TRANSIENT SYNCHRONY AMONG MOTOR CORTICAL NEURONAL ENSEMBLES DURING OLFACTORY STIMULUS ANTICIPATION IN A GO/NOGO TASK

By

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To AS1, AS2, CS1 and nameless others who sacrificed a lot and taught me the patience to keep persevering
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<td>Primary forelimb motor cortex</td>
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<tr>
<td>pPIR</td>
<td>Posterior piriform cortex</td>
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<tr>
<td>JSE</td>
<td>Joint spike event</td>
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Considerable evidence suggests that perceptual, cognitive and motor information processing occurs not only by means of neuronal firing rate modulations, but also through finer-scale temporal modulations involving spikes and slower potentials. One variety of temporal coding, transient spike synchrony among neurons, is thought to encode information about the timing of an anticipated stimulus that will signal the animal to perform a specific movement, or to be related to movement preparation per se in these tasks. In several previous experiments, spike synchrony was shown to increase in the primary motor cortex as the stimulus approached in time, indicating that the monkeys should perform a previously instructed directional movement. Increased temporal precision in synchrony was also observed as the stimulus approached. We sought to verify whether this increased synchrony and temporal precision was observed in a similar task, an olfactory Go/NoGo discrimination task, in a different species, rats. Furthermore, we sought to test whether such synchrony, as seen in past experiments, was more likely related to stimulus anticipation or timing, on the one hand, or movement planning on the other. To address these questions, we employed a relatively novel, recently validated analytical technique which had the advantages of being able to detect
synchrony among groups of neurons greater than two, and being more robust under conditions of low or high firing rates and low trial numbers. We recorded multiple single units from the primary motor cortex and the posterior piriform cortex in three rats during the odor anticipatory period as well as during a control period mostly containing locomotion, in which less neural synchrony was expected. The intra-regional synchrony results in the primary motor cortex didn't support our hypotheses in a straightforward way either when viewed alone or when the control period was included in the analyses. Increasing synchrony and temporal precision during the anticipatory period alone and when compared to the control period were observed only in some analyses in some rats as the stimulus approached, but in some other cases, more synchrony was observed in the control period. These results raise the question of whether monotonically increasing synchrony and temporal precision are actually observed as stated by previous groups particularly because only one study (Kilavik et al., 2009) included whole-ensemble statistics. The findings were significant in that a new technique was used that was robust with low trial numbers and succeeded in detecting transient synchrony among neural ensembles possibly as large as 20 or more. We discuss ways in which hypotheses about how the amount and fineness of temporal synchrony related to cue anticipation and movement preparation could be better addressed.
CHAPTER 1
INTRODUCTION

Information processing in the brain is thought to be distributed across various areas and it is now fairly well established that perceptual, cognitive and behavioral processes once thought to be carried out in single brain regions in fact depend on the coordinated activity across many brain regions. For example, syntactic processing in humans is no longer thought to take place solely within Broca’s area, but rather, it appears to require distributed processing across the anterior, middle and superior areas of the temporal lobe (Kaan and Swaab, 2002; Grodzinsky and Friederici, 2006). Moreover, temporal area activation is also seen in both hemispheres during syntactic processing (Meyer et al., 2003). Perceptual functions such as face perception in humans have been shown to be mediated in a distributed cortical network involving not only the fusiform gyrus, as first reported, but the hippocampus, amygdala, and the orbitofrontal cortex, with bilateral activation in many of these areas (Ishai et al., 2005).

The performance of certain visual working memory tasks is associated with activation of multiple areas that are widely distributed such as the posterior visual cortices, motor cortices and the prefrontal cortex (Courtney et al., 1997; Haxby et al., 2000). In other working memory tasks, activation in the medial temporal lobe, the inferotemporal cortex and the prefrontal cortex and bidirectional coupling between these regions have been shown to correlate with increasing load, thereby implicating a distributed network. Some working memory tasks have even been shown to engage networks of spinal interneurons, which inhibit motor output until a cue for the animal to signal its choice is given (Prut and Fetz, 1999).
Studies such as the above raise important questions about how different areas in the brain may be connected structurally and functionally and how they might interact with each other to process information. Connections might exist between brain regions in a random fashion or a highly regular fashion, each having their own advantages. Small world networks, a compromise between randomly connected and regularly connected networks, incorporate high clustering between adjacent nodes and short paths between clusters of nodes so that high speed connectivity between far away nodes is accomplished leading to fast signal processing. (Watts and Strogatz, 1998; Sporns and Zwi, 2004). Other notable advantages of small world networks include the facilitation of coherent oscillations and synchronization across nodes (Strogatz, 2001; Lago-Fernandez et al., 2000). While small world network properties (characterized by the amount of clustering and the average node length) have been recognized in other real world phenomena such as electric power grids, the neural network of Caenorhabditis elegans and social influence networks, they have also been applied to the vertebrate brain to explain probable connections among areas that are involved in distributed information processing. Computer simulations of networks made of Hodgkin-Huxley neurons incorporating small world properties have been shown to have a fast system response with coherent oscillations across neurons (Lago-Fernandez et al., 2000). Network topologies have been applied to anatomical connections of cortical areas in the macaque monkey and the cat using computational approaches and the cortical networks have been shown to be organized into densely intra-connected clusters of areas, that reflect functional connections (Hilgetag et al., 2000). Functional magnetic resonance imaging studies (Achard et al., 2006; He et al., 2007) have shown
during resting states that cortical areas are connected in a small world fashion with local clustering. The greater intraregional connectivity of the mammalian brain suggests that intraregional synchrony (e.g. among populations of neurons in the motor cortex) will more likely be achieved and perhaps be used more often in information processing than will synchrony among more distantly located neurons. The next two sections elaborate upon the modes by which information may be processed and propagated throughout the central nervous system.

**Action Potentials and Local Field Potentials**

Neurons fire generating action potentials in a mostly binary fashion. Graded action potentials have been long been known to occur in invertebrates (Juusola et al., 1997; Haag and Borst, 1998; Simmons, 1999; Manor et al., 1997), and have more recently been demonstrated in mammals¹. While dendrites from a given cell are known to contact dendrites and cell bodies from other neurons, action potentials (spikes) are thought to be the primary means of communication across neurons, especially across long distances (Laughlin and Sejnowski, 2003). This is thought to arise from constraints on energy usage and neuron sizes affecting transmission speeds. Spikes are high frequency signals in the range of 0.6 - 3 KHz, whereas local field potentials (LFP) are low frequency signals in the range of approximately 0.05 - 500 Hz (Buzsáki and Draguhn, 2004). LFPs are considered to be the average activity reflecting the subthreshold membrane oscillations (Kamondi et al., 1998). Some others (Hasenstaub et al., 2005) explain a certain set of high frequency oscillatory activity as an effect of inhibitory post-synaptic potentials. LFPs are also thought to arise from double current

¹ For review see Alle and Geiger, 2008.
dipole activity of neurons in anatomical layers, for example in the hippocampus (Holsheimer et al., 1982; Buzsáki, 2002; Montgomery et al., 2009). Spikes and LFPs are usually the most commonly recorded in studies about brain activity using extracellular recordings, although middle-range oscillations and other activity are increasingly being recorded and analyzed (Bullock, 1997). While LFPs reflect average activity over a large area, typically a 0.25 - 1 mm sphere around a given electrode tip, the actual information about how individual neurons interact with each other is seen better with spikes recorded from multi-electrode implants, which typically record spiking activity in a ~100 µM sphere around each electrode tip. Nonetheless, it should be kept in mind that LFPs influence spiking in multiple ways. For instance, LFPs have been shown to have causal influences on individual neurons (Weiss and Faber, 2010), at least under certain circumstances, and other studies have shown that LFPs might play a major role in determining spiking timing (Gray et al., 1989; Radman et al., 2007; Buzsáki, 2002; Denker et al., 2010). LFPs have been used to study many psychological processes including perception (Wilke et al., 2006), working memory (Pesaran et al., 2002), and attention (Taylor et al., 2005). However, there remains considerable debate over the relationship between LFP oscillations in specific frequency ranges and specific cognitive processes (e.g. Tallon-Baudry, 2009).

Our current focus, however, centers on how information about an upcoming stimulus, whose time of onset the animal “knows” at some level, might be coded in motor cortical neurons. Our study addresses limitations in many previous reports indicating that motor cortical units indeed encode this information. Moreover, there is a long and rich history of studying how information is coded in the nervous system with
spike recordings, with the motor cortex serving as one of the primary sites for these investigations. Information processing as revealed by relationships among spikes is particularly important where motor encoding and decoding is concerned because of the long distances over which information from the brain’s motor networks, including motor cortical information carried by the corticospinal and rubrospinal tracts, must travel to reach motor neurons and the muscle fibers they innervate. The remainder of this report will therefore focus mainly on information coding in motor cortical spikes.

**Spikes: Rate and Temporal Coding**

It is relatively well-established that LFP oscillations facilitate communication over long distances, for instance between prefrontal and visual cortex during attention (Gregoriou et al., 2009), and across a frontal-occipital-hippocampal network during visual object processing (Sehatpour et al., 2007). Despite considerable investigation (e.g. Mainen and Sejnowski, 1995; Gawne and Richmond, 1993), however, information encoding, communication and decoding using spikes is not very well understood (Gawne and Richmond, 1993; Salinas et al., 2000). There is evidence that spikes encode information using each of two broad classes of coding: rate coding and temporal coding. Rate coding refers to information contained in spike firing rate averaged within temporal binwidths ("bins") of 10-25 ms or even longer – some researchers analyze their data using bins of 100-200 ms (e.g. O'Keefe, 1976). Temporal coding, on the other hand, refers to any type of information encoding involving more precisely timed activity (O'Keefe and Reece, 1993). It includes interspike interval coding, in which information is contained in the timing between individual spikes from a given neuron (Bialek et al., 1991), coding using oscillatory spike activity, coding contained within activity across
multiple neurons, and other types of spike firing synchrony or temporally consistent interspike or interneuronal firing relationships.

There is considerable evidence that many types of information processing in the nervous system involve rate coding (e.g. Shadlen and Newsome, 1998; Kumar et al., 2010). Some researchers even argue that only rate coding exists, or rate coding combined with modulation of levels of noise in the nervous system (e.g. Rolls and Deco, 2010). Rate coding models don't generally include inter-neuronal relationships. But there is evidence that population coding, which is encoding by multiple neurons firing in the temporal and spatial vicinity of each other, encodes more information along with rate coding than rate coding alone as seen in visual systems (van Rossum et al., 2002; Shadlen and Newsome, 1998). In fact Shadlen and Newsome (1998) show that rate coding can be estimated by an ensemble of neurons with just one interspike interval (10 - 50 ms). They argue that individual neurons do not encode information with temporal coding alone, but rather that small populations of neurons (50 - 100) encode and transmit information. Their argument is consistent with the results of several studies showing that despite trial-to-trial variability in individual neurons' responses to stimuli, the same stimuli tend to activate the same neurons, whose number (at least as found in extracellular multi-electrode studies) is roughly in the above range\(^2\) (~20-100 neurons).

Further studies implicate other forms of temporal coding as crucial modes of information encoding and transmission in the nervous system, either alone or in combination. For instance, temporal and rate coding in place cells have been shown to correspond to different aspects of exploratory behavior in rats (speed of movement vs.

\(^2\) For review see Harris, 2005 and Kumar et al., 2010.
the animal’s location in the place field) (Huxter et al., 2003). Moreover, ERP studies in vivo show variation in response over a large area (frontal negativity) to different stimuli as early as 150 ms after stimulus presentation (Thorpe et al., 1996). This seems to suggest that rate coding alone might not be responsible for processing stimuli. That is, because rate coding encodes information over a large time interval, this time interval might not be available online for animals to perform real time processing of sensory stimuli and make decisions.

One form of temporal coding that theoretically requires very little time to encode information is interspike intervals, which have been shown to have more than sufficient information capacity required to encode inputs (Victor and Purpura, 1996). Some studies have provided strong correlative evidence that cortical neurons make use of interspike interval coding. For example, precisely timed multi-neuronal spiking in the neocortex has been shown to faithfully encode incoming information about sensory stimuli (Mainen and Sejnowski, 1995). While the evidence overall is mixed on whether cortical neurons make use of interspike interval coding, this type of coding has been fairly convincingly demonstrated in the mammalian brainstem (Di Lorenzo et al., 2009; Di Lorenzo et al., 2003) with causal manipulations, and in the insect nervous system (Rieke et al., 1991).

Temporal coding includes pairwise and higher-order relationships between firing neurons, including transiently synchronous firing. In the insect nervous system, landmark studies have demonstrated that synchronous spike oscillations are crucial to the decoding of the identity of perceptually similar stimuli by neurons in a downstream brain area (Stopfer et al., 1997; Perez-Orive et al., 2002). Going back to the small world
hypothesis, firing synchrony across neuronal networks might facilitate faithful communication of information between distant brain regions (aided by LFP oscillations). The lag between neurons firing might be zero or non-zero. Uhlhaas et al. (2009) propose that spike synchrony within or across networks might support encoding of information about stimuli in two ways: (1) the modulation of strength of synchronization across multiple neurons and (2) the fineness of temporal precision or proximity between neuronal firing during such synchronization. A long line of studies has supported cortical neurons’ use of these types of spike synchrony, which creates the foundation for our experiments and is reviewed in the next section.

**Spike Synchrony Encoding Stimulus Anticipation, Time-To-Stimulus, or Movement Preparation**

Synchronization in oscillatory activity (Fries et al., 2000; Murthy and Fetz, 1996) and non-oscillatory spike firing (Steinmetz et al., 2000; Super et al., 2003; Oliveira et al., 1997; Konig, 1994) has been studied using cross-correlogram techniques. Transient increases in neuronal synchrony have been found to correlate with a variety of psychological processes. For example, synchrony in oscillatory activity in cortical area V4 increased when the animal attended to behaviorally relevant stimuli (Fries et al., 2000). Another example is that states of attention are accompanied by a general increase in synchronous neural activity (Steinmetz et al., 2000; Ress et al., 2000; Murthy and Fetz, 1996).

Many of these studies have concerned the role of spike synchronization in encoding temporal intervals or stimulus anticipation when the time-to-stimulus is predictable. The general idea that timing might be encoded in neuronal firing has been examined by Brody et al. (2003) in a somatosensory parametric working memory task
and they found that firing rates in the prefrontal cortex were proportional to time during the delay period. Furthermore, Leon and Shadlen (2003) have shown that responses in the posterior parietal cortex signal changes in the animal’s perception of elapsed time, in addition to spatial processing and sensorimotor integration.

Given that some neurons might encode timing information and some others fire differentially when the animal attends to a stimulus, it is possible that stimulus expectation or anticipation might also be encoded. Oliviera et al. (1997) studied this in a direction discrimination task. The monkey was initially presented with a stationary pattern, the trial started after it touched a central bar. A moving white bar pattern appeared at a random time after the trial was initiated with the monkey continuing to touch the central bar. The monkey had to determine the direction and touch the appropriate bar denoting the direction. The authors showed that there was increased neuronal spike synchronization in the macaque visual areas MT and MST before the stimulus (moving white bar pattern) was presented, which appeared to relate to stimulus anticipation before signaling a response. Riehle et al. (1997) attempted to design a task to separate processing related to “purely internal, cognitive” events from that related to specific stimuli or the directions of movement they signaled the monkey to make. In their delayed-response center-out task, different visual stimuli signaled the direction in which the monkey was to move its hand, before the presentation of a reliably timed second visual stimulus, the Response Signal (or “Go” cue). In our task however both Go odors and NoGo odors were Response Signals as well as differential cues like the Preparatory Signals. The authors found that some task-related information such as stimulus identity or movement direction was encoded in motor cortical unit firing rates.
In contrast, neuronal synchrony increased such that some motor cortical spikes fired to within one millisecond of each other in the last 100 ms before Response Signal presentation. Though the authors meant to isolate pure stimulus expectation from other factors, their task design may have confounded stimulus anticipation with the planning of movement direction, and in a later publication using a similar task this group (Riehle et al., 2000) suggested that the increased neuronal synchrony might instead be encoding movement preparation, though their task continued to confound these two factors. A number of other studies have found increased spike synchronization in the motor cortex during the interval of stimulus anticipation and potential movement planning (e.g. Grammont and Riehle, 1999). A general finding of these studies, however, remains that spike firing synchrony increases in its precision as the Response Signal presentation approaches.

**Motivations for Our Experiment**

The last several studies discussed above are intriguing in how they consistently implicated interneuronal spike firing synchrony – and synchrony whose precision increases as the time to the Go signal nears – in the motor cortex. However, there are several limitations to the interpretations one can make from them. One limitation is the fact that the initial visual cue signaled which direction of movement would be performed before the Go signal had been presented. We attempted to completely de-confound stimulus anticipation and movement planning using a different task design such that either of two cues indicating either of two actions would be presented after a fixed delay from the time the animals' snouts crossed an infrared beam (i.e. a location the animals could learn) as they inserted them fully into a nosepoke port. We looked for stimulus anticipation during the nosepoke-to-odor-arrival time period, when the well-trained
animal was unsure at some level which cue would be presented and therefore which movement it would be making. The Go response entailed making a lever press with the contralateral forepaw within 1 second while the NoGo response entailed abstaining from lever pressing for 10 seconds. Note that we use the term “anticipation” loosely here, as we are not making any claims about the animal’s anticipation being conscious. The term “anticipation” is only used as shorthand for what neural processing might be taking place in the animal’s brain when it is waiting for a stimulus.

Another limitation concerns the generalizability of the results. In all the above studies visual stimuli were used, as were macaques. In our study we employed rats in a Go/NoGo task, in which the Go and NoGo cues were olfactory.

A third limitation was the lack of a control period in many of these studies, which could be analyzed to determine if the degree of spike synchronization was specific to the experimental period rather than being a baseline value. We decided to analyze a control period where the animals wouldn’t presumably be expecting or anticipating a stimulus, or preparing for movement, but rather were actually performing a well-learned movement, locomotion. The control period we chose was of the same duration as the experimental (stimulus anticipation) period, and behaviorally it corresponded to the average time during which the animal was locomoting from the response lever to the reward delivery apparatus. We provide further details on the rationale for choosing this control period in the Methods section.

The last motivation for this study was to improve upon the analytical methods used in the prior studies. Many studies used cross-correlogram analyses to obtain spike synchrony values between neurons. One of the disadvantages with this method was
that a large number of successful trials needed to be averaged to obtain significant results. This would add jitter to the analysis because various conditions (e.g. the animal’s state) are unlikely to be identical across trials. There is also evidence that neuronal ensembles form and dissolve very quickly, on the order of a few hundreds of milliseconds or less (Bressler and Kelso, 2001; Thorpe et al., 1996), and the same neurons might not participate in these neuronal ensembles on each trial. This dynamic would not be captured with cross-correlogram analysis, despite the fact that correlated firing might be a critical part of information encoding, because recordings across trials are usually performed from the same set of neurons. Finding significance with cross-correlogram analyses also requires that there be a constant (whether zero or non-zero) time lag between neurons, which would likely not be the case if different neuronal ensembles participated in the information encoding across trials. Finally traditional cross-correlogram analyses only examine pair-wise correlated firing, whereas information is likely to be encoded across much larger groupings of neurons (Welsh and Schwarz, 1999; Shadlen and Newsome, 1998; Thorpe et al., 1996). The result of these limitations is that studies of spike synchronization using cross-correlogram analyses have likely underestimated many types of correlated, information-laden firing patterns that encode a certain behavior.

We therefore did not employ cross-correlogram analyses in this study. For spike synchronization analyses, two more recently developed methods are considered computationally more sophisticated. Riehle et al. (1997) developed a method called Unitary Event (UE) analysis to address some of these limitations with cross-correlogram analyses. This method, explained graphically in Figure 1-1 has the advantages of
maintaining the high, trial-specific temporal resolution while searching for coincidences in spike trains, but only on a pairwise basis. Spike trains are shifted and coincident timestamps across two spike trains are counted towards the synchrony measure. The raw coincidence rates are calculated for the entire spike train for two neurons. Then the neurons are modeled as Poisson processes and the expected coincidence rates for these modeled neurons are calculated. The raw and the expected coincidence rates are transformed into a surprise value and based on a threshold, the coincidences are determined to be significant or not. While there are advantages to this method, one disadvantage is that the method is parametric and hence is only as accurate as the model it uses (stationary Bernoulli process or a Poisson process) (Roy et al., 2000; Pipa et al., 2008). This modeling doesn't take into account the auto structure, which is encountered, in real neuronal firing (Pipa et al., 2008). Another disadvantage is that at low firing rates, the interpretation of changes in spike synchrony is problematic (Roy et al., 2000). Finally, as with traditional cross-correlograms, only pairwise synchrony relationships are examined.

**NeuroXidence**

Pipa et al., (2008) developed a novel, non-parametric technique NeuroXidence for analyzing precise and consistent temporal relations between discharges of simultaneously recorded neurons. Because of this technique’s many improvements over Unitary Event analysis as well as cross-correlograms, we use this technique for determining spike synchrony. Among this technique’s advantages are the ability to characterize synchrony among many more than two neurons, its trial-by-trial analyses (as with UE analysis), its robustness in the face of low and high firing rates and low trial...
numbers, and its maintenance of the spike trains’ autostructure in forming a surrogate dataset for computing statistical significance.

Given an example of spike trains as in Figure 1-2, a joint-spike event (JSE) is defined by the set of neurons that exhibit coordinated firing (synchronous spiking of at least two neurons with millisecond precision, synchrony time window as determined by user-set Tau_C). The amount of jitter corresponds to the size of a kernel, which is applied to all the spike trains as a part of the pre-processing (Figure 1-3). Application of a kernel makes the procedure robust in cases where two neurons fire near-simultaneously but be considered to fire in different bins in a technique such as Unitary Event analysis.

This kernel is multiplied with the spike train and two or more transformed spike trains are compared. The amount of overlap of the kernels determines the JSE as shown in Figure 1-4.

The complexity of the JSE is defined by the total number of neurons that are participating in a synchronous spike pattern. The order of correlation is defined as the number of neurons that are directly coupled and are the primary causes of the coordinated firing. The complexity might include neuronal firing that doesn’t necessary participate in the JSE, i.e. they might be spurious firing that is not from neurons that are directly coupled with each other. The order, on the other hand is more difficult to determine as it reflects the number of neurons that are primarily coupled. Causal manipulations with techniques such as optogenetics would be required to extract the information of what neurons are actually coupled (order) from the set of all neurons that are observed to fire synchronously (complexity).
NeuroXidence was designed to perform robustly given the following facts about neuronal activity, and validated with computational and in vivo studies:

1. There exists a high degree of variability of neuronal firing due to noise, changes in neuronal response properties and the dynamics of the information processing.

2. Neuronal states linked to information processing last only a hundred milliseconds or so (Bressler and Kelso, 2001; Thorpe et al., 1996).

3. The auto-structure of the signal needs to be conserved when significance testing for spike synchrony is performed, or the comparison dataset for testing significance may have the wrong statistical distribution.

4. The same neurons may not be involved in the temporal code at different times and even large-scale recording likely undersample the neurons that participate in the neural code.

In our study we addressed the aforementioned limitations such as the frequent lack of comparison between a stimulus period and a control period, use of confounded behavioral tasks and analytical methods and a focus on only one phylogenetic order (primates). In our experiment with rats, we hypothesized that relative to the control period we would find increased spike synchronization during the movement preparatory period/anticipatory period as well as increasingly precise synchronization during that time. Each hypothesis has two parts. In both hypotheses, the degree of synchronization was evaluated in the experimental period alone, and when compared to synchronization in a control period. Hypothesis 1A) There would be an increase in the degree of synchronization (quantified by the number of synchronous events) in the experimental period as the stimulus approaches. Hypothesis 1B) The increase in synchronization in the experimental period would be higher than the synchronization during the control period. Hypothesis 2A) There would be an increase in the temporal precision of synchronization (quantified by the jitter window) in the experimental period as the stimulus approaches. Hypothesis 2B) The increase in the temporal precision in the
experimental period would be higher than the temporal precision of synchronization during the control period.
Figure 1-2. Spike trains of three neurons. Each box represents a timestamp determined by the sampling frequency, NeuroXidence. Adapted by permission from Pipa et al., 2008. NeuroXidence: reliable and efficient analysis of an excess or deficiency of joint-spike events (Page 67, Figure 2). J Comput Neurosci 25:64-88

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Figure 1-3. Kernel applied to single spike, NeuroXidence. Adapted by permission from Pipa et al., 2008. NeuroXidence: reliable and efficient analysis of an excess or deficiency of joint-spike events (Page 67, Figure 2). J Comput Neurosci 25:64-88

\[
G^{+/−} = (-2, 1)
\]

Replacement of each spike by kernel G

\[\begin{array}{cccccc}
1 & 1 & 1 & 1 & 1 & 1 \\
\end{array}\]

\[\begin{array}{cccccc}
0 & 1 & 1 & 1 & 1 & 0 \\
\end{array}\]
Figure 1-4. Spike trains multiplied by kernel, JSE determined. NeuroXidence, Adapted by permission from Pipa et al., 2008. NeuroXidence: reliable and efficient analysis of an excess or deficiency of joint-spike events (Page 67, Figure 2). J Comput Neurosci 25:64-88
CHAPTER 2
THE EXPERIMENT

Rationale

In our study, rats were trained on a two-odor, Go/NoGo olfactory discrimination task, with a stereotypically produced lever press required for reward on Go trials and abstention from pressing the lever for 10 seconds required for reward on NoGo trials. During task performance, spikes were recorded from many single units in the primary forelimb motor cortex (M1) and the posterior piriform cortex (pPIR). For the present purposes, we analyzed neural data only from the forepaw motor cortex and only from correctly performed Go trials from sessions on which the animals’ Go-trial performance was significantly above chance. The experimental period we analyzed was from the time of infrared beam breaking as the snout entered the noseport until the calculated time of Go odor delivery, a period lasting 600 ms on average. The control period we analyzed was 600 ms during which the animals had touched down from lever pressing and were locomoting to the pellet dispenser. We performed our primary analyses with NeuroXidence, as reviewed in the Introduction and tested Hypotheses 1A, 1B, 2A, 2B as described there. Our first hypothesis was that with NeuroXidence we would find greater synchrony among motor cortical units during the stimulus approach period than the control period. Our second hypothesis was that the temporal precision of this synchrony would increase as the stimulus approached.

Methods

Subjects

Four female Long-Evans rats aged 3-4 months were purchased from Harlan Laboratories and pair-housed until electrode implantation surgery was performed. After
surgery they were singly housed with cage covers to reduce the chance that the electrode headcap would be damaged during fighting or play. Rats were housed in a room with a 12 hour:12 hour light/dark cycle, and the animals were trained and tested exclusively during the rats' nocturnal cycle. All housing, training and testing procedures were approved in advance by the Institutional Animal Care and Use Committee at the University of Florida.

**Apparatus**

Training and testing were conducted in an automated behavioral testing chamber (MedAssociates, St.Albans, VT), which has multiple inputs and outputs. The testing apparatus included a house light, a recessed nose poke, a stimulus light, a lever press and a reward dispenser. The house light (an output device, henceforth referred to as output) was an incandescent bulb that served as an indication of trial starting and ending. The recessed nosepoke housed a light emitting diode (LED) light (output), an InfraRed (IR) beam break detection device (input) and two ports that allowed for odorant mixed with air to flow in and out. The activation, inactivation or other use of these devices (e.g. lever depression to a certain angle) were programmed to send timestamps both to a spreadsheet and to the neural data acquisition file, and were synchronized to within 2 ms of neural data acquisition (when neural data were simultaneously being collected). The LED light was used to signal when the nosepoke port was active. The IR beam break detection device provided timestamps for the rat's nose entry into and exit out of the nosepoke. The two ports were connected to an olfactometer to precisely deliver odorant mixed with air and a vacuum to scavenge it. The lever (an input device, or “input”) was a spring-loaded device which, when depressed with a load of ≥ 25 grams, yielded a timestamp in the behavioral (and during recording sessions, the
neural) data files. The reward dispenser (output) dispensed 45 mg chocolate pellets (Bio-Serv, Frenchtown, NJ) immediately after lever press at the end of each successful trial. All devices were activated by the MedPC® interface package (MED-SYST-8) in turn controlled using MedPC® IV software and the MedState Notation procedures. All devices could be controlled by a Windows general-purpose computer and the timestamps were accurate to a resolution of 2 ms.

The nosepoke was connected to a flow-dilution olfactometer (MedAssociates, St. Albans, VT), which could dispense odorants at different concentrations and rates of flow. All tubing was Teflon®-coated on the inside to minimize residual odor contamination across different trials. The odor-dispensing solenoids were controlled by outputs from the MedPC® interface package. An air pump provided a constant flow of air to the olfactometer where it was desiccated, filtered and rehydrated before being mixed with the odorant and sent to the nosepoke port in the operant chamber.

A consequence of the experimental apparatus setup was that the odorant-air mixture would take a non-zero amount of time to reach the nosepoke port after it had been released from the olfactometer. Because the odorant-air mixture flow dynamics were non-deterministic, we calculated an approximate, average time of odor arrival at the nosepoke port. We estimated the arrival time based on the length (1.3 m) and inner diameter of the tube (4.7 mm) and the pressure of the air pump (2.5 L/min), resulting in an arrival time of approximately 540 ms. After it reached the nosepoke the rat would be exposed to the odorant-air mixture for as long as the odor was released in the olfactometer (500 ms) and then it would be scavenged using a vacuum immediately. The odorants used were strawberry-banana and vanilla scents dissolved in oil. Based
on odor preference tests performed with rodents (Mandairon et al., 2009; Bensafi et al., 2007), pleasant odors were chosen and were oil-based formulations in order to avoid nociceptive activation (i.e. the odorants were non-trigeminal).

The layout of the various components in the operant chamber took either of two configurations depending on which forepaw the rat used for lever pressing. For rats that pressed the lever with their left forepaw, the lever was located immediately to the right of the nosepoke port and the pellet dispenser was located further to the right of the nosepoke port; for right-forepawed rats, both the lever and pellet dispenser were located to the left of the nosepoke port.

**Behavioral Training and Testing**

Rats were screened against anosmia and checked for handedness through training up through the initial stages of the Go/NoGo task, with the criteria that they had to begin to use the same forepaw to press the lever in a stereotyped manner within 1-2 training sessions and had to begin to show a differential response to the Go and NoGo odors within 4-5 sessions/ 400-500 trials after starting training on that task. The handedness determined the hemisphere of electrode implantation. The rats were trained on a Go/NoGo olfactory discrimination task, though for the purposes of this experiment, data from the well-learned Go trials alone where the rat’s Go performance was above chance were processed and analyzed.

Rats were trained on a series of increasingly complex tasks leading to learning of the Go/NoGo task. First the rat was introduced to the operant chamber, being allowed to acclimate to it for about 10 minutes or until the animal had habituated to the features of the new environment as indicated by its ceasing to explore them. After the rat had acclimated to the operant chamber, training was started where the rat learned to
respond to the houselight first. In the first stage, during each trial, the houselight was turned on for 15 seconds. This stimulus was made salient by rewarding the rat at the end of each trial. Approximately 20 trials were performed at this stage. Once the rat was trained to respond to the houselight, the nosepoke action was introduced. The rat was trained to poke its nose in the odor port (nosepoke port) when the odor port light was switched on simultaneously with the houselight, with filtered air being released into the bottom of the noseport and scavenged from the top during nosepokes. A nosepoke of any duration led to a reward being dispensed. Approximately 200 trials were performed at this stage. After this stage, the rat was trained to hold its nose in the odor port for at least \( \geq 300 \) ms, with a reward being dispensed after each successful nosepoke of this duration. After 200 trials of this stage, the rat was trained to press the lever after having performed the nosepoke action for \( \geq 300 \) ms. Once the rat performed a few hundred trials, the Go odor (strawberry-banana scent mixed with air, International Flavors & Fragrances (IFF), Jacksonville, FL) was introduced for 500 ms in all trials immediately after the rat had performed the nosepoke action for 300 ms. The rat was trained for 3 or 4 sessions of approximately 200 trials each until it performed asymptotically at a high rate (~80%-90%) of success.

Then training on the Go/NoGo task commenced (Figure 2-2). The rat was trained to press the lever after being exposed to the Go odor as explained above. The No-Go odor (vanilla scent mixed with air, IFF, Jacksonville, FL) was introduced pseudo randomly on 50% of the trials and the rat had to abstain from lever pressing for 10 seconds on those trials to get a reward. An 8 second intertrial interval (ITI) was used to optimize the rats’ motivation, as indicated by the number of trials of each type obtained.
with this ITI. The rat was trained on this stage until it performed asymptotically at a high success rate of ~80%-90% correct on both trial types (Go and NoGo). We note that we had planned to run twice the number of animals in this study, and were going to use vanilla as the Go cue and strawberry-banana as the No-Go cue for the further rats in the sample.

Electrode implantation surgery (see below) was performed after this stage of training. Following recovery from surgery, animals were retrained on the Go/NoGo tasks up to ~80%-90% correct on Go trials, then combined neural and behavioral recordings commenced. The Go/NoGo task described above (Figure 2-2) was used for these sessions. The Go odor (strawberry banana) and the NoGo odor (vanilla) were presented to the rat in a pseudorandom manner with 50% probability.

Electrode Implantation Surgery

Aseptic survival surgery was performed to implant electrodes for recording action potentials in the primary motor cortex and the posterior piriform cortex. Rats were given Baytril® (5 mg/kg) in their food for three days before and after the surgery to minimize the risk of infection. The diluted solution was added to sweetened, flavored food pellets so that the rats would ingest it. Before implantation, rats were anesthetized in an induction chamber with a 5% isoflurane-oxygen mixture. After initial loss of reflexes and every hour for the duration of surgery rats were injected with atropine sulphate (0.5 mg/kg) to prevent fluid build-up from the anesthetic. The rat was transferred to a nosecone anesthetic delivery system, the isoflurane was lowered to 2.5%, and the rat’s head was secured by means of ear bars, a bite bar and a nosecone clamp. Puralube® ointment was placed over the eyes to ensure that they did not dry out during surgery, and was reapplied as needed during the surgery. The scalp was cleaned three times
with alternating use of iodine and alcohol preps, then the scalp was shaved to expose the skin; any hairs remaining in the scalp area were removed with masking tape. After determining that the rat had no corneal, ear pinch or hindpaw reflexes (the anesthetic concentration was adjusted upward, if needed, until these reflexes were lost), an incision was made in the anterior-posterior direction and the skin was pulled back and held by four hemostats. The anesthetic level during this part of the surgery was increased (as this was the most painful procedure in the entire surgery) and at other times the anesthetic was lowered and maintained to ensure that corneal, ear pinch and hindpaw reflexes were present and the rat was unconscious. The exposed area was cleared of blood and cleaned with three swabs of 70% isopropyl alcohol and iodine by using radial motions to disinfect and minimize potential infection later. With the aid of a surgical microscope, reference points including bregma and lambda were marked using a cauterizer. The stereotactic equipment (Kopf Instruments, Tujunga, CA) was used to level the skull by looking at the respective z-axis coordinates of the bregma and lambda points and adjusting the nosecone screw until the differences between the z values was less than 0.05 mm. During the procedure, the rat’s breathing and general reflexes were monitored and maintained at a level conducive to electrode implantation and electrophysiological verification, with typical breathing rates of 55-60 breaths/minute and isoflurane concentrations of 1 – 1.5%. Teflon®-coated, 50 µm diameter stainless steel electrodes (Neuro Biological Laboratories, Denison, TX) were used and the ends of the electrodes were cut to expose a small area for recording neuronal activity at the desired depth alone and to reduce interference at other depths.
An 8-wire bundle of electrodes was implanted in the rat’s posterior piriform cortex (2.3 mm posterior, 5 mm ipsilateral from bregma) at a depth of 7.8 - 8.1 mm from the surface. An array of electrodes (1x8 or 2x8 wires), with 200 µm spacing between adjacent wires, was implanted in the rat’s caudal primary motor cortex (0 mm anterior, 4 mm contralateral from bregma) in the forelimb mapped area at a depth of 1.5 - 1.6 mm from the surface. Spikes and local field potentials were recorded using a Multi-neuron Acquisition Processor (MAP) recorder (Plexon, Inc., Dallas, TX) as the electrodes were lowered into the brain. The electrodes were lowered slowly at the rate of 100 µm/minute to minimize cell damage (Welsh and Schwarz, 1999). The final depth values were determined by approximate concordance with cortical thickness in that area of the rat brain (Paxinos and Watson 4th ed.), the presence of many channels with high-amplitude spikes on them (as apparent from the oscilloscope and audio recordings) indicative of layer V pyramidal cells, and the presence of complex spiking with a “ch-ch” (crackling) sound and a burst of activity upon touching the forepaw skin (Hermer-Vazquez et al., 2004). The somatosensory response occurs because the rat caudal forelimb M1, unlike some other rat motor cortical sub regions, receives dense layer IV somatosensory afferentation (Hermer-Vazquez et al., 2005). After both electrode implants were in place, the remaining brain tissue exposed by the craniotomies was covered with Gelfoam® (Pfizer, New York, NY) and a headcap fashioned out of dental acrylic (Jet dental acrylic) was constructed to preserve the position of the electrodes and to cover the wound.

Once the dental cement cap had set, the rat was taken off the anesthesia and allowed to wake up. Post-operative care was provided. A topical analgesic, Neosporin®
(Bacitracin) was applied at the headcap-skin junction, to prevent infection. A painkiller Buprenorphine (0.05 mg/kg) was administered intraperitoneal. The bedding was made with paper towels so that the rat wouldn’t get infected by the woodchip bedding. Sugary treats were provided as food until the rat was able to eat standard chow. Baytril® (5 mg/kg) was added to the food. These procedures were performed every 8-12 hours for three days. The headcap was checked daily for bleeding and/or infection and the rat was allowed to recover for a week or until when it was back to behaving normally. The rat was checked to see if it was bright, alert and responsive, regained its presurgical weight, proper eating and grooming behaviors, and if there were any unnatural changes, or any complications from the electrode implantation surgery.

**Neural Recordings**

After rats had been trained on the Go/NoGo task, undergone and recovered from surgery, and received retraining on the Go/NoGo portion of the task, neural recordings during performance of the Go/NoGo task commenced. Action potentials and local field potentials were recorded using a second MAP recorder (Figure 2-3) through a pre-amplifier connected to the headcap connectors and run through a commutator to prevent the wires from the headcap from tangling when the rat moved around in the operant chamber. This neural recorder is capable of recording up to four units on each of 128 channels, along with 16 local field potentials (LFPs). The electrodes we used generally had impedances in the 200-350 kΩ range, and were capable of recording spikes from neurons within ~125 µM of each electrode tip and LFPs from a ~500 µM diameter sphere around each electrode tip. We therefore recorded LFPs from one out of each two channels in both areas, leading to 8 LFPs from M1 and four from the pPIR. The spikes were high-pass filtered at 6 kHz and sampled at 40 KHz, while the local field
potentials were low-pass filtered at 140 Hz and sampled at 1 KHz. Each electrode can record up to four units that produced action potentials, its bandwidth limited by the MAP DSP board, and action potentials from putatively different neurons were sorted using waveform discrimination based on PCA (Principal Components Analysis) and K-means clustering, which were implemented in Sort Client, a part of the MAP’s RASPUTIN server software package (Plexon Inc., Dallas, TX). Once the spikes were sorted, the timestamps of their occurrence were stored and this was the format of the data that was further processed using NeuroXidence (Pipa et al., 2008) as described below.

**Video Recording and Scoring**

The rat’s behavior was recorded using a video camera (CinePlex, 640x480, 30fps) that was synchronized with the neural recording software. A special video card enabled the time from the neural recording clock to be stamped on each frame of the video. Behavioral data was recorded to determine the time intervals in each trial that would be used for synchronization analysis using NeuroXidence. The videos of the Go trials were analyzed to determine whether the trials were valid. A trial was considered valid if the rat held its nose in the nosepoke port long enough to detect the odor and the rat used its contralateral paw to press the lever. The timestamps of events, nosepoke, removal of nose from nosepoke and lever press were coded automatically, whereas the timing of single sniffs of the odor was coded with video analysis. Determination of the post-lever press/pre-reward interval for the control data period was also performed with manual video coding analysis.
**Data Processing**

**Behavioral Data**

Because we were interested in well-learned successful Go trial periods, data from those periods were processed and analyzed. Above-chance Go sessions were detected by looking at ratio of successful trials to total trials and determining whether these ratios were above chance using binomial tests (the default binomial probability was 0.5, because Go and NoGo trials occurred with 50% probability each). Because data was obtained from rats with an above-chance Go trial performance, we focused on Go trials alone, even though one rat (out of three rats that we obtained data from) didn’t perform above chance on the NoGo trials. The time period of interest was chosen to be the time bounded by when the Go odor was released at the olfactometer (which was virtually simultaneous with the rats’ having broken the IR beam) to when the odor would have theoretically reached the odor port and the rat’s nose, as determined by our calculations. During this period, the rat having initiated a nosepoke and having triggered the odor release at the olfactometer was assumed to be anticipating the odor at some level. Trials were excluded if during this interval the rat chewed or made other movements such as using its paw on the nosepoke or removing its nose from the odor port or other forelimb movements that would likely produce mechanical artifacts. All exclusion of trials took place before any neural data analysis.

**Spike Data**

We performed separate NeuroXidence analyses on the Go trials from individual above-chance recording sessions and those trials’ respective control periods. Before discussing the analysis procedure used on each time period (experimental or control), we describe our rationale for choosing the control period we used and how we
determined its starting and ending times. The control time interval was chosen to be of equal length to that of the odor anticipation (experimental) interval; for the discrete analyses, each consisted of 600 ms of spike train data from multiple M1 units, and for the moving window analyses, 700 ms (as explained further below). We selected the post-lever press/pre-reward interval as our control period for the following reasons. First, several studies have shown that motor cortical spike synchrony appears to be reduced during repetitive movements relative to newly learned or skilled movements because repetitive movements are thought to be mediated by downstream structures (e.g. Murthy and Fetz, 1992), though these studies were not performed with such relatively unconfounded methods as NeuroXidence. Nonetheless, we thought that we might observe less motor cortical synchrony during this period than during the odor anticipatory period, and less increase of synchrony over the 500 ms. Second, we expected later to analyze whether olfactory-motor synchrony increased during olfactory stimulus anticipation, and we found no support in the literature for this type of sensorimotor synchrony during locomotion (though there was circumstantial support for somatosensory-motor synchrony). Through manual videorecording analysis, we determined that the period from 0.6 – 1.2 seconds after automatic registration of the lever press was commonly when the rat was ambulating to the pellet dispenser and safely before it grasped and began chewing the reward. Thus we used this time period for the control period data analyses.

For analyses with NeuroXidence, Go trials from recording sessions that were found to be above-chance were temporally aligned to the time when the noseport beam break was registered. For the present purposes, neurons in the primary forelimb motor
cortex alone were considered for the spike synchronization analysis. Spike trains from all primary motor cortex neurons on each trial were interleaved such that temporal patterns of unit firing could be easily determined. Interleaving is the process where all unit spike timestamps are combined in ascending order (moving forward in time) such that each timestamp has the specific neuron ID associated with it. The same procedure of interleaving spike trains as mentioned above, starting from 0.6 seconds after each trial’s lever press, was followed in preprocessing the spike data from the control time period.

The degree of spike synchronization was determined using the program NeuroXidence designed and implemented by Pipa et al. (2008) introduced earlier. The jitter time (Tau_C) for the analyses was set at 5 ms. This jitter time fixed the size of the kernel used to determine if two spikes were close enough to each other, to be considered synchronous. Four sets of analyses were performed on the experimental data interval and the control data interval for each rat. Each interval was chosen to be 600 ms (500 ms + 100 ms) for the discrete window analysis and 700 ms (500 ms + 200 ms) for the moving window analysis. For the experimental “anticipatory” period, this interval time was such that the odor reached the noseport approximately at the end of this time, and for the control period, ambulation, rather than potential skilled movements relating to the end of the lever press or chewing of the reward, was likely occurring. We performed auto-correlational and cross-correlational analyses on the spike data to check for line noise and signal overlap between wires in the experimental and control period. This would account for any spurious synchrony that was a result of measurement artifacts. The first two analyses were highly discrete in that they were
performed by dividing the entire interval into non-overlapping 100 ms bins and then the
degree of spike synchronization was determined in each of these bins (Figure 2-4). For
the first highly discrete (henceforth discrete) analysis, the degree of spike
synchronization was determined with a constant amount of jitter value (set at 5 ms) over
the entire time interval. The window size of 100 ms was chosen based on a tradeoff
between the window size and the number of data points we would obtain about patterns
of change in the experimental or the control period. This analysis helped test our first
hypothesis, that there would be more spike synchrony overall during the experimental
period than during the control period. The second discrete window analysis was
performed with decreasing jitter values over time over these 100 ms bins, with Tau_C
values of 5 ms, then 4 ms, then 3 ms, then 2 ms, then 2 ms and another 2 ms for the
progressive 100 ms windows. This helped us test our second hypothesis that the
precision of spike synchrony would increase among M1 units in the experimental period
relative to the control period.

For the next two analyses we used a moving window technique to determine
finer variations in the degree of spike synchrony, also using them to test the above two
hypotheses (Figure 2-5). For these analyses, we used a window size of 200 ms at the
beginning of the interval and determined spike synchrony. The window size of 200 ms
was chosen so as to capture a larger area across time, which would amplify the
variations in spike patterns. Then this window was moved by 5 ms to the right and the
procedure was repeated across a total of 700 ms of spike train data (each window
overlapped by 195 ms). This would also increase the overlap ratio between windows
such that finer variations in JSEs between windows would be captured. For the first
moving window analyses, as with the first discrete analysis, the jitter time was kept constant at 5 ms. For the second moving window analysis, as with the second discrete analysis, the same moving window technique was used but the jitter time was constantly decreased gradually as the window moved to the right. The jitter time was decreased uniformly across the 200 ms-window analyses that occurred every 5 ms so that the Tau_C value decreased from 5 ms at the beginning of the interval to 2 ms at the end of the interval.

Many results can be obtained and plotted with NeuroXidence, including the total number of joint spike events per trial or across trials (i.e. the apparent number of synchronous events within the specified jitter value, before significance testing), and the significant joint spike events on each trial or across trials (after significance testing). NeuroXidence determined which JSEs are statistically significant by generating surrogate datasets and analyzing/evaluating coordinated firing events with these datasets. These surrogate datasets were generated by temporally shifting the N individual spike trains by random and independent values as a way of preserving the autostructure of the data. Joint spike events were then detected for these surrogate datasets. The null hypothesis was that these coordinated firing events across spike trains in the original dataset happened by chance. By comparing the frequencies of joint spike events in the original and surrogate datasets using a Wilcoxon rank test, NeuroXidence determined if the coordinated firing events were significant or not at the alpha level we chose of 0.05. If there were more joint spike events in the original datasets than in the surrogate datasets significantly, the units were considered to be
coordinated in firing. Note that a significant joint spike event would consist of two or more neurons firing within the chosen synchrony (or jitter) window.

**Interpretation of Results from NeuroXidence**

The results were plotted in the following template format (Figure 2-6). The left column of graphs shows the significant spike pattern results for each analysis on the experimental interval, while the right column of graphs show the same analysis on the control interval. The three graphs show the number of significant JSEs in the top, the total number of JSEs in the middle and the ratio of number of significant JSEs to the total number of JSEs in the bottom. In the experimental period, the reference for all trials is the Go odor release at $t = 1$ second. The reference for all trials in the control period is the lever press at $t = 0.5$ seconds. During the experimental period the rat is holding still in the nose port, while in the control period, the interval of interest is the rat’s movement from the lever press to the pellet dispenser (this occurs approximately from 600 ms to 1200 ms after the lever press).

While two of our rats had high trial numbers, one rat had lower trial numbers and one of the strengths of NeuroXidence is that it is robust with low trial numbers and a wide range of firing rates.

We couldn’t perform group statistics because of the small number of animals and also because the quantities computed from these analyses couldn’t be grouped in a meaningful way. We meant to perform quantitative analyses of significant JSEs across trials but did not have time to extract them for analysis. So, we will show the results for each rat and discuss them and try to draw general conclusions.
Figure 2-1. Overall experiment workflow. After checking for handedness, the rats are trained and electrodes are implanted in M1 and pPIR. Post-surgery retraining is performed and spikes and LFPs are recorded when the rat performs the Go/NoGo task. The spikes from different units are processed using NeuroXidence to determine the amount of synchronization.

Figure 2-2. Go/NoGo task workflow for trials used in this study. The houselight is turned on for 15 seconds total during which the rat has to perform the trial. The rat has to nosepoke for 300 ms. Once the rat holds this position for 300 ms the odor is released for 500 ms. The Go odor is released in 50% of the trials. In case of the Go odor, the rat has to press a lever, if NoGo odor is delivered, the rat should inhibit lever press. In both these situations, the rat gets a reward.
Figure 2-3. Neural recording setup – Connectors on the rat’s head pass LFPs and spikes through the headstages, the preamp into the MAP. The behavior is captured and track by a video camera system. The video feed is synchronized with the MAP recording and both are acquired and recorded by the computer. Reprinted by permission from http://www.plexon.com/product/Multichannel_Acquisition_Processor__MAP__.html

Figure 2-4. Discrete window analysis with constant Tau_C and decreasing Tau_C values in the six intervals spanning 600 ms. The constant Tau_C values are 5 ms, while the decreasing Tau_C values are 5 ms, 4 ms, 3 ms, 2 ms, 2 ms and 2 ms.
Figure 2-5. Moving window analysis with constant Tau_C and decreasing Tau_C values. Each window (200 ms length) is moved by 5 ms. Constant jitter values (Tau_C) are 5 ms, whereas decreasing jitter values are 5 ms, 4.97 ms, 4.94 ms… 2 ms.
Figure 2-6. Comparison of JSEs in the experimental period and the control period. The top row shows the significant JSEs (that are significant across all trials), the middle row the total JSEs (summed across trials within an analysis window) and the bottom row the ratio. Experimental period: odor release was at 1 second and the interval is 1-1.6 seconds (1-1.7 seconds) for the discrete window (moving window) analysis. Control period: lever press was at 500 ms and the interval is 1.1-1.7 seconds (1.1-1.8 seconds) for the discrete window (moving window) analysis.
CHAPTER 3
RESULTS

Out of eight rats that completed training up through the Go/NoGo task and underwent chronic electrode implantation surgery, only three provided usable behavioral and neural data. In general the rats with usable data were run toward the end of the experiment. Earlier on, one rat died during surgery, for instance, and another suffered a stroke two days following surgery. Other rats failed to perform the Go/NoGo task at above-chance levels during recording sessions.

For each animal we chose files for analysis with the first criterion being a high level of performance on Go trials, and the second criterion being as high a NoGo trial performance level as possible. Ideally for this experiment, all rats would have performed both Go and NoGo trials at a high level, so that they could be anticipating a stimulus without knowing yet which movement to prepare. Unfortunately, the first two rats presented below performed both the Go and NoGo trials above chance, as determined by binomial tests, while the last rat performed Go trials above chance but only performed NoGo trials at a marginally significant level. We present the results on an individual rat basis, evaluating our hypotheses relative to each one’s behavioral and neural findings. First we present the behavioral results, followed by qualitative trial-by-trial analyses of total and significant joint spike events (JSEs), finally followed by quantitative, whole-file analyses of significant JSEs, the total number of JSEs, and the ratio of significant to total JSEs. Regarding the whole-file analyses, we present the results pertaining to our Hypothesis 1B – that there would be more significant JSEs (i.e. more spike synchrony) during the stimulus anticipation period as a whole than during the control period – first, followed by results pertaining to the Hypothesis 2B – that the
temporal precision of spike synchrony would increase as the time-to-stimulus approached, and would not increase during the control period.

**Rat 1**

**Behavioral Results**

This rat performed 78 Go odor trials out of which 77 were successful (Figure 3-1; binomial $p < .001$). Out of 79 NoGo trials, the rat performed 74 trials successfully (binomial $p < .001$). This rat's behavioral data therefore allowed us to evaluate whether, if more synchrony or increasing temporal precision was found during the experimental than the control period, it was more likely related to stimulus anticipation (because the animal “knew” the meaning of the Go and NoGo cues but did not know which was coming) versus movement planning.

**Neural Results**

This rat’s M1 implant recorded 17 units that were found not to overlap in their waveform profiles and to have a spike rate of $\geq 1$ spike/s. The results from our NeuroXidence analyses, which were only performed on data from correct trials, are shown below. Spike trains for all units from M1 were grouped for all trials and the JSEs are plotted. The significant JSEs are also plotted. Units found not to participate in any JSEs before significance testing were excluded from further analyses. Figure 3-2 shows trial-by-trial total JSEs (left) and significant JSEs (right) for 600 ms of the experimental period, and Figure 3-3 shows the same two plots for 600 ms of the control period.

Comparing the raster results for the experimental and control periods, there are some commonalities. All 17 units showed JSEs before significance testing during both periods, and similarly small numbers of units showed no significant JSEs. During the experimental period, units 6, 8, 10 and 14 participate in high numbers of significant
JSEs, whereas during the control period, units 10 and 14 participate in high numbers of significant JSEs, although overall, there seems to be higher significant JSEs in the experimental period. However, the significant JSE raster plots show no obvious complexity relationships that would indicate which neurons participated in a true JSE in a given time period and there is no obvious increase in the density of significant JSEs over the course of the experimental period.

We now present the results pertaining to our hypothesis that there would be more synchronous events with a constant Tau_C of 5 ms in the experimental period relative to the control period. Figure 3-4 shows significant JSEs (top), total JSEs (middle) and the ratio of the two (bottom) for the experimental and control periods (left and right, respectively). We note that we present the total JSEs and ratios because the number of significant JSEs is likely a function of the total JSEs, and these numbers sometimes vary by condition or rat.

In Figure 3-4, the total number of JSEs was similar across conditions, with slightly more occurring during the experimental period. However, if anything, there were fewer significant JSEs in the experimental period (N=24) than the control period (N=34). There is a sharp drop to 0 significant JSEs in the 1.3-1.4 second analysis window of the experimental period. This possible aberration creates the appearance that there is a somewhat marked increase in significant JSEs in the experimental period from 1.3 seconds to the end of the analysis window, in contrast to the control condition. This may provide indirect support for Hypothesis 2. The top plots for significant JSEs show only JSEs that were found to be significant across all 77 trials, which is why the numbers are so low despite the relatively high density of significant JSEs in the raster plots. With
such low numbers and with no obvious complexity relationships in the raster results, it is unlikely that further analyses of complexity would extract much more data for interpretation of the results.

The moving window analyses with a constant Tau_C (Figure 3-5), appear to support Hypothesis 1. The total number of JSEs is similar across the two periods, but there is a sharp increase in the number of significant JSEs from the middle to the end of the analysis window for the experimental period only. Because the denominators are similar across the two periods, the ratio plots show this relative increase as well.

With Tau_C decreasing from 5 ms to 2 ms across the analysis window, in both periods the total number of JSEs decreases slightly, whereas the number of significant JSEs actually increases (Figure 3-6). Hypothesis 2 is at best somewhat supported by these results.

As with the moving window results for Hypothesis 1, the moving window results for the decreasing Tau_C analyses appear to support Hypothesis 2. The total number of JSEs decreases slightly for both periods, but only for the experimental period does the number of significant JSEs increase.

**Summary for Rat 1**

Rat 1 performed the Go/NoGo task with high accuracy on both trial types and performed many trials. The discrete neural analyses (with separate 100 ms windows) produced similar results for the odor anticipation and control periods. However, the moving window analyses appeared to show an increase in the total number of JSEs as well as an increase in their temporal precision during the latter half of the odor anticipation window, while they remained relatively constant throughout the control window. The moving window although not the discrete analysis results appeared to
support both our hypotheses in an animal whose behavioral performance allowed us to favor the concept of stimulus anticipation over response preparation during its experimental period.

Rat 2

Behavioral Results

This rat, like Rat 1, performed many trials of both types, although it performed more NoGo trials than Go trials despite the fact that they were presented with 50% probability. Many Go trials had to be omitted after videocoding revealed they were invalid. Also like Rat 1, because this rat performed both Go and NoGo trials at an above-chance level (Figure 3-8, binomial p-values both < .01), it allowed evaluation of the stimulus anticipation vs. movement planning hypothesis. However, Rat 2 was still in the learning phase of the NoGo part of the task, not in the “overtrained” or “highly trained” phase unlike Rat 1.

Neural Results

This rat’s M1 implant recorded 20 units that were found not to overlap in their waveform profiles and to have a spike rate of ≥ 1 spike/s. The results from our NeuroXidence analyses, which were only performed on data from correct trials, are shown below. Spike trains for all units from M1 were grouped for all trials and the total number of JSEs as well as the significant JSEs were plotted. Units found not to participate in any JSEs before significance testing were excluded from further analyses. Figure 3-9 shows trial-by-trial total JSEs (left) and significant JSE(right) for 600 ms of the experimental period and Figure 3-10 shows the same two plots for 600 ms of the control period.
Comparing the raster results for the experimental and control periods, overall, more units appear to participate in higher total JSEs and also significant JSEs in the control period. However, the set of units displaying significant JSEs seems to differ between the control and experimental periods. While all 20 units showed JSEs before significance testing in both periods, in the experimental period units 3, 4, 6 and 17 participated in a large number of JSEs, whereas in the control period units 1, 3, 4, 6, 7, 11, 14, 17 and 19 participated in a large number of significant JSEs. However, no complexity relationships stand out and again there isn’t an obvious increase in significant JSEs over time.

Our Hypothesis 1B was that there would be more synchronous events with a constant Tau_C of 5 ms in the experimental period relative to the control period. Figure 3-11 shows significant JSEs (top), total JSEs (middle), and the ratio of the two (bottom) for the experimental and control periods (left and right respectively).

In Figure 3-11 relating to our first hypothesis the number of significant JSEs was slightly higher in the experimental condition (N=58) than in the control period (N=48); however, the total number of JSEs was also somewhat greater. These results do not support Hypothesis 1 either when considering the experimental period alone or with including the control period.

The moving window analyses with a constant Tau_C do not appear to support Hypothesis 1. Both the total number of JSEs and the number of significant JSEs are higher in the control period than in the experimental period. The ratio seems to have stayed constant over both periods because of the increase in both the numerators and the denominators. Indirectly relevant to Hypothesis 2, with Tau_C constant at 5 ms
there is no obvious increase in the number of synchronous events over the experimental period relative to the control period.

With Tau_C decreasing in both periods from 5 ms to 2 ms across the analysis window, the total number of JSEs appears to be decreasing in both periods. The number of significant JSEs, however, appears to be increasing towards the end of the interval in the experimental period alone. As a result the ratio seems to be increasing towards the end of the interval in the experimental period but not in the control period. This finding appears to support Hypothesis 2.

The decreasing Tau_C moving window analyses show a similar trend to the constant Tau_C moving window analyses. Overall, the total number of JSEs and the number of significant JSEs are higher in the control period than in the experimental period. Furthermore, this analysis revealed that the number of significant JSEs increased over time and as Tau_C decreased during the control period but not the experimental period, even as the total number of JSEs sharply declined in the control period. These moving window results from Rat 2 therefore do not support Hypothesis 2.

**Summary for Rat 2**

Rat 2 performed the Go/NoGo task with high accuracy on the Go trials, with lower but still significant accuracy on the NoGo trials. The discrete neural analysis (with separate 100 ms windows) for constant Tau_C produced similar results for the experimental and control periods. The constant Tau_C moving window analyses showed an overall increase in significant and total JSEs in the control period compared to the experimental period, the opposite of what was predicted in Hypothesis 1. Similarly, the moving window analyses with decreasing Tau_C revealed an increase in temporal precision over time in the control period relative to the experimental period,
again contradicting Hypothesis 2. Only the discrete neural analysis with decreasing Tau_C supported the second hypothesis.

Rat 3

Behavioral Results

This rat performed fewer trials of each type than the first two rats, performing only about a third as many Go trials as Rat 1 and half as many as Rat 2. As with Rats 1 and 2, its accuracy on Go trials was above chance (binomial p-value = 0.0205), whereas its accuracy on NoGo trials only showed a trend of being above chance (Figure 3-15). Because this rat’s NoGO performance was not strictly above chance, it would not allow a clear test of the stimulus anticipation vs. movement preparation hypothesis.

Neural Results

This rat’s M1 implant recorded 29 units that were found not to overlap in their waveform profiles and to have a spike rate of ≥ 1 spike/s. The results from our NeuroXidence analyses, which were only performed on data from correct trials, are shown below. Spike trains for all units from M1 were grouped for all trials and the total number of JSEs as well as the significant JSEs were plotted. Also as before, units found not to participate in any JSEs before significance testing were excluded from further analyses. Figure 3-16 shows trial-by-trial total JSEs (left) and significant JSEs (right) for 600 ms of the experimental period and Figure 3-17 shows the same two plots for 600 ms of the control period.

Comparing the raster results for the experimental and control periods, it appears that for total JSEs but not significant JSEs, all units are participating in both periods. The number of units participating in significant JSEs appears to be greater in the experimental period than in the control period. This could support Hypothesis 1 that
units are more synchronous during the experimental period than in the control period. However, once again there are no notable complexity patterns and the number of significant JSEs does not increase in an overt way during the experimental period.

Considering our first hypothesis that there would be more synchronous events in the experimental period than in the control period, we present results that show the total number of JSEs and the number of significant JSEs for a constant Tau_C of 5 ms. Figure 3-18 shows significant JSEs (top), total JSEs (middle), and the ratio of the two (bottom) for the experimental and control periods (left and right respectively).

In Figure 3-18, relating to our hypothesis, the number of significant JSEs in the experimental period (N = 26) was more than double the number occurring during the control period (N = 11), while the total number of JSEs was similar in both cases. This result, along with those seen in the raster plots, appears to support Hypothesis 1.

The moving window analyses with a constant Tau_C (Figure 3-19) also appear to support Hypothesis 1. While the total number of JSEs is initially higher in the experimental period, it is in the same range as the total number of JSEs in the control period towards the middle and end of the interval. The number of significant JSEs, however, is higher towards the middle and end of the interval in the experimental period compared to the control period. This supports the first hypothesis and further seems to be relevant to the second hypothesis. Similarly, there is an increase in the ratio of significant to total JSEs during the middle and end of the interval in the experimental period relative to the control period.

With Tau_C decreasing in both periods from 5 ms to 2 ms across the analysis window (Figure 3-20), the total number of JSEs appears to be decreasing, not
increasing, in both periods, as is the case for significant JSEs. If anything, these results contradict the second hypothesis.

In the decreasing Tau\_C moving window analyses (Figure 3-21), the number of significant JSEs shows an increase in the beginning of the interval in the experimental period relative to the control period. But they remain at a similarly constant, relatively low number during the middle and the end of the interval across the two periods. The total number of JSEs decrease gradually across the experimental and control periods, and the ratio remains relatively constant across the two periods except for an upward spike right at the end of the control period the experimental period (there are a few peaks). Though these moving window results from Rat 3 do not dramatically contradict Hypothesis 2, they do not support it either.

**Summary for Rat 3**

Rat 3 performed the Go/NoGo task with lower but significant accuracy on the Go trials and non-significant accuracy on the NoGo trials. Regarding Hypothesis 1, the discrete neural analysis (with non-overlapping 100 ms windows) for constant Tau\_C as well as the moving window analyses with constant Tau\_C produced a higher number overall of significant JSEs for the experimental period relative to the control period but with similar total number of total JSEs for both periods. These results supported the first hypothesis. In contrast, the decreasing Tau\_C discrete and moving window analyses showed overall similar results for the experimental and the control period. For this rat, the decreasing Tau\_C results did not provide any clear support of our second hypothesis.
Overall Summary of Results

Table 3-1 encapsulates the results of each analysis for each rat. The penultimate column describes whether the experimental results, if considered alone as in previous studies, would appear to support, be neutral to, or contradict each hypothesis. The last column describes whether the results from the experimental and control periods considered together relate to each hypothesis.
Figure 3-1. Behavioral results for Rat 1. Percent correct, number of trials performed, and binomial p-values for the Go/NoGo task for Rat 1.
Figure 3-2. Raw results from NeuroXidence for Rat 1 over a 600 ms experimental period interval. A) total JSEs. B) significant JSEs. The rat broke the nosepoke port’s IR beam at t = 1 second and the odor was estimated to arrive ~540 ms later. For each unit (denoted by Neuron ID number), the results are shown as a raster plot with the first trial at the bottom and the last trial at the top. Coordinated firing events are indicated by red squares. Statistical significance was determined as described in the methods section with alpha = 0.05.
Figure 3-3. Raw results from NeuroXidence for Rat 1 over a 600 ms control period interval. A) total JSEs. B) significant JSEs. The lever press was at \( t = 0.5 \) seconds in the graph (with locomotion beginning on average at 1.1 seconds). Coordinated firing events are indicated by red squares. Statistical significance is determined as described in the methods section with alpha = 0.05.
Figure 3-4. Discrete window analysis of Rat 1 data with constant Tau_C. A) experimental period. B) control period. Interval length = 100 ms. Significant JSEs plotted in row 1. Total JSEs plotted in row 2. Ratio of sig. JSEs to total JSEs in row 3. N = 77 trials. #M1 units = 14, Tau_C (jitter) = 5 ms.
Figure 3-5. Moving window analysis of Rat 1 data with constant Tau_C. A) experimental period. B) control period. Interval length = 200 ms. Window moved by 5 ms. Significant JSEs plotted in row 1. Total JSEs plotted in row 2. Ratio of sig. JSEs to total JSEs in row 3. N=77 trials, #M1 units =14, Tau_C (jitter) = 5 ms.
Figure 3-6. Discrete window analysis of Rat 1 data with decreasing Tau_C. A) experimental period. B) control period. Interval length = 100 ms. Significant JSEs plotted in row 1. Total JSEs plotted with row 2. Ratio of sig. JSEs to total JSEs in row 3. Tau_C (jitter) = 5 ms, 4 ms, 3 ms, 2 ms, 2 ms, 2 ms over the six time intervals.
Figure 3-7. Moving window analysis of Rat 1 data with decreasing Tau_C. A) experimental period. B) control period. Interval length = 200 ms. Window moved by 5 ms. Significant JSEs plotted in row 1. Total JSEs plotted in row 2. Ratio of sig. JSEs to total JSEs in row 3. Tau_C (jitter) = 5 ms, 4.97 ms, 4.94 ms… 2 ms over 100 moving windows.
Figure 3-8. Behavioral results for Rat 2. Percent correct, number of trials performed, and binomial p-values for the Go/NoGo task for Rat 2.
Figure 3-9. Raw results from NeuroXidence for Rat 2 over a 600 ms experimental period interval. A) total JSEs. B) significant JSEs The rat broke the nosepoke port's IR beam at $t = 1$ second and the odor was estimated to arrive $\approx 540$ ms later. For each unit (denoted by Neuron ID number), the results are shown as a raster plot with the first trial at the bottom and the last trial at the top. Coordinated firing events are indicated by red squares. Statistical significance was determined as described in the methods section with alpha = 0.05.
Figure 3-10. Raw results from NeuroXidence for Rat 2 over a 600 ms control period interval. A) total JSEs. B) significant JSEs. The lever press was at t = 0.5 seconds in the graph (with locomotion beginning on average at 1.1 seconds). Coordinated firing events are indicated by red squares. Statistical significance is determined as described in the methods section with alpha = 0.05.
Figure 3-11. Discrete window analysis of Rat 2 data with constant \( \text{Tau}_C \). A) experimental period. B) control period. Interval length = 100 ms. Significant JSEs plotted in row 1. Total JSEs plotted in row 2. Ratio of sig. JSEs to total JSEs in row 3. \( N = 63 \) trials. #M1 units = 20, \( \text{Tau}_C \) (jitter) = 5 ms.
Figure 3-12. Moving window analysis of Rat 2 data with constant Tau_C. A) experimental period. B) control period. Interval length = 200 ms. Window moved by 5 ms. Significant JSEs plotted in row 1. Total JSEs plotted in row 2. Ratio of sig. JSEs to total JSEs in row 3. N=63 trials, #M1 units =20, Tau_C (jitter) = 5 ms.
Figure 3-13. Discrete window analysis of Rat 2 data with decreasing Tau_c discrete window. A) experimental period. B) control period. 
Interval length = 100 ms. Significant JSEs plotted in row 1. Total JSEs plotted with row 2. Ratio of sig. JSEs to total JSEs in row 3. Tau_C (jitter) = 5 ms, 4 ms, 3 ms, 2 ms, 2 ms, 2 ms over the six time intervals.
Figure 3-14. Moving window analysis of Rat 2 data with decreasing Tau_C. A) experimental period. B) control period. Interval length = 200 ms. Window moved by 5 ms. Significant JSEs plotted in row 1. Total JSEs plotted in row 2. Ratio of sig. JSEs to total JSEs in row 3. Tau_C (jitter) = 5 ms, 4.97 ms, 4.94 ms... 2 ms over 100 moving windows.
Figure 3-15. Behavioral results for Rat 3. Percent correct, number of trials performed, and binomial p-values for the Go/NoGo task for Rat 3.
Figure 3-16. Raw results from NeuroXidence for Rat 3 over a 600 ms experimental period interval. A) total JSEs. B) significant JSEs. The rat broke the nosepoke port’s IR beam at $t = 1$ second and the odor was estimated to arrive $\sim 540$ ms later. For each unit (denoted by Neuron ID number), the results are shown as a raster plot with the first trial at the bottom and the last trial at the top. Coordinated firing events are indicated by red squares. Statistical significance was determined as described in the methods section with alpha = 0.05.
Figure 3-17. Raw results from NeuroXidence for Rat 3 over a 600 ms control period interval. A) total JSEs. B) significant JSEs. The lever press was at t =0.5 seconds in the graph (with locomotion beginning on average at 1.1 seconds). Coordinated firing events are indicated by red squares. Statistical significance is determined as described in the methods section with alpha = 0.05.
Figure 3-18. Discrete window analysis of Rat 3 data with constant $\text{Tau}_C$. A) experimental period. B) control period. Interval length = 100 ms. Significant JSEs plotted in row 1. Total JSEs plotted in row 2. Ratio of sig. JSEs to total JSEs in row 3. $N = 22$ trials. #M1 units = 16, $\text{Tau}_C$ (jitter) = 5 ms.
Figure 3-19. Moving window analysis of Rat 3 data with constant $\text{Tau}_C$. A) experimental period. B) control period. Interval length = 100 ms. Significant JSEs plotted in row 1. Total JSEs plotted in row 2. Ratio of sig. JSEs to total JSEs in row 3. $N = 22$ trials. #M1 units = 16, $\text{Tau}_C$ (jitter) = 5 ms.
Figure 3-20. Discrete window analysis of Rat 3 data with decreasing $\tau_C$. A) experimental period. B) control period. Interval length = 100 ms. Significant JSEs plotted in row 1. Total JSEs plotted with row 2. Ratio of sig. JSEs to total JSEs in row 3. $\tau_C$ (jitter) = 5 ms, 4 ms, 3 ms, 2 ms, 2 ms, 2 ms over the six time intervals.
Figure 3-21. Moving window analysis of Rat 3 data with decreasing Tau_C. A) experimental period. B) control period. Interval length = 200 ms. Window moved by 5 ms. Significant JSEs plotted in row 1. Total JSEs plotted in row 2. Ratio of sig. JSEs to total JSEs in row 3. Tau_C (jitter) = 5 ms, 4.97 ms, 4.94 ms… 2 ms over 100 moving windows.
Table 3-1. Summary of results of three rats and whether they support or contradict the hypotheses or if they are inconclusive

<table>
<thead>
<tr>
<th>Hypothesis 1</th>
<th>Hypothesis 2</th>
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<tbody>
<tr>
<td>Experimental period alone</td>
<td>Experimental and control periods</td>
</tr>
<tr>
<td>Discrete analysis inconclusive, moving window supports the hypothesis</td>
<td>Discrete results inconclusive or they contradict the hypothesis, Moving window results support the hypothesis</td>
</tr>
<tr>
<td>Discrete results inconclusive or do not support; moving window inconclusive</td>
<td>Discrete results inconclusive or do not support hypothesis, Moving window results contradict the hypothesis</td>
</tr>
<tr>
<td>Discrete results inconclusive or support; moving window results support</td>
<td>Discrete results more clearly support hypothesis; moving window results support</td>
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<tr>
<td>Rat 1</td>
<td>Rat 2</td>
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Discrete and moving window analyses appear to support the hypothesis

Discrete results support the hypothesis, Moving window results contradict the hypothesis

Both discrete and moving window results inconclusive or contradict the hypothesis
CHAPTER 4
DISCUSSION

Our first hypothesis was that in the experimental period alone, the number of significant JSEs would increase as the time of odor release approached, and that relative to the control period, there would be more significant JSEs overall and more of an increase over time. Our second hypothesis was that both in the experimental period alone and in that period compared to the control period, the temporal precision of significant JSEs would increase over time. We evaluated each hypothesis using two types of NeuroXidence analyses, one employing discrete windows and the other employing moving windows slid in 5 ms increments across the experimental and control periods. Unfortunately, when viewed across rats and analysis types, none of our hypotheses were supported in a clear manner. This was the case when we looked primarily at the graphs for significant JSEs, which was arguably the simplest measure we could have used for judgment. It was also the case when we considered the ratio graphs, which took into account factors such as a declining number of total JSEs across an analysis window combined with a flat graph of significant JSEs, which would lead to an increase over time in the ratio values.

It is possible that some of this variation occurred because not all three rats performed the behavioral task at the high training level of monkeys in similar studies whose results led to our current hypotheses, particularly for the experimental period when considered alone. The first rat was the only rat to perform Go and NoGo trials at a very high level, as with Riehle et al.’s monkeys in their 1997 report. (In their subsequent papers this research group has not always reported their animals' behavior performance levels. However, with chance performance of 50% or less, the monkeys in Riehle et al.
(1997) and Kilavik et al. (2009) all performed at >80% correct, so we assume animals in their other studies performed a high level as well.). In the moving window analyses for this rat, Hypotheses 1 and 2 were supported when considering the experimental period considered alone and when evaluating it in light of the control results. The second rat performed Go trials at a similarly high level, though while it performed NoGo trials significantly above chance, it did not perform them at its pre-operative, near-perfect level. For this rat neither analysis type supported Hypothesis 1 strongly, though both the discrete and moving window results with decreasing Tau_C provided some support for Hypothesis 2, at least for the experimental period alone. The results thus appeared to be less clear for Rat 2 than Rat 1; possibly varying as a function of how highly trained they were on each trial type. The results for Rat 3, however, do not fit into this scheme well. It performed at above-chance but not high levels on Go trials, and only showed a trend toward remembering how to perform the NoGo trials. One might expect, therefore, that its results would be the least conclusive, but in fact both analysis types supported both parts of Hypothesis 1. Hypothesis 2, however, was not supported by the results of either analysis type. Thus, there was some support for both hypotheses in the most behaviorally competent rat, Rat 1, and support for the first hypothesis in the least behaviorally competent rat, Rat 3, but not any clear support for the hypotheses in Rat 2. For several reasons, we think that the 200 ms, moving window analyses are likely to be better than the 100 ms discrete analyses. First, a 100 ms window is considered the lower limit for robust results with NeuroXidence. Second, a 200 ms window would cover more data for detecting and determining significant JSEs and would result in a better estimate of the significance. Third, moving the 200 ms window slowly by 5 ms after
each iteration would guarantee that abrupt changes in degree of synchrony would go undiscovered. But even looking at the moving window results alone, they were inconsistent across rats and neither hypothesis was clearly supported. These findings also underscore that our set of results cannot easily be reconciled by differences between our two analysis types, or by our inclusion, unlike most previous studies of this type, of a control period. We discuss what could be the possible reasons for not seeing a clear agreement with the hypotheses below.

The analyses were performed with constant Tau_C and decreasing Tau_C to determine if there was increasing synchrony and increasing temporal precision. While the constant Tau_C analysis is a clear test of whether there is increasing synchrony by means of checking for increased number of JSEs, it doesn't say anything about the possibly transient nature of the ensembles that produce the JSEs. The monotonic increase hypothesis, which wasn't supported in our raster plots or our across-trial significant JSE graphs, was based on work by Riehle et al., in their published reports with monkeys, along with their presenting examples of pairs of neurons displaying such an increase as the time of the stimulus to execute movement approached. We formulated our hypotheses based on the information presented in their graphs and the text. Though their work seemed to indicate a monotonic increase in synchrony, their analyses except those in Kilavik et al. (2009) do not appear to have been performed comprehensively over the entire set of neurons, in contrast to ours. It is therefore possible that our results show a different perspective.

This notion is further supported by the fact that they do present a few graphs showing that certain pairs of neurons reach their synchrony peak not at the end of the
anticipation interval, but rather at different times in the interval. These graphs indicated peak levels of UEs that in fact did not bear any clear relationship to the timing of the upcoming response stimulus or any other task variables. Given these instances and the fact that they didn’t present statistics for increasing UEs across all pairs or larger groups of neurons, it is possible that during their anticipation or movement-planning window, different subgroups of neurons increased their synchronous spiking at different times. It is hard to assign a task-related or other functional interpretation to that finding. With such low significant JSEs and no obvious clustering in the raster plots, it is unlikely that further analyses evaluating unit subgroups would have supported our results. A main reason for the low number of significant JSEs in Pipa et al. (2008) is that NeuroXidence only counts JSEs that are significant across all trials, which seems to be an overly severe standard. Given the amount of noise in the nervous system, it is probably too strict to expect consistent neuronal coupling across all trials. Even though we can determine trends in overall synchrony over smaller windows using the current results, the problem of determining synchrony variations in subgroups of neurons (that we recorded from) requires expanding the JSE results from NeuroXidence into clusters. But it is possible that if the rasters had instead been plotted for the 200 ms moving window analyses, and if the statistical significance criterion were relaxed to not require significance in all trials, such subgroups would have been detected and could have been further analyzed.

On the other hand, if the monotonic synchrony increase were true, our failure to replicate them may have been caused by the lower number of neurons used in our analyses. They typically recorded from > 200 units across two monkeys’ M1 whereas
we recorded from approximately 65 across our three rats. It is possible that despite using one more animal than their and Pipa’s groups typically did, we undersampled the needed diversity of neuron activity patterns.

Our decreasing Tau_C analyses were intended to combine increased temporal precision with traversing the interval. We decided to look at the synchrony as the Tau_C decreases. The assumptions were that neurons would participate in JSEs with increased precision and that the number of JSEs with increased temporal precision would increase as the interval progressed. A better analysis would be to look at a matrix of values of synchrony across varying Tau_C and the time across the interval. This would provide some insight into how synchrony and temporal precision varies across the interval. Also, this would be helpful in teasing apart shorter ensembles that participate in synchrony.

Nevertheless based on the current analysis, it is possible to look at the last three windows of the discrete window analyses with decreasing Tau_C where the value was constant at 2 ms. Similarly in the moving window analyses with decreasing Tau_C the Tau_C value varies slowly compared to the window traversal across the interval and looking at the latter half of the interval could give a better idea about whether more units engage in JSEs. Rats 1 and 2 show increasing number of significant JSEs and the ratio in the last three discrete windows with decreasing Tau_C in the experimental period and also when compared to the control period. Rat 3 doesn’t show increasing temporal precision in the discrete window decreasing Tau_C analysis. Given the above-chance performance of Rats 1 and 2 on both Go and NoGo trials, these results support the idea that more neurons engage in increased temporally precise synchrony. In the decreasing
Tau_C moving window analyses, the rate of decreasing Tau_C is low compared to the window traversal rate. This means that Tau_C changes by only 0.03 ms steps as each window is moved by 5 ms. If transient synchrony was expected to occur over a shorter interval, it would reflect in an increase in synchrony followed by a decrease within a shorter interval than in the current analysis. We could look for temporally local increases in synchrony over a relatively short interval (about 365 ms (200 +33*5)) as Tau_C changes from 5 ms to 4 ms. If there is an increase in synchrony, it would mean that more units are engaging in JSE of a certain Tau_C. The comparison could be performed over small variations of Tau_C (~0.5 -1 ms) to determine if temporal precision of synchrony increases. This is based on the assumption that prior studies by Riehle et al. did show an increase in temporal precision towards the end of the interval.

One important difference between our experiment and Riehle et al.’s experiments is that the onset of the response signal is fixed in time. For instance in Riehle et al.’s 2000 study, until the first time point the animal is anticipating the response signal and the signal is presented at 600 ms or 1200 ms with equal probability. Once the first time point had passed (600 ms) and the response signal was not presented, the response signal had a 1.0 probability of being presented at 1200 ms. While the animal is not expected to resolve conditional probabilities, it remains that the amount of jitter in this experiment was very controlled in that the response signal occurs at only two exact time instants. In contrast, the arrival of the odor plume in our experiment was dependent on many factors such as the air pressure provided by the air pump, the dissipating ability of the odor plume that arrives at the nose port etc. Though we tried to minimize variability in odor arrival time with precise instrumental control over these factors, there was still
likely considerable jitter in the time instant the odor plume was first available to the rat. Rats well trained to discriminate odors can do so with only one sniff of ~150 ms (Uchida and Mainen, 2003). In defense of our instrumentation, in our video analyses of sniffing we too found that rats often took only one sniff after the calculated odor arrival time before leaving the nosepoke port to make a response. It remains true, however, that it was less clear in our behavioral task how soon rats recognized the odor and how synchrony might have played a role in the recognition process indirectly affecting movement preparation. This was one of the motivations for positing a monotonic increase in temporal precision during the interval, i.e. because the rats presumably recognized at some level that the probability of odor arrival was increasing over at least the first 500 ms of the analysis window. In a few cases we saw a drop in significant JSEs at the end of the experimental period and it is possible that that was because the odor had often arrived by that point and the animals had begun moving.

The auto-correlogram and more specifically the cross-correlogram analyses of units we performed on the experimental and control periods to check for line noise also shed light on a strange finding in Rat 2’s NeuroXidence results. One thing that stood out is that Rat 2 has more total JSEs and synchronous JSEs in the control period in both moving analyses with constant Tau_C and decreasing Tau_C. Similarly, this was observed in the cross correlation analyses with more peaks around zero in the control period than in the experimental period. This was surprising given that prior studies (Murthy and Fetz, 1992; Riehle et al., 1997; Riehle et al., 2000) had pointed to a decrease in synchrony when the rat engaged in movements, especially also in stereotyped movements. This led us to believe that the control period could be defined
better and recoded for Rat 2 and possibly for the other rats too. We tried to include only
the time the rat had touched down from lever pressing and was locomoting toward the
pellet dispenser. However, I only had time to check that the rat hadn’t obtained the
pellet early and begun chewing in about 20% of Rat 1’s and Rat 2’s Go trials. It is
therefore possible that some artifactual “significant JSEs” arose in the control period
especially for Rat 2. If I had had more time I would have checked the videos more
closely for the control intervals, as I did with all experimental intervals.

A related possibility for why our experimental period did not have clearly more
significant JSEs across the three rats is that in the control period more neurons
engaged in synchrony at the unit level though not at the LFP level, during movement
than previously appreciated. In fact, in Rat 1 we initially tried using as a control period
the time 500 ms earlier to the registration of a lever press, and again found more
significant JSEs in the control relative to the experimental period. Also, some of the
example unit pairs shown by Riehle et al. actually do show high synchronization during
movement performance (although, their verbal account implies that synchrony during
movement reliably decreased). There have been recent reports of gamma oscillations in
LFPs recorded in M1 during movement (Szurhaj et al., 2005; Muthukumaraswamy,
2010), and LFPs are thought partly to reflect synchronous unit activity. So I believe it is
not out of the question that we found synchronous activity related to movement, despite
Riehle et al.’s claims that units desynchronize on movement in their 1997 and 2000
papers. However, this interpretation would run counter to what is currently believed in
field of neuroscience, and our results don’t clearly show more synchronization among all
rats during movement than stimulus anticipation. Rat 2’s control results could then be
accounted for parsimoniously by the accidental inclusion of some trials with mechanical artifacts.

Finally, the most parsimonious interpretation of why we didn’t find consistent increasing synchrony results as the olfactory stimulus approached is that the experiment conducted by Riehle et al. really examined M1 units during movement preparation rather than stimulus anticipation alone. The monkeys already knew what movement they would be performing during the analyzed period (the direction to move was already known, the monkeys waited until the response stimulus was presented) where the group reported increases in overall synchrony and its temporal precision. Our task was more likely comparable to the start- to- preparatory signal period of their task, Figure 1-1, in which the animal held a fixed position with its contralateral forelimb (in our task, there was no forepaw movement during the odor release – to – odor arrival period). During that time, the animals were anticipating a stimulus, but did not know yet which movement the stimulus would indicate. In fact, as their graphs show (Figure 1-1), the joint surprise values for the -250 ms to preparatory signal are mainly significantly below chance. Perhaps, including more rats with similar above-chance behavioral performances on Go and NoGo trial types would have demonstrated that the type of synchrony we assessed was more likely related to movement planning.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Sridhar Srinivasan received his Bachelor of Engineering in Electronics and Communication Engineering from Government College of Technology, Coimbatore, India in the spring of 2004. His undergraduate thesis was in object motion detection in a series of images. He continued in a similar line of research in the laboratory of Dr. Clint Slatton in the adaptive signal processing area of the Department of Electrical and Computer Engineering at the University of Florida. After receiving his Master of Science in Electrical and Computer Engineering in the summer of 2006, he worked on a gait analysis program suite in the Brain Rehabilitation Research Center at the Veterans Affairs hospital in Gainesville, Florida. In the fall of 2007 he was accepted into the laboratory of Dr. Linda Hermer-Vazquez in the behavioral neuroscience area of the Department of Psychology at the University of Florida to study electrophysiological measures of decision-making in rodents. This project led to the research for his thesis and he received his Master of Science in Psychology from the University of Florida in the spring of 2011.