Taylor Redmond

The role of state-dependent oscillators within the olfactory tubercle during olfactory discrimination

Drew University

Abstract:

The olfactory tubercle (OT) remains an understudied olfactory region which is vastly different from olfactory regions such as the main olfactory bulb (MOB) and the piriform cortex (PC). Regions such as the OT can be investigated by analyzing populations of neurons using local field potentials (LFPs). Two state-dependent oscillators, beta (15-35 Hz) and gamma (40-120 Hz) have been associated with roles in olfactory discrimination and can be used to study how the OT processes olfactory information. While gamma is well-understood, beta oscillations are not, although it is predicted that beta assists in processing tasks associated with higher cognitive difficult. LFPs were recorded from rats (n = 2) to investigate the role beta and gamma oscillations have in olfactory discrimination within the OT. It is found that beta oscillations occur later in the OT than the MOB, beta oscillations may be associated with odor valence/odor identity, and that beta increases in power within a more difficult cognitive task than an easy cognitive task. Due to noise, results for gamma oscillations were more inconclusive, but odor valence did not seem to have an effect on gamma power, while an easier cognitive task was associated with an increase in gamma power. Beta and gamma oscillators may therefore perform different roles in encoding the difficulty of the task performed. Limitations within the experiment may be affected the results; noise present within the recordings, possible errors in noise removal, and the absence of histology suggest that these relationships should be further investigated.

Table of Contents:

1. Introduction (pgs. 1 - 16)

2. Materials and Methods (pgs. 17 - 19)

3. Results (pgs. 19 - 34)

4. Discussion (pgs. 34 - 40)

5. References (pgs. 41 - 43)

Introduction

The processing of olfactory information begins in the nasal cavity, where bipolar olfactory receptor neurons (ORNs) and nasal epithelium form the main olfactory epithelium (MOE) (Firestein 2001; Franks and Isaacson 2006; Kaupp 2010). Within the MOE of mammals, the dendrites of the ORNs form dendritic knobs which grow cilia into the mucus of the nasal cavity. The cilia contain receptors which detect odor molecules. When an odor molecule makes contact with the mucus of the neuroepithelium, it is carried to its receptor where it binds and a second messenger cascade induces the depolarization of the neuron (Firestein 2001; Kaupp 2010). The cascade begins when odor-bound receptors undergo a conformational change to activate an olfactory-specific G-protein, G_{olf} (Kaupp 2010). Adenylyl cyclase, activated in turn by G_{olf}, converts adenosine triphosphate (ATP) into cyclic AMP (cAMP). Within the ORNs, cAMP binds to cyclic-nucleotide-gated channels (CNGCs), which causes an influx of extracellular sodium and calcium ions. Chloride channels are then activated, releasing chloride ions within the cell (Kaupp 2010). If enough receptors are activated, the cell depolarizes from -65mV to its threshold potential (-20mV), and generates an action potential. This pathway of signaling is beneficial for the depolarization of the ORNs, as an odorant can cause a large depolarization easily due to the amplification effect of a second messenger cascade: one receptor can activate multiple cAMP molecules, opening many CNGCs and calcium channels.

All ORNs within the MOE synapse with second-order neurons located within a primary olfactory structure, the main olfactory bulb (MOB) (Kaupp 2010). The action

potentials which are generated in the ORNs are transported through their axons to the first layer within the MOB, the glomerular layer (Imamura and Greer 2009; Nagayama, Homma, and Imamura 2014). In this layer of the MOB, the axons of ORNS and dendrites of neurons within the MOB converge into a glomerulus, the initial site for olfactory information processing (Franks and Isaacson 2006; Imamura and Greer 2009). The neurons within the MOB, mitral/tufted cells (M/T cells), have apical dendrites which extends downward into the glomeruli, while lateral dendrites extend in a horizontal plane within the external plexiform layer (EPL) of the MOB, allowing reciprocal connections across M/T cells (Imamura and Greer 2009; Nagayama et al. 2004). Due to the network of glomeruli and M/T cells, each odorant activates action potentials in its own spatial and temporal pattern; the number of cells activated, the group of cells activated, and the firing rate of the cells are different based on the odorant received (Sosulski et al. 2011; Stettler and Axel 2009).

Data has suggested that mitral and tufted cells decode different aspects of the chemical features of olfactory stimuli, such as the chemical structure or purity of an olfactory compound (Giessel and Data 2014; Nagayama et al. 2004). Tufted cells, for instance, respond to more odorants than mitral cells (Nagayama et al. 2004). The primary role of M/T cells, however, is to act as projection cells to olfactory regions. The axons of M/T cells come together to form the lateral olfactory tract through which olfactory information is sent to primary olfactory regions which form the primary olfactory cortex (Nagayama, Homma, and Imamura 2014; Giessel and Data 2014).

The primary olfactory cortex spans multiple brain regions within the forebrain, including the piriform cortex (PC), the olfactory tubercle (OT), anterior olfactory nucleus, tenia tecta, lateral entorhinal cortex, and cortical amygdala (Giessel and Data 2014; Wilson and Sullivan 2011). These structures have feedback projections to the MOB in order to modify incoming olfactory information. Each of these regions except the lateral entorhinal cortex has three layers, performing different roles in olfactory processing. Layer one contains a plexiform layer which contains the M/T axons from the lateral olfactory tract (LOT) (Nagayama, Homma, and Imamura 2014). As part of the LOT, mitral cells project to all regions of the olfactory cortex, while tufted cells project to the anterior olfactory nucleus, parts of the piriform cortex, and the OT (Imamura and Greer 2009; Nagayama et al. 2004). Layers two and three of these regions, primarily consisting of pyramidal cells and interneurons, receive olfactory information for the first layer for further processing.

The piriform cortex, the largest component of the primary olfactory cortex, is used as a model for other regions involved in olfaction. Although past studies reported that the axons of the LOT enter only the first layer of the PC, imaging studies have shown that the branches of the LOT enter the PC and project diffusely throughout the region, with axonal boutons located along the tract (Wilson and Sullivan 2011; Sosulki et al. 2011). While glomeruli within the MOB are spatially segregated into groups, dependent on their preferred odorant, the same topography is not found within the PC (Poo and Isaacson 2011). Ensembles of semilunar and spinal pyramidal neurons within the PC are activated by olfactory stimuli, but they are not clustered together (Poo and Isaacson

2009). Instead, these cells receive innervation from intracortical connections. The PC uses these connections to form association networks, which assists the PC in encoding olfactory information despite its spatially segregated topography (Haberly and Price 1978; Poo and Isaacson 2009). Inhibitory signals within this network also prevent the PC from becoming over stimulated due to the large amount of activity (Poo and Isaacson 2011; Suzuki and Bekkers 2010).

The glutamatergic spiny neurons and semilunar neurons within these networks have been studied to understand their responsiveness to odorants. Electrophysiological and histological studies have shown that neurons within the PC can be responsive to multiple odorants; studies showed that the majority of neurons within the PC were tuned to most presented odorants – referred to as broadly tuned cells - while a smaller amount of cells were more narrowly tuned (Giessel and Data 2014; Zhan and Luo 2010). Other studies have shown that mixtures of odorants elicit different groups of neurons than monomolecular or un-mixed odorants (Giessel and Data 2014).

Studies of the PC give insight into the morphology and functions of the olfactory cortex. The regions within the cortex are adept at processing individual components of stimuli with ensembles of cells, while exhibiting different ranges of tuning. Although the OT is part of the olfactory cortex, studies have shown that the morphology and circuitry of the OT is very different from that of the PC and other olfactory regions. This implies that their functions may be different in the perception of olfaction.

The olfactory tubercle (OT) is located in the posterior-ventral side of the cerebrum; in olfactory-driven animals such as rats, it is a relatively large region which

resides under the PC (Carriero et al. 2009). It has a trilaminar structure, divided into a molecular layer, a dense cell layer (DCL), and a multiform layer (Carlson, Dillione, and Wesson 2014; Giessel and Data 2014). The molecular layer receives input from the LOT and regions such as the PC, similar to how the first layers of the MOB and PC received the most input from their respective precursors (Carriero et al. 2009). However, histological research has shown that this trilaminar structure is only present within the anterior portion of the OT. Within its ventral-dorsal locations, the dense cell layer undulates, forming gyri and sulci (Giessel and Data 2014; Wesson and Wilson 2011). Above this layer, the OT contains unique clusters of cells termed "islands of Calleja" which innervate a large portion of the OT (Bayer 1985). The islands of Calleja receive innervation from dopaminergic neurons associated with reward and motivation including the substantia nigra and the raphe nuclei (Talbot, Woolf, and Butcher 1988; Wesson and Wilson 2011).

The islands of Calleja and other regions of the OT contain feedforward and feedback projections to other olfactory regions, allowing for the modulation of signals between regions (Giessel and Data 2014; Wesson and Wilson 2011). The OT has feedback projections to MOB, AOC, PC, and amygdala for the modulation of olfactory information. Feedforward projections to areas such as the thalamus allow for higher cognitive processing of sensory information (Rampin, Bellier, and Maurin 2012). The OT receives large innervation from regions involved in reward processing - including the ventral tegmental area, nucleus accumbens, and medial forebrain bundle - while projecting to the lateral habenula, a region which detects a punishment or absence of

reward (Ikemoto 2007; Wesson and Wilson 2011). The OT is also innervated by areas involved in behavior and memory, including the hippocampus, locus coeruleus, and raphe nuclei (Wesson and Wilson 2011). These afferent and efferent projections implicate the OT in the modulation of state-dependent reward and reinforcement behaviors.

The cellular morphology and function of the OT are not as well understood. Due to its location on the ventral surface of the brain, it is a difficult region to analyze using equipment specialized to study cell activity such as electrodes. Research has given us some insight, however. The principal cells of the OT, medium spiny neurons, reside in the DCL and project into the multiform layer and other regions such as the ventral striatum (Millhouse and Heimer 1984). Other cells found within the OT include are crescent cells, which reside in the DCL and multiform layer, as well as pial, radiate, and small spine-rich neurons (Wesson and Wilson 2011). The functions of these cells are not fully understood. Electrophysiological studies investigating the activity of cells within the DCL and multiform layers have found that activity of these cells can be categorized into regular-spiking, intermittently-spiking, and bursting activity, but these have not been tied to certain morphology (Owen and Halliwell 2001). While these cells communicate with each other, as seen when stimulation of the multiform layer causes a response within the DCL, an association network within the OT has not been found (Wesson and Wilson 2011). In terms of odor responsivity, one study by Chiang and Strowbridge (2007) showed that OT neurons seem to have similar tuning profiles and response magnitudes as the PC which is unusual due to the differences in structure, but small odor sets and the

complex morphology of gyri and sulci may hinder the ability of researchers to record a proper response in cell populations (Giessel and Data 2014).

The differences in structure, cell morphology, and projections from other olfactory regions such as the PC call for the OT to be more thoroughly investigated. As previously mentioned the projections the OT receives from areas such as the ventral tegmental area, amygdala, and hippocampus suggest that it is involved in reward and learning behavior in addition to its role in olfactory processing. Behavioral, cellular and electrophysiological studies have supported this viewpoint. Rampin, Bellier, and Maurin (2012) exposed anesthetized rats to odors with a positive or negative biological relevance, such as fox urine. Odors with both a positive or negative connotation (valence) was associated with an increase in the firing rate of OT cells. Another study found that stimulation of neurons in the OT during odor presentation was associated with a preference to that odorant in a preference test (FitzGerald, Richardson, and Wesson 2014). Neurons within the OT are recruited to process characteristics of odorants, such as positive or negative connotations (valence), and as a result, synapses between neurons are strengthened and weakened. These changing connections lead to a different psychological or physiological response; the subject has learned the characteristics of an odorant.

Studies such as Murata et al. (2015) and Gadziola et al. (2015) have studied odorant learning within the OT using Go/No-Go (GNG) tasks; GNG tasks are a learning-based behavioral task where one stimulus, such as an odorant, is associated with a reward (positive stimulus), and another stimulus is associated with a punishment such as a foot-

shock or absence of reward (negative stimulus). The subject over time learns which stimulus is the positive stimulus and which one is the negative stimulus. The subject may be prone to errors such as attempting to receive a reward when the negative stimulus is presented or by not receiving the reward when a positive odor is presented. An outline of an olfactory GNG is given in Figure 1 below.

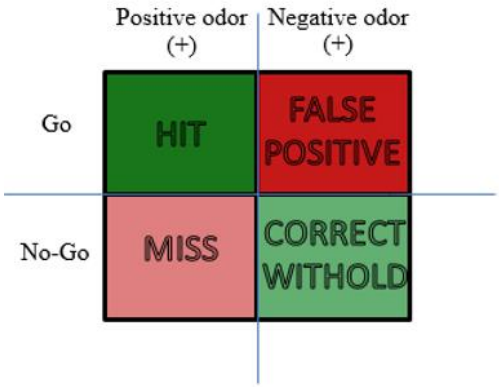

Figure 1: A diagram of responses a rat could make during the Go-No/Go (GNG) task. Rats were trained to make a nose-poke for the positive odorant ("Go") and to not make a head-entry if a negative odor was presented ("No Go"). Rats were trained to do this by associating a reward (sucrose solution) with the positive odorant, and the absence of a reward and a task time-out for the negative odorant. The diagram above shows the response outcomes that can occur during a Go/No-Go task. Hit = the rat made a head-entry upon presentation of the positive odorant and received the sucrose solution reward. False positive = The rat made a head-entry upon presentation of the negative odorant and did not receive a reward, and was unable to continue with the task for 500 ms. Correct withhold = The rat did not make a head-entry upon the presentation of the negative odorant. Miss = The rat was presented with the positive odorant, but did not make a head-entry to receive the sucrose solution reward.

Gadziola et al. (2015) found that during a GNG task, the firing rate of neurons in the OT increased when the odor was presented, whether or not they received the reward during presentation, but increased more when an odor with a positive valence was presented. Murata et al. (2015) found that after being trained in a GNG task, different areas of mouse OT were activated depending on whether the odorant had a positive or negative valence. Studies such as these support the idea that the OT is involved in olfactory learning; different neurons were recruited in order to process an odor with a positive or negative valence, however, neurons in one study increased in firing rate

despite odor valence. The OT may encode characteristics related to reward behavior, such as valence, but also may be an important region for the discrimination of odorants and processing tasks involved in odorant discrimination.

The method used in these studies to measure OT responsivity is the recording of single-unit activity or multi-unit activity. Both single-unit and multi-unit activity are electrical representations of action potentials generated by either a single neuron (single-unit) or multiple neurons (multi-unit). Analyzing the action potentials within a population of neurons can lead to conclusions about their responsivity; cells may increase or decrease their firing rate in response to a stimulus. Experiments such as the above have shown that single unit activity changes when presented with an olfactory stimulus, for instance.

Although investigating the single-unit activity present within the OT gives insight into the responsivity of the OT, another way to study the neural encoding of the OT is through monitoring local field potentials (LFPs). While single-unit activity represents the action potential triggered by a neuron, LFPs are a representation of the electrical activity of a large population of neurons within a small area of tissue, roughly 200-1000 μm^2 (Kajikawa and Shchroeder 2012). Influxes of ions in the intracellular space cause the neurons to depolarize (triggering an action potential) or to hyperpolarize and become inhibited. LFPs are the representation of the sum of this ion flow (Buszaki 2006). The electrical potential regulated by this ion flux can determine whether the cells are closer to depolarization or hyperpolarization due to a stimulus. While single unit activity can determine the response of a relatively small number of cells to a stimulus at one moment

in time, LFPs show the changes in activity of a large population of neurons over an extend period of time as the ion flux changes (Buszaki 2006). LFPs also travel farther and have a longer duration than single unit activity, which may make them more useful in studying the responsivity of a population of neurons

The information represented within LFPs is used by the cortex in order to transfer information efficiently, as outlined by Buszaki (2006). The ion flux found within LFPs is caused by a large neuronal population synchronously changing their membrane potential and then resting by mutual entrainment. The electrical potential caused by this flux presents itself as a continuous oscillatory wave, with information being encoded in each period of oscillation. As information is encoded during this period, regions transmit information using not only an electrical signal but a temporal organization, called "binding by synchrony". One populations of neurons will change their membrane potential at a similar time – this would cause one large, synchronous change in the local field potential. However, the timing of this change may differ from another population of neurons encoding different stimuli information. Changing which populations are active to encode different aspects of stimuli allows a special temporal and electrical signal to be activated for that quality of the stimuli. LFPs are also a rather energetically favorable process, in that changes within the LFP do not use a large amount of cellular energy.

Temporal coding of information within the LFP occurs through the length and size of the period of oscillation. The period of oscillation itself is determined through the wavelength and frequency in the LFP (Buszaki 2006). LFPs with a long wavelength and low frequency, for instance, would have a longer period of oscillation than LFPs with a

short wavelength and high frequency. Buszaki argues that LFP signals with a large wavelength and low frequency can carry more information and encompass a large population of neurons. These LFPs may involve input from multiple brain regions and may travel across regions. A small wavelength and high frequency LFP signifies processing in a small, localized area with less neuronal activity.

Different frequencies within an LFP may encode information specific to its frequency range. Although these ranges tend to be arbitrarily defined, they are present within the mammalian brain, including olfactory regions (Buszaki 2006). These frequency bands are referred to as "oscillators" (Buszaki 2006). Although there are many oscillatory bands depending on the region, the oscillators within olfactory regions are defined as: theta (2-12 Hz), beta (15-35 Hz), and gamma (40-120 Hz). These oscillators have been studied extensively in the MOB and in recent years have been investigated in the OT.

These oscillators - theta, beta, and gamma – are state-dependent oscillators; their activity is dependent on the cognitive state of the subject. Studies have shown that while theta remains relatively unchanged between subjects that are under anesthesia, gamma and beta oscillations are reduced in power when rats are under anesthesia (Fontanini and Bower 2003; Kay 2003; Manabe and Mori 2013). Gamma and beta oscillations, then, are used within regions such as the OT while subjects are alert to process different and changing stimuli within the environment. This can be seen in behavioral assays such as the GNG task, where beta and gamma are elevated during the processing of odorants, which is in contrast to their absence when subjects are under anesthesia.

Each oscillator has also been associated with a function in the processing of olfactory stimuli in studies involving the MOB and PC. Oscillations within the theta range are associated with sniffing behaviors and respiration. Theta has been shown to be entrained with the respiration activity of rat, driven by somatosensory input into the nose (Carlson, Dillione, and Wesson 2013). It may inform olfactory regions about the different qualities of the respiration, such as frequency and duration. Beta and gamma oscillations, in contrast, are involved in learning and reward behaviors, such as discrimination between odorants and emotional valence.

Gamma oscillations tend to be smaller in power than the other two oscillators, but are essential for the local integration of stimuli, as implied by its high frequency range (Beshel, Kopel, and Kay 2007; Buszaki 2006). Gamma oscillations in sensory regions such as the visual cortex tend to increase about one-hundred-and-fifty milliseconds after stimulus presentation – within the window of time for synaptic changes to occur – and last for hundreds or thousands of milliseconds before returning to baseline (Buszaki 2006; Leposez and Lledo 2013). This can be seen in the olfactory system, where gamma oscillations are most active during the end of a respiration cycle, and are often tied to the firing of mitral cells within the nasal epithelium (Kay and Beshel 2010; Carlson, Dillione, and Wesson 2013; Manabe and Mori 2013).

These localized increases in cell activity, occurring within such a critical period of synaptic transmission, support the idea that gamma oscillations are important for learning and discriminating between different stimuli (Buszaki 2006). Blocking gamma oscillations in insects impairs the ability to discriminate against odorants which are

chemically similar to each other, while not affecting the ability to discriminate against dissimilar odorants (Laurent 2002). Beshel, Kopel, and Kay (2007) observed increased gamma oscillations in the MOB and PC during a Go/No-Go (GNG) discrimination task; gamma oscillations in the MOB tended to increase in power with odorants that are similar in structure while not increasing with dissimilar odorants. Gamma also increased over the course of the task session. Recent studies have confirmed that gamma is necessary for this discrimination, across GNG tasks and another behavioral task, a 2-alternative choice task (Kay and Beshel 2010)

Evidence suggests that the gamma oscillator can be further separated into two bands: low frequency gamma (40 – 64 Hz) and high frequency gamma (65-120 Hz). Differences in when they occur within odor sampling and what roles they play in olfaction have been observed in experiments. Manabe and Mori (2013) found that high frequency gamma oscillations increase in power the most earlier in the odor sampling period and Kay (2003) found that these frequencies are associated with sniffing behaviors and odor discrimination tasks. Increases in lower frequency gamma occur later in the odor sampling period, and are associated with times where the subject is alert but immobile (Manabe and Mori 2013; Kay 2003). These two types of gamma activity alternate depending on the behavioral state of the subject. Therefore, higher frequency gamma may be more involved in odor discrimination than low frequency gamma.

The beta oscillator is a less understood frequency band. Beta oscillations, as a lower-frequency range, have been associated with interregional cortico-cortical processing rather than localized processing (Buszaki 2006; Kay and Beshel 2010;

Fontanini and Bower 2005). Beta has been shown to remain relatively consistent in power across regions, for instance (Frederick et al. 2016; Kay and Beshel 2007; Kay and Beshel 2010). Removing MOB feedback from other brain regions, including the piriform cortex, is associated with a lower or abolished beta power (Kay and Beshel 2010). Frequencies within the beta range were previously determined to be critical for the act of odor discrimination, and that gamma and beta oscillations performed different tasks within odor discrimination; as gamma would increase in power during odor discrimination, beta would decrease (Kay and Beshel 2010). The power of beta would also increase across tasks while gamma remained unchanged. The authors suggested that beta oscillators may be associated with the actual task performed rather than the discrimination.

A more recent study by Frederick et al. (2016) supports this view; beta oscillators may have much different state-dependent roles than previously indicated. Beta and gamma oscillations were investigated in the MOB of rats during a GNG discrimination task and a 2-alternative choice task (TAC). While gamma oscillations increased in power within one or two inhalation periods, beta oscillations increased in power much later (0.5 s) in the sampling period. The study also found that beta was not necessary for odor learning – rats performed well on both tasks if only gamma was present; however, the rats performed better statistically if the rats performed the task long enough for beta to occur and increase. Because of these two results the role of beta oscillations in olfactory discrimination is debated. Frederick et al. (2016) suggests that because of its role in system-wide olfactory processing and its delay in olfactory tasks that beta oscillations

may not be completely involved in olfactory discrimination, but rather are associated with actions which are more difficult cognitively. Instead of being involved in the discrimination of the odorant, for example, beta may be associated with decision making in a GNG task (Frederick et al. 2016).

Despite its direct connections from the MOB, its complex functions, and the large size of the structure in rodents, few LFP studies have been performed in the OT as it is difficult to access using bipolar probes. The previous studies mentioned have either been performed in the MOB and/or the PC. Furthermore, the role that gamma and beta oscillators have within olfactory discrimination need to be investigated within this region. One study from Carlson, Dillione, and Wesson (2013) investigated beta and gamma within the OT, and found that beta and gamma power increase upon odor presentation and the LFP within the OT was entrained with the MOB LFP. While this study gives insight into the OT, further research must be necessary to confirm the changes seen in beta and gamma power in the OT. This is especially true because of the suggested role that beta oscillations have within olfactory discrimination tasks. The relationship between the cognitive difficulty of the task is a new relationship to explore and will give insight into how olfactory regions designate cellular activity to process olfactory information.

Therefore, in my experiment, I studied the changes within the oscillators of the OT to presented odorants during a GNG task. Because previous studies have suggested that beta oscillations may be associated with the difficulty of the cognitive task, the main goal of my experiment was to view the changes in power of beta across a low cognitive load and high cognitive load task.

My main hypothesis is:

1. Beta oscillations will have a higher increase in power from the pre-stimulus period to the post-stimulus period during a high difficulty olfactory task than a low cognitive load task. "Difficulty" in this experiment will be represented by how familiar the rat is with the odorant. Gamma oscillations will not have this difference between tasks.

Other hypotheses of this study are:

2. As found in previous studies, both beta and gamma oscillations will increase in power after odor presentation in comparison to the pre-odor period. The valence of the odorant will not have an effect on beta or gamma oscillation power.
3. Beta and gamma oscillations will occur earlier within the MOB than the OT, due to its proximity to the M/T cells of the neuroepithelium.

Materials and Methods

Surgical procedure and animal care

Male Sprague-Dawley rats (n=2; 200-300 g) were given isofluorane as the primary anesthetic (0.1 mL of 1% in distilled water). The absence of the hind-limb reflex was used to confirm anesthesia. The rats were mounted in a stereotaxic frame, and body temperature was maintained using a heating pad (36.0 ˚C). The skull was exposed, craniotomies were performed, and a tungsten bipolar electrode was implanted into the MOB and the OT (MOB: 6.7mm antero-posterior and 1.5mm medio-lateral relative to bregma; OT: 0mm antero-posterior and 2mm medio-lateral relative to bregma; Paxinos and Wesson 1996). An adaptor was connected to the electrodes and cemented to the head.

Rats were placed in separate cages for recovery for one week and given a daily topical antibiotic until healed.

Behavioral task and electrophysiological recordings

After full recovery, rats were deprived of food and kept at 85% free-feed body weight. Within a behavioral chamber, rats were taught to activate the deposit of sucrose solution (20%) based on the insertion of their nose into an odor port, where limonene (2 s, 350 ppm) was presented. After at least forty correct hits in a session, rats were trained in a go-no-go (GNG task). Subjects were taught a low cognitive load GNG task (30 minute sessions) where isoamyl acetate or 3-hexanone were presented in a pseudo-random order (2 s, 350 ppm). Subjects were taught that the presentation of isoamyl acetate would produce the sucrose solution reward. 3-hexanone would not produce the reward and cause a 500 ms delay in the task. After achieving criterion (>85% correct response), LFP data acquisition began.

Data acquisition occurred by placing rats in the behavioral chamber. The head adaptor of each subject was attached to a 16 channel amplifier (1000x gain) and subjects were acclimated to the environment. LFP from both the MOB and OT were recorded from the rats while performing the low cognitive load task for one hour. Due to equipment restraints, MOB and OT LFPs were recorded in separate sessions rather than simultaneously. Data acquisition for the high cognitive load task was performed on a different day. Rats were presented with the previous odor set as well as a new odor set consisting of heptanal and propyl butyrate as the positive and negative odors respectively. The four odorants were presented to the subjects in a pseudo-random order at 350 ppm

for 2 s. LFP data for the MOB and OT were recorded in separate one-hour sessions on different days.

Data Analysis

Local field potential data was extracted using the Spike2 program and filtered using low pass and high pass filters to remove noise. Using a power-within-band expression located in Spike2, the LFP was separated into the three frequency bands discussed in this study: beta, gamma 1, and gamma 2 (frequency resolution: 0.977Hz). These data, along with the marker data for odor events, were imported into NeuroExplorer for further data analysis. Due to the amount of noise present within the bandwidth, the gamma 2 oscillator was excluded from data analysis.

A bin count was taken for each trial within frequency band across odor identity, odor valence, odor familiarity, and brain region. Bin counts divide values found within a data set into a series of intervals and counts them by putting them in order. Trial bins were set at 100 ms, and so a series of intervals was taken every 0.1 s. Trial bin counts took 6s epochs, with data centered at odor presentation at t = 0 s (3 s pre-stimulus and post-stimulus windows). Time of odor presentation was determined to occur at nosepoke. Results were sent to Microsoft Excel where the average for each 0.1 s bin was taken. Using visual inspection, trials within each session which seemed to be affected by noise were manually removed. Standard deviation and standard error were calculated for each average. For statistical comparisons, a t-test was calculated between bin averages. To combat the high error associated with multiple t-tests, the p-value for the t-tests was set to less than 0.001 for statistical significance. Due to variability within subjects (due to electrical noise) and variability between subjects (due to different electrode placement),

data was not averaged across subjects. Statistical significance for the odor sampling period was determined to occur if there were 5 statistically significant time points between -0.5 s and 1.0 s during the odor sampling period.

Results

Behavioral training

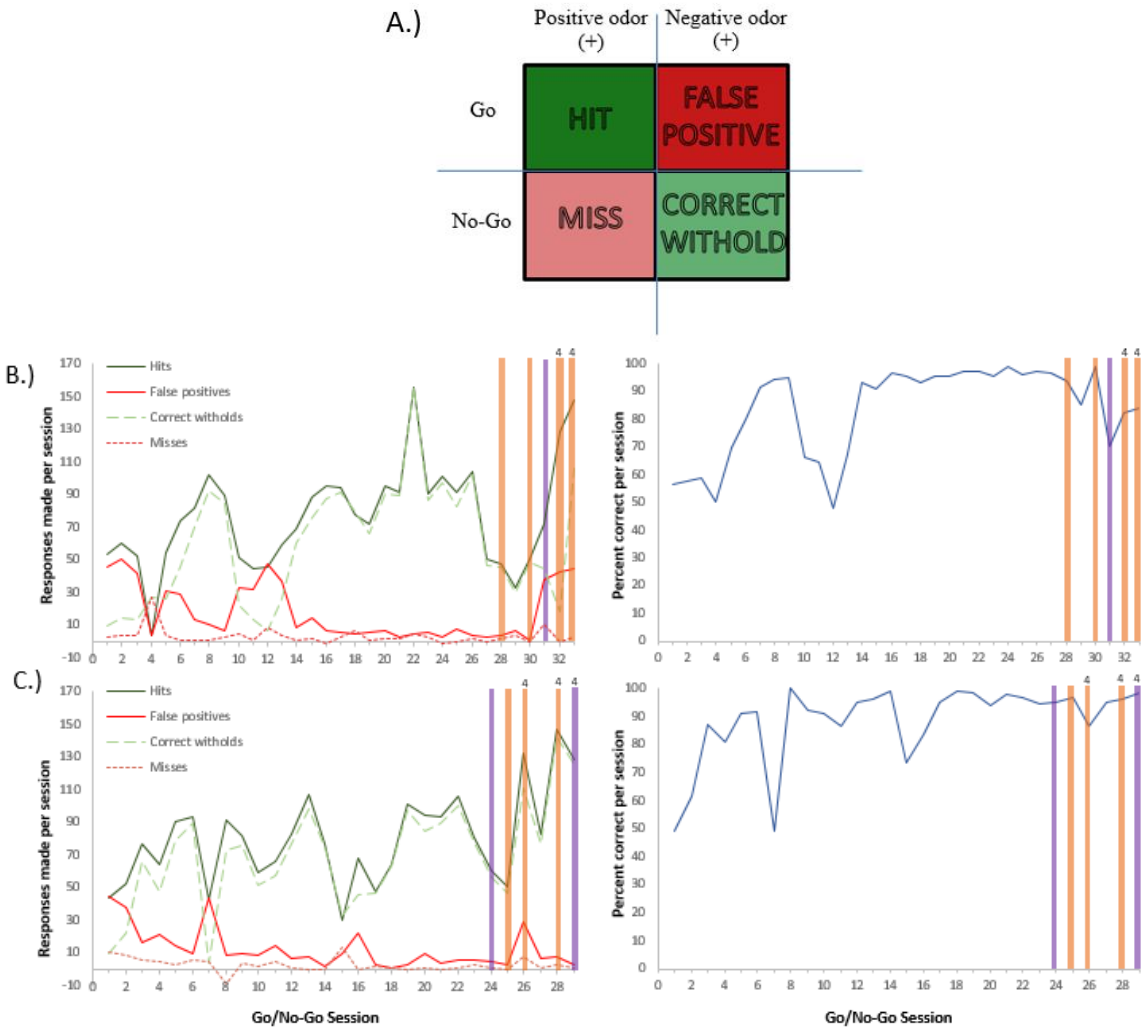

Figure 2: A.) A diagram of responses a rat could make during the Go-No/Go (GNG) task. Rats were trained to make a nose-poke for the positive odorant ("Go") and to not make a head-entry if a negative odor was presented ("No Go"). Rats were trained to do this by associating a reward (sucrose solution) with the positive odorant, and the absence of a reward and a task time-out for the negative odorant. The diagram above shows the response outcomes that can occur during a Go/No-Go task. Hit = the rat made a head-entry upon presentation of the positive odorant and received the sucrose solution reward. False positive = The rat made a head-entry upon presentation of the negative odorant and did not receive a reward, and was unable to continue with the task for 500 ms. Correct withhold = The rat did not make a head-entry upon the presentation of the negative odorant. Miss = The rat was presented with the positive odorant, but did not make a head-entry to receive the sucrose solution reward.

B.) Responses across the GNG sessions and the percent correct within sessions for R1. Orange bars across graphs represent recordings performed in the OT. Purple bars represent recordings performed in the MOB. A "4" above a bar shows

that the recording was performed during a 4-odor GNG session. Decreases in performance could be due to hunger level of subject or adjustment to equipment during the 4-odor sessions.

C.) Responses across the GNG sessions and the percent correct within sessions for R2.

Rats were first trained to complete a Go/No-Go task in order to record local field potentials during the discrimination task. In Figure 2, A describes how responses are coded for the Go/No-Go Task. The graphs on the left in B and C show the responses within a GNG task across sessions for R1 and R2. Overall, the number of hits and correct withholds increased across sessions, while the number of false positives and misses decreased. Rats were more likely to make a hit than a correct withhold, and make a false positive than a miss. Misses in general were extremely low (<10 responses) across trials. This suggests that in the 2-odor trials, subjects preferred to make a response to obtain a reward than potentially miss the reward. Decreases in performance can be seen at certain points of the 2-odor task (such as session 12 for R1 and session 7 for R2). A decrease in performance during the 2-odor sessions could be due to lack of hunger or a delay between sessions due to the unavailability of the researcher. When recording sessions began, a decrease in performance was also found. This decrease is most likely due to their head adaptor being connected to the equipment. The unfamiliar sensation may have caused the rats to respond less.

When first introduced to the 4-odorant task, rats had a decrease in correct withholds and an increase in false positives and misses. Hits also tended to slowly increase over sessions. This behavior is most likely due to the introduction of the two new odorants. Rats continued to nosepoke during new odorants in order to receive a reward, based on previous knowledge. Over sessions, false positives and misses

decreased. R4 had a high rate of false positives throughout the four odor sessions, but this trend was not found in R5.

The graphs on the right show the correct responses within a session in a percentage format. These graphs confirm that the rats learned the task, reaching criterion (>85% correct) within 8 sessions for R1 and 3 sessions for R2. Overall, R2 reached criterion earlier and performed at criterion more than R1.

LFP acquisition

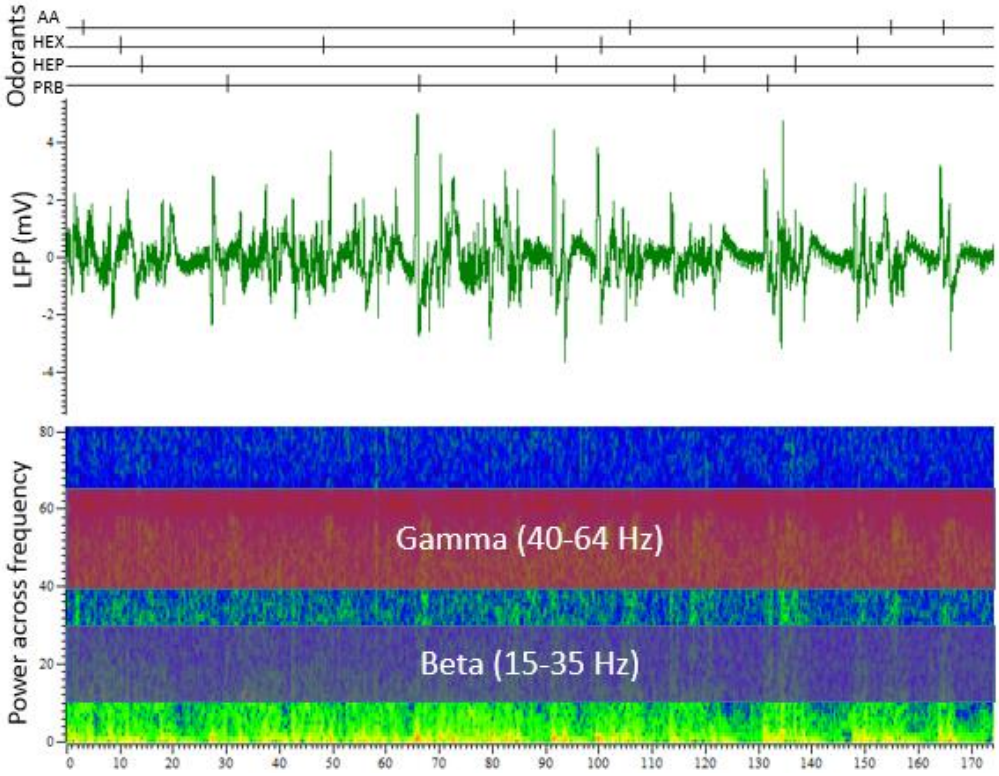

Figure 3: Odor presentation markers (top); First marker = isoamyl acetate; Second marker = 3-hexanone; Third marker = heptanal; Forth marker = propyl butyrate. An LFP signal is shown (middle), with a sonogram showing the frequencies within the LFP and the power of the frequencies displayed in the sonogram (bottom). The sonogram shows greater power (more activity) with warmer colors such as green and yellow. Colored bands on the power spectra denote the two frequency bands within the study, beta and gamma, and the general power within band can be seen by viewing the peaks within these colored bands.

After reaching criterion, LFPs were recorded from rats during a 2-odor or 4-odor trial GNG trial. Figure 3 gives an example of the data collected during LFP recordings. The raw LFP signal is shown in the middle of the figure with a sonogram showing the

power of each oscillator and markers indicating nosepokes at the top. The largest amount of activity occurs in the theta frequency band (2-12 Hz), but as this oscillator has been well studied and associated with sniffing behavior, it was not analyzed in this experiment. A large amount of activity is found in the 15-35 Hz range, representing beta activity. While much lower in power, gamma activity between 40-80 Hz can also be seen. Large increases within both oscillators occur with sharp increases or decreases in the raw LFP signal. These spikes in the LFP signal, and the increase in power within beta and gamma, occur during odor presentations. Beta and gamma therefore increase in power during odor sampling, signifying that they are used to process olfactory information.

An issue noted across sessions is that in some sessions, the power within beta and gamma across subjects has a 10-fold increase. This difference is most likely not due to animal performance or brain activity, but changes made to the amplifier between recordings.

Total power of oscillators during olfactory discrimination

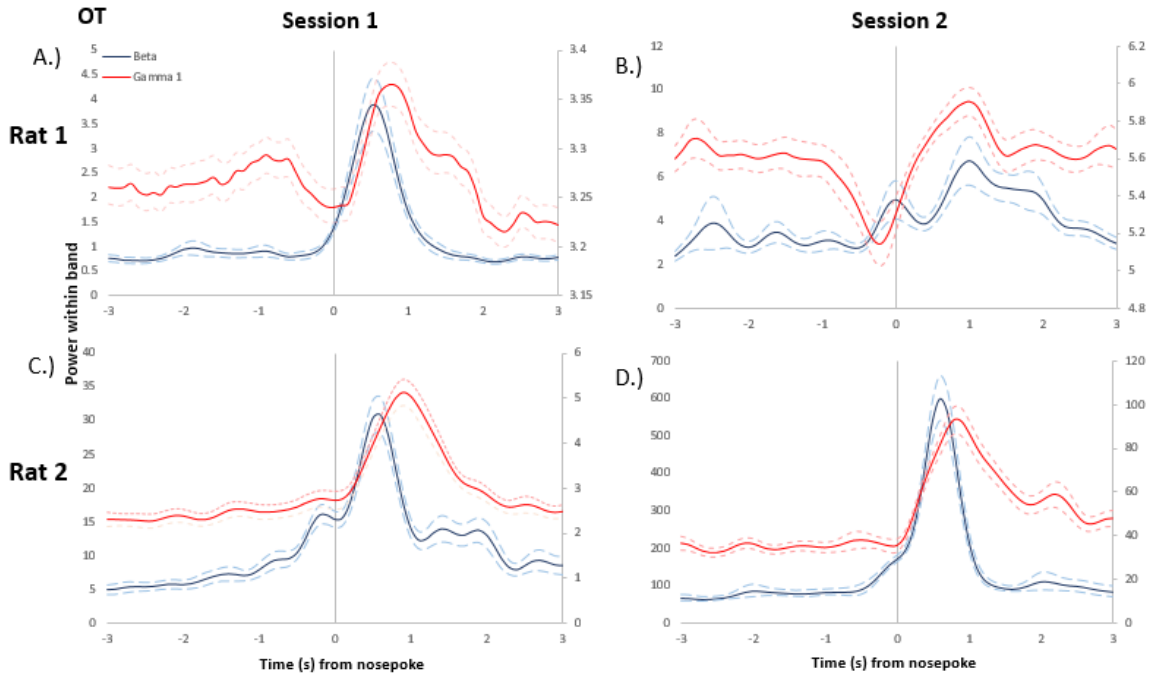

Figure 4: Total power, with error bars signified by dotted lines, in band of beta and gamma oscillators within the OT during 2-odor GNG sessions. Letters correspond to different recording sessions. Subject recorded from in the session is noted in the upper right corner of each graph. Beta power corresponds to the y-axis located in the left of the graph; gamma 1 power corresponds to the left axis of the graph. Gamma 1 was added to a secondary axis in order to compare the time where beta and gamma exhibited the largest increase in power.

The local field potential signal was separated into the three oscillators discussed in this experiment – beta (15-35 Hz), gamma 1 (40-64 Hz), and gamma 2 (65–80 Hz) and trial bin counts were taken of the data to provide power within band. Beta 1 and gamma 1 were used for this study; unfortunately, the quality of the gamma 2 signal was not clear enough to analyze and was excluded from this results section. This may be due to electrical noise or the size of the electrodes used for recording. Overall, every recording was affected by the presence of electrical noise and it significantly altered the power of both beta and gamma 1 between recordings. Therefore, it is difficult for graphs across subjects to have a similar y-axis without compromising the visibility of the data. Beta was also significantly larger than gamma 1 across most recordings. In graphs which

compare beta and gamma power, a secondary axis which is scaled to gamma 1 power has been added to the right side of the graphs for this reason.

Figure 4 displays the total power of beta and gamma across 2-odor GNG sessions for each subject with the OT. Overall, beta power seems to be higher than gamma both before and after odor presentation. In 4C for example, beta power increases to around 35; gamma increases only to around 5. This can be seen in 3 out of the 4 sessions recorded. In terms of when power peaks, beta tends to increase in power earlier than gamma, around 0.5 s in comparison to around .75 s to 1.0 s. Beta power also starts to increase about 0.1 s before odor presentation, where the same trend is not found within gamma power. This can be seen in C and D specifically. Nose may affect the outcome of these graphs, especially gamma, as noise is present in the pre-stimulus period. Noise artifacts may account for the large decrease seen in gamma in A and B around the 0.0 s mark.

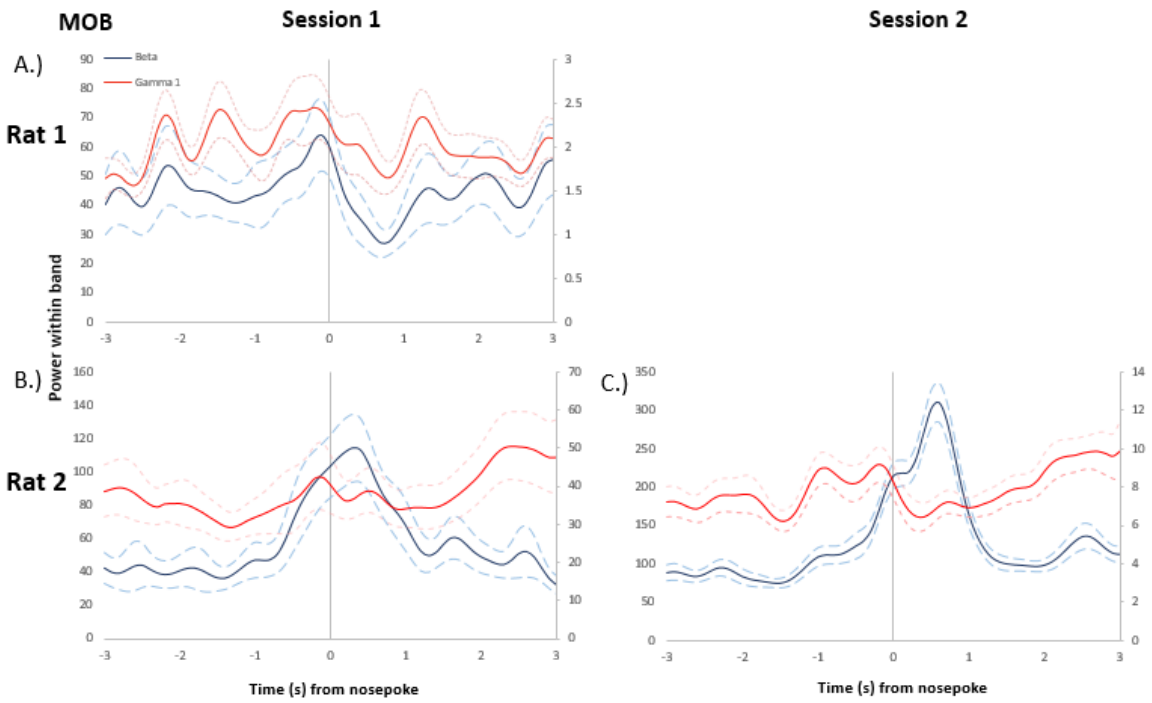

Figure 5: Total power, with error bars, in band of beta and gamma oscillators within the MOB during 2-odor GGN sessions.

Figure 5 shows the total power of beta and gamma within the MOB across GNG sessions; although confident on the shape and size of the beta power across sessions, the gamma power seems to be affected by noise and the relationship between beta and gamma is hard to interpret. A trend found within all three sessions is that within the MOB, beta still has a much larger power overall than gamma. According to the data, gamma tends to reach its peak in total power earlier than beta; about 0.5 to 0.3 s before odor presentation. Beta tends to begin increasing around this time period, but its largest increase in power occurs in the post-odor period, around 0.5 s. Because noise has affected the shape of gamma, solidified conclusions cannot be made about the time-dependent relationship between beta and gamma.

The two oscillators can be compared between the MOB and OT. As can be seen by comparing Figure 4 to Figure 5, both oscillators have their largest increase in power at different times during odor presentation. Increases within gamma in the MOB tend to occur slightly before or during odor presentation (t = 0 s). In contrast, gamma power within the OT tends to occur much later in the post-odor period. For beta within the MOB, increases begin around 0.5 s before odor presentation, and have a slow and broad increase to its peak. The time of this peak varies across subject and session. Beta within the OT has a much sharper increase overall than beta within the MOB and regularly has its highest power value at around 0.5 s. While gamma peaks later than beta in the OT, gamma peaks earlier than beta in the MOB. Once again, because of the noise within the gamma range, conclusions about gamma power peaks within the MOB and OT are tentative.

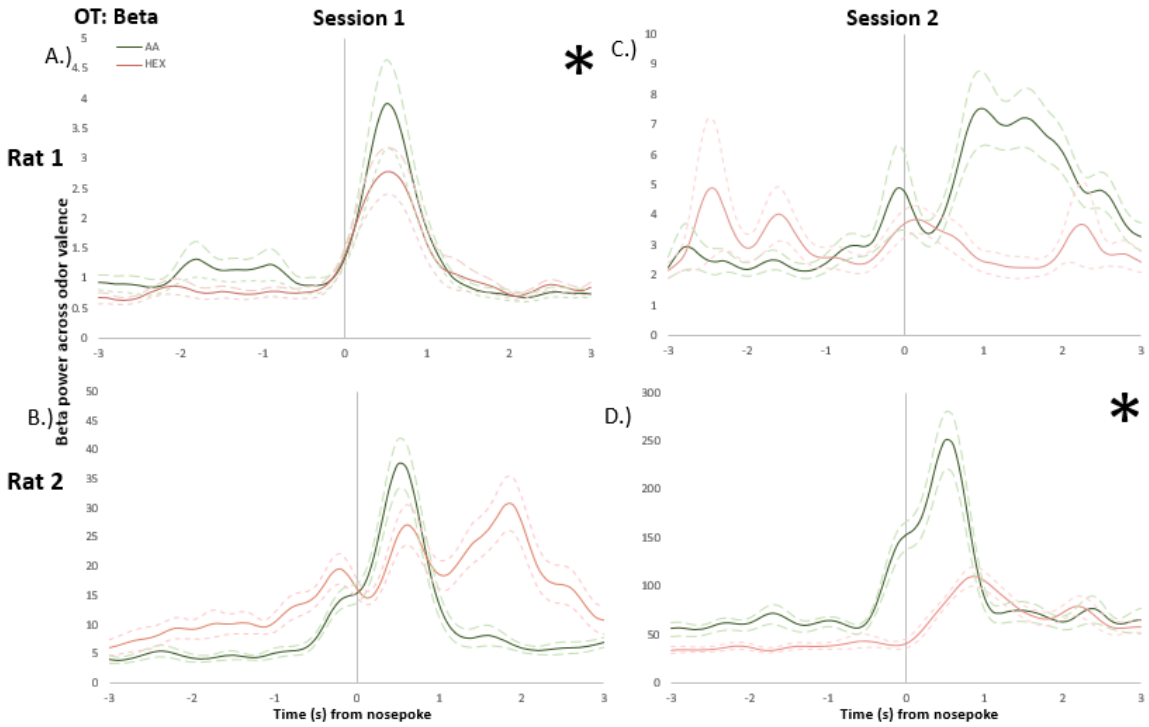

Figure 6: Beta power according to odor valence: isoamyl acetate (AA; positive) or 3-hexanone (HEX; negative) within the OT during 2-odor GNG sessions. Statistically significant ($p < 0.001$) differences between odor valence within the -0.5s – 1.0 s time period are marked by asterisks.

Within the OT, beta and gamma oscillators were compared across odor valence/identity. Because odorants were not counterbalanced due to time restrictions, effects shown may be due to identity or valence. Figure 6 shows the results for the effect of odor valence on beta power within the OT. Results of the analysis are inconclusive, although the trend is that the positive odorant, isoamyl acetate, caused a larger increase in beta power than 3-hexanone. While all sessions found a difference between the two odorants, the time of the increase in beta and the whether there are statistical differences in the -0.5 - 1.0 s. time period differs. In sessions A, C, and D, odorant AA (positive valence) has a larger increase in beta power than the negative valence odorant, HEX. However, only A and D contain statistically significant data based on the experiment

parameters. Within A and D, the statistical difference and highest increase in beta power occurs at around 0.5 s. Session C is not significant as the increase in beta occurs outside of the defined parameters:1.0 – 2.0 s. In Session B, 3-hexanone is associated with a larger beta power but is not statistically significant. There is a trend that the positive odorant tended to cause a larger increase than the negative odorant, but this occurs in only half the recordings. Strong conclusions cannot be made about this data, especially because results could be confounded by odor identity. Electrical noise may have also affected the results of the analysis.

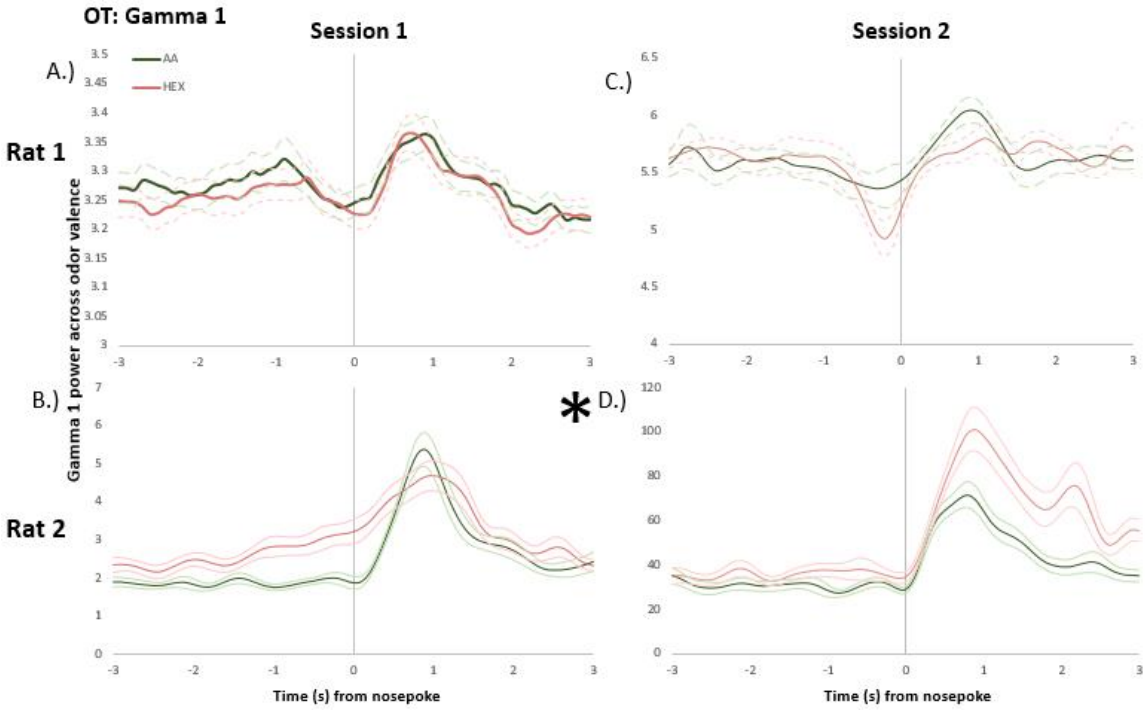

Figure 6: Gamma 1 power according to odor valence: AA (positive) or HEX (negative) within the OT during 2-odor GNG sessions. Statistically significant ($p < 0.001$) differences between odor valence within the -0.5s – 1.0 s time period are marked by asterisks.

For gamma 1 power, only one trial had a statistically significant difference between power of the positive odor and the negative odor; this is found in session B. B is the only conclusive session, where an increase in gamma for both odorants occurs 0.8 s after odor presentation, and the positive odorant is associated with a higher gamma 1 power. While other sessions had power differences, such as session C and D, a statistical difference was not found. As only one session overall contained a statistical difference, odor valence does not seem to have an effect on gamma 1 power. As noise may have affected the overall gamma 1 results, it can be assumed that it had a similar effect with the odor valence results. Once again, odor valence could be confounded by odor identity.

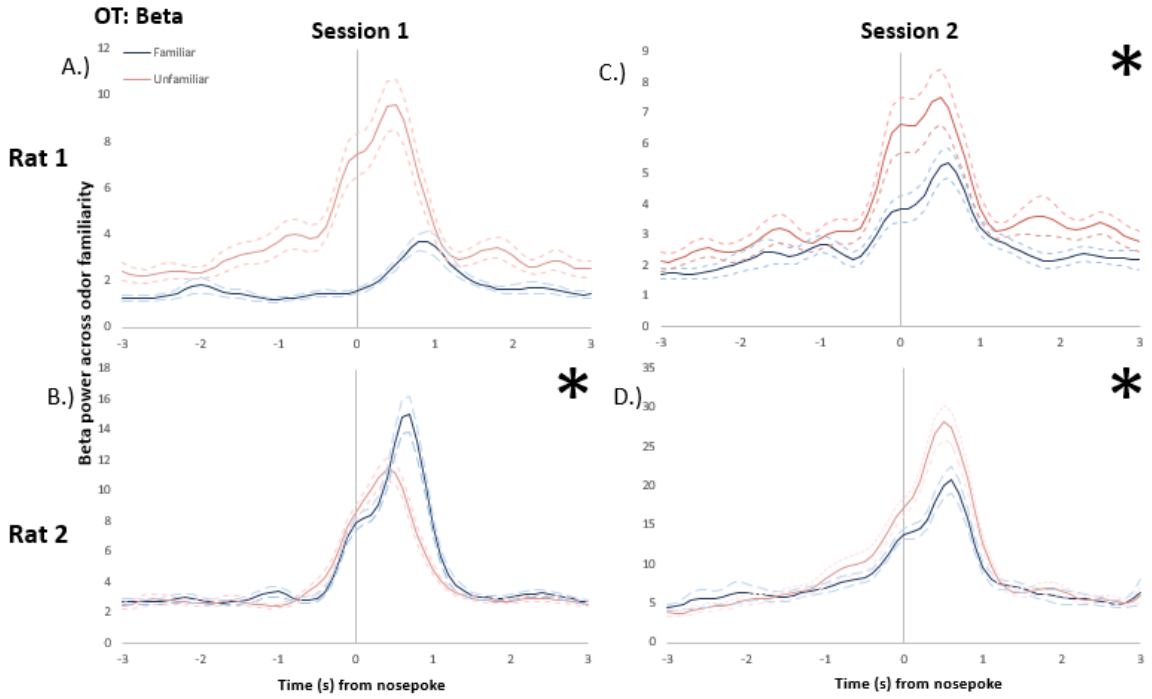

Figure 7: Beta power according to odor familiarity within the OT during 4-odor GNG sessions. Statistically significant ($p < 0.001$) differences between odor familiarity in the -0.5 – 1.0 s time period are indicated with an asterisk. Graphs A and B are sessions in which the subject was first exposed to the unfamiliar odorant set. C and D are the second session in which the subjects are exposed.

In order to determine the effect of the cognitive load of a task on oscillators within the OT, cognitive load was associated with the familiarity of the subject with the odorant. Data from the 4-odor GNG sessions were used to evaluate the effect of odor familiarity on the power of beta and gamma oscillators. The data for the first session for each subject is found in A and B in Figure 7. Within session A, a large difference between the familiar and unfamiliar odorants was found but was determined to not be statistically significant. In session B, it was determined that the familiar odorants caused a statistically significant increase in beta power in comparison to the unfamiliar odorants.

The second sessions C and D, however, have different results. Both show that the unfamiliar odorant set caused a larger increase in beta than the familiar odorant set. The relationship between sessions B and D, which were the first and second sessions for R2,

suggest that while on first exposure the unfamiliar odorant set may not cause a significant increase, which upon the next exposure beta power may increase greatly in comparison to the previous session. The power associated with the familiar odorant set remains relatively the same across sessions within subjects; around 4 for R1 and around 16 for R2. It is the power associated with the unfamiliar odorant set which changes. The peak of the increase in power tends to occur earlier for the unfamiliar odorant set than the familiar odorant set as well.

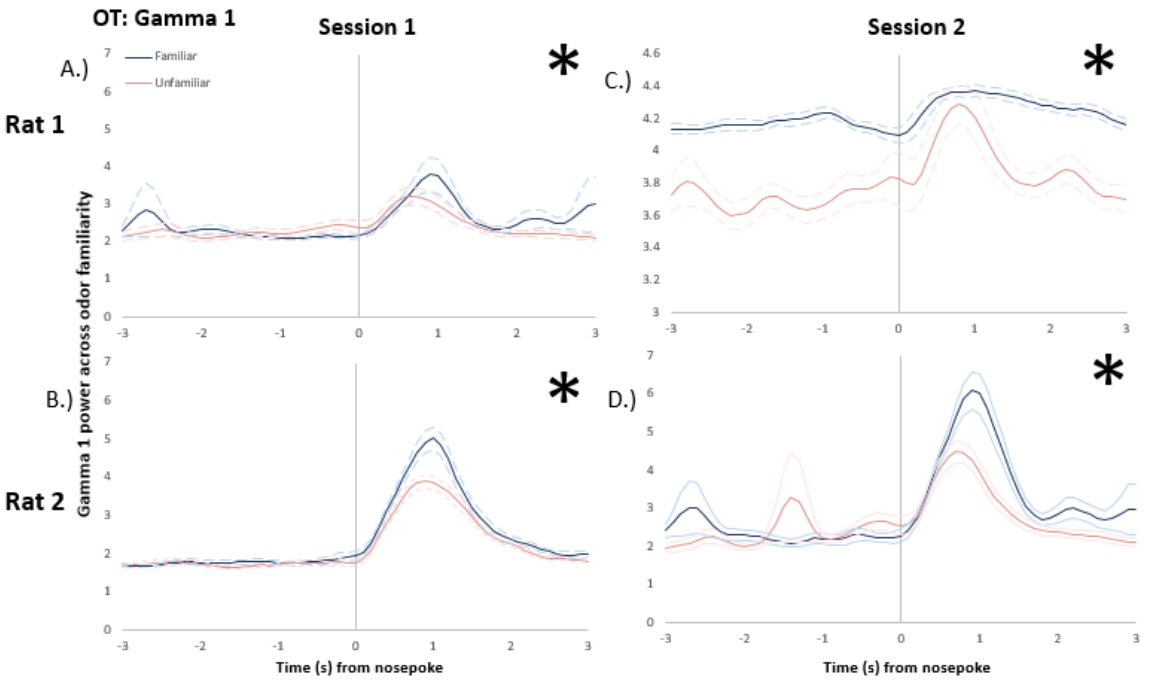

Figure 8: Gamma 1 power according to odor familiarity within the OT during 4-odor GNG sessions. Statistically significant ($p < 0.001$) differences between odor familiarity in the -0.5 – 1.0 s time period are indicated with an asterisk. Graphs A and B are sessions in which the subject was first exposed to the unfamiliar odorant set. C and D are the second session in which the subjects are exposed.

For the gamma band, a relationship was found between odorant familiarity and gamma power. Across all sessions and subjects, the familiar odorant set was associated with a significant increase in gamma 1 power in comparison to the unfamiliar odorant set. This relationship occurred upon the second exposure of the unfamiliar odorant set, in contrast to what was the found with beta power. However, the gamma data seems to have noise artifacts which could not be removed by manual inspection; this can be seen in the oscillatory behavior in the pre-odor period in sessions C and D. The t-test to establish significance may have been affected by noise, especially in session C where the data do not have the appearance of the other graphs. In this data, there was statistical significance across the entire 6.0 s odor presentation period; this is a very unlikely result and has not been seen in other studies. Noise has most likely increased the familiar odorant beta

power through the entire session and altered the results. Similar to the OT, the peak of the power of the unfamiliar odorants occurs slightly earlier than the familiar odorants.

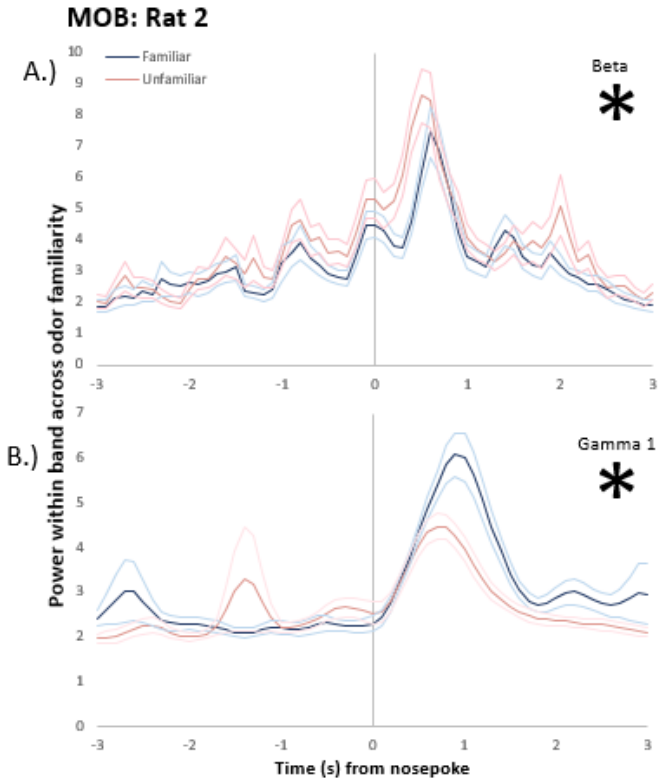

Figure 9: Beta and gamma 1 power according to odor familiarity within the OT during 4-odor GNG sessions. Statistically significant ($p < 0.001$) differences between odor familiarity in the -0.5 – 1.0 s time period are indicated with an asterisk. Graphs A and B are sessions in which the subject was first exposed to the unfamiliar odorant set. C and D are the second session in which the subjects are exposed.

The effect of odorant familiarity was also investigated in the MOB in both the beta and gamma oscillators. This data is presented in Figure 12, with beta power results shown in A and gamma power results shown in B. This data was recorded from R2 and this session was its third exposure to the unfamiliar odorants. The results of the MOB analysis are similar to the results from the OT. For beta, the unfamiliar odor set is associated with a larger increase in beta power than the familiar odorant set. Meanwhile for gamma 1, the familiar odor set is associated with a larger increase in beta power. In both oscillators, the unfamiliar odor set is associated with an increase in power which

occurs earlier than the familiar odor set. Noise artifacts are present in this data set, however, and may have an effect on the data.

Histology

Electrode placement is typically confirmed in electrophysiological studies through a histological process. Due to time and equipment restraints, however, histology could not be performed.

Discussion

In this experiment, the effect of odor valence, brain region, and the familiarity of an odorant on beta and gamma oscillations was investigated using a Go/No-Go (GNG) behavioral task. The main prediction of this experiment was that beta oscillations would increase based on the "cognitive load" of the task. It was established in this experiment that cognitive load would be represented by the familiarity of the odorants within the GNG task. Rats were familiarized with one odor set by reaching performance criterion and then exposed to the same task with a new odor set. Both beta and gamma power were compared between the old odor set and the new odor set. It was determined that familiarity has an effect on both beta and gamma power.

Effect of region

Previous LFP research within the MOB has shown that beta oscillations tend to peak at around 0.5-0.75 s after odor presentation, while gamma oscillations tend to peak at around 0.25 s (Frederick et al. 2016). The relationship between oscillator and time course may be explained by their roles in olfactory discrimination. Gamma oscillations and beta oscillations are associated with early and late odor processing respectively

(Buzsaki 2006: Frederick et al. 2016). Gamma, associated with odor discrimination and activity of mitral-tufted cells, occurs earlier in the time course as an oscillator dedicated to encoding basic olfactory information (Manabe and Mori 2013). Beta, associated with the task demand and olfactory learning, encodes information which requires longer processing time (Fredeirck et al. 2016). It therefore occurs later in the sampling period.

Contrary to this idea and the results found in the MOB by Frederick et al. (2016), it was found that within the OT, beta oscillations tend to occur before gamma oscillations. Beta oscillations tended to peak at around 0.5 s, while gamma oscillations tended to increase at around 0.75 s – 1.0 s. In the MOB, however, gamma oscillations occurred before beta oscillations. Previous studies have suggested that gamma and beta are adjusted independently based on the task and odorants, and may differ between experiments based on the task. The MOB and PC may be regions which rely more heavily on gamma for odor discrimination and encoding olfactory stimuli. The OT however may rely on beta as the primary oscillator used; the OT may be a region more involved in decoding aspects of the task demands or more complex olfactory information. As behavioral tasks used in these studies tend to differ, however, differences in the behavioral tasks may also have an effect on beta and gamma oscillation levels (Frederick et al. 2016).

Furthermore, while beta and gamma power levels tend to differ between regions such as the MOB and the PC, there has been no documentation on whether regions have an increase in power at different times over the course of odor presentation and recognition (Frederick et al. 2016; Kay and Beshel 2010). In this study, it was found that

beta and gamma oscillators tended to increase in power within the MOB earlier than within the OT. A similar effect as mentioned for the time of beta and gamma may be occurring; the MOB and other regions receive most olfactory information before it is passed on to other olfactory areas within the primary olfactory cortex. Perhaps due to its roles within the primary cortex, and its involvement in higher cognitive processes, the OT may have a delay in processing olfactory information in comparison to structures such as the MOB and the PC.

Within these results, however, we must account for noise which has affected the shape of the gamma data. The results found here are tentative due to the influence of noise, and future work must address this issue.

Effect of odor valence

Effect of odor valence was also investigated within the OT. In the MOB, odor valence has not been shown to have an effect on beta or gamma power (Kay 2003; Kay and Beshel 2010; Frederick et al. 2016). The effect of odor valence on beta power within the OT was inconclusive, as only two recordings contained statistically significant changes in beta power. However, the trend within those sessions that was the positive odorant isoamyl acetate (AA) was associated with a larger increase in beta. Odor valence on gamma 1 power. Noise or other artifacts may have affected the beta power results, as sessions tended to have very different results from the other. The same can be said for the results for gamma power, although the results for beta suggested that the odor with a positive valence, isoamyl acetate, may have had an effect. Odors were not

counterbalanced however and therefore any results could be due to odor identity and not the valence of the odorant.

Effect of odorant familiarity (cognitive load)

The main hypothesis of the experiment was that beta oscillations within the OT would increase during exposure to the unfamiliar odorants, which represented a higher cognitive load within the 4-odor GNG task. As beta oscillations are not necessary for odor discrimination, are associated with state-dependent changes, and have been linked to olfactory learning in the MOB, beta oscillations were predicted to increase when learning the two novel odors more than the beta power increase when exposed to the familiar odorants (Frederick et al. 2016). It was predicted that this change would be found within the first session of exposure to the unfamiliar odorants. Although this relationship was not found in the first 4-odor session, data suggested that over multiple odor sessions, beta oscillations tended to increase in power with exposure to the unfamiliar odorant set. While not the expected result, the change in beta power suggests that within the OT, beta oscillations may be used during higher cognitive process or more difficult tasks in order to process olfactory information (Frederick et al. 2016). As the subjects began to learn the new odorant set through more exposure, more cognitive power and coordination across olfactory regions was present in order to categorize the odorants based on chemical structure or reward, for example. While this assists in identifying the role of beta

oscillations in olfactory discrimination, it also assists in identifying the role of the OT in olfactory processing.

In contrast to the results of the beta power analysis, gamma oscillation power tended to increase in response to the familiar odorant set rather than the unfamiliar odorant set. While familiarity was not expected to have an effect on gamma power, gamma within the OT may be associated with discrimination known odorants from each other based on their chemical characteristics (Buszaki 2006; Frederick et al. 2016). As gamma oscillations are associated with the first sniffs during odor presentation, gamma oscillations may represent the processing of known odorants or relatively easily cognitive processes (Buszaki 2006). As this is quick and relatively local processing, these results support previously mentioned ideas that gamma oscillations and beta oscillations are state-dependent oscillators which, while both present during olfactory tasks, may play entirely different roles in olfactory processing (Kay and Beshel 2010; Frederick et al. 2016). This also suggests that each region may use gamma or beta oscillations at different power levels and times based on overall structure and function.

What does this suggest for the OT? The results found in this experiment contrast with many of the results found in the MOB and the PC, which are often used as representatives of other regions in the primary olfactory cortex. Based on the results of this study, the OT may not encode the valence of olfactory information and may be a region that is more involved in higher cognitive processing than the MOB or PC. As information within the cerebrum is often processed hierarchically – some receiving stimuli more quickly than others and processing simple aspects of stimuli, while other

regions receive information later and process and integration information from many regions – these results may suggest the OT communicates with other olfactory regions and processes complex olfactory information like the association with a task or the familiarity of an odorant.

It must be pointed out, however, that similar results were found in this experiment within the MOB. The changes within beta and gamma may not be due to the brain region, but rather the task. It may be that the entire primary olfactory cortex adjusts the power of beta across regions in order to process olfactory information which is contained by unfamiliar stimuli. More studies within the MOB, the PC, the OT, and other olfactory regions must be done with an odor set which is unfamiliar to the subjects to make firm conclusions about the role of beta in olfactory discrimination and the role of the OT in olfactory processing. A drawback of local field potential studies is that any changes in

There are aspects of the methodology which may have affected the results of this experiment. Only two subjects were used for the experiment; while studies such as Frederick et al. (2016) have used as few as four subjects, two subjects may not properly represent the population. Although electrical noise was manually removed, there might be noise from the movement of the commutator during recording which could not be seen or properly removed without altering the data. This electrical noise, especially with a small subject size, may have affected the shape and temporal range of the power of the oscillators. Manually removing this noise may have also introduced bias by the research removing trials using visual judgement and not through a computer program such as MATLAB. Electrode placement also could not be confirmed. In the future research, these

aspects must be addressed by a larger subject size, grounded equipment thoroughly, and developing a better method of removing noise. This experiment could be furthered by investigating the power of beta across cognitive difficulty across multiple tasks: for instance, GNG and 2-alternative choice tasks as seen in Frederick et al (2016) and Kay and Beshel (201). This might shed insight as to whether the increase in power is due to the cognitive difficult found within the task or aspects of the task itself, and would allow the results of the experiment to be compared more thoroughly to past research.

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