Hz to 6 kHz.

Once the electrode array was in position, the final preparations of the injection system were made. The injections for this experiment were performed using a syringe pump (New Era Pump Systems NE-1000). The pump was programmed and controlled using the WinPump Control software. Before the start of experiment, the syringe, tubing, and internal cannula were filled with artificial cerebrospinal fluid (aCSF) (the solution was comprised of 150 mM Na, 3.0 mM K, 1.4 mM Ca, 0.8 mM Mg, 1.0 mM P, and 155 mM Cl) and all air bubbles were removed from the system. Immediately before the start of each muscimol injection, the volume of drug solution to be injected was frontloaded into the system using the syringe pump. The internal cannula was then inserted into the array’s guide cannula and locked in place. The location of the injection was controlled by the position of the guide cannula which was positioned during the building of the array to ensure that the injection center occurred at the same level as the electrode recording sites.

Before any injections were performed, a baseline recording of neural activity on all four channels was collected for 30 minutes using the TDT system. After the baseline was recorded, an injection of only aCSF was performed as a control to verify that the aCSF flush used later in the experiment had no effect on neural activity. The duration of the control injection was chosen to match a typical drug injection experiment in order to keep the affected volume similar. The flow rate for all injections in this experiment was set at 0.5μL/min. This flow rate was chosen to minimize the risk of back flow along the injection cannula. (Chen, Lonser, Morrison, Governale, & Oldfield, 1999).
After the control injection, the GABA agonist, muscimol, was loaded into the injection cannula and then injected into the brain. The neural activity was recorded throughout the injection process and continued after the initial drug injection concluded. After pausing for several minutes to record the initial neural activity after the muscimol injection, the syringe, tubing and internal cannula used to inject the muscimol was swapped with similar setup containing only aCSF. This was done to prevent any contamination of the aCSF flush with muscimol due to diffusion. The swapping of the two cannula systems typically was done in under five minutes. The aCSF flush was started and continued until the end of the particular experimental run.

After the experiment was done, the spikes were detected using threshold ($Thr_{spike}$) set by the following equation:

$$Thr_{spike} = 4 \times median\left\{ \frac{|x|}{0.6745} \right\}$$  \hspace{2cm} (1)

where $x$ is the band passed filtered neural recording (Quiroga, Nadasdy, & Ben-Shaul, 2004). The threshold was calculated for each 10 second segment of data. A spike occurred when the neural recording crossed this adaptive threshold and the spike times were recorded.

The spike times were then binned in 1 ms bins and the instantaneous firing rate was calculated using the method developed by Nawrot, Aertsen, & Rotter (1999). In order calculate the instantaneous firing rate a triangular smoothing kernel ($K(t, \sigma)$) was created using the following equation:

$$K(t, \sigma) = \frac{1}{6\sigma^2} \left( \sqrt{6\sigma} - |t| \right)$$  \hspace{2cm} (2)
The smoothing parameter for the kernel (\(\sigma\)) was set to 20ms as recommended by Nawrot et al. The interval of time the kernel was calculated over was \([-\sqrt{6}\sigma, \sqrt{6}\sigma]\). The critical properties of the kernel are that its values are all positive to avoid negative firing rates and that the integral of the kernel has a unit area in order for the integral of the instantaneous firing rate to equal the number of spikes detected over the same time span in the original discrete data. The kernel (K) was convolved with the binned spiked data in order to calculate the instantaneous firing rate.

**Results**

**The effects of different aCSF solutions on neural activity.** While the experiments were being run, a noticeable change in neural activity was observed both in the audio of the neural activity and the real time display of the raw recording signals during the injection of the aCSF flush. In many cases, the overall magnitude of the recording signals was reduced significantly and the neurons observed at each recording site were nearly completely silenced over the course of flush. As the flush spread, the audio from the neural recording at each site changed from one having characteristic popping of neurons to sound best described as white noise.

The changes in neural recordings over the course of aCSF flush could have several possible explanations. First, the neurons could have stopped firing. This cessation of firing could be due to the lack of any component in the aCSF to supply energy to the neurons such as glucose. In order to test this idea, three different aCSF solutions were compared – a solution of just aCSF (aCSF solution), an aCSF solution with lactate added (aCSF+lactate solution), and aCSF solution with glucose added (aCSF+glucose). A second potential explanation for the change in neural firing is that the
injection changed the geometry of the tissue around the electrodes by mechanical
deformation. The third possible explanation is that the aCSF does not carry the current
from neural spikes to the recording electrode the same way due to differences in the
impedance value between it and the natural extracellular fluid initially found between the
neuron and the recording electrode.

Because the overall magnitude of the recording signals appeared to be decreasing
over the course of the aCSF flush, it did not make sense to use a stationary threshold set
at the start of the experiment to detect neuron spikes. Using a stationary threshold could
give the appearance that the neuron firing rates were decreasing but in reality the neurons
may be firing at the same rate with an overall spike magnitude below the threshold.
Instead, an adaptive threshold for spike detection was created by dividing the recording
data into 10s segments. A new threshold was calculated for each segment using equation
(1) from the Methods section of this chapter. Since the equation uses the median, the
changes in threshold also illustrate the overall changes in the magnitude of the recording
signal over the course of the each experiment. The change in magnitude could be a partial
confirmation that the observed recording changes are due to changes in the impedance
that affects current transmission especially if the firing rate of the neurons stays constant
despite changes in threshold.

Figures 1-3 illustrate the changes in the threshold over the course of the three
different types of flush. Only channels where neurons were noted to be present during
the start of the experiment are shown in the plots. The red color portions of the plot
indicate when threshold dropped below three standard deviations of the pre-flush average
threshold value which was calculated using the threshold values before 0 min point.
indicating the start of the flush. For all experiments, channel 3 is the recording site placed right next to the injection site. Channel 2 and 4 are approximately 1 mm from the injection site and channel 1 is approximately 2 mm from the injection site. Ideally, if the changes are due to the spread of the flush the order of the changes should follow the arrangement of the site from the closest to furthest. As can be seen in the example threshold, these changes do not perfectly follow this ideal order.

Using these adaptive thresholds, neuron spikes were detected. Since the overall magnitude of the recording signal appears to change, a question arose about how the overall shape of the neuron spikes were changing over time for the different type of flushes. Figure 4 shows average neuron spike waveforms created by averaging all the spikes detected during 10 min intervals of the flush experiments. The three subplots of the average spike waveform came from the same runs as the three sets of thresholds shown in Figures 1-3. The shaded region shows the area that is within +/- 1 standard deviation of the average waveform for the time period before the start of the flush. As can be seen in the figure when the waveform shape did change noticeably, it was always in the direction of the decreasing magnitude. This corresponds to the observations made during the running of the experiment.

In order to further analyze the effects of different aCSF solutions, a fast Fourier transform (FFT) was performed over one minute intervals of each run of the flush experiment. The resulting power spectrum was divided into 50 Hz frequency bands where the power over each band was averaged. By calculating the FFT this way, the change in the power spectrum over time could be analyzed. Figure 5 shows an example of the resulting analysis of the frequency spectrum for one run of the flush experiment at
the recording site nearest to the injection site. The frequency bands start at 300 Hz because the high pass filter for the recording was set at that frequency. The bands stop 1000 Hz because the most of the signal power is found below this frequency and the changes in power over time were consistent across bands. As can be seen by the figure, the flush tends to decrease the power of the signal as it progresses. This decrease in power occurs across most bands of the frequency spectrum but the magnitude of the decrease also varies between frequency bands.

Looking at the changes in power across the frequency bands, two questions arise. First, does the magnitude of the change in power between the frequencies band differ in significant way? Second, does the magnitude of the changes in power vary between the three types of flushes? In order to answer these two questions, the percentage change in the power for each frequency band was calculated by comparing the average power before the start of the flush with the minimum value of the power after the start of the flush. This calculation was performed on all the runs of the flush experiment. Runs of the experiment using the same type of flush were then grouped together and the mean value of the percentage change was taken for each frequency band for each type of flush. After checking the data were normally distributed for each flush type and frequency band, a 95% confidence interval was calculated for each average. Figure 6 shows the resulting average percent change and the confidence intervals for channel 3, the recording site nearest to the injection cannula. Though the percent change is greater for the aCSF+glucose solution across all the frequency bands, almost all averages fall within the confidence intervals of the other flush types for corresponding frequency band which indicates that the differences between flush types are not statistically significant. The one
exception is the mean for the aCSF solution at 400-450 Hz which does not fall within the confidence interval of the aCSF+glucose solution. At the same time, the mean of the aCSF+glucose solution at the same frequency band does fall within the confidence interval for the aCSF solution. Overall, magnitude of the change in power does not appear to significantly differ based on the type of flush used.

Results from the analysis of the next two recording sites are shown in Figure 7 (channels 2 and 4). These site are both approximately 1mm from the injection though on opposite sides. Since they are same distance from the recording site and the injection should spread radially from the injection site, the results for the two channels were combined to create Figure 7. In this figure, the aCSF solution creates the largest percentage change in the signal power and the aCSF+glucose the least which is opposite ordering when compared to Figure 6. Also several of the aCSF+glucose means are significantly different from the aCSF solution means when looking at the 95% confidence intervals.

Finally, the changes in firing rate during the course of the three different flushes were compared. Figures 8, 9, and 10 illustrate the changes that occurred in the instantaneous firing rate for the aCSF, aCSF+lactate and aCSF+glucose solutions respectively. The firing rate plots are from the same runs of the flush experiment as the threshold plots in Figures 1, 2, 3 and also the average spike waveforms in Figure 4. In order to determine when a decrease in firing rate was significant, the average and standard deviation of the instantaneous firing rate before the flush was started (before the 0 min point in the plots) was found. This average value is shown as the blue line drawn through the instantaneous firing rate waveform before 0 min mark. A decrease in firing
rate was considered to be significant when it dropped below a threshold set at three standard deviations below the average of the firing rate before the flush started. These times when the firing rate dropped below this threshold are shown in red in each plot. In order to compare the magnitude of the drop between the channels and the runs of the experiment, the average firing rate value for each continuous region of the plot below the threshold was found. These average values are shown as straight red lines on each plot if the run had any regions below the threshold.

Comparing the firing rate plots to their respective threshold plots, the changes in firing rate tend to be a much sharper decrease. Decreases in the threshold do tend to correspond to decreases in the firing rate but there are exceptions for example channel 1 in Figures 1 and 8. When the average spike waveforms in Figure 4 are compared to the instantaneous firing rate, one finds that in many cases, smaller average spike magnitude did correspond to the lower firing rate regions.

To determine the duration of the effects, several experiments were performed in which the flush was stopped when the decrease in recording magnitude was first observed on the electrode closest to the injection site (channel 3). Figure 10 shows one such injection using aCSF+glucose solution. The decrease in recording magnitude was first noticed at around 10 minutes into the flush at which point the flush was stopped as shown in the figure. Even though the flush was stopped, the firing rate stayed below the original baseline firing rate for the majority of the remaining recording and never approached the peak or even the average firing rate found during the baseline period. The threshold values shown in Figure 12 show a similar stability on channel 3 after the
initial drop. Figure 13 show that average spike waveform on channel 3 maintained a lower magnitude even after the flush was stopped.

Figure 14 shows the instantaneous firing rate plots for each run of the flush experiments and plots bars with respect to the time where each channel’s instantaneous firing rate dropped below the threshold of three standard deviations below the average pre-flush firing rate. The thickness of the bar is proportional to the percent difference between the average firing rates of the region below threshold and the pre-flush region. These averages are highlighted in Figures 8-10 as the horizontal red lines for the below threshold averages and the horizontal blue lines for the pre-flush averages. The greater the thickness of the colored bar in Figure 14 then the greater the difference between the two averages. For each run, the channels with neurons are indicated with asterisks that match the color assigned to the channel.

Figure 14A shows the results from the aCSF and aCSF+lactate solution runs and Figure 14B show all of the aCSF+glucose solution runs. There are greater number of aCSF with glucose runs because after the initial study comparing the three type of aCSF, an aCSF+glucose flush was done before every muscimol injection at new recording location. Figure 14A appears to have more channels with significant deactivations and larger changes across those channels than Figure 14B. This result agrees with the power analysis observed in Figure 7.
The effects on neural activity of small volume muscimol injections followed by an aCSF flush. The first goal of these experiments was to determine if using small volumes of low concentration muscimol solutions could lower the activity of neurons in a limited volume of cortex. The second goal of the experiment was to determine if following this muscimol injection with an aCSF flush could return neuron activity rapidly back to its original level before the injection.

In order to analyze the results of these experiments, a definition for a successful experiment run needed to be created. A successful run occurred when the instantaneous firing rate decreased significantly below the pre-injection average firing rate. A decrease in firing rate was considered significant when the firing rate decreased below a threshold of three standard deviations below the pre-injection average firing rate. This decrease also had to occur during the time period of the injection. In addition, the firing rate must return to normal firing rate after this initial decrease which occurred when the firing rate once again crossed above the three standard deviations threshold.

Using the definition of a successful run, the injection results then could be categorized for additional analysis. Figure 15 shows the eight injection runs that were categorized as successful. Figure 16 shows the seven injection runs that were categorized as unsuccessful. The color bars on the plot once again indicate the time period of the experiment runs where the instantaneous firing rate dropped below three standard deviation of the pre-injection average firing rate. The thicker the bar the greater the magnitude of the decrease in firing rate when compared to the average pre-injection firing rate. The molarity and volume of the muscimol solution injected is also indicated.
for each trial. For both plots, the color purple represents channel 3 which is the site closest to the injection cannula.

Looking at the successful trials in Figure 15, a significant decrease in the firing rate occurred during the injection as shown by the purple bar falling between 0 min and the line representing the end of the injection. The one questionable run is W11 run #2 but when examined, the decrease in firing did occur shortly before the end of the injection. With the exception of Y11 run #4, the initial decrease firing rate occurs over range of 1.56 min with the earliest time starting at 1.11 min after the injection and the latest time occurring at 2.68 min. Y11 run #4, the one outlier, the decrease starts at 4.91 min. All of the unsuccessful runs failed to show any decrease in the firing rate during the actual injection of muscimol.

Figures 17, 18, and 19 show an example of the one of the successful runs. In this particular run, the spike size and threshold levels on both channels with neurons present decreased throughout the injection and flush. The firing rate on channel 3 did decrease significantly while the firing rate of channel 4 stayed the same and even increased. Despite the threshold continuing to decrease, channel 3 did return to a firing rate within three standard deviations of the pre-injection average firing rate.

Figures 20, 21, and 22 show a successful run in subject Z11. In this example, the spike size on the channel 3 before the start of the injection and the spike size in final 5 minutes of the experiment are similar in size and shape but the average spikes between these two times have decreased in magnitude. The significant depression in firing rate and return from that depression occurred during the time period covered by the lower magnitude average spikes and before the average spike shape returned to its original size.
In addition, these results were repeated multiple times in the same location in Z11 as can be seen in Figures 23 and 24. The time between each consecutive injection run was about 15-20 minutes which was used to reset the pumping system and check the condition of the subject.

Discussion

The overall goal of this chapter was to verify the modeling work done by Stukel et al. (2008), which showed that the combination of micro-volume low concentration drug injections when followed by an aCSF flush could produce effects that were spatially limited and short in duration. This set of experiments does suggests that the normally long lasting effects of muscimol can be reduced using this injection technique, but further work on determining ideal concentrations and perfecting the injection system is needed to increase the reliability of the results. In addition, this study shows that the solution the drug is dissolved in can have significant and wide spread effects on the behavior of neurons that may need to be accounted for when doing drug injections into neural tissue.

The effects of aCSF on neural activity. During the course of the experiment, a clear change in the magnitudes of neural activity was observed when injecting a solution of only aCSF. The ionic make-up and pH of the aCSF solution was chosen to closely mimic naturally occurring cerebrospinal fluid but still these changes in magnitude of neural recordings and firing rate appeared to occur ("Preparation of Artificial CSF", 2014). There could be several potential causes to this changes. The primary ones being mechanical deformation due to the injection, changes in impedance of the solution surrounding the recording electrode, and the solution causing the neurons to stop and/or change their firing rate.
The mechanical deformation explanation does not seem likely based on the result. First, the slow inject flow rate (0.5 µL/min) was specifically chosen to minimize the pressure of the injection. Higher flow rate and thus higher pressure injections rather than deform the tissue tend to take the path of less resistance and back flow through opening in the tissue surrounding the injection cannula (Chen et al., 1999). In addition, the pressure should reduce as the injection spreads further from the injection site making the effects less likely to occur on the further away recording site. This is not the case for the aCSF and aCSF+lactate solutions which showed similar magnitudes of changes in firing rate across several channels. Finally since the same injection flow rate was used in all experiments, the results should be consistent across different injection solutions which is not the case for the aCSF+glucose solution when compared to the other two types (Figure 14).

Another explanation for the changes in neural activity is that aCSF changes the impedance of the extracellular fluid which changes how the electrical signals from neurons are broadcasted through surrounding tissue to the recording electrode. There is some evidence that at least partially support this view. First, the changes in the spike detection threshold magnitude seen during the flush correspond to the changes in the median value of the signals being recorded which indicates the overall level of background “noise” is changing. This background noise partially reflects the summation of activity from more distant neurons which transmission would be affected by changes in impedance, but an overall change the firing of neurons over the surrounding patch of neural tissue could also be the cause some of this reduction. If a change in the neural activity was the sole cause of changes observed then the frequency spectrum most likely
would not show such consistency in the size of the decrease as seen in the Figures 6 and 7 because changes in neuron activity should preferentially effect certain frequencies. A caveat to this conclusion is that the neurons may not be firing at a high enough rate before the flush to have an effect on the overall baseline frequency spectrum compared to other sources. Finally, the change in average spike waveform magnitude observed throughout these experiments indicate a change in signal transmission.

The signal transmission explanation’s one flaw is the changes instantaneous firing rate seen over the course of these flushes. Since the adaptive spike detection threshold should adjust proportionally to the change in the overall signal magnitude due to impedance, the firing rate should stay constant because the magnitude of the neuron spikes waveform should also be changing by a proportional amount and keep being detected by the threshold. The threshold is only updated every 10s so some of the firing rate changes could be due to the threshold not adapting as fast as the signal magnitude is changing and missing neuron spikes. In almost every flush experiment, the changes in magnitude eventually reach an equilibrium point at which the threshold stays more constant. At this point, the firing rate should return to the original value if the only change is a change in signal transmission. This is not the case as can be seen by the instantaneous neuron firing rate plots during the flush. As a result, a change in impedance changing signal transmission appears to only partially explain the observed changes in the neural recordings.

The fact that the firing rate still changes despite adjusting the threshold leads to the third explanation that the actual neuron firing changes or stops as a result of the flush. There are at least three potential causes for a change in firing rate. First, the ionic
concentrations could be different enough from the natural extracellular fluid to cause changes in the voltage across the membrane hyperpolarizing the cells. The second possibility is that the cells in the injection area may have been deprived of energy because the aCSF solution initially did not contain any energy source. A final possibility is that the threshold method may not be adequate enough to detect the spikes out of the background noise when the overall signal magnitude dropped and there was no real change in firing rate but rather change in our ability to detect.

The effects of changes in the extracellular fluid on neurons is complex and still not fully understood. The research is often done in vivo using cell cultures and slice preparations which may not accurately capture all the factors that affect neuron behavior. The activity of neurons at it is core is about the difference in extracellular and intracellular concentrations of ions so it does not seem unperceivable that the aCSF solution could affect activity and research does support this fact. In the case of potassium ions and calcium, concentrations lower than normal decrease neuron excitability and increases of concentration above normal increase excitability in slice preparations (Balestrino, Aitken, & Somjen, 1986; Czéh, Obih, & Somjen, 1988). In the case of magnesium ions, the relationship is reversed and slight increases in the magnesium concentrations above normal will lower excitability of neurons (Wang, Wang, Cottrell, & Kass, 2004). There are two cautions with these slice prep studies. First, the normal concentration appears to be defined as the concentration of the bath solution during the control experiments not necessarily based on physiological based in vivo concentrations. Second, they also focused on hippocampal neurons not the cortex where these experiments were conducted.
Overall, these studies suggest that some of the effects observed in response to the aCSF flush could be the result of changes in ion concentrations. Since the effects consistently depress neural activity, it is most likely not the result of inaccuracies in the measurement of compounds when making the solutions but rather the result of a consistent difference between the ionic make-up of the aCSF and the extracellular fluid. Ion concentration alone cannot explain the differences in effect between the aCSF, the aCSF+lactate and the aCSF+glucose solutions. In the case of aCSF and aCSF+lactate solutions, the inhibitory effects are more widespread as indicated by significant decreases in firing rate across multiple channels in Figure 14.

This difference does raise questions about whether inclusion of glucose is necessary to best minimize the aCSF effects. Neuron activity is a highly energetic process and as expected glucose deprivation in slice preparations hyperpolarizes neurons (Muir, Lobner, Monyer, & Choi, 1996; Spuler, Endres, & Grafe, 1988). There is also evidence that excitatory post-synaptic potentials become depressed but inhibitory post-synaptic potentials show little change in magnitude when deprived of glucose thus making neurons more sensitive to inhibition (R. L. Martin, Lloyd, & Cowan, 1994). To further complicate the situation, energy metabolism in the brain also involves complex and not fully understood interactions between neurons and astrocytes. These same astrocytes are involved with homeostatic control of extracellular fluid which relies on some amount of active transport and this reliance increases the larger the disparity between the actual concentration and the normal desired concentrations (Amédée, Robert, & Coles, 1997). The lack of an energetic metabolite such as glucose in the aCSF may prevent the astrocytes from carrying out these homeostatic functions. There is also indications that
Astrocytes use glucose to produce lactate to be used by the surrounding neurons which could mean reduced glucose levels could have additional effects on neuron activities (Pellerin & Magistretti, 1994).

There are few studies that have indicated aCSF and saline solution have effects on neural activities in vivo. Endeline et al (2002) showed that 1 μL saline injection can significantly depress neural activity for up to 15 minutes at the injection site though the level of depression was less than what occurred when injecting the same volume of 8.7 mM muscimol. Another experiment showed that aCSF control injection in the striatum of rats actually affected behavior in a T-maze task and that these effects could be reversed by following the aCSF injection with an injection with glucose in the striatum (Canal, Stutz, & Gold, 2005).

Overall these aCSF experiments indicate that careful analysis is needed when doing drug injections in the brain. These effects in some case may not significantly confound experimental results especially if a long lasting inhibitory drug like muscimol is being used. A problem may arise when drugs that effect neurons differently than the particular aCSF solution are used for example drugs that block dopamine receptors or excite neuron activities. Problems may be more significant when the drug effects have similar duration as the aCSF effects. In these cases, the aCSF may confound the results more significantly. This may be something researchers should look in to when drug injection results are in conflict with each other or other parallel research findings.
Assessing the effectiveness of the muscimol drug injection method. In order to first assess the muscimol injection experiments several factors need to be considered. The first one is the electrode location. Attempts were made to ensure the spacing between the electrodes and the guide cannula for the injection were kept consistent between each array, but because the arrays were being constructed by hand, each array will have some unavoidable variation. In general, channel 3 was within 1 mm of the injection site. Channel 2 and 4 were approximately 2 mm from the injection site and channel 4 was approximately 3 mm from the injection site. In addition, the process of driving electrodes in the brain can cause bending and create another source of variation in the distances. This variation needs to be considered when looking at the timing of the significant changes in firing rate shown in Figure 15. Since each subject used a different array, there will likely be some variations in the timing between subjects.

The second factor is the predicted spread of the initial injection over time since this will give some indication of whether the observed start times in the successful injection runs are reasonable. This predicted spread time, $t_s$, can be roughly estimated using the following equation:

$$t_s = \frac{4\pi r^3}{3 q_v}$$  \hspace{1cm} (3)

where $r$ is the distance from the injection site, $\alpha$ is the extracellular volume fraction and $q_v$ is the volumetric flow rate of the injection which 0.5µL/min for these experiments (Stukel et al., 2008). The volume fraction represents the amount of overall tissue volume that is extracellular space where the injection can flow into. The typical extracellular volume fractions are between 0.18 to 0.3 for neural tissue (Nicholson, 2001). Estimated
times for the injection to reach each recording site is shown in Table 1 which should give some ideas of the minimum time muscimol effects should appear. These estimates do not take into account diffusion and drug spreading that will lower the muscimol concentration and delay or prevent neuron inhibition by muscimol at the recording site. The estimates also assume a spherical spread and that the tissue will not deform and change the value for the extracellular volume fraction. Unfortunately in reality, these assumptions appear to not completely hold true. Injections will spread in a more oblong shape even when performed at low flow rates and interfaces between gray and white matter will further distort the flow pattern (Allen et al., 2008; Chen et al., 1999; J. H. Martin, 1991).

Despite these assumptions, the estimates should give some indication of whether the starting times of the observed injection effects are reasonable. For the successful muscimol injections shown in Figure 15, significant decreases on channel 3 occurred within two minutes of the start of injection for seven out of eight trials which agrees with the estimated times for channel 3 in Table 1. In the eighth trial, the decrease occurred fewer than 5 minutes from the start of the injection. Considering the possible variations in electrode position discussed previously, the difference could be caused by the electrode being 1.25-1.50 mm from the injection site instead the estimated 1 mm.

The third factor to consider is the predicted duration of the muscimol effects. Using the model results in Stukel et al (2008), the effects 2.2 or 4.4 µM muscimol should end between 15 to 50 min after the start of the flush. This estimate is based on 1 µL injection volume so it is likely that higher volumes would result in longer durations. In addition, these estimates have only a two minute pause period between the injection and
start of the flush which is much shorter than the majority of our trials. The longer pause period will result in greater diffusion and larger decrease in concentration of muscimol but it will also cause the muscimol to spread further before the flush starts. The model also predicts at these concentrations that the effects should be observed on the next closest set of electrodes (channel 2 and 4) in about 5 minutes. For 1 µL muscimol injection, this would amount to total injection time (muscimol injection plus flush time) of about 7 minutes. It is foreseeable that for large muscimol injection volumes, effects on these channels would show up sooner. All of predictions are based on estimated values that describe the properties of the tissue and it is expected that the actual values will vary. The other important estimated value is the minimal effective concentration of muscimol. When the concentration of muscimol drops below this threshold, the effect on neuron activity should be insignificant. Differences from the predicted value of 150 nM could result in significant changes in the estimates (Stukel et al., 2008).

The actual durations of the successful muscimol injections are not nearly as neat as the predicted ones which is it to be expected with the complicated nature of the experiment. For channel 3, the durations for the muscimol effects are close to the predicted durations but not all are completely within the range. Unfortunately, there is not a consistent variation in the duration across experimental runs to determine if there are inaccurate model values.

Some of this may be due to variations in electrode distances between subjects. The results of subject Z11 indicate this may be the case. Three successful runs were achieved. The first two trials used the same concentrations but the second trial had a larger volume and correspondingly longer duration. The second and third trials injected
same volume but the third trial had a larger concentration giving longest duration of the three trials.

The predicted effects on channels 2 and 4 are not present in the actual experimental results. The only trial where effects on channel 2 potentially fit the model is Z11 Run #4 where a significant decrease occurs about 8 minutes into the injection. The effects on these channels are more sensitive inaccuracies in estimated effective muscimol concentration. If the actual value is slightly higher then it is possible no effects will be seen on these channels. Also about 0.5 mm increase in distance from the injection site for channel 2 and 4 would cause no muscimol effects to be seen according to the model.

The fourth consideration is whether the effects are due to muscimol or the aCSF solution. When the decreases in firing rate are looked at for the three type aCSF solutions, the firing rate on channel 3 stays depressed and does not return to normal levels in almost all of the trials. This is not the case for the successful muscimol injection where every single one returns to normal firing rate on channel 3 even while the flush is continuing.

The final consideration is the unsuccessful trials shown in Figure 16. Three of the seven unsuccessful trials were from the same subject and notes indicate there were issues with getting quality neural recordings throughout the trials. The 1µL and 2 µL volumes used in three other unsuccessful trials are more prone to loading and injection errors because of their small volumes.

Overall, these muscimol injection experiments indicate that it is possible to reduce the duration and spread of drug effects by using low concentrations. In previous
muscimol injection studies using higher concentrations (8.7 mM), significant changes in neural activity were observed beyond 3 mm from the injection site based on both single unit neural recordings and cell activity measured using glucose utilization with the changes lasting from 2-7 hours (Edeline et al., 2002; Hikosaka & Wurtz, 1985; J. H. Martin, 1991). Using technique first proposed by Stukel et al. (2008), the effects were kept limited to between 1-2 mm from the injection site and 7 out of 8 successful trials neurons returned to normal firing rate in less than 60 minutes after the start of the flush. Further reduction in concentration should reduce the spread and duration even further. The one tradeoff between higher concentration muscimol injection and this study is that level of inactivation in those other studies appears to be much greater than this technique which may make the effects on the behavior and other areas of the brain more subtle.
Figure 1. The threshold changes over the course of an aCSF solution flush. The parts of the line in red are areas where threshold value dropped below three standard deviations from the average threshold value before the start of the flush at 0 min mark.
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Figure 15. The timing of significant decreases in the instantaneous firing rate for eight successful muscimol injection runs. A successful run occurred when the instantaneous firing rate on channel 3 dropped significantly below the baseline firing rate (the average firing rate before 0 min) during the injection of the muscimol and then returned to the original baseline firing rate at some point during the course of the experiment.
Figure 16. The timing of significant decreases in the instantaneous firing rate for eight unsuccessful muscimol injection runs. For the runs for X11, Y11 and Z11, the flush began immediately after the injection.
Figure 17. The average spike waveforms over selected period of a successful injection trial (Y11 Run #3).
Figure 18. The threshold levels over a successful injection trial (Y11 Run #3). The threshold levels used to detect neuron spikes is shown. The part of the plot in red are three standard deviations below the average value before the start of the injection. The green dotted line (..) indicates the start of the muscimol injection at the time 0 min. The purple dashed line (-) indicates the end of the injection. The blue dashed line (--) marks the time the flush started. The flush continued the duration of the experiment.
Figure 19. The instantaneous firing rate over the course of a successful injection trial (Y11 Run #3). The part of the plot in red are three standard deviations below the average value before the start of the injection. The green dotted line (..) indicates the start of the muscimol injection at the time 0 min. The purple dashed line (-.) indicates the end of the injection. The blue dashed line (--) marks the time the flush started. The flush continued the duration of the experiment.
Figure 20. The average spike waveforms over selected period of a successful injection trial (Z11 Run #3)
Figure 21. The threshold levels used during the course of a successful injection experiment (Z11 Run #3). The part of the plot in red are three standard deviations below the average value before the start of the injection. The green dotted line (..) indicates the start of the muscimol injection at the time 0 min. The purple dashed line (-) indicates the end of the injection. The blue dashed line (--) marks the time the flush started. The flush continued the duration of the experiment.
Figure 22. The instantaneous firing rate of over the course of a successful injection experiment (Z11 Run #3). The part of the plot in red are three standard deviations below the average value before the start of the injection. The horizontal red lines in these areas indicate the average firing rate during the drop. This average is compared in Figure 15 with the average baseline firing rate indicated by the horizontal blue line before time 0 min. The green dotted line (..) indicates the start of the muscimol injection at the time 0 min. The purple dashed line (-.) indicates the end of the injection. The blue dashed line (--) marks the time the flush started. The flush continued the duration of the experiment.
Figure 23. The instantaneous firing rate for a successful injection run (Z11 Run#4). This injection was performed within 15-20 minutes of Run #3 for Z11 and in the same location as Run #3. Run #3 is shown in Figure 22.
Figure 24. The instantaneous firing rate during the course of a successful injection run (Z11 Run #5). This injection started 15-20 minutes after Run #4 ended and also was in the same location as Run #3 and #4 of subject Z11. These previous runs are shown in Figures 22 and 23.
<table>
<thead>
<tr>
<th>Channel (Distance)</th>
<th>Time to reach ((α=0.18))</th>
<th>Volume injected ((α=0.18))</th>
<th>Time to reach ((α=0.3))</th>
<th>Volume injected ((α=0.3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Channel 3 (~1mm)</td>
<td>1.5 min</td>
<td>0.75 µL</td>
<td>2.5 min</td>
<td>1.3 µL</td>
</tr>
<tr>
<td>Channel 2 &amp; 4 (~2mm)</td>
<td>12.1 min</td>
<td>6.05 µL</td>
<td>20.1 min</td>
<td>10.1 µL</td>
</tr>
<tr>
<td>Channel 1 (~3mm)</td>
<td>40.7 min</td>
<td>20.4 µL</td>
<td>67.9 min</td>
<td>34.0 µL</td>
</tr>
</tbody>
</table>

Table 1. Estimated time for injection to reach each electrode site.
CHAPTER 3
IDENTIFYING PATTERNS OF NEURAL ACTIVITY USING SELF-ORGANIZING MAPS

Introduction

In behavioral studies, the identification and quantification of patterns of neural activity is simplified by the structured nature of behavioral tasks. Certain cues or actions in a task can be used to synchronize neural recordings in order to see patterns of neural activity in the form of raster plots and cumulative neuron spike activity over multiple repetitions of a behavioral sequence. What if there are no cues or controlled behaviors to assist in the detection of these patterns? In this section, we propose to use a self-organizing map (SOM) to identify, detect and quantify patterns of neural activity without the use of behavioral cues. In the future, these identified patterns can then be paired with negative or positive reinforcement signals either externally such as a food reward or internally using electrical stimulation of the neural circuits associated with reward. The end goal will be to shape neuron firing itself, not just external behavior, and then to measure the changes in neuron behavior as a result of this shaping over time.

An SOM is a form of neural network that carries out two functions on the data it analyzes. First, it takes a set of high-dimensional data vectors and projects them onto a two-dimensional plane (though in some cases a three-dimensional volume is also used). Second, it clusters similar data vectors (Kohonen, 1982; Si, Kipke, Witte, Lan, & Lin, 2002).

In order to do these functions, a two-dimensional map or mesh of interconnected nodes is created. The arrangement of these nodes is typically either rectangular with each
node having four neighboring node connections or hexagonal with each node having six neighboring node connections (see Figure 25). This interconnected mesh of nodes makes the two dimensional projection of the data possible.

In addition to this two-dimensional location, each node has a corresponding position in the high-dimensional space of the training data. The position of each node in this space is represented by a vector array the same length or number of sample points as the training vectors or patterns used train the map. During the training process of the SOM, it will be the node’s high-dimensional position that will be shifted in order to cluster the data but its two-dimensional arrangement, based on the other nodes that it is connected to, is maintained throughout the training process.

As with most neural networks, the SOM self-organizes by using a set of training data vectors. For this experiment, each data dimension of the data vector represents a value of the instantaneous firing rate at a particular moment of time, and each data vector is the firing pattern of a particular neuron over a fixed time interval. In the general case of SOMs, each time point is also a coordinate on one of the axes in the high-dimensional space. The training vectors are either fed into the map algorithm individually or as a batch. For each data vector, the SOM’s algorithm calculates the Euclidean distance of that data vector from each node in the high dimensionality space. The node that is closest to each data vector is considered the winning node for that vector. The high dimensional position of this node is then shifted towards the data vector some distance that is determined by a learning rate function. In addition, the nodes neighboring the winner on the two dimensional map are also shifted in the high dimensional space using a neighborhood function which determines the amount of the shift based on how many
nodes away the node is from the winner. This can be visualized as movements of the
nodes in the two-dimensional space, as in Fig. 2, but that is really a representation of the
proximity of each of the nodes to the others in the high-dimensional space. This shifting
of neighboring nodes makes the SOM algorithm different from many other clustering
algorithms such as K-means which shifts only the winning node. It also ensures that the
neighborhood constraints of the two dimensional mesh are maintained (e.g. nodes which
are next to each other stay next to each other).

The training algorithm cycles through the data until the map reaches a pre-set
level of optimization. At this point, the trained map can them be used to categorize other
data vectors by matching each vector to its best matched node: the node with the smallest
Euclidian distance from the data vector. If the map is well trained, all vectors that share
the same best match node should share similar characteristics with the exception being
nodes that collect data vectors with minimal amounts of neural activity occurring on
them. In our analysis, the vectors that are matched to the same node are considered to
represent the same pattern of activity.

In this chapter, SOMs are used to analyze data vectors consisting of snippets of
single unit instantaneous firing rate data. By organizing the data into an SOM, we
describe a set of “typical” patterns of neural activity represented by the nodes of this map.
We then describe the overall neural activity using the distribution of pattern occurrence
on theses nodes and make comparisons between data sets using these distributions. In the
following sections, the methods for implementing the SOM on neural data are discussed
and several example implementations are shown.
Methods

Single unit neural recording data was obtained using the methods described in Chapter 2. They were spike sorted using M-Sorter software in Matlab (Yuan, Yang, & Si, 2012). After the recordings were spike sorted, one well isolated neuron spike was selected. The instantaneous firing rate was calculated using the method developed by Nawrot, Aertsen, and Rotter (1999) and previously discussed in the methods section of Chapter 2.

The initial ten minutes of the instantaneous firing rate data were selected out of the complete data set, and two different methods were used to create the neural firing patterns needed to train the map. In the first method, the selected data were split into patterns 1000 data points in length representing one second in time. Each one second sample served as one training pattern for creating the SOM, giving a total of 600 training patterns.

The second method sorted the training data by the level of firing activity before using it to create the SOM. The training data were broken into neural firing patterns by sliding a one second (1000 data point) window at 100 ms increments. The level of firing activity of each pattern was then categorized by using an adjustable threshold. The threshold value was set using the average instantaneous firing rate of the entire training data set plus a multiple ($\varepsilon$) of the standard deviation of the instantaneous firing rate using equation (4)

$$F_{threshold} = F_{avg} + \varepsilon F_{std} \quad (4)$$

The multiple, $\varepsilon$, was decremented from 2.5 to 1 by 0.02. A high activity pattern was a pattern that had at least $P_{min}$ percentage of the pattern’s data points above the threshold,
Like $\varepsilon$, $P_{\min}$ was also adjusted downward from a starting point of 50\% in order to achieve a minimum number of high activity training patterns which was set to be 500 high activity patterns in this experiment. During the training set creation process, $P_{\min}$ was only decremented after $\varepsilon$ reached the minimum value of 1 and 500 high activity training patterns were not found. When this occurred, $P_{\min}$ was reduced by 0.1\% and $\varepsilon$ was reset to 2.5 and process was repeated until either a minimum of 500 high activity training patterns were found or $P_{\min}$ reached the minimum level of 20\%. In addition to high activity training patterns, low activity ones were identified as patterns where none of their data points crossed the threshold, $F_{threshold}$. An example of how the threshold was used to determine the quality of the pattern is shown in Figure 27.

The training set for the SOM was created by combining the high activity training patterns with a randomly selected sampling of the low activity training patterns. The number of low activity patterns included was set to be 10\% of the number of high activity arrays. This makeup of the training set was chosen to reduce the amount of patterns with little or no neural activity on them in hopes that the majority of the nodes of the SOM would be trained to recognize more active patterns of neural firing.

Once the training set was created, the SOM was implemented using the SOM toolbox in MATLAB. This toolbox was developed by the Laboratory of Information and Computer Science at the Helsinki University of Technology and released as a free software add on in 2000 (Vesanto, Himberg, Alhoniemi, & Parhankangas, 1999). The toolbox implements many of the algorithms necessary for training, analyzing and visualizing SOMs. The patterns of the training set were loaded into the SOM toolbox function for training the map. The training function creates a two dimensional hexagonal
grid of nodes: each node is connected with six neighboring nodes except nodes on the edges which will only have 2-3 connections. The number of map nodes (N) is initially based on the number of training patterns (K) and calculated using the following equation:

\[ N = 0.5\sqrt{K} \]  \hspace{1cm} (5)

The numbers of nodes can also be manually set, but increasing the number of nodes increases the risk of over-fitting the data to the map. In this experiment, the number of nodes was either kept at N or was reduced to 25% of N in the case of the small map implementation. The number of nodes in each row and column on the two dimensional map was determined by the ratio of the two largest Eigen values (\(\lambda_{\text{max}}\) and \(\lambda_{\text{max-1}}\)) calculated using the patterns in the training set. The Eigen values and the Eigen vectors used later in the creation of the SOM were calculated by performing a principle component analysis on the training pattern set using MATLAB’s built in Eigen vector function. The ratio (\(\lambda_{\text{ratio}}\)) was calculated using equation (6).

\[ \lambda_{\text{ratio}} = \sqrt{\frac{\lambda_{\text{max}}}{\lambda_{\text{max-1}}}} \]  \hspace{1cm} (6)

This ratio was chosen since the Eigenvectors for these two Eigen values will be used to linearly initialize the node positions in the high dimensional space of the data in the next step (Vesanto & Alhoniemi, 2000). The formula for determining the number of nodes in the larger dimension is

\[ D_x = \text{Round} \left( \sqrt[\frac{N}{\lambda_{\text{ratio}}}]{0.75} \right) \]  \hspace{1cm} (7)
where $D_x$ is the number of nodes in the in $x$-dimension. The $\sqrt{0.75}$ is used because the hexagonal map compresses the nodes together by a factor of $\sqrt{0.75}$ in $y$-dimension resulting in the two dimensions not being directly proportional to each other. The number of nodes in the $y$-dimension ($D_y$) is then found.

$$D_y = \text{Round} \left( \frac{N}{D_x} \right)$$  \hspace{1cm} (8)

The position of the map nodes are linearly initialized in the two dimensional map plane by equally spacing them over the range of -1 to 1 in both the $x$ and $y$ directions. The training set of patterns was then used to calculate a set of Eigenvectors and Eigenvalues using the standard procedure for principle component analysis. The two Eigenvectors with the highest Eigenvalues were then selected ($\mathbf{v}_{\text{max}}$ and $\mathbf{v}_{\text{max}-1}$). In addition to the Eigenvectors, the training set was used to calculate a mean value for each sample point of the pattern ($\bar{x}$). The position of the map nodes in the high dimensional space ($m_i$) was then initialized using the following equation:

$$m_i = \bar{x} + \mathbf{v}_{\text{max}} n_{i,y} + \mathbf{v}_{\text{max}-1} n_{i,x}$$  \hspace{1cm} (9)

where $n_{i,x}$ and $n_{i,y}$ represent the $x$ and $y$ position of the $i^{\text{th}}$ node in the 2-dimensional map plane.

Once the position of the map nodes are initialized the training process is started. The SOM toolbox implements a batch training algorithm running the whole training set at one time and then adjusting the map node positions. The first step in training is determining the best match node for each training patterns. The best match node ($m_c$) or
the node that detects the pattern is the node closest to the selected training pattern \((x_j)\)
defined as:

\[
c(j) = \arg\min_i \{\|x_j - m_i\|\}
\]  

(10)

where the argument sets \(c(j)\) equal to the index \(i\) of the best match node, and \(\|x - m_i\|\)
is the Euclidean distance calculation between the training pattern \(x\) and each node’s
current location in the high-dimension space, \(m\).

After the best match node is determined for each pattern in the training set then
the batch training can be performed using the following equation:

\[
m_i(t + 1) = \frac{\sum_{j=1}^{K} h_{ic(j)}(t) x_j}{\sum_{j=1}^{K} h_{ic(j)}(t)}
\]  

(11)

The neighborhood function, \(h_{ic(j)}(t)\), determines what nodes will be adjusted and by how
much based on their proximity to the winning or best matched node \(m_{c(j)}\). Overall the
batch training equation is a weighted average using the neighborhood function to
determine the weights for each training pattern.

There are several types of neighborhood functions, but for this particular
implementation, a Gaussian neighborhood function was chosen and was calculated using
the following equation:

\[
h_{ic(j)} = \exp \left( -\frac{\|n_c - n_i\|^2}{2\sigma^2(t)} \right)
\]  

(12)

where \(\sigma(t)\) is the radius of the neighborhood function and \(n_c\) is the position of the best
match node on the two-dimensional map where each node is one unit of distance from its
six nearest neighbors on the hexagonal map (see Figure 25).
The training of the map goes through two stages where the batch training algorithm (equation (11)) is iterated through multiple times, updating the map locations during each iteration. The first stage is rough training, in which the initial value of the radius in the neighborhood function (equation (12) when using linear initialization (equation (9)) is determined using equation:

\[
\sigma_{\text{initial}} = \left\lceil \frac{D_y}{8} \right\rceil
\]

(13)

where \( \lceil \cdot \rceil \) indicates taking ceiling of the value (round up) and \( D_y \) is the value previously calculated in equation (8). The initial radius value is then linearly decreased based on the number of iterations of the batch training algorithm and the final value of the radius set for the rough training stage. The final radius (\( \sigma_{\text{final}} \)) is determined using the initial radius

\[
\sigma_{\text{final}} = \frac{\sigma_{\text{initial}}}{4}
\]

(14)

If \( \sigma_{\text{final}} \) is less than one then the value is set to one instead. The number of iterations performed during the rough training stage is

\[
l_{\text{rough}} = \left\lceil 10 \frac{N}{K} \right\rceil
\]

(15)

The second stage is the fine tuning training. The final radius during the rough training is used as the initial radius for the fine tuning stage. The radius linearly decreases to a value of one during this stage. The main difference between the rough and fine tuning training stage is that number of iterations is increased during the fine tuning training.
After the map has gone through the fine tuning iterations, the SOM is considered trained.

After the SOM was trained, the remaining data set was then broken into patterns by sliding a one second window at 100ms intervals. Once these neural patterns were created, the SOM was used to categorize the patterns based on each pattern’s best match node on the SOM which was determined using equation (10).

**Evaluation of node characteristics.** In order to evaluate the quality of each node and the patterns it detected, several metrics were calculated. The first was the overall magnitude of the node waveform itself using the Euclidean norm equation below:

$$\|m_i\| = \sqrt{m_i \cdot m_i}$$

where $m_i$ is the vector representation of the node waveform. The magnitude of the node was chosen as a metric because a higher magnitude should correspond to a higher level of neural activity being detected by that node.

A second metric for determining quality of each node is the average cross-correlation value between the patterns detected by the node and the node waveform. The cross-correlation ($R$) was calculated for each pattern:

$$R(j) = \frac{1}{L} \left( \bar{x}_j - \bar{\bar{x}_j} \right) \cdot \left( \bar{m}_{c(j)} - \bar{\bar{m}}_{c(j)} \right)$$

where $L$ is the number of dimensions/samples in the pattern and node waveform. The average value of the selected pattern ($x_j$) and the best match node for that pattern ($m_{c(j)}$) are represented by $\bar{x}_j$ and $\bar{m}_{c(j)}$ respectively. $\sigma_j$ and $\sigma_{c(j)}$ are the standard deviation of selected pattern and its corresponding node. The cross-correlation values for each pattern

$$l_{fine} = \left\lceil \frac{40N}{K} \right\rceil$$

After the map has gone through the fine tuning iterations, the SOM is considered trained.
were then sorted by their best match node and then the mean taken to get an average for cross-correlation value for each node. The closer the cross-correlation value is to one the more similar the pattern and the node are to each other.

The third metric to determine the quality of the node and/or map is the distance between each pattern and its best matched node using their positions in the high-dimensional space. This distance is calculated using the equation for Euclidian distance. Nodes with a smaller average distance between its detected patterns \(x_j\) and itself \(m_{c(j)}\) indicate that the node is more similar to the patterns it is detecting than a node with a larger average distance. Maps developed using the same data set can be compared using overall of average distance between all patterns in the data set and their best matched node. Maps that have a smaller overall average distance have a set of nodes that are better able to describe the typical patterns in the data set. Caution must be taken because a small overall average distance may indicate the map is over-fitting the data set by creating a set of nodes too specific to particular patterns and not robust to small variations in the data. This risk is increased in likelihood if the number of nodes exceeds the value calculated in equation (5).

A final measure of the overall quality of the map is topographical error. As previously mentioned, the SOM has a two-dimensional arrangement of the nodes in addition to the nodes location in high-dimensional space (Figure 26). Each node has set of nearest neighbor or adjacent nodes. In the case of rectangular map, each node has four adjacent nodes unless it is on the edge or corner of the map in which case they have three or two adjacent nodes respectively. For the hexagonal map, most nodes have six adjacent nodes except for nodes on the edges or corners. The topographical error (TE) measures
the percentage of patterns where the second closest node based on Euclidean distance in high-dimensional space to the pattern is not adjacent to the pattern’s best matched node.  

\[ TE = \frac{K_{\text{nonadjacent}}}{K} \times 100\% \] (19)

For equation (19), K is the total number of patterns in the data set and \( K_{\text{nonadjacent}} \) is the number of patterns in the data set where its second nearest node is not adjacent to the best matched node. A lower percentage of topographical error indicates a greater degree of spatial organization was preserved in the two-dimensional map during the training process and the SOM is considered to be better than an SOM with higher percentage created with the same data set. In an ideal SOM, there should not be any patterns where the closest and second closest nodes are not neighbors in the 2-D map. In reality, SOM like other neural nets seldom reach the ideal due to the training process and the nature of the training set. In the case of the SOM, the best matched nodes are determined in the high dimensional space which can result two non-neighboring nodes ending up being the two closest nodes for a particular pattern due to preferences among nodes for particular dimensions.

**Results**

An SOM was created using the procedure described in the methods section. The spikes were sorted using the M-sorter software (Yuan et al., 2012). Figure 28 shows a typical example of a well isolated neuron used to train the SOM. The individual neuron spike waveforms shown in the figure were randomly selected from the complete data set along with the template that was created by the spike sorting software. When selecting
neurons to train, we tried to choose neurons with variations from the template similar to
the one shown.

Using the same ten minutes sections of instantaneous firing rates, two different
types of training sets were created. For the first set, the non-selective training set, the ten
minutes of data were split into 600 one second patterns (1000 samples each). For the
selective training set, a set consisting of 570 training pattern arrays was created (519 high
activity patterns and 51 low activity patterns) with each pattern one second in length
(1000 samples). The activity level was determined using the thresholding method
described in methods section of this chapter. A 10s segment of the continuous
instantaneous firing rate signal used to train the SOM and the corresponding discrete
times when the neuron fired is shown in Figure 29. Because of the nature of the
algorithm used to calculate instantaneous firing, peaks of 20 spikes/s indicate only a
single neuron spike occurred during the time span of the kernel used to calculate
instantaneous firing rate. As can be seen in Figure 29, peaks greater than 20 spikes/s
correspond to a cluster of multiple spikes. These peaks tend to occur at the center of these
clusters. On this same 10 s segment, an example of a high and low activity pattern is
also shown in Figure 29. The high activity pattern has multiple peaks greater than 20
spikes/s corresponding to several distinct clusters of multiple spikes. The low activity
pattern includes mainly areas of no spike activity or single spike activity. In the case of
the non-selective method, each one second interval on the 10s segment would be one
pattern used to create the map. Looking at the 10s example segment, over half of the
patterns created using the non-selective method will have only one or two multiple spike
cluster.
A map was created for both the non-selective and selective training set using data from 13 different neural recording sessions resulting in 13 maps for each type of training set. Examples of two sets of maps created using these two different training set methods are show in Figure 30. Each waveform on the map is the same number of samples and is measured using the same units (spike/s) as the training patterns. The waveform also represents the location where the node is found in the high-dimensional space. This location is used to calculate the Euclidean distance between each pattern array and the set of nodes in order to determine which node the selected pattern is best matched or detected by. When comparing the two types of maps in the figure, the selective training set appears to create nodes with larger magnitudes than the non-selective set. Since the magnitude of the SOM node waveforms is also in terms of spike/s, higher magnitude node waveforms indicate that these nodes will attract patterns with higher spike activity.

Once each map was trained, the topographical error and the average node magnitude was calculated for each map. The 10 min segment of instantaneous firing data originally used to create the two types of training set was then re-divided into 6000 patterns by sliding the one second window at 100 ms intervals. The two types of SOM's were then used to detect patterns in this set finding each pattern's best-matched node. The average distance and the correlation value between the nodes and their detected patterns was then calculated. The results were then compared between the maps using the two different types of training sets. The results of this comparison are show in Table 2. The results verify that the perceived increase in magnitude seen in Figure 30 is statistically significant. In addition, the selectively trained maps show a statistically significant improvement in the spatial arrangement of the map nodes based on the decrease in the
value of the topographical error. The lack of statistically significant changes in the
distance between patterns and their best matched node and the correlation values indicate
we are not gaining nor losing anything in the ability of individual nodes to describe the
patterns they detect since these two values measure the amount of similarity between the
node and the patterns detected by it.

Figure 31 shows an example of a fully trained map using the selective training
method. Each hexagon is one node which is identified by a number shown Figure 31A
and has a representative waveform shown in Figure 31B which is the same length as the
training pattern array. This particular map will be used throughout the remainder of the
remainder of results section discussing the characteristics of the nodes and their
corresponding patterns. As can be seen in the figure, there is the expected spatial
organization to the map with the waveforms gradually transitioning to different shapes
when moving from node to node. Figure 31C and D shows the number of high and low
activity patterns from the training set detected by each node of the map. Nodes 8 and 15
on the map are the nodes that collected all of the low activity patterns in the training set
and none of the high activity patterns. This leaves the high activity patterns to be
detected by the rest of the SOM. As can be seen by the number of patterns detected by
each node, low activity patterns tend to greatly outnumber the high activity ones. Yet as
was seen in Figure 29, low activity patterns are not very interesting consisting of only an
occasional isolated spike with larger spaces of no activity where as high activity have
clusters of multiples spikes. Collecting low activity patterns in one or two nodes should
help to ensure that the rest of the map is detecting patterns with more interesting activity.
The quality of the node can then be assessed using the correlation value between node and the patterns it detects. A set of 5990 patterns was run through the detection process using the SOM previously trained from a smaller subset of the same data (Figure 31). Figure 32 shows the number of patterns detected by each node from this set. About 30% of the 5990 patterns are collected by nodes 5 and 18 which were previously shown to collect low activity patterns. From this sorting of the pattern set, the correlation values for each detected pattern and the node that detected it was calculated. Figure 33 illustrates the relationship between individual detected patterns, the node’s waveform and the corresponding correlation value between the node and the pattern. The patterns with high correlation values do appear to be more similar to the node waveform. In particular, areas where the node reaches a peak or valley tend to correspond closely to the same peak and valley areas of the patterns. Even in the high correlation cases, the patterns do not trace the same shape as the node waveform but fluctuate around the values of the node waveform. This illustrates the idea that the node waveform is the high dimensional location of the cluster center for the patterns it detects.

The individual correlation values for each pattern were then averaged for the node that detected them. The averaged correlation values are shown in Figure 34. The nodes 8 and 15 have the lowest correlation value which is a desirable result since these nodes are collecting all the patterns that are low in activity and their purpose is not to detect a specific pattern of activity. The nodes with the highest average correlation values were nodes 5, 6, 12, 18 and 28.

The relationship between the average pattern and the node is shown in Figure 35. Figure 33 illustrates that individual patterns tend to fluctuate around node waveforms.
because the node is ideally at the center of the cluster of patterns it detects. Figure 36 further illustrates this idea because when all patterns detected by each node are averaged together, the resulting averaged waveform is typically very similar to the waveform of the detecting node. The average of the patterns barring outliers should be the centrally located over the range of pattern values which is also where the node location should be. When the histogram of spike occurrence is compared to node waveform, the peaks correspond to centers of spike cluster of spike activity. Figure 36 pulls out several nodes and their detected patterns for closer examination. The raster plot shows each time when the neuron fired for each individual pattern detected by the node. The individual patterns used calculated the average pattern in Figure 35 are also plotted and once again show that the individual patterns can vary considerably from the node waveform.

The SOM can be used to describe the characteristics of the overall data. One way to do this is look at the frequency of pattern occurrence across the SOM. Changes in the overall characteristics of the data set should result in changes of in the pattern percentages. These changes can be compared using the Spearman rank correlation coefficient which was chosen because the frequency at each node is categorical data and a result non-parametric. The correlation coefficient is calculated between two sets of pattern that we wish to compare with each other. For this analysis, the data over the time interval used to train the SOM was chosen as the baseline to compare with other sets of data. Figure 37 shows the percentage of the patterns that each node detected over the training time interval. These percentages will be the baseline to compare with other sets of patterns. Examples of pattern occurrences for several of the data sets that were compared with this baseline are shown on the left side of Figures 38 and 39. The pattern
occurrence was determined using the same SOM as the baseline comparison case. In the case of Figure 38, the three sets of data came from the same recording subject and session used to create the SOM and the baseline set, but not from the same time interval as the baseline. These sets in theory should have similar recording characteristics and little change from the baseline. On the right side of Figure 38, the baseline and comparison set pattern frequencies are paired together by node and plotted. The corresponding Spearman correlation coefficient (R) is also indicated. The closer the value of R is to 1, the greater the similarity between the data. In this case, the same subject R-values are quite high. Figure 39 shows the exact same types of plots as in Figure 38 but the three sets of patterns used to create the plots in this figure came from data recorded from three different subjects. There is considerable variation in the R-values between these three sets of data. Despite the data coming from a different subject, it is possible to get similar R-value as were achieved within the same subject.

Because of the similarities in the correlation coefficient when comparing between different recording subjects, the effects of firing rate on these values were examined. For each of the 13 SOMs previously created, the corresponding data was broken into comparison pattern sets that covered five minute time intervals of the data. Each of the pattern sets were then run through the 13 different SOM’s previously created and frequency of pattern occurrences for each node was found. These frequencies were then compared to the map’s baseline created using the training interval pattern set for that particular SOM (the first 10 minutes of each recording session). The difference between the average firing rate of the comparison set and the baseline set was also calculated. Figure 40 shows how differences between the average firing rate of the comparison set
and the baseline set affect the resulting correlation values. As the difference in firing rates grow larger, the correlation values between pattern frequency of the comparison and baseline set decrease and in some cases become negatively correlated. This indicates that the reason for similar results when comparing the distributions of two different subjects using the same SOM was most likely the result of similarities in firing rate and not in the maps inability to detect changes in the distribution.

**Conclusions**

In this chapter, a method for classifying typical patterns of cortical activity was developed using an SOM. The instantaneous firing rate of a neuron over time was broken up into pattern segments some of which were used to train the SOM. The nodes of the SOM represented a location in a high dimensional space shape that correspond to a typical pattern of neuron firing. The peaks of these node waveforms relate centroids of neural firing as can be seen by the various raster plots in the Results section. A method of pre-sorting the training data helped to create maps that focused on patterns with higher magnitudes.

These maps once trained can then be used to detect similar patterns of neural activity over the course of a recording period. Distributions of pattern occurrence at each node can also be created and compared in order to detect changes in the overall neural activity. This work could be expanded to ensemble of neurons to detect changes in overall activity.
Chapter 3 Figures

Figure 25. Examples of the layout for the two main types of two dimensional meshes. A. In the hexagonal layout, each hexagon represents a node and neighboring nodes share sides. B. Nodes only have four neighboring nodes in the rectangular mesh.
Figure 26. An illustration of the SOM self-organization process. The best match node (BMN) is shifted towards the training vector position (X) along with its neighboring nodes. The shift is greatest for the BMN with the neighboring nodes are shifted a smaller amount based on their proximity to the BMN. The new node positions and mesh shape is represented by gray points and the dashed lines. (Vesanto et al., 1999)
Figure 27. The three examples of high and low activity patterns. A and B are high activity patterns. In order to be a high activity pattern, the pattern need to have at least 250 (25%) of its data points above the threshold. C is a low activity pattern that had no data points above the threshold.
Figure 28. A selection of fifty of the sorted spikes used to train the SOM. The spike template shown and its corresponding detected spikes were used create the SOM shown in Figure 30. The red dashed line is the template used by the sorter to identify corresponding spikes. Once the spikes were sorted, the times of spike occurrence were used to calculate the instantaneous firing rate which was used to train the SOM to find patterns in the activity of the isolated neuron.
Figure 29. A 10s segment of the instantaneous firing rate signal used for training the SOM and detecting patterns. The corresponding times when the neuron fired are shown in the raster below the curve. The highlighted red portion of the waveform is one of the low activity patterns used to train the map. The highlighted blue portion is one of the high activity patterns used in training. For this particular set of data, the high activity patterns had an instantaneous firing rate that was higher than the threshold of one standard deviation above the average firing rate for 20% of the 1s length of the pattern. The low activity patterns remained below the same threshold for the entire time span of the pattern.
Figure 30. The node waveforms for four different SOM’s. A and C are maps created using the non-selective training set. A has an average node magnitude of 506.9 spikes/s and B has an average node magnitude of 733.2 spikes/s. Maps B and D use the same time span of data to create the training set but the selective algorithm was used before training to sort patterns by their level of activity. C has an average node magnitude of 646.4 spikes/s and D has an average node magnitude of 843.8 spikes/s. Maps A and B and maps C and D used the same scale plot the node waveforms.
<table>
<thead>
<tr>
<th></th>
<th>Non-selective training set</th>
<th>Selective training set</th>
<th>P –value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Distance between nodes and patterns</strong></td>
<td>356.03±128.28 spikes/s</td>
<td>395.13±101.77 spikes/s</td>
<td>0.398</td>
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<tr>
<td><strong>Topographical error</strong></td>
<td>6.12±1.45%</td>
<td>3.79±2.50%</td>
<td>0.00777</td>
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<tr>
<td><strong>Node magnitude</strong></td>
<td>406.32±214.20 spikes/s</td>
<td>581.02±209.68 spikes/s</td>
<td>0.0463</td>
</tr>
<tr>
<td><strong>Correlation value</strong></td>
<td>0.429±0.082</td>
<td>0.437±0.078</td>
<td>0.682</td>
</tr>
</tbody>
</table>

Table 2. The average values for four measures used to compare the maps created using the non-selective and selective training sets. The p-values were calculated using a t-test. There was significant difference for the topographical error and node magnitude.
Figure 31. The SOM training results for one set of data. A. The numbering scheme for each node on the SOM. B. The waveform that represents each node on the SOM. The vertical and horizontal axis of the waveform corresponds to instantaneous firing rate and time respectively. C. The number of high activity patterns from the training set that was best matched to each node. D. The number of low activity patterns from the training set that was best matched to each node.
Figure 32. Patterns detected by each SOM node for on pattern set. The figure shows the number of patterns detected by each node from the 5990 member pattern set.
Figure 33. Comparison between individual patterns detected, neuron spike occurrence and the node waveform for several nodes of an SOM. Individual patterns (red line) detected by the node (blue line) are shown. For each node shown below, the top, middle, and bottom set of plots correspond to the patterns detected by the node with the highest, mid-value and lowest correlation values respectively. The value $C$ is the correlation value of the pattern to the node waveform and the value $D$ is the Euclidean distance between separating the pattern from the node in the high dimensional space. In addition to the instantaneous firing rate, the times when the neuron fired is plotted in the raster below each pattern waveform.
Figure 34. The average correlation value of the patterns detected at each node. The closer the correlation value is to 1 the greater the similarity between the shape of the node waveform and the patterns it has detected.
Figure 35. The node waveform compared with the average of the patterns detected by each node of an SOM. The waveform for each node is shown in blue along with the mean of the patterns detected by the node in red. The pink shaded region is the area that is within one standard deviation of the mean. The histogram below each plot is the sum of the spike occurrence of the patterns detected by the node. The scale each histogram was set to be the same across all the nodes and was averaged by the number of patterns detected by the node. The value N is the node number which corresponds to the numbering in Figure 31. The value M is the magnitude of each node in spikes/s.
Figure 35 (cont.)
Figure 36. Several examples of nodes and their detected patterns. The top raster plot shows the time of spike occurrence for each pattern detected by the node. The middle plot is the sum of spike occurrence for each time point. The bottom plot show the actual patterns detected from the instantaneous firing rate. The red dotted dash line is the average of the all patterns detected by the node and the blue dashed line is the waveform representing the node location. The value C is the average correlation value for the node and its detected patterns.
Figure 37. The percentage of patterns detected by each node from the same data set used to train the SOM. The SOM used to get this distribution is in Figure 31.
Figure 38. The pattern distribution using data from the same subject used to train the original SOM. The bar graphs on the left shows the pattern percentages by node for three different sets of data from the same recording subject whose neural data was used to train the SOM. These patterns sets do not include any of the patterns used to train the SOM. The scatter plots on the right matches the pattern percentages for the training set with pattern percentages of the comparison set on the left. The value, R, corresponds to the Spearman correlation coefficient for each set of data.
Figure 39. Comparing the SOM pattern distribution of test data from different subject with the original training pattern distribution. The plots on the left illustrate the pattern percentage and the plots on the right show compares the percentages with the original training pattern set in Figure 37. Unlike Figure 38, the patterns are not from the same recording subject used to train the SOM but three different recording subjects.
Figure 40. The effects of the difference in average firing rate between the training set and the comparison set on Spearman correlation coefficient for the SOM pattern distribution. Using 13 different subjects and their corresponding SOM, the difference between the average firing rate of the comparison set and the training set was calculated. The Spearman coefficient calculated for each of these comparisons was the plotted against these difference.
CHAPTER 4
REINFORCEMENT OF CORTICAL PATTERNS USING MEDIAL FOREBRAIN BUNDLE STIMULATION

Introduction

As the field of neural engineering advances, the complexity of the tasks we will want to carry out in brain machine interfaces (BMI) will increase. As a consequence, the challenges of training or tuning the brain to interact successfully with these interfaces will also increase. Currently, we rely on the traditional method of operant conditioning in order to improve the quality of this tuning. The subject learns to better perform the task by repetition with successful tasks receiving some form of reward reinforcement. In the case of animals, the reward may take the extrinsic form of food or juice. In human subjects, the reward may be more intrinsic such as the satisfaction of successfully completing a task or the feeling of greater independence experienced by a totally paralyzed person being able to control a computer cursor with their mind.

Ultimately, this traditional form of training does have drawbacks. Frustration can set in when the task is overly difficult and successful completion comes infrequently. As the tasks become more complex or varied, training time will increase because more repetitions will be needed to adequately tune the brain to successfully interact with the machine interface. Also the more complex the task, the longer it may take to complete a single repetition. Fatigue limits the amount of training that can occur since training requires the subject to stay focused on the task at hand.

The entire process of operant conditioning has a neural basis. The behavior is represented by a spatiotemporal pattern of neural activity taking place in various parts of
the brain depending on the nature of the behavior. The reception of the reward that reinforces this behavior is represented in other areas brains by a phasic neural signal (Schultz, Apicella, & Ljungberg, 1993; Schultz et al., 1997). The combination of the desired pattern corresponding to behavior and this reinforcing signal over time causes changes in the brain making the desired behavior occur in a more regular and typically increasingly efficient manner.

Imagine if it were possible to harness these internal mechanisms of operant conditioning directly without the need of external behavior and repetitive training exercises. With such a system, neural signals could be pre-tuned to better interact with the BMI before the subject even begins to directly use it. The process of learning could be sped up making it less intense and reducing the limitations due to fatigue and frustration.

In order to create such a system, an understanding of the structures and functions of the areas of the brain involved in operant conditioning is needed. Since the operant conditioning area of brain must be able to influence the behaviors being carried out by the organism, it should have connections to regions of the brain that initiate action such as the motor cortex. In addition these operant conditioning areas must also receive inputs about rewards and these signals must alter its behavior. The basal ganglia appear to fulfill these requirements for an area involved in operant conditioning (Yin & Knowlton, 2006).

The anatomical relationship of the basal ganglia with the cortex indicates that it has the ability to analyze and subtly influence the activity of the cortex. The striatum is the main input nucleus of the basal ganglia receiving axon projections from throughout
the cortex. These inputs converge greatly but still keep a similar functional organization though not necessarily spatial organization as they enter into the striatum (Alexander & Crutcher, 1990; Graybiel, Aosaki, Flaherty, & Kimura, 1994; Parent & Hazrati, 1995a). The basal ganglia maintain this functional organization throughout its nuclei and then feed the signals to the same functional areas in the cortex. The projections from the basal ganglia are especially dense to the frontal cortex areas involved in executing action orientated cognitive, behavioral and motor activities (Middleton & Strick, 2000). The signal that is fed back to these cortical areas from the basal ganglia via the thalamus is dispersed over numerous neurons rather than a focused signal targeting a few select neurons (McFarland & Haber, 2002). These dispersed signals could alter cortical behavior by nudging relevant cortical signals out of the noise of competing activity.

At the neuron level, even though these inputs converge greatly, a cortical axon seldom forms more than a few synapses with any single medium spiny projection neuron (MSN) in the striatum (Cowan & Wilson, 1994). Since a typical MSN has 5,000 to 15,000 cortical synapses (Kincaid et al., 1998), these neurons need highly correlated inputs from numerous cortical neurons in order to be activated. This organization makes MSN a possible cortical pattern detector capable of analyzing the firing activity of a large number of cortical neurons. The patterns detected by an individual MSN can be shaped via synaptic plasticity. In addition, MSN connections with interneurons and other MSN may cause activated MSN to hyperpolarize surrounding MSN thus not only detecting but selecting the pattern from other competing ones in the surrounding functional region (Kawaguchi, Wilson, Augood, & Emson, 1995; Tepper, Wilson, & Koós, 2008). This ability to detect and select patterns of cortical activity is essential for operant
conditioning since there must be a method to select behaviors represented by this activity that are associated with reward.

Synaptic plasticity in the basal ganglia appears to require the neurotransmitter dopamine to trigger long-term potentiation (LTP) and depression (LTD) (Calabresi, Picconi, Tozzi, & DiFilippo, 2007). The presence of dopamine is not enough to control synaptic plasticity. Its timing in relation to the activity of the cortical input at the synapse and the corresponding MSN is essential in determining whether plasticity occurs and whether it is LTP or LTD (Wickens, Begg, & Arbuthnott, 1996). It also appears to be important in maintaining dendritic spines on MSN in the striatum since dopamine denervation reduces the number of spines (Ingham, Hood, & Arbuthnott, 1989). The importance of dopamine in striatal plasticity becomes even more relevant when the behavior of dopaminergic neurons in the substantia nigra compacta (SNC) is studied.

Dopaminergic neurons in the SNC provide the reinforcing signal corresponding to reward that is necessary to carry out operant conditioning at the neural level. When subjects receive unexpected rewards such as food, these neurons typically fire in phasic bursts (Mirenowicz & Schultz, 1994). In operant conditioning, this dopamine signal shifts to the earliest stimulus predicting reward as the task becomes learned and the reward predictable (Romo & Schultz, 1990). In these same tasks, the firing rate of these neurons will become depressed if the expected reward does not occur (Schultz et al., 1993).

The above features when combined together begin to suggest a possible method of internal operant conditioning. Using synaptic plasticity mechanisms, it should be possible to train neurons in the striatum to recognize a pattern of cortical activity when
paired with a properly timed dopamine signal. Through this pairing, the basal ganglia should learn to recognize a pattern in the same way as an external behavior that leads to reward. The basal ganglia should then begin to influence the cortex in a similar way in order to optimize the “perceived reward” signaled by the dopamine.

One key component of this method is the creation of the dopamine signal. In the early 1950’s, rats were shown to learn to press a lever by reinforcing this behavior with electrical stimulation of certain areas of the brain in a technique called intracranial self-stimulation (ICSS) (Olds & Milner, 1954). One of the areas where this effect occurs is the medial forebrain bundle (MFB). This bundle of axons extends from the dopaminergic neurons in the VTA and substantia nigra compacta (SNc) to the striatum and the frontal cortex. Stimulation of this bundle has been shown to release dopamine into the striatum when measured using cyclic voltammetry (Kuhr, Wightman, & Rebec, 1987). In addition, drugs that block dopamine receptors have been shown to reduce the rate of lever pressing during ICSS (Fouriezos, Hansson, & Wise, 1978). These studies show that MFB stimulation releases dopamine and even has the ability to reinforce behavior which is central to our hypothesis about this technique.

In this chapter, a method is described for removing the external aspects of operant conditioning, thus shifting the focus to internal neural signals. Instead of looking for a desired external behavior to pair with a reward, the internal neural activity was used. Using signals recorded from the motor cortex, a spatial-temporal pattern of neural activity was chosen. The external reward was replaced by an internal reward signal created by electrically stimulating the MFB resulting in the release of dopamine. When the selected pattern occurs, this reward signal will be triggered attempting to create an
internal version of the operant conditioning paradigm. It is hypothesized that this method should cause an increase in the rate of occurrence of the randomly selected pattern of neural activity in the motor cortex.

Methods

**MFB electrode implantation.** The initial step of this experiment was to implant the stimulating electrodes in Sprague Dawley rats. All animal protocols were approved by the Arizona State University IACUC. Rats were initially placed under anesthesia using ketamine xylazine cocktail (100 mg/ml ketamine and 5mg/ml xylazine). The subject was then placed in the stereotaxic unit. An incision was made in the skin on the top of the skull and all tissue was removed from the bones of the skull around the implantation area. Once the bones of the skull were revealed, the stereotaxic system was adjusted to make the top portion plane of the skull as level as possible through the anterior posterior axis.

The coordinate location of Bregma was then determined using the drill bit attached to the drilling arm on the stereotax. Drill holes were made at coordinate locations determined to be over the medial forebrain bundle (MFB) (2.5 mm posterior and ±1.8 latterly from Bregma). The holes on both sides of the rat skull in order to target the MFB in both hemispheres of the brain. The choice to implant bilaterally was made in order to increase the success rate of the MFB stimulation. The drilling process was done gradually in order to get an accurate measure of skull thickness. The dura was carefully punctured at both sites using a pair of forceps. This was done because the stimulating electrodes were unable to puncture through the dura on their own and would instead bend. After drilling the holes for the MFB stimulation electrodes, an additional hole was
drilled posterior of each stimulation site for the placement of the screws to hold the headcap in place. The screws were then placed in these holes.

Once all the holes were drilled and the screws placed, one stimulating electrode was then placed in the implanter arm and care was taken to make sure the electrodes wires were straight. The stimulating electrodes used were bipolar platinum wire electrodes made by Plastics One. The location of Bregma was found once again using the electrode instead of the drill bit and the electrode was centered over the MFB using these new Bregma measurements. If all measurements were done properly, the electrode should be placed in the center of the previously drilled hole. If this was not the case measurements were retaken and if electrode was still not placed over the hole, the hole was widened using rongeurs. The dorsal/ventral position of the skull surface was then measured at the implantation site and the electrode was then inserted to the depth based on the previous measured skull thickness and the depth from the dorsal/ventral coordinates for MFB (-7.8mm from dura). If the electrode started to bend during the initial part of the implantation, the electrode was raised and another attempt was made to puncture the dura using the forceps.

Once the electrode was implanted, it was attached to the screw immediately posterior to it using a small amount of acrylic. The acrylic was allowed to harden before the implanter arm was removed from the electrode. The entire implantation process was then repeated for the stimulating electrode on the other hemisphere of the brain. The electrodes could not be implanted at the same time due to the implanting arms bumping into each other. Once the second electrode was implanted, the two sites were connected together using acrylic being careful not to allow the acrylic to flow beyond the Bregma
location on the skull since recording electrode array would be placed anterior of this point if the stimulating electrodes were shown to successfully stimulate MFB. After the head cap was formed, the incision made through the skin was stitched up around the headcap and the rat was allowed to recover from the surgery.

**MFB stimulation testing.** In order to determine whether MFB stimulation was successfully releasing dopamine and the corresponding parameters for that release, two tests were carried out. The first test measured the number of circular rotations made by the rat during stimulation. The test was done using a train of electrical stimulation (10s pulse train consisting 1ms square cathodal pulses at 50Hz) applied to one of the two implanted electrodes (Arbuthnott & Ungerstedt, 1975). The magnitude of the stimulation started at 50µA and was increased by 50µA increments until it reached a maximum level of 300µA. After each stimulation, the rat was allowed to rest two minutes before the next one was triggered. Previous studies have shown that the number of rotations of rat in the contraversive direction of the selected electrode during stimulation is related to the level of dopamine being released by the stimulation (Arbuthnott & Crow, 1971; Crow, 1971). The electrode with the highest rate of contraversive rotation during stimulation was selected for the next test. If neither electrode could trigger rotations or if ipsilateral rotations were triggered then the rat was removed from the study.

The second test was an intracranial self-stimulation task (ICSS). The rat was placed in a behavioral box with lever. When the rat pressed the lever, it was electrically stimulated using the electrode selected in the previous rotation test. The electrical train was 500 ms in duration and consisted of 1ms pulses occurring at a rate of 141Hz with a 500ms lockout period occurring after each stimulation (Carlezon & Chartoff, 2007). The
magnitude of stimulation was increased from 50\(\mu\)A to 300\(\mu\)A by 50\(\mu\)A increments until at rate of 30 lever presses per a minute was achieved. The current level corresponding to this rate was then used in the next stage of the experiment to reinforce the selected pattern of cortical activity. The test was stopped if there were signs of a motor artifact being produced by the stimulation or if the stimulation appeared to cause discomfort in the rat.

**Recording array implantation.** The rats with properly placed electrodes next had a recording array implanted anterior to the previous headcap for the stimulating electrodes on the ipsilateral side of the best performing stimulating electrode. The exact location for each subject varied slightly in order to avoid the electrode array hitting the previous headcap during implantation. The typical location was approximately 3.0 mm anterior to Bregma, 2.0 mm lateral from midline and 2.0 mm below dura placing the array in the motor cortex of the rat. In some cases excess headcap material was carefully filed away before the surgery to give more clearance. The recording array used in this experiment was a sixteen channel Tucker Davis Technologies (TDT) micro-wire array with the electrodes arranged in two rows of eight.

The surgery was performed under anesthesia using a chronic implant technique similar to the one described for the stimulating electrodes with several changes. First, the drilled holes at the implant site were enlarged using a set of rongeurs being carefully to avoid damaging the dura. Second, the electrodes were driven into the brain without removing the dura since the electrode wires were able to penetrate without bending. In addition to creating a craniotomy for the array, a screw hole was drilled in the frontal plate of the skull on the contralateral side. A ground wire was tucked into the hole and anchored in place by a stainless steel screw. The older headcap created during the
stimulating electrode implantation was incorporated into the new acrylic head cap that held the recording array in place.

**Reinforcement of cortical patterns.** After the recording array was implanted and the rat was allowed to recover from the surgery, the experiment to reinforce cortical patterns of activities was conducted. The rat was placed in a restraint tube that prevented it from rotating and entangling the wires during the experiment. In order to keep the rat content in the tube, a small drop of chocolate milk was dispersed at random intervals unrelated to any part of the actual experiment.

Once placed in the tube, an initial recording of the cortical activity was collected using the TDT RX5 recording system. During the initial recording, the quality of the neural signals from the two electrodes that were across from each other in each row were compared and the electrode with the better quality signal was selected for use in the experiment. This selection process reduced the number of electrodes used in the experiment from sixteen channels down to eight channels.

Once the channels used in the experiment were selected an initial recording was taken. Rather than recording single neural activity during the experiment, the multiunit activity signal (MUA) was recorded instead (Stark & Abeles, 2007). The raw neural recording signal from each electrode was first band pass filtered from 300 Hz to 6 kHz. The initial sampling was set at 24.414 kHz. The MUA signal is basically the root mean square (RMS) of the raw neural recording. The square of each data point was calculated. Then these values were ran through a smoothing filter that low passed filtered the signal and then down sampled by a factor of 48 to get a final sampling rate of 508.62 Hz. The
smoothing filter is the mean portion of the RMS. Finally the square root of each down
sampled data point was taken.

In order to determine possible patterns to stimulate, ten minutes of neural
recordings were taken. The resulting MUA data for the previously selected eight
channels was broken in ~100 ms chunks (50 data points per channel). The chunks on
each channel corresponding to the same time span were then combined to for a single
pattern consisting of 400 samples (50 samples from each of the eight channels). The
resulting set of patterns was then used to train a self-organizing map (SOM) using the
SOM toolbox discussed previously in Chapter 3. Once the map was trained, the node
that detected each training pattern was found and the average Euclidean distance between
the detected patterns and the node was calculated.

In order to ensure the pattern template did not describe a pattern that occurs too
infrequently, only nodes with an above average number of detected patterns when
compared to the other nodes were chosen. From these above average nodes, the node
with the smallest average Euclidean distance between it and its detected patterns was
selected to create the pattern template. The smallest average distance was used because it
indicates that training array points are close together and should be more similar as a
result. The pattern template was created using the patterns detected by the selected node.
The points that made up each detected pattern and the corresponding node were broken
back into channels and the minimum and maximum value of each channel’s detected
patterns for each time point was used to create the lower and upper thresholds
respectively. During the remainder of the experiment, the pattern being detected
occurred when the MUA signal fell between the lower and upper thresholds on all eight channels.

Once the pattern template was created, it was loaded into the TDT software. An initial baseline recording was done where the patterns were detected using the newly created template but no stimulation occurred. If the patterns did not occur between 1-2 times per a minute during the baseline recording period, a new pattern was selected using the previously created SOM and another baseline recording was taken.

After achieving a baseline recording with a regularly occurring pattern, the pattern was then paired with MFB stimulation upon detection. The stimulation parameters were the same as the previously discussed ICSS task. The current was set to the level where the rate of lever pressing was the closest to 30 presses per a minute on the previous ICSS task. If the maximum rate during the ICSS task never reaches this level then the current level with the highest rate will be chosen. The MUA signals and the times of pattern detection were recorded.
Results

Evaluating the effectiveness of MFB stimulation. As was stated in the methods sections, each rat used for the pattern reinforcement experiment underwent two surgical procedures. The first surgical procedure implanted two MFB stimulating electrodes one in each hemisphere of the subject. Afterwards, the ability of the MFB stimulation to release dopamine was first evaluated using the turning behavior test. Figure 41 shows an example of a successful turning behavior test. The number of contraversive rotations were counted during the 10 s stimulation of the MFB. The linearly increasing contraversive rotations indicate that the release of dopamine is being triggered the MFB stimulating electrode.

Once the release of dopamine was verified using the turning behavior test, the level of current necessary to reinforce behavior was determined using the ICSS task. Figure 42 show an example of the number of lever presses over the course of time for an ICSSS training session using four different current levels. The lever pressing rates show signs of increasing as the current levels are raised which is further confirmed when the average lever pressing rate was plotted in Figure 43. This increasing rate as current increases indicates successful stimulation of dopamine axons in the MFB since increased current should stimulate a larger number of axons increasing the amount of dopamine released.
**Pairing cortical patterns with MFB stimulation.** Once the current level for pattern reinforcement was determined using the ICSS task, the rat was implanted with a cortical array. A self-organizing map (SOM) was created using a 10 minute portion of the MUA and the algorithm described in Chapter 3. Unfortunately, the recording and computer system could not directly use the SOM to detect patterns in real time. As a result, an alternative approach to the one described in Chapter 3 was taken where an upper and lower threshold was created. A pattern was considered detected when the MUA signal fell completely within the threshold on all eight channels. These detected patterns were then paired with MFB stimulation.

Figure 44 shows an example of an experimental trial where the rate of pattern occurrence was increasing while paired with MFB stimulation. In order to determine whether the pattern was increasing, a linear regression on the pattern rates shown in Figure 44 was performed to find the slope and the p-value of the slope. A trial with increasing pattern occurrence was defined as a trial with positive slope and p-value less than 0.05. After performing the regression, several checks were done on the regression which are shown in Figure 45. For the difference between the predicted and actual value shown in Figure 45A, the difference should be zero in a regression that perfectly fit the data. In a less than perfect fit, the data should not show any signs of oscillation and appear to be normally distributed around a difference of zero. For this regression, there are few outliers but there does not appear to be major issues with fit. These outliers were the two points at end of the trial. For Figure 45B, the closer the points mapping the actual and predicted values are to the line then the better the fit. Issues with the regression would be indicated by points curving away from the line which is not the case for this
trial. Overall, the regression fit appears to be acceptable and the $R^2$ value indicates the regression describes around 70% of the variation in the data. Out of the 40 trials, there were 8 trails that showed significant positive increases in pattern occurrence.

The problem is that there are also eight trials that show significant decreases in pattern occurrence. Two examples are shown in Figures 46 and 48. Figure 46 and its corresponding checks shown in Figure 47 show fairly continuous decrease in the rate of pattern occurrence. The fit of the linear regression is not as good at the one shown in Figure 44 but it appears to be acceptable based on the checks in Figure 47. Figure 48 poses additional problems since there is a clear oscillation in the rate of pattern occurrence as can be seen in Figure 49. The fact that there is 10-20 min period where there appears to be increases in the rate of pattern occurrence despite the general decreasing trend is particularly troubling since it could indicate that the successful runs are more a matter of timing than being paired with the MFB stimulation.

The summary of the overall results of the regression analysis are shown in Table 3. The decreasing trials had a higher average rate of pattern occurrence than both the constant and increasing trials though there is a larger variation in the data for the decreasing trials. The high average could be indicative of a higher starting pattern rate as seen in Figure 48 making these trials more prone to decrease. The average magnitudes of the slope are similar which again may indicate the positive effects are more random in nature.

Figures 50 and 51 look more closely at the nature of the patterns detected on two different trials. The upper and lower thresholds for these two trials are indicated by the dashed line. The waveform that results from averaging all the detected patterns together...
is indicated by the solid line. The shaded area is within +/-1 standard deviation from the average at each time point. Both example are from trials where there pattern occurrence show a significant increase. Ideally the results should look something like Figure 50 where the average waveform shows variations that match the variations of the threshold. Unfortunately, Figure 50 is the exception for the increasing and decreasing trials and Figure 51 is the more typical of the patterns detected by the thresholding method. The lack of variation in the average is indicative of the threshold being less than selective. The trials with constant pattern occurrence had slightly more results like Figure 50.

In order to further determine the selectivity of the pattern detection threshold, the 50 data point pattern detection window was slid through each MUA data point checking how many points fell within upper and lower values of the threshold over the length of the window. Since there are eight channels, a detected pattern (one that would trigger MFB stimulation) would have 400 data points falling within the upper and lower threshold. Figures 52 and 53 show distribution of the number points fell within the detection threshold window over all the MUA data points during a trial. Figure 52 is the result of the trial whose threshold and average patterns are shown in Figure 50. As previously stated this particular set of threshold appears to be more selective. Figure 53 corresponds to the threshold and average pattern results shown in Figure 51 which was a less selective threshold. The two distributions appears to confirm these thoughts about selectivity. Figure 52 distribution has a peak further from maximum of 400 data points when compared the peak seen in Figure 53. This indicates that activity captured by the threshold is more unique for the trial shown in Figures 52 and 53.
Performing this analysis on all 40 trials, the average number of data points within threshold window over was calculated for the increasing, constant and decreasing rates of pattern occurrence. These averages and their corresponding standard deviations are shown in Figure 54. Both the decreasing and increasing rates, had similar averages for the number points that fell within the threshold window. The fact that these averages are higher than the constant rate trials indicate the results where there significant changes in the rate occurred may not be as selective.

Conclusions

In this chapter, a method of reinforcing patterns of cortical activity was attempted. Though there were trials where the rate of pattern occurrence increased, the overall results appear to be less than conclusive. The patterns detected appear not to be very different from the overall neural activity based on the results in Figure 54. The average number of points that fell within the pattern detection window. The reason for the lack of specificity was most likely do to the method used to detect the patterns. Rather than directly use the SOM to detect the pattern, a thresholding method was used due to the limitations of recording systems.

Even if the patterns were selective, there is a high probability that patterns may have been too complex to start out with. The patterns were relatively long lasting occurring over a time period of almost 100 ms and requiring control over eight channels extending over a 2 mm long stretch of cortex. In addition, the multiunit activity signal is a noisier signal than an instantaneous firing rate based on an isolated single unit which could make the MUA more difficult to control in this method.
In the future, attempts should be made to simplify the experiment. One suggestion would be to focus on controlling the activity of a single neuron. This experiment could be designed to stimulate the MFB when the instantaneous firing rate of the neuron falls within a certain range similar to the experiments of Fetz (1969). In his experiments, the target neuron firing rate was reinforced using food pellets which would be replaced with MFB stimulation in this proposed experiment. The other difference is that Fetz’s experiment gave the subject visual and/or auditory feedback about the neuron firing rate which would not be provided in the proposed experiment.

This experiment may also be a good candidate for the use of optogenetics. Dopaminergic neurons in VTA have already been modified to be stimulated using channel rhodopsin (Tsai et al., 2009). Unlike electrical stimulation, only the dopaminergic neurons would be stimulated making the effects of the results clearer interpret. In addition, light stimulation would eliminate the electrical artifact that prevents the recording of neural activity of the cortical and striatal neurons during stimulation. This could give additional information about the learning process.

Overall, this technique should work if a proper pattern is used. Brain machine interface at the core is about the control of neural activity but based on using external outcomes. MFB stimulation uses the internal reward signals to shape external behavior. The use of MFB stimulation to shape neural activity is not that far of a leap.
Chapter 4 Figures

Figure 4. Rotation rates caused by MFB stimulation.

Figure 41. Rotation rates caused by MFB stimulation.
Figure 42. ICSS lever press rates over time at different stimulation current levels. The histograms show the number of lever presses during each minute of a typical ICSS experiment. Each histogram represents a different current level used in the experiment. The current level was changed in an ascending order. The continuous nature of the lever pressing indicates the MFB electrode is properly placed and is reinforcing the pressing of the lever.
Figure 43. The average rate of lever pressing for each current level used in the ICSS experiment. The positive correlation between the current level and the rate is another indication of proper MFB electrode placement.
Figure 44. An example of the rate of pattern occurrence during a trial with an increasing rate and the resulting regression line. The $R^2$ value was 0.519 and the slope was 0.289 patterns/min$^2$ ($p<0.001$)
Figure 45. The checks performed on the regression analysis shown in Figure 44. A. The difference between the actual and predicted values. B. The predicted value vs actual values.
Figure 46. An example of the rate of pattern occurrence during a trial with a decreasing rate and the resulting regression line. The R² value was 0.349 and the slope was -0.106 patterns/min² (p<0.001)
Figure 47. The checks performed on the regression analysis shown in Figure 46. A. The difference between the actual and predicted values. B. The predicted value vs actual values.
Figure 48. An example of the rate of pattern occurrence during a trial with an oscillating rate and the resulting regression line. The $R^2$ value was 0.522 and the slope was $-0.257$ patterns/min$^2$ ($p<0.001$).
Figure 49. The checks performed on the regression analysis shown in Figure 48. A. The difference between the actual and predicted values. B. The predicted value vs actual values.
<table>
<thead>
<tr>
<th></th>
<th>Duration (min)</th>
<th>Patterns detection rate (patterns/min)</th>
<th>Slope (Patterns/min²)</th>
<th>P-value for slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreasing Occurrence (n = 8)</td>
<td>53.0±20.2</td>
<td>7.51±5.79</td>
<td>-0.113±0.103</td>
<td>0.0036±0.0045</td>
</tr>
<tr>
<td>Constant Occurrence (n = 16)</td>
<td>32.0±16.5</td>
<td>2.98±3.57</td>
<td>0.0176±0.0525</td>
<td>0.329±0.278</td>
</tr>
<tr>
<td>Increasing Occurrence (n=8)</td>
<td>43.3±17.4</td>
<td>3.93±1.82</td>
<td>0.126±0.118</td>
<td>0.0068±0.0129</td>
</tr>
</tbody>
</table>

Table 3. The average and standard deviation values for decreasing, constant and increasing pattern occurrence data sets. Duration is the average duration of the experiments. Patterns detected is the average number of patterns detected during the experiment. Slope is the average slope that was found by performing linear regression.
Figure 50. An ideal example of the pattern detection threshold used and the average waveform of the patterns detected. The dotted line represents the upper and lower threshold used to detect patterns to stimulate. When all fifty data points of the MUA signal fell within the threshold for each channel, MFB stimulation was triggered. The solid line represents the average waveform for the patterns detected during the trial and shade region represents the area within one standard deviation of the average.
Figure 51. A typical example of the pattern detection threshold used and the average waveform of the patterns detected. The average pattern waveform from the same trial as the ones seen in Figure 44.
Figure 52. The distribution indicating how many MUA data points of each 50 point sliding window fell within the pattern detection threshold. This distribution was created using the MUA data and threshold for the trial shown in Figure 50.
Figure 53. The distribution indicating how many MUA data points of each 50 point sliding window fell within the pattern detection threshold. This distribution was created using the MUA data and threshold for the trial shown in Figure 51.
Figure 54. The average number of points that fell within the pattern detection window.
CHAPTER 5

CONCLUSIONS

The beginning of this research started from a desire to understand the role of the basal ganglia in learning during the control of movement. The basal ganglia like every other brain structure is part of a complex interconnected system that adapts over time. The first two parts of this dissertation aimed to develop tools to study the two realities of interconnectedness and adaptation. The first technique was the localized short duration microinjections which will be useful for manipulating one part of the brain in order to see how these effects propagate through its connections thus gaining insights in how these connections influence other parts of the brains. The second technique applied a self-organizing map (SOM) to determine typical patterns of neural activity. The map was then used to create a frequency distribution of these patterns allowing for comparisons of the neural activity at different times periods and making it a measure of the amount of change in neural activity that is occurring. Though these tools were designed for use with the basal ganglia in mind, they are applicable for other areas of the brain because of the commonality of these issues. The third experiment was designed specifically to test a hypothesis on basal ganglia function and its role in learning reward related tasks that was developed by the modeling work of Hunn (2008) in our lab.

Designed Injections

One of the messages that I hope this research emphasizes is that researchers cannot inject drugs into the brain and just assume they are only targeting their desired area. Research has shown this is not typically the case (Allen et al., 2008; Edeline et al., 2002; J. H. Martin, 1991). Careful considerations of factors such as the volume of the
injection, concentration of the drug, the injection flow rate, and the size of the injection cannula must be made in order to ensure the desired results are achieved from the injection. In addition, the properties of the tissue and the surrounding regions need to be looked at.

The mathematical model of Stukel et al (2008) demonstrated the power of taking such a design approach to injections. By considering tissue properties, concentrations of drugs, and the convection diffusion characteristics of an injection, a method was devised to give much greater control over the effects of the injection. In Chapter 2, a method to alter the behavior of small volumes of neural tissue for a limited amount of time using drug injections was tested in vivo. This experiment demonstrated the principles highlighted in the previous mathematical model are correct. By taking a design approach, the multiple hour effects of muscimol when using traditional concentrations were reduced to less than an hour in most cases (Edeline et al., 2002; Hikosaka & Wurtz, 1985). In addition, the significant neural effects were contained to region far smaller than most typical muscimol injections (Arikan et al., 2002; Heiss, Walbridge, Astagiri, & Lonser, 2010; J. H. Martin, 1991).

Drug injections into the brain have been a traditional method for understanding how regions of the brain interact with each other and affect behavior but the typically large spread may complicate the interpretation of the results. For example, the striatum of the basal ganglia is divided into very distinct regions that receive input from very different functional regions of the cortex (Middleton & Strick, 2000; Parent & Hazrati, 1995a). A study interested in the role of striatum in motor control may injection region of the striatum receiving inputs from the primary motor cortex. Now if the injection
spreads and also inactivates a large area of the striatum receiving prefrontal cortex inputs then questions may arise about whether the results are solely due to the influence of basal ganglia on the motor cortex or are they also the result of affecting judgment or reward valuation which the prefrontal cortex plays a role in. By limiting injection size, the effects of this technique should be much easier to interpret since results will be less prone to be confounded by unintended spread of drug effects into neighboring regions of the brain.

There are several areas of future work that could be done to improve this study. Because of design limitations of the combination injection cannula and electrode array, the study was forced to use an acute preparation rather than a chronic one. As a result, the number of experimental runs performed was very limited. In addition, the array did not have a high amount of spatial resolution which limits the ability to fully quantify the effects over distance. At the lowest concentrations, the effects could be limited to a radius within 1 mm from the injection site or less which would be on the very edge of the closest recording site to the injection in the current array and may not be detectable. Based on conversations, there are companies designing recording arrays with injection cannula but as of yet, they do not appear to be readily available. It would be useful to run more variations of this experiment if an improved chronic array setup became available. A systematic exploration of drug concentration, volume, and delay time between the injection and the flush would be useful to better quantify their effects on the spread and the duration of the drug effects and would allow for better design of the injections in future studies of brain function.
The Effects of aCSF and Other Solutions Used in Drug Injections

This work also highlights that the solution the drug is dissolved in may have its own effect on neural activity. When researching drug injections in the brain, it becomes clear that there is not an agreed upon solution medium for injection. Experiments will use a variety of mediums including saline solution, phosphate buffered saline, and variety of aCSF recipes. This is despite the fact that ion concentration of the extracellular fluid is a critical factor in neuron behavior (Somjen, 2002). The assumption seems to be that the neural system of glia and neurons will be easily able to overcome local differences in ion concentration. Yet, this research and several other studies seem to contradict that assumption (Canal et al., 2005; Edeline et al., 2002). Researchers need to consider this fact when designing and interpreting their results. In terms of experimental design, it is essential to do control injections of just solution medium along with the drug injections. When interpreting results, researchers should beware the results could be a combination of the both drug and medium effects. This could be especially important when comparing experiments with conflicting results.

In the future, there are several areas that could warrant further investigation. The first is optimizing the aCSF solution for minimal effects on in vivo neural tissue. As mentioned previously, a better combination electrode and injection cannula array would make this kind of study much more possible. A second area is increased understanding of interrelationship between astrocytes and neurons in maintaining extracellular fluid homeostasis in vivo. Much of what we know about astrocytes seems to be from either in vitro cell cultures or slice preparations. These experiments greatly simplify the system and are missing key components. They also tend to focus on the effects of global
changes in the solution medium bathing the cells or slices rather than local changes. The challenge with in vivo studies is developing methods to manipulate and measure astrocytes behavior without directly affecting the surrounding neurons. This is especially difficult since they have evolved from the same endothelial ancestor and share some of the same receptors and channels (Nedergaard & Verkhratsky, 2012). There are several newer techniques being developed that could help in studying these interactions. One possibility is the use of voltage sensitive dyes which have previously been used to determine changes in membrane potentials of neuron cells in vivo (Baker et al., 2005). Similar techniques could be used to measure the flow of ions into astrocytes when changes in extracellular ion concentrations are occurring. The inserting of channel rhodopsin into astrocytes is another method that is being developed and will make it possible to perturb the membrane potentials of astrocytes without directly affecting the behavior of neurons in the process (Figueiredo et al., 2011). Another parallel technique that is currently being developed but not yet perfected is the use of mutated rhodopsin that no longer has channels for ions to pass through but will fluoresce at varying amounts based on the membrane potential (Kralj, Douglass, Hochbaum, Maclaurin, & Cohen, 2012). This technique could make it possible to optically image the electrical activity of astrocytes under different conditions and give a better idea about how astrocytes adjust ion concentrations.

**The Use of Self-Organizing Maps to Analyze Changes in Neural Activity**

Chapter 3 used a self-organizing map to find typical patterns of neural activity and then used this map to create distributions of pattern frequency. The demonstration of this technique was limited to using only a single neuron and a rat under anesthesia. At
same time this technique should be scalable to ensembles of neurons with relatively little
modification with the main limiting factor being the time to train the map. Future work
will hopefully demonstrate this possibility using data from awake and behaving subjects
to study changes in these pattern distributions as a subject are learning/performing a task.

Because of the limited application in this dissertation, the full potential of this
technique was not realized. Technology is allowing researchers to record ever increasing
numbers of neurons and other forms of neural activity. Labs like the Sensorimotor
Research Group also collect data from motion capture systems, pressure readings as
subjects grip objects, and directions of brain machine interface objects among other
things. Finally, as understanding of the brain increases, the complexity of the
experiments are increasing. Yet, many of the methods used to analyze neural data and
other data have not fully adjusted to these realities. The application of machine learning
techniques such as SOMs and other big data mining techniques may play a critical role in
getting the most out of huge quantities of data being collected in most neuroscience labs.
Finally, they may help researchers see subtle patterns that may not be as apparent using
traditional analysis techniques.

**Practical Lessons From Attempting to Reinforce Cortical Patterns Using Medial
Forebrain Bundle Stimulation**

The idea for the experiment in Chapter 4 came from earlier modelling work by
Hun (2008). His model suggested that the medium spiny projection neurons in the
striatum were well suited for detecting specific patterns from the cortical inputs they
receive. The patterns they learn to detect were the ones associated with positive
outcomes. This association was formed through synaptic plasticity triggered by the convergence of cortical inputs and dopaminergic inputs on the same striatal neurons. The goal of the experiment in Chapter 4 was to test this explanation of basal ganglia function by pairing patterns of cortical activity with dopamine release in the striatum triggered by medial forebrain bundle (MFB) stimulation. The hypothesis was that the selected pattern would increase in frequency over time. The experiment was less than successful resulting in a mix set of outcomes. The most likely reason for the failure of this experiment was a poor method for detecting the patterns that resulted in a lack of selectivity. Because of this, the results of the experiments do not yet confirm or deny the validity of the proposed hypothesis. Future attempts to do this experiment must make sure the pattern used is salient.

The practical lesson from this set of experiments is to start with the most basic proof of concept and then increase the experimental complexity in relationship with successful results. Future experiments attempting this technique may want to start out by controlling a single neuron’s firing rate and then gradually increase number of neurons or the length and complexity of the pattern. If the initial single neuron experiment works, then there are several interesting questions that could be tested. First, what is the maximum length of the pattern before reinforcement ceases? Second, what is the maximum distance between pattern occurrence and the MFB stimulation before the pattern rate does not change which would indicate learning is no longer occurring? Third, how does the activity of cortex change in between pattern detection? This technique could also be paired with targeted drug injections in the cortex, striatum and other areas of the basal ganglia to get further understanding of the role different parts of
the brain in reward learning. If this technique can be perfected, then it could be a very useful method to probe learning in the brain.
REFERENCES


Hunn, D. R. J. (2008). *A theory of basal ganglia function using non-specific output implemented in a large-scale computational model*


Parks, J. P. (2006). *Drug injection modeling for basal ganglia recordings*


APPENDIX A

ANIMAL PROTOCOL APPROVAL
Animal Protocol Review

ASU Protocol Number: 10-1129R
Protocol Title: Reinforcement of Cortical Patterns Using Medial Forebrain Bundle Stimulation
Principal Investigator: Stephen Helms Tillery
Date of Action: 04/26/2010

The animal protocol review was considered by the Committee and the following decisions were made:

☐ The original protocol was APPROVED as presented.
☒ The revised protocol was APPROVED as presented.
☐ The protocol was APPROVED with RESTRICTIONS or CHANGES as noted below. The project can only be pursued, subject to your acceptance of these restriction or changes. If you are not agreeable, contact the IACUC Chairperson immediately.
☐ The Committee requests CLARIFICATIONS or CHANGES in the protocol as described in the attached memorandum. The protocol will be considered when these issues are clarified and the revised protocol is submitted.
☐ The protocol was approved, subject to the approval of a WAIVER of provisions of NIH policy as noted below. Waivers require written approval from the granting agencies.
☐ The protocol was DISAPPROVED for reasons outlined in the attached memorandum.
☒ The Committee requests you to contact ____________________ to discuss this proposal.
☐ A copy of this correspondence has been sent to the Vice President for Research.
☐ Amendment was approved as presented.

REstrictions, Changes or Waiver Requirements:

Total # of Animals: 61 Pain Level: D Species: Rats

Signature: ____________________________ Date: 9/26/10
IACUC Chair or Designee

Original: Principal Investigator
Cc: IACUC Office
     IACUC Chair
Nathan A. Baldwin was born and raised in Kewaunee, Wisconsin where he attended school until he graduated from high school in May 2000. Despite Kewaunee School District’s small size, he was fortunate to have several outstanding science teachers that created numerous hands-on lab experiences helping to spark his interest in science and engineering. He went on to attend Milwaukee School of Engineering where he studied biomedical engineering. The choice of biomedical engineering stemmed in part from his experiences as a childhood leukemia survivor. After graduating from Milwaukee School of Engineering with a B.S. in Biomedical Engineering in May 2004, he went on to attend graduate school at Arizona State University where he continued to study biomedical engineering. He was initially a member of the lab headed by Dr. Jiping He. He went on to join the Sensorimotor Research Group (SMoRG) when it was started by Dr. Stephen Helms Tillery. At SMoRG, he worked on developing a lab for neural implantation and recording in rodents with the assistance of members of Dr. Jennie Si’s lab and performed the current work. He was an NSF IGERT fellow in 2006 and received MS in Bioengineering from Arizona State University in 2010. For the last three years, he has been a teaching assistant for Biomedical Engineering Capstone Design. He was also a visiting professor at DeVry University.