Analysis of Rat Event Related Potentials in Frontal and Parietal Lobes as a Possible Neural Correlate of Attention in Passive Oddball and Active Go/No-Go Paradigms

Allicia Imada
aimada@wellesley.edu

Follow this and additional works at: http://repository.wellesley.edu/thesiscollection

Recommended Citation
Analysis of Rat Event Related Potentials in Frontal and Parietal Lobes as a Possible Neural Correlate of Attention in Passive Oddball and Active Go/No-Go Paradigms

Allicia Imada
Advisor: Michael Wiest
Wellesley College

Submitted in partial fulfillment of the prerequisite for honors in the Neuroscience Program

May 2013

Copyright, 2013, Allicia Imada and Michael Wiest
Acknowledgements

Thank you very much to:

Mike Wiest for being my wonderful advisor on this project and helping me learn and grow throughout this process;

Other members of the Wiest Lab including: Linnea Herzog ’12, Sophia Gauss ’12, Caroline Dodge ’13, Kia Salehi ’13, Allyn Morris ’14, Farah Mahmud ’14, and Sam Peterson;

Pat Carey and Val LePage for helping to maintain the health of our rats in the Animal Facility;

My thesis committee members Bevil Conway, Sharon Gobes, and Connie Bauman for helping me make my project the best that it could be;

The Dean’s Office and the Neuroscience Program for allowing me to have the opportunity to do this research.
# Table of Contents

Abstract ............................................................................................................. 4  

Introduction

  I. Attention ........................................................................................................ 5  
  I.1. Top-Down and Bottom-Up Attention ...................................................... 6  
  II. Neural Correlates of Attention ................................................................. 8  
  II.1. Functional Imaging ................................................................................... 8  
  II.2. Single Units ............................................................................................ 11  
  II.3. Event Related Potentials (ERPs) ............................................................ 11  
  II.3.a. P300 ..................................................................................................... 12  
  II.3.b. P300 – P3a and P3b ............................................................................. 15  
  III. Hypothesis ................................................................................................ 17  
  III.1. Passive Approach .................................................................................. 18  
  III.2. Active Approach ................................................................................... 19  
  III.3. Human vs. Rat Active and Passive Comparisons ................................. 19  

Materials and Methods ....................................................................................... 21  

Results

  I. Passive Oddball Rare Tone Enhancements .................................................. 32  
  II. Go/No-Go Active ERPs ............................................................................ 36  
  III. Active and Passive Paradigm Comparisons ............................................. 45  

Discussion ........................................................................................................ 48  

Literature Cited .................................................................................................. 61
Abstract

Event related potentials (ERPs) are often used to examine normal human attention and to assess attentional impairments in neurological and mental disorders (Linden, 2005, Jahshan et al., 2012). To better understand sensory and attentional processing in the rat, ERPs were recorded from medio-dorsal frontal and posterior parietal areas while the animals were exposed to passive and active auditory attentional paradigms. The passive oddball paradigm was designed to target bottom-up stimulus-driven attention, while the active go/no-go paradigm was designed to evoke top-down goal-driven attention. In the passive paradigm rare-tone enhancements were seen in frontal and parietal N1, P2, N2, P3E, P3L peak amplitudes that overall could not be accounted for by stimulus specific adaptation (SSA). In the active paradigm N2 peaks showed a trend toward being larger during lick trials in general as well as specifically in false alarms, while P2 in hit trials and P3 in miss trials showed trends toward being larger. Licking might therefore affect ERP peaks by increasing N2, while attentional processing in the active paradigm might manifest itself as larger P2 on hit trials and perhaps as larger P3 on misses and N2 on false alarms. The active paradigm produced larger amplitude ERP components in general than the passive paradigm, in agreement with other studies (Shinba, 1997, Sambeth et al., 2003, Wronka et al., 2008, Hattori et al., 2010). Active N2 and P3 peak latencies were longer than those in the passive condition, which is also in agreement with other studies (Ritter et al., 1983, Katayama and Polich, 1999, Folstein and Van Petten, 2008, Wronka et al., 2008). Active and passive tasks do seem to elicit different ERP peaks, but the similarities between these differences in rats and humans is still unclear. Overall our results help extend the correspondence between human and rat sensory processing, while also beginning to identify species differences.
Introduction

1. Attention

Attention has been a large focus in the fields of psychology and neuroscience for many years. One of the reasons for this is that attention plays a large role in cognition and performance (Posner, 1980). The Encyclopedia Britannica defines attention as “the concentration of awareness on some phenomenon to the exclusion of other stimuli.” Animals use this process in many aspects of survival, including hunting, foraging, and escaping prey. Humans must attend to books for reading and must attend to traffic while driving. Attention, according to William James, is something that everyone knows. “It is taking possession by the mind, in clear and vivid form, of one out of what seem several simultaneously possible objects or trains of thought. It implies withdrawal from some things in order to deal effectively with others” (James, 1890).

Attention can be fractioned into sub-processes: attentional orientation (direction of attention to a particular stimulus), selective attention (giving priority to one stimulus over another), divided attention (dividing attention between stimuli), and sustained attention (attention to a stimulus over a long period of time) (Coull, 1998).

James referred to the idea of a limited capacity attentional system, which was examined by Cherry (1953) in his experiments looking at the “cocktail party problem” (Cherry, 1953). He proposed that some kind of “filter” is implemented in mental processing enabling humans to pull out and understand one person’s voice while other people are speaking at the same time. In looking at speech recognition, subjects were presented with auditory messages in each ear and instructed to attend to either the right or left message. Subjects were able to correctly reproduce messages that they attended to, but they failed to notice extreme changes in the unattended messages. However, physical attributes of the unattended messages were recalled, such as the gender of the speaker. Questions about why certain things were remembered while others were not prompted further research into attentional processing.

Broadbent (1958) proposed a Selective Filter Theory of Attention, which has since been questioned and updated with further research (Lachter et al., 2004). In his model, processing occurs on all of the sensory features presented (color, pitch, and orientation). These features are temporarily stored in working memory. Processing of semantic features based on the meaning of the object, such as reading a word, is restricted by capacity limitations and requires implementation of a selectivity filter, similar to Cherry’s (1953) idea. Broadbent proposed that
selection occurs on the basis of attending to a physically definable stream or “channel” of information, while sensory information outside of this “channel” is not processed beyond the physical attributes required to separate the “channels.” This theory was supported by data from the partial report paradigm, where subjects were presented with a large number of stimuli and were cued to identify only certain elements (Sperling, 1960). Not all stimuli could be reported, but subjects were able to successfully complete the task and report cued stimuli correctly. Therefore there seemed to be some kind of bottleneck effect filtering cued and non-cued elements, letting only cued elements through.

A useful way to quantify attention in research is to use behavioral tasks. Behavioral tasks can be used because attention lowers detection thresholds, improves behavioral performance, and decreases reaction time (Laberge et al., 1970, Posner, 1980, Stelmach and Herdman, 1991).

Stelmach and Herdman (1991) presented two visual stimuli to their subjects instructing them to keep track of temporal order after directing their attention toward one stimulus or away from both stimuli. They found that directed attention influenced temporal order. When both stimuli were presented at the same time, the attended stimulus seemed to occur first. They proposed that this was because the attended stimulus reached the “temporal comparator” first, since speed of transmission through the perceptual system is faster with attended stimuli. Cuing techniques also produced biases in reaction times in Laberge et al.’s (1970) study using colors and tones. Cues not pertaining to the actual target stimulus increased reaction times and errors.

### I.1. Top-Down and Bottom-Up Attention

Attention is a single word, but it can refer to distinct psychological phenomena (Styles, 1997), which may have distinct physiological implementations. For example, attention can be defined as bottom-up or top-down. Bottom-up, or stimulus-driven, attention occurs when attention is automatically driven to some kind of sensory stimulus, such as a fire alarm. The alarm is not expected and seems to occur abruptly. Conversely, top-down attention, or goal-driven attention, is cued by interest in or expectation of a stimulus. For example, runners apply top-down attention at the start of races as they are expecting to hear the starting gun. Bottom-up attention is driven by properties inherent in the stimulus, while top-down attention is driven by knowledge about the current task. Both kinds of attention are thought to contribute to the filter applied in sensory processing (Lachter et al., 2004).
Both types of attention have been separately analyzed and compared by using varying stimuli. A basic example can be seen in Figure 1 (Kastner and Ungerleider, 2000). Figure 1A would show a condition driven by bottom-up attention if a subject was presented with the stimulus, since the target stimulus (vertical line) is saliently different. The vertical line grabs bottom-up attention quickly identifying the target stimulus. Figure 1B would require cuing so that the subject could search and locate the target vertical line. The top-down condition would require more time for the subjects to identify the target.

![Figure 1: Visual stimuli eliciting top-down or bottom-up attentional processing](image)

**Figure 1: Visual stimuli eliciting top-down or bottom-up attentional processing.** When visual stimuli are presented to the visual system, different kinds of attentional processes can be recruited. Bottom-up attentional processing is stimulus-driven and can be engaged by salient stimuli, such as the vertical line in A. Top-down attention is used by subjects trained to seek or respond to specific stimuli. For example, this kind of attention would be active when a subject is cued to identify the target vertical line in B (Kastner and Ungerleider, 2000).

Buschman and Miller (2007) used visual stimuli under two conditions to analyze top-down and bottom-up attention in monkeys. A target was presented in an array of four stimuli, differing in the relationship between the targets and distractors under the two conditions. Their “pop-out” condition presented targets differing from identical distractors on two dimensions (color and orientation), while their “search” condition involved distractors that independently differed from the target requiring the animal to identify it based only on its remembered appearance (Buschman and Miller, 2007). Each of the distractors in the “search” condition could be distinguished from the target by only one dimension. The “search” condition required more time for the monkeys to react than the “pop-out” condition. My thesis examined functional
mechanisms of bottom-up and top-down attention using passive and active attentional tasks in rats.

Competing models in psychology have prompted further research into the physiological processes underlying attention. Breakthroughs in neuroscience techniques, especially recordings of specific neural activity, have allowed scientists to see and try to understand the neural underpinnings of attention and what changes in the brain when attention is engaged vs. not engaged. Neuroscientists have focused on trying to find the neural correlates of attention, or the neural processes that allow us to attend to certain sensory aspects of our environment while ignoring others. My thesis focuses on certain neural correlates of attention using a rat model.

II. Neural Correlates of Attention

Varying approaches have been taken to look into the neural correlates of attention including functional brain imaging, single unit recordings, and event related potentials. The correlates being looked at include “consistent and significant associations between particular cognitive functions and either temporal patterns of neural firing, levels of activity in discrete brain regions, or levels of activity of neuromodulatory neurotransmitters which can influence these other two systems” (Coull, 1998).

II.1. Functional Imaging

Imaging studies use non-invasive ways to record neural activity and are much more common in human studies than invasive spiking studies. Imaging also allows for the visualization of the whole brain, rather than just specific areas implanted with electrodes in direct electrophysiological recordings. Functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) have helped identify brain regions that are involved in attention.

Frontal and parietal brain areas have been shown to be involved in attentional processing, as observed through these functional imaging techniques. Increased activity in these areas has been shown during sustained attention tasks (Coull, 1998, Corbetta et al., 2002, Linden, 2005). Coull (1998) used PET and fMRI to show increased activity in the posterior parietal area during an attentional orienting task. Another study using fMRI found that frontal areas are involved in allocation and maintenance of attention (Corbetta et al., 2002). Pardo et al. (1990) showed through PET that the anterior cingulate cortex was activated more in the Stroop attentional
conflict paradigm requiring more selective attention (Pardo et al., 1990). In this paradigm, one condition displayed words as color names that were the same as the color they were displayed in. In the other conflict condition the words were shown as color names different from the color they were displayed in. Subjects were instructed to identify the color that the words were displayed in. Frontal and parietal activation also has been shown to increase with attentional load in an fMRI study (Culham et al., 2001).

These findings are supported by lesion studies that found that frontal and parietal lesions led to attentional deficits (Posner et al., 1984, Hu et al., 2013). Hu et al. (2013) showed that patients with focal frontal or parietal lesions showed deficits in a visual reaction time attentional test involving different kinds of cues and targets. They concluded that brain damage selectively impaired attentional networks. Posner et al. (1984) similarly found that parietal lesions impaired sensory attention when targets were presented on the side contralateral to the lesions.

Connections between frontal and parietal areas have been shown through retrograde tracing and stimulation experiments in animals. Reep et al. (1994) showed that connections exist between the posterior parietal cortex and frontal regions including the angular cortex and ventrolateral and medial orbital areas in rats using fluorescent retrograde axonal tracing (Reep et al., 1994). Another study similarly found connections between frontal and parietal regions using retrograde tracing in monkeys (Marconi et al., 2001). Golmayo et al. (2003) found that electrical stimulation of somatosensory neurons in rats caused activation of neurons in the prefrontal cortex (Golmayo et al., 2003). Primary sensory areas are involved in early processing, while late processing occurs in the frontal cortex, which shows that attention seems to involve a progression of neural activation (Mole 2009). Thus frontal and parietal areas seem to function as an attention/detection network.

Posner and Peterson (1990) proposed an attentional system separated into two sub-systems in frontal and parietal areas (Posner and Petersen, 1990) (Figure 2). In this system areas involved in posterior parietal function such as the cortex itself, the superior colliculus, and the thalamic lateral pulvinar are involved in attentional orienting to locations. Anterior areas, including the anterior cingulate, are thought to be involved in target detection. The medio-dorsal frontal cortex is also thought to be involved in attentional processing (LaBerge and Buchsbaum, 1990), which is one of the areas that we looked at in my thesis. Posner and Peterson (1990) also
hypothesized that the brainstem reticular system has a hand in both of these systems in terms of setting the arousal state.

**Figure 2: Posner and Peterson’s (1990) neuropsychological model of attention.** Frontal and parietal systems seem to be involved in orienting and target detection, respectively (Posner and Petersen, 1990).

Other brain areas have also been implicated in attentional processing related to the sensory modality involved in particular tasks. Brefczynski and DeYoe (1999) showed that cortical activity was enhanced in monkey V1 areas that correspond to retinal areas of attentional focus (Brefczynski and DeYoe, 1999). Janke et al. (1999) used fMRI to show that the primary auditory cortex (A1) was activated more in an auditory detection task where patients were required to respond to specific tones (‘detection’) compared to when they were just required to listen (‘attention’). Both paradigms evoked larger activity than the ‘no attention’ condition where patients were instructed to look at their feet while the tones were played (Jancke et al., 1999). Another study using PET showed that primary somatosensory cortex (S1) was activated in a tactile attentional task involving discriminating between pairs of gratings differing in roughness or duration of contact (Burton et al., 1999). However, frontal and parietal areas are active during sustained attentional tasks for all modalities (Pardo et al. 1991, Corbetta et al. 1993, Coull et al. 1996, Coull & Nobre 1998).
II.2. Single Unit Recordings

Another method used to look into attentional processing is direct electrophysiological single unit recordings. Looking at individual neuron activity has been beneficial in attention-related research because it allows scientists to look into underlying cellular mechanisms. These direct recordings of individual neuron activity can be readily used in animal models. The opportunity to do direct recordings in humans is limited to rare cases involving neurosurgery. Early on, Hubel (1959) found that certain neurons in the cat auditory cortex seemed to respond to auditory stimuli only if the cats were attending to the stimuli (Hubel et al., 1959). For example, certain units produced no firing when the researcher crumpled paper in his hand, but if the cat turned its head to look at the researcher in response to the paper crumple a large burst of firing would occur. Single units in the sensory cortex seemed to fire more than others in accordance with levels of attention.

Other studies have also shown that spiking activity is increased due to attention. Moran and Desimone (1985) recorded spiking activity in prestriate area V4 of the inferior temporal cortex in rhesus monkeys during a visual attention task (Moran and Desimone, 1985). The normal response of cells in these areas to an attended stimulus increased, while the response to the unattended stimulus decreased. Cohen and Maunsell (2009) found further that attention-related spiking activity of cortical neurons in area V4 was reflected primarily as decreasing the correlations in “trial-to-trial fluctuations” between pairs of neurons, and therefore neuronal interactions may be important when looking at population spiking activity evoked by sensory stimuli (Cohen and Maunsell, 2009). In general, attention increases the selectivity of neurons thereby displaying the filtering effect of irrelevant stimuli.

Single unit recordings have proven to be a valuable tool in the search for neural correlates of attention. These recordings are used to examine neural mechanisms of attention and can help us understand how attentional processing works.

II.3. Event Related Potentials (ERPs)

Functional imaging studies have good spatial resolution and have identified areas activated during attentional tasks; however, they lack the temporal resolution needed to measure neural activity on the time-scale of action potentials. Imaging studies show activity seconds at a time, while action potentials actually occur in one or two milliseconds (Linden, 2005).
Electroencephalograms (EEGs), on the other hand, detect changes in electric fields due to movements of charged ions during an action potential or a post-synaptic potential with greater temporal resolution. This technique measures synchronized activity of large neuronal populations in brain tissue because individual action potentials and postsynaptic potentials represent transient small changes in voltages. EEG lacks the spatial resolution of functional imaging studies, but gives scientists the temporal resolution that those techniques lack.

Event related potentials (ERPs) represent the average EEG response with respect to an “event” or stimulus. Therefore, an ERP shows the stimulus-driven summed postsynaptic potentials of multiple neurons. EEG responses are manipulated so that the segment of time around the stimulus is extracted, and then averaged over all trials in a session. This thereby reveals activity evoked by the stimulus. ERPs generally are composed of characteristic peaks named for their latency and voltage sign: N1, N2, P1, P2, and P3. Using these constraints, ERPs can be compared. ERPs evoked during EEG measurements have been used to examine normal human attention and to assess attentional impairments in neurological and mental disorders (Linden, 2005, Jahshan et al., 2012).

Rats are often used in ERP studies since invasive electrophysiological recordings can be done readily to examine the mechanisms of attentional processing. In my thesis local field potentials (LFPs) are recorded from frontal and parietal areas using chronically implanted multi-electrode arrays (Buzsaki et al., 2012, Wiest et al., 2007). LFPs are analogous to human EEG signals except that they are recorded from within brain tissue rather than at the scalp, so they reveal activity that is not visible at the scalp. They detect postsynaptic activity from large groups of nearby neurons. LFPs contain both action potentials, as well as other fluctuations in membrane potentials in small neuronal volumes. ERPs are extracted from these LFP signals, and ERP components are compared between paradigms and brain areas. The multi-electrode arrays also detect spikes from individual neurons that are not analyzed in my thesis.

II.3.a. P300

One component in particular, the P3 (also known as the P300 because it appears approximately 300 milliseconds post-stimulus in humans (Wronka et al., 2008)), is a component of the ERP response to sensory stimuli that is believed to be related to attention, decision-making, and memory updating (Zenker and Barajas, 1999, Wronka et al., 2012). P3 is altered in
a number of neurological disorders, such as schizophrenia, Alzheimer’s disease, Parkinson’s disease, and attention deficit disorder (Wiersema et al., 2005, Polich, 2007, Jahshan et al., 2012). Using changes in P300 to examine neural mechanisms of mental disorders is becoming more popular because it can readily be completed through simple paradigms, and it does not necessarily require behavioral responses. P3 latency seems to be correlated with speed of mental functioning in that shorter latencies correspond to faster cognitive performance. P3 latency decreases in normal child development and increases with dementia and normal aging (Wright et al., 2001, Polich, 2007). ERP studies in animal models also show a P3-like component with variable latencies and amplitudes in various tasks (Yamaguchi et al., 1993b, Shinba, 1997, 1999, Broussard and Givens, 2010, Hattori et al., 2010). Further research into the neural mechanisms of changes in P300 could lead to better treatments of cognitive diseases or attentional deficits.

P3 is thought to reflect memory processing as well as attentional resource allocation. Increases in task difficulty, for example by increasing memory load, have been shown to increase P3 amplitude and latency (Donchin et al., 1986, Kok, 2001). Increasing memory processing in these tasks seems to alter attention and change P3 amplitude and latency. According to this theory, increasing difficulty of active paradigms may increase P3 amplitude and latency by increasing attentional demands.

One of the theories describing the P3 response is the context-updating theory (Polich, 2007). Figure 3 shows this framework, stating that an updating process comparing incoming stimuli to previous stimuli stored in working memory evokes a P3 response if the new stimuli are comparatively different. All sensory stimuli evoke certain “obligatory” sensory components, such as N100, P200, and N200. When a stimulus comes in, a comparison process is implemented evaluating the new stimulus in working memory to see if it is the same or if some kind of stimulus attribute is different. If no changes are detected then just the sensory components are recorded, but if changes are detected the stimulus representation is “updated” with a P3 component. P3 is produced as an updated change in the response due to a detected change in the stimulus.
On the other hand, recent studies in rats and cats have suggested that larger amplitude responses to rare tones, also referred to as the oddball effect, may be explained by stimulus specific adaptation (SSA) to repeated stimuli. The idea behind these studies is that neurons that respond to the frequent standard tones may get fatigued relative to neurons responding to the infrequent oddball tones. On the other hand, “true deviance detection or novelty detection,” in which an enhanced response signals an implicit comparison between current and previous stimuli in a series, cannot be achieved by SSA alone (Farley et al., 2010, Taaseh et al., 2011). If only adaptation is in effect, then adding a frequently repeated standard tone to a sequence of rare oddballs can only further fatigue the neurons that respond to the oddball tone, leading to equal or smaller oddball responses than would be expected in the absence of standards. SSA is manifested by auditory cortical neurons (Squires and Donchin, 1976) and thalamic neurons in rats (Anderson et al., 2009), but not by thalamic neurons in cats (Ulanovsky et al., 2004b). Conflicting results have been found as to whether SSA of neurons in the auditory cortex can account for the oddball effect. Ulanovsky et al. (2003) concluded that SSA could account for the oddball effect that they found in group recordings of cat auditory cortex (Ulanovsky et al., 2003), while other studies found that SSA could not account for the deviance detection they observed in rat (Farley et al., 2010, Taaseh et al., 2011) or human (Farley et al., 2010) ERPs.
In humans active attentional tasks in which subjects are required to respond to stimuli evoke larger amplitude ERP components, including P3, compared to passive oddball tasks in which subjects are not required to respond (Oades et al., 1995, Bennington and Polich, 1999, Zenker and Barajas, 1999). In rats Hattori et al. (2010) found that their active paradigm evoked larger P3s than their passive paradigm. They also found that P3 latency was similar to humans in that the hippocampal P3 occurred after the cortical P3. Sambeth et al. (2003) found that P3 was larger in active vs. passive conditions (Sambeth et al., 2003). They did the same study with humans, and the rats showed components with the same order and polarities as the human ERPs. They concluded that rats might have similar neural generators of this attentional ERP component compared to humans. Examining the properties of P3 in my thesis may reveal more information about mechanisms of P3 and similarities between rats and humans in searching for the neural correlates of attention.

II.3.b. P300 – P3a and P3b

P3 in humans can be divided into distinct subcomponents based on latency that respond differently in different types of attentional tasks. The early P3a subcomponent (220-240 milliseconds) is exhibited in fronto-central locations, and has been suggested to be associated with an automatic bottom-up (involuntary) alerting process. The late P3b (250-500 milliseconds) is detected in posterior parietal locations, and has been associated with indexing voluntary attention and top-down attention (Polich, 2007, Wronka et al., 2012).

Both the frontal and parietal cortex have been implicated in attentional processing in these subcomponents. Damage to the temporal-parietal junction reduces parietal P3 (Polich, 2007). Patients with frontal lobe lesions show decreased P3a amplitudes, but no decrease in P3b (Polich, 2007). P3a has been localized more anteriorly, while P3b seems to be localized more posteriorly (Volpe et al., 2007). Rare stimuli are thought to elicit frontal lobe activity when enough attention is engaged. Temporal-parietal areas are thought to be elicited in tasks that require behavioral action, such as those requiring covert or overt responses to stimuli (Wronka et al., 2012).

Neuropharmacology studies have indicated that dopamine and norepinephrine pathways may contribute to P3 and its subcomponents. Studies have shown that P3a may be mediated by frontal dopaminergic activity, while P3b may be mediated by temporal-parietal norepinephrine
circuitry (Polich, 2007). In a three-tone oddball paradigm with two non-target tones, EEG activity was recorded in controls, restless leg syndrome patients, and Parkinson’s disease patients (Figure 4) (Polich and Criado, 2006). They found that P3a was decreased in restless leg syndrome and was virtually absent in Parkinson’s disease. P3b also showed the same trend of decrease, although less so than in P3a. Dopamine levels are thought to be reduced in restless leg syndrome, and are greatly reduced in Parkinson’s disease. Therefore Polich and Craido (2006) concluded that dopaminergic activity contributes to P3a and parts of P3b.

![Figure 4: EEG topographic mapping of distractor and target P3 responses in controls, restless leg syndrome, and Parkinson’s disease patients. Seven subjects, matched by age, sex, and education, were subjected to a three-stimulus oddball paradigm. P3a was decreased in restless leg syndrome and was virtually absent in Parkinson’s disease. P3b also showed the same trend of decrease, although less so than in P3a. Dopamine levels are reduced in restless leg syndrome and are greatly reduced in Parkinson’s disease. Therefore dopamine seems to contribute to the generation of P3a and parts of P3b (Polich and Criado, 2006).](image)

Norepinephrine has also been implicated in P3b generation in the parietal lobe (Nieuwenhuis et al., 2005). P3 exhibits one of the important processing functions of the locus-coeruleus-norepinephrine system, which is to “potentiate the response to motivationally significant events.” A study using a drug called clonidine, a norepinephrine antagonist which reduces the release of norepinephrine from axon terminals, decreased parietal P3 in an auditory
oddball paradigm in squirrel monkeys (Swick et al., 1994). Thus norepinephrine seems to be implicated in the generation of P3.

Glover et al. (1988) found that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) completely abolished P3 in monkeys, thereby showing that depletion of dopamine and norepinephrine greatly affects P3 (Glover et al., 1988). Of the five monkeys treated, two of them recovered behaviorally after about a month, and P3 re-emerged in these animals only. The drug also eliminated early components, but the components were recovered after a couple of weeks in all animals. These findings do not show which of the two neurotransmitter systems contributes on its own, but they do show that one or both of them greatly influences P3.

Many of these studies were done in animal models. Specifically rats are used in my thesis as a possible model of human ERP attentional processing. The latency of P3 subcomponents has proven to be more variable in animal studies compared to human studies. Sambeth et al. (2003) mentioned that latency of P3 varies between rat studies. Some studies call a component between 250-500 milliseconds after the onset of a stimulus the P3, such as Shinba (1999) and their own study, while others call an earlier component 220-240 milliseconds after the onset of the stimulus the P3 in rats (Yamaguchi et al., 1993a). They attribute the differences to unclear P3 morphology compared to other components and to non-standardized reference electrode localization and choice of reference electrode. Many of these studies do not attempt to distinguish early and late P3 peaks (Yamaguchi et al., 1993b, Shinba, 1997, Hattori et al., 2010).

**III. Our Hypotheses**

The aim of this study was to look into the potential analogs of human ERP components in rats using active and passive attentional tasks to distinguish between bottom-up and top-down attention. LFPs were recorded from rat frontal and parietal cortex during auditory passive oddball and active go/no-go paradigms. Behavioral paradigms are shown in Figure 5, and explained in **III.1** and **III.2**. In the passive paradigm (Figure 5A), the rats were not required to respond to auditory tones. In the active go/no-go paradigm the rats were required to distinguish between and correctly respond to target and distractor tones (Figure 5B). Bottom-up attention is thought to be engaged in the passive oddball paradigm, while top-down attention was used in the go/no-go paradigm. Component amplitudes and latencies were compared between brain areas as well as between paradigms.
III.1. Passive Approach

The passive oddball paradigm was used to examine cellular mechanisms of bottom-up attention. Our hypothesis was that rare-tone response enhancements may be caused by SSA. Rats were placed in behavioral boxes, and a series of beeps were played consisting of standard and oddball beeps. Standard beeps occurred more frequently and were of a consistent pitch, while oddball beeps occurred less frequently and were played at a different pitch. Pitch frequencies in matched recording sessions were switched to control for pitch differences. No action was required from the rats. ERP components were compared after standard and oddball tones.

III.2. Active Approach

The active condition required the rats to distinguish between and correctly respond to two types of beeps. This condition was used to test the hypothesis that P3 and/or other components were larger when attentional processing was used to: (a) correctly respond to target beeps or (b)
correctly inhibit action after distractor beeps. Our null hypothesis was that P3 is a correlate of licking. One tone activated the water pump in response to a lick, and the other pitch yielded no water reward. The active paradigm required top-down goal-driven attention, since the rats were trained to expect and respond to the appropriate beep. Evidence for this is that the rats did discriminate between the two beeps and could complete the task. ERP component amplitudes and latencies were compared on different types of trials.

Hits were successful trials where the rat successfully responded to the beep corresponding to the water reward (target), and correct rejections occurred when a rat correctly did not try to get water after the non-water (distractor) beep. Misses were when the rat did not get water after the target tones, and false alarms were when the rat tried to get water after the distractor beeps. These types of trials are restated in Table 1.

<table>
<thead>
<tr>
<th>Trial Type</th>
<th>Water/no water</th>
<th>Attentional/Response Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hits (H)</td>
<td>Water</td>
<td>Good attention and perception</td>
</tr>
<tr>
<td>Correct Rejections (CR)</td>
<td>No Water</td>
<td>Good attention and response inhibition</td>
</tr>
<tr>
<td>Misses (M)</td>
<td>No Water</td>
<td>Poor attention and response inhibition</td>
</tr>
<tr>
<td>False Alarms (FA)</td>
<td>No Water</td>
<td>Poor attention and response inhibition</td>
</tr>
</tbody>
</table>

Table 1: Trial types in the go/no-go task. Hit trials warranted a water reward. Attentional interpretations are shown on the right.

III.3. Human vs. Rat, Active vs. Passive Comparisons

We compared our ERP components with other rat and human studies in active and passive tasks. We expected to see components similar in latency in both the active and passive conditions, with larger amplitude (Oades et al., 1995, Bennington and Polich, 1999, Zenker and Barajas, 1999) and longer latency (Donchin et al., 1986, Kok, 2001) components during the active condition if our results were consistent with other rat and human studies. The active condition required the rats to successfully complete learned tasks, and therefore might have exhibited both early (P3E) and late (P3L) P3 peaks if similar to humans. Hit trials may have shown larger P3Ls (300-500 milliseconds) since the rats would have successfully completed the task, whereas miss trials may have shown smaller P3Ls since the rats did not successfully manipulate the tasks to receive a water reward. Passive paradigms should mainly have exhibited
P3E (200-240 milliseconds) effects since that subcomponent in humans is associated with involuntary attention to stimulus differences (Wronka et al., 2008). P2 has also been implicated in modulating attention, so examining differences between passive and active P2 may help expose similarities and differences between rat and human ERPs (Picton and Hillyard, 1974, Crowley and Colrain, 2004). P3 early and late components may have been localized to frontal or parietal areas similar to P3a and P3b in humans. Go/no-go studies in humans have showed that N2 is increased during ‘no-go’ response inhibition trials in frontal and central areas (Pfefferbaum et al., 1985, Kiefer et al., 1998, Bruin and Wijers, 2002), while N2 is increased in response to rare visual targets over parietal, temporal, and occipital areas (Ritter et al., 1983, Folstein and Van Petten, 2008). Therefore looking specifically at ‘no-go’ correct rejection trials in frontal areas may reveal additional similarities in ERPs between rats and humans.

Overall, we looked into how various ERP components contribute to attentional processing in rats. Analysis of active and passive ERPs may help clarify the significance of P3, as well as other components, in relation to attention. If we saw early and late P3-like components with comparable functional properties to the human P3a and P3b in the active and passive tasks, we would help establish the rat as a model of attention and perception analogous to that of humans. If these subcomponents also had higher amplitudes in frontal or parietal lobes (P3a in frontal and P3b in parietal), we could show further similarities between rat and human P300s. If rats and humans are similar in these processes, the more invasive methods possible in rat models could reveal cellular mechanisms of attention, such as neural adaptation.
Materials and Methods

I. Animals

Four male Long-Evans rats, *Rattus norvegicus* (500-700 grams), were purchased from Charles River Laboratories (Wilmington, MA). Rats were housed in pairs before surgery and individually after surgery in the Wellesley College Animal Care Facility on a 12:12 light/dark schedule (6am-6pm light/6pm-6am dark). The rats were trained once a day (Monday-Friday) either during a morning session (8 am-12 pm) or an afternoon session (1 pm-4 pm). The rats were given access to free water for 15-20 minutes 1 hour after training. They were also allowed free access to water from Fridays after training until Sunday afternoons. Weights were monitored daily to make sure that rats did not drop below 85% of their *ad libitum* weights, measured on Sundays after 48 hours of free water access. The rats were allowed food *ad libitum*. Animals were monitored daily by lab members as well as the Wellesley College Animal Care Facility staff. The Wellesley College Institutional Animal Care and Use Committee approved all procedures, in accordance with the guidelines set by the American Association for Accreditation of Laboratory Animal Care (AAALAC) International.

II. Experimental Paradigms

Local field potential (LFP) recordings took place during the day in a standard operant chamber (8003NS, Lafayette Instrument) housed in an actively ventilated sound-attenuating outer chamber (Figure 6).
II.1. Passive Exposure Paradigms

The passive exposure paradigms did not require responses from the rats, and therefore were thought to target mechanisms of bottom-up attention. No training was required in these paradigms. Two variants of the paradigm were used in my thesis.

II.1.a. Two-Tone Paradigm

In the two-tone oddball paradigm, 2 different types of auditory tones were played in each session. Rats were placed in the standard operant chamber and exposed to a series of standard and oddball beeps generated by ABET-II software for 15-30 minutes from a speaker mounted on the upper left side of the front wall of the operant chamber. Rats were discouraged from sleeping and “drowsiness,” as seen by large amplitude 10 Hz mu-like rhythms in LFPs, by occasional manual offering of water and door opening. Oddball beeps occurred less frequently (16.67% of

Figure 6: Standard operant chamber used for rat training and recording. Rat behavioral training sessions and neural recordings were completed in a standard operant chamber (80003NS, Lafayette Instrument). The nosepoke was not used in these experiments (Reprinted with permission from Herzog, 2012).
trials) compared to standard beeps (83.33% of trials) for 50 or 100 milliseconds each depending on the session. Tones occurred at different pitches and intensities; either 3000 Hz, 68 dBA or 1500 Hz, 83 dBA. Standard and oddball pitch frequencies were switched in matched recording sessions to control for pitch differences. Average inter-tone intervals (ITIs) were 1 or 2 seconds depending on the session. ITIs were either random or rhythmic. We did not see a difference between the responses to random or rhythmic ITIs, so we pooled the sessions. The front wall of the standard operant chamber was covered with cardboard to ensure that ERPs were not influenced by the rats trying to lick for water.

II.1.b. Single-Tone Paradigm

Single-tone sessions were completed to see if stimulus specific adaptation (SSA) could account for the oddball effect. These sessions were the same as their respective two-tone sessions except that either the standard or oddball beeps were omitted (Figure 7). Therefore sessions consisted of standard-only or oddball-only beeps. Tones in this paradigm both occurred at 3000 Hz, 68 dBA.

Oddball Only:

Standard Only:

Figure 7: Auditory single tone oddball-only and standard-only paradigms. Stimuli are labeled in red. Single tone sessions examine the influence of SSA on the oddball effect.

II.2. Go/No-Go Active Paradigm

While the passive oddball paradigms were thought to target bottom-up attention, the go/no-go paradigm used here targeted top-down goal-driven attention. The active paradigm required training. The rats learned to distinguish between and correctly respond to two types of
beeps, targets and distractors. The target tones were at 3000 Hz, 75.8 dBA and occurred 50% of the time. Distractor tones were at 1500 Hz, 75.7 dBA and occurred 50% of the time. Tones were played for 100 milliseconds. The target tone activated the water pump in response to a lick, while the distractor tone did not yield a water reward. Premature licks and false alarm trials resulted in time out periods where the next trial would not start for an additional 5 seconds. Licks during the penalty period, as well as initial false alarms or premature licks yielded either air puffs or a buzzing noise. The buzzing noise consisted of alternating sets of 32 pulses at 1500 Hz for 5 milliseconds with sets of 32 pulses at 1000 Hz for 5 milliseconds. The buzzing noise overall occurred at 80.2 dBA. The air puffs have more recently replaced the buzzing noise to deter incorrect trials in 4 of the 10 sessions analyzed here. Air puffs adjacent to the lickometer occurred for 200 milliseconds each at 15 psi. After each beep, the rats had 3 seconds to respond (or not respond). Table 1 lists the different types of trials.

Training sessions lasted 40-60 minutes. Once training performance levels reached appropriate levels according to a chi-squared test of hits/targets and false alarms/distractors, surgery was completed. The chi-squared test was used to determine whether the rats were responding differently to target tones and distractor tones. Specifically, their performance was deemed good if this difference showed that the rats were correctly licking for water during the target beeps and ignoring distractor beeps with a $p$-value of less than 0.05.

**III. Animal Surgery**

Rats that performed at acceptable behavioral levels were given water *ad libitum* for at least 3 days before surgery. Mike Wiest performed the electrode implantation surgeries. Rats were anesthetized with isoflurane (1-2% in O$_2$). They were then placed in a stereotaxic apparatus. Chronic 32-microelectrode arrays (Innovative Neurophysiology, Inc.) were inserted into right medio-dorsal frontal (2.0 mm anterior to bregma, 0.75 mm dextrolateral to the midline and 1.5 mm beneath the brain surface) and right posterior parietal (4.15 mm posterior to bregma, 3.5 mm to the right of midline and 1.2 mm beneath the brain surface) cortex in accordance with the coordinates set by Paxinos and Watson’s Rat Brain Atlas (2008) (Figure 8). Both arrays had inter-electrode spacing of 150 micrometers and row spacing of 300 micrometers. The frontal array was a 2 X 6 grid, and the parietal array was a 4 X 8 grid. Dental cement was used to hold arrays in place. Three skull screws in contact with the brain surface at left frontal, left parietal,
and right occipital locations were used as attachment sites for ground wires from each array. Antibiotic ointment was used to clean the incision site. Bupivacaine (0.125%, 2 mg/kg, 0.16 mL/100 g) was applied under the scalp before the incision as a local anesthetic. Post-operative pain was reduced by administration of Buprenorphine (0.01-0.05 mg/kg) before incision and after surgery for 48 hours. Rats were allowed to recover for 1 week after surgery while being monitored daily for signs of pain with access to food and water ad libitum.

**Figure 8: Placement of chronic microelectrode arrays in right frontal and right parietal cortex according to Paxinos and Watson’s Rat Brain Atlas (2008).** The frontal array is colored in red, and the parietal array is colored in blue. The image models the arrays in a sagittal section from the right side of the brain of a rat (Adapted from Herzog, 2012).

**IV. Electrophysiological Recordings**

Isoflurane (1-2%) was used to anesthetize the rats briefly while plugging recording headstages into their implants. After 10-15 minutes of anesthesia recovery or after normal locomotion was seen, Cerebus Data Acquisition System (Blackrock Microsystems) was used to record LFP activity during active and passive oddball sessions at 1000 Hz sampling rate. A lowpass 250 Hz filter was applied to the LFPs to filter out high frequency artifacts or noise. NeuroExplorer was used to transfer LFPs into MATLAB for data analysis.
V. Data Analysis

LFPs were selected and used to calculate oddball and SSA effect sizes for each LFP component. Component latencies were also analyzed.

V.1. Pre-processing

LFP recordings were transferred to MATLAB using NeuroExplorer. MATLAB was used to analyze the LFPs using custom routines. LFPs were chosen based on visual inspection of raw-evoked LFPs to exclude poor quality channels or channels with severe artifacts. Trials were defined by segmenting LFPs from 250 milliseconds before the stimulus onset to 500 milliseconds after the stimulus in the passive paradigm and 500 milliseconds before the stimulus to 3 seconds after the stimulus in the active paradigm. Artifacts seen as signals above 1500 microvolts or as flat lines were automatically discarded in MATLAB, while other artifacts that passed the cut were manually discarded (Figure 9A). Manual rejection sorting rules included: trials with impossibly steep inclines or declines (180°), trials with flat horizontal Event related potentials (ERPs), and trials exhibiting large mu rhythm-like signals at 10 Hz. Fontanini and Katz have shown that 7 to 12 Hz activity reflects a disengaged state and a withdrawal from experimental conditions (Fontanini and Katz, 2005). After artifact rejection, sessions with fewer than 75 trials were omitted from the analysis.

Figure 9: Examples of single trial LFPs. Some single trials required manual rejection (A), while others did not and were accepted into ERPs (B). Trials were manually thrown out if they displayed impossibly steep inclines or declines (180°), flat horizontal lines, or large 10 Hz mu-like signals. The tones occurred at 500 milliseconds. This particular session from R1 contained 419 trials before filtering and 367 after manual sorting.
ERPs were then generated by averaging over LFP trials. ERPs generated by each type of beep in the passive paradigm (oddball, standard, oddball-only, and standard only), as well as each type of trial elicited by target (hits and misses) and distractor (correct rejections and false alarms) tones in the active paradigm were calculated. Grand average ERPs were generated across sessions by averaging 1 LFP for each area from each session.

**V.2 ERP Peak Constraints**

Peak amplitudes and latencies from ERPs for each session were calculated in MATLAB. Component latency windows were defined based on visual inspection of grand average ERPs as well as those of individual sessions. In other words, the go/no-go and passive oddball ERP components were constrained to occur in specified time periods after the beeps. These latency windows for each peak are shown in Table 2. In the passive oddball paradigm N1 was defined between 30 and 100 milliseconds, but before the latency of P2. P2 was between 48 and 140 milliseconds, N2 was between the latency of P2 and 300 milliseconds, P3E was between 140 and 300 milliseconds, and P3L was between 300 and 500 milliseconds. In the go/no-go paradigm N1 was constrained between 10 milliseconds and the latency of P2, P2 was between 300 milliseconds and 2 seconds, N2 was between the latency of P2 and 1 second, and P3 was between 300 milliseconds and 2 seconds. Definitive early and late P3 peaks were not seen in the go/no-go ERPs (Figure 10), so a larger single window was defined.

<table>
<thead>
<tr>
<th>Component</th>
<th>Go/No-Go</th>
<th>Passive Oddball</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>10 ms - P2</td>
<td>30 - 100 ms (&lt; P2)</td>
</tr>
<tr>
<td>P2</td>
<td>300 ms - 2 s</td>
<td>48 ms - 140 ms</td>
</tr>
<tr>
<td>N2</td>
<td>P2 - 1 s</td>
<td>P2 - 300 ms</td>
</tr>
<tr>
<td>P3E</td>
<td>NA</td>
<td>140 - 300 ms</td>
</tr>
<tr>
<td>P3L</td>
<td>300 ms - 2 s</td>
<td>300 ms - 500 ms</td>
</tr>
</tbody>
</table>

**Table 2: ERP component constraints for go/no-go and passive oddball paradigms.** Windows were defined based on visual inspection of individual session and grand average ERPs. Early and late P3 components (P3E and P3L) were seen in passive oddball ERPs, but they were not seen in go/no-go ERPs.
V.3. Oddball and SSA Effect Calculations

The oddball and SSA effect sizes were calculated separately. The oddball effect size was calculated as the difference between the peak amplitude in response to the tone presented as an oddball vs. the amplitude in response to the same tone presented as a standard: $\Delta\text{ODD} = \text{AMP}(\text{High-pitched oddball}) - \text{AMP}(\text{High-pitched standard})$. This subtraction method controls for the likelihood that different pitches elicit different response amplitudes even when the different pitches are equally probable (Knight et al., 1985).

Single-tone recordings were analyzed similarly to examine the effects of ITI on amplitude. SSA effect size was calculated as the difference between the response amplitude when the tone was presented rarely (oddball-only session) vs. the response amplitude when the same tone was presented frequently (standard-only session): $\Delta\text{SSA} = \text{AMP}(\text{High-pitched lone oddball}) - \text{AMP}(\text{High-pitched lone standard})$ (Imada et al., 2013).

VI. Statistics

In the passive paradigm paired two-tailed $t$-tests were used to see if effect sizes were significantly different from 0, and a three-way ANOVA was used to compare oddball and SSA

Figure 10: Go/no-go paradigm does not seem to exhibit early and late P3 peaks. Latencies of maximum ERP amplitudes between 300 milliseconds and 2 seconds after tones are shown for 7 sessions in 4 rats. Beep onsets occurred at 0 seconds. Yellow bars denote frontal latencies, while blue bars denote parietal latencies.
effect sizes. *T*-tests were performed according to the Bonferroni correction criterion with significant *p*-values less than 0.005. The factors in the ANOVA were brain AREA (frontal and parietal), CONTEXT (two-tone and single tone paradigms), and PEAK (N1, P2, N2, P3E, and P3L) with within-subjects levels of context.

A three-way repeated ANOVA was used in the active paradigm to compare ERP amplitudes of the different trial types. The factors were PEAK (N1, N2, P2, and P3), AREA (frontal and parietal), and TRIAL TYPE (hits, misses, correct rejections, and false alarms) with within-subjects levels of all three factors. Comparisons of grand average ERPs in different conditions were assessed by two-tailed paired *t*-tests at the point of maximum difference between ERPs for the indicted trial types to get an idea of their potential significance, although the proper way to statistically compare the conditions is the three-way ANOVA. Similarly, we compared peak amplitudes as defined above (V.2) for different trial types with two-tailed paired *t*-tests based on the variability across sessions.

**VII. Comparisons**

ERP components were compared within and between both paradigms. Analysis of ERPs in the passive paradigm may help reveal information about mechanisms involved in bottom-up attention, while analysis of ERPs in the active paradigm may help reveal mechanisms of top-down attention.

**VII.1. Passive Analysis: SSA and Rare Tone Enhancements**

High frequency oddball tone ERP peak amplitudes were compared with high frequency standard tone ERP peak amplitudes to see if ERPs were enhanced by infrequent tones. Oddball and SSA effect sizes were then calculated and compared using the ANOVA described above (VI) to see if SSA could account for rare-tone enhancements.

**VII.2. Active Analysis: Neural Correlates of Licking**

In our go/no-go paradigm, the physical action of licking may have affected ERP components. This alteration may also have been due to other involved processes, such as activation of taste receptors on the tongue, motor planning, or activation of muscles involved in swallowing. We did not encounter this licking confound in our passive oddball paradigm since
no licking was involved. Licking or the processes involved in tasting and swallowing reward water may have affected these components rather than actual attentional processing. Motor planning may also have altered ERP peaks that were evoked before licking responses. If this lick hypothesis is correct, components in hit and false alarm trials would be greater than respective components in miss and correct rejection trials, (though in principle lick effects could be cancelled by attentional effects).

**VII.3 Active Analysis: Neural Correlates of Attention**

Previous studies have found evidence that P3 and other ERP components are involved in attentional processing. Therefore we completed comparisons to test this hypothesis by comparing various trial type amplitudes in the go/no-go paradigm.

The potential licking confound was avoided first by comparing trials that did not involve licking. We compared peak amplitudes during miss and correct rejection trials, both that did not involve licking. Misses signified poor attention, while correct rejections showed good attention for response inhibition. If P3 was larger for correct rejections than misses, it could signify the behavioral inhibition that prevents licking. Other components, such as P2 and N2, were also analyzed and may contribute to attentional processing for response inhibition.

We also wanted to compare other types of trials that did involve licking to investigate P3, as well as other peaks, as a neural correlate of attention. We compared hits and false alarms, since the rats licked in both types of trials. Hits showed good attention for responding, and the rats got a water reward. False alarms showed poor attention for response inhibition. In false alarm trials the rats licked, but did not get a water reward. A larger P3 on hit vs. false alarm trials could support P3 as being a correlate of attention, but might also reflect correlates of receiving a water reward.

Finally we wanted to compare trials with licking and without licking, which were hits vs. misses and correct rejections vs. false alarms. These comparisons may reveal attentional effects of behavior on changes in ERP component amplitudes to the extent that we can rule out or eliminate components caused by licking.
IV.4 Human vs. Rat, Active vs. Passive Comparisons

We compared our ERP amplitudes and latencies with other rat and human studies in active and passive tasks. Studies have shown that active conditions elicit larger amplitude and longer latency components (Oades et al., 1995, Bennington and Polich, 1999, Zenker and Barajas, 1999). Respective active and passive peak latencies have also been shown to be similar between rats and humans (Sambeth et al., 2003). We expected to see early and late P3 peaks in the active condition with hit trials evoking larger P3Ls (300-500 milliseconds) and miss trials evoking smaller P3Ls since P3b in humans is associated with voluntary top-down attention (Polich, 2007, Wronka et al., 2012). Passive paradigms should mainly exhibit P3E (200-240 milliseconds) effects since that subcomponent in humans is associated with involuntary attention to stimulus differences (Wronka et al., 2008). P3 early and late component area localizations were also examined since in humans P3a is localized in frontal cortex while P3b is localized in parietal cortex (Volpe et al., 2007), so we might have seen localization of early and late P3 peaks in these respective areas.
Results

To look into how various event related potential (ERP) components contribute to attentional processing in rats ERPs were recorded from medio-dorsal frontal and posterior parietal cortex while awake rats were exposed to passive oddball or go/no-go auditory paradigms.

I. Passive Oddball Rare Tone Enhancements

Bottom-up stimulus-driven attentional mechanisms were assessed in the passive paradigms. Two-tone oddball sessions were recorded to see if ERPs show rare-tone enhancements. Single-tone sessions were also recorded to isolate the effect of stimulus specific adaptation (SSA) on these enhancements. Two-tone averages were obtained from 61 session pairs (high-pitched oddball and low-pitched oddball) in 14 rats (31 frontal session pairs, 30 parietal session pairs). Single-tone averages were obtained from 32 session pairs (frequent single-tone and infrequent single-tone) in 9 rats (18 session pairs each for frontal and parietal cortex). Alternating standard and oddball pitches and analyzing data from just the high-pitched oddball and standard tones together controlled for pitch differences.

To look into whether individual components manifest larger amplitudes in response to rare tones in a background of frequent tones in the two-tone oddball paradigm, we compared means of grand average differences between standard and oddball ERPs in both frontal (A) and parietal (B) areas (±SE) (Figure 11 left). Since varied latencies between animals could have skewed grand averages, component amplitudes derived from peaks at different latencies in different sessions were also calculated. These averages of peak amplitudes derived from individual session ERPs of each of the peaks in the two-tone paradigm in both areas and are shown on the right (±SE). Peaks in both frontal (A) and parietal (B) cortex show trends toward larger amplitude oddball tones compared to standard tones in the grand average ERPs (left). These trends were shown to be significant in individual session peak amplitudes (right) with Bonferroni correction criterion in all of the peaks except for frontal P2 and parietal N1 (two-tailed t-tests, \( p < 0.005 \)). These differences are not due to variations in pitch because our methods compared responses from matched paired sessions where the tones of the standard and oddball were switched.
Figure 11: Grand average ERPs (left) and individual session peak amplitudes (right) for the two-tone oddball paradigm (±SE). ERPs were averaged over session-pairs in 14 rats in frontal (A) and parietal (B) cortex. Traces from the oddball tone stimuli are shown in red, and traces from the standard tone stimuli are shown in blue. Shaded regions show 1 standard error. Beeps occurred at 0 seconds. Asterisks denote significant rare-tone enhancements (two-tailed t-tests, \( p < 0.005 \)). Both frontal and parietal areas show evoked responses that were significantly larger toward oddball tones compared to standard tones in grand average ERPs (left) as well as individual session amplitudes (right).

Grand average ERPs (Figure 12A and B left) and individual session peak amplitudes (Figure 12A and B right) were also calculated for single-tone sessions to examine the effects of SSA on rare-tone enhancements. In single-tone sessions background tones were omitted so that only adaptation to that specific tone was seen. Oddball repetition and separate standard repetition rates were presented in paired sessions. Therefore the response amplitude to the tone in the two
contexts can be attributed to the different ITIs in the two paired single-tone sessions, but not to any putative memory comparison of the current stimulus with a recent history of different pitches, such as might occur in the two-tone paradigm. The same N1, P2, N2, P3E, and P3L peaks were seen in grand averages of single-tone sessions (±SE) (left). Both areas show grand average ERPs that showed trends towards being larger in response to the infrequent tones (left). Individual session peak amplitudes (right) showed significant differences between oddball and standard tones in frontal P3E, frontal P3L, and parietal P3E (two-tailed t-tests, p < 0.005).

![Figure 12](image)

**Figure 12:** Grand average ERPs (left) and individual session peak amplitudes (right) for the single-tone oddball paradigm (±SE). ERPs were averaged over session-pairs in 9 rats in frontal (A) and parietal (B) cortex. Traces from the oddball repetition rate are shown in red, and traces from the standard repetition rate are shown in blue. Shaded regions show 1 standard error. Tones occurred at 0 seconds. Asterisks denote significant rare-tone enhancements (two-tailed t-tests, p < 0.005). Significant differences between oddball and standard tone frequencies were seen in frontal P3E, frontal P3L, and parietal P3E individual session peak amplitudes (right).
Effect sizes were calculated in both frontal (A) and parietal (B) areas for single (red) and two-tone (blue) conditions (Figure 13). Effect sizes were calculated as the difference between the response amplitude to a tone presented as the oddball and the response amplitude to the same tone presented as the standard. This calculation was reversed for the negative peaks so that enhanced peak amplitude responses to the oddball tone correspond to positive effect sizes for positive and negative peaks. Statistically significant oddball enhancements in the two-tone paradigm (blue) were seen in both frontal (A) and parietal (B) cortex in all of the five peaks, as seen through the Bonferroni correction criterion (two-tailed $t$-tests, $p < 0.005$), except in frontal P2 and parietal N1 (Figure 13). These rare tone enhancements may play a role in involuntary neural allocation of attention to rare stimuli.

![Figure 13: Oddball and SSA effect sizes (±SE)](image)

**Figure 13: Oddball and SSA effect sizes (±SE).** Average “oddball” effect sizes from the two-tone paradigm were calculated in blue for frontal (A) and parietal (B) cortex. Average “SSA” effect sizes were calculated for the single-tone paradigm in red for both areas. Positive effect sizes correspond to larger responses to infrequent tones for both positive and negative components. Asterisks next to the blue bars denote significant oddball enhancements from the two-tone paradigm, and asterisk next to the red bars denote significant oddball enhancements in the single-tone paradigm based on Bonferroni-corrected two-tailed $t$-tests ($p < 0.005$). Averages were obtained across 61 two-tone session pairs and 36 single-tone sessions. A three-way ANOVA revealed a significant main effect of tone context (i.e. blue bars > red bars) on rare-tone response enhancements ($dof = 484, F = 7.9, p = 0.019$), showing that the response enhancements cannot be accounted for by SSA alone.

35
Average effect sizes from single-tone sessions were compared with effect sizes from the two-tone context to tell if SSA could account for rare-tone enhancements. If oddball effects are greater than their corresponding SSA effects, than SSA is not the only cellular mechanism contributing to enhanced responses to rare tones. Figure 13 shows this comparison with mean oddball (blue bars) and SSA (red bars) effect sizes plotted together by component peaks for frontal and parietal cortex. Asterisks adjacent to blue bars show significant rare tone-enhancements in the two-tone paradigm, while asterisks adjacent to red bars show significant enhancements in the single-tone paradigm (two-tailed t-tests, \( p < 0.005 \)).

To statistically compare oddball and SSA effect sizes we applied a three-way ANOVA. The factors were brain AREA (frontal and parietal), CONTEXT (two-tone and single-tone paradigms), and PEAK (N1, P2, N2, P3E, and P3L) with within-subjects levels of context. There was a significant effect of context \((dof = 484, F = 7.9, p = 0.019)\), showing that the oddball effect was significantly larger than the SSA effect. The effect of brain area was not significant \((p = 0.07)\), but the trend was toward larger effect sizes in the parietal cortex. There was a significant effect of peak \((p = 10^{-9})\). We did not pursue the peak effect because the purpose of our comparisons was to look at differences between the contexts of the oddball and SSA effects. These results show an overall greater rare-tone enhancement for the two-tone context, but this difference was not localized to frontal or parietal cortex, or to a particular latency window. We can nevertheless note that the largest difference between SSA and oddball effect sizes was at the parietal P3E.

II. Go/No-Go Active ERPs

The active go/no-go condition was used to test the hypothesis that P3 and/or other components were larger when attentional processing was used to: (a) correctly respond to target beeps or (b) correctly inhibit action after distractor beeps. Go/no-go ERP grand averages and individual session peak amplitudes were obtained from 10 sessions in 5 rats, with some sessions omitted in certain comparisons due to low trial numbers (i.e. < 10 trials for any trial type).

Although we were interested in attentional effects of ERPs in the go/no-go paradigm, the physical action of licking may have altered ERP components. This alteration may also have been due to other involved processes, such as activation of taste receptors on the tongue, motor planning, or activation of muscles involved in swallowing. To test this “lick hypothesis” that
licking affects ERPs, grand averages for trials that involved licking (hits and false alarms) were calculated and compared with trials that did not involve licking (misses and correct rejections) in frontal (A) and parietal (B) cortex (±SE) (Figure 14 left). Individual session peak amplitudes were also calculated for both areas (Figure 14A and B right). In the grand average ERPs of both frontal (A) and parietal (B) areas, lick trials showed a trend toward larger N2 components (left). A histogram of lick times (Figure 14C) showed that most licks occurred between 0.2 and 0.6 seconds, which is about the latency of N2. P3 in both brain areas seems to show a trend towards being larger for no-lick trials in the individual session peak amplitudes (right); however this trend conflicts with the trend seen in grand average ERPs (left) showing lick trials producing larger P3s.

Although the proper way to statistically compare different conditions without incurring a multiple comparisons problem is the ANOVA described later, we applied two-tailed t-tests to certain pair wise comparisons to get an idea of their potential significance. The increases in N2 in lick trials did show significance using this method in frontal ($p = 0.03$) and parietal ($p = 0.02$) areas. The P3 trends in the individual session peak amplitudes were not significant (frontal $p = 0.13$, parietal $p = 0.09$).
Figure 14: Grand average ERPs (left) and averages of peak amplitudes (right) for lick trials (blue) vs. no-lick trials (red) (±SE). ERPs relative to beeps at 0 seconds were averaged over 7 sessions from 4 rats in frontal (A) and parietal (B) cortex during the go/no-go active attentional paradigm. A histogram of lick times relative to beeps at 0 seconds is also shown (C). Lick trials include hits and false alarms. No-lick trials include misses and correct rejections. Shaded regions and error bars show 1 standard error. Asterisks denote significant differences between trial types (two-tailed t-tests, \( p < 0.05 \)). N2 peaks show trends toward being larger to lick trials in both areas’ ERP averages (left). P3s in both areas show trends toward being larger in no-lick individual session peak amplitudes (right), but this trend is contradicted by an opposite trend in the ERPs (left). A majority of licking occurred during the latency of N2 (C).
To avoid the possible licking confound we initially compared trials that did not involve licking, but signified good and poor attention to test the hypothesis that these ERP components may be neural correlates of attention. Any effect elicited by licking would not be seen in these trial types since no licking was involved. We compared grand average ERPs during miss and correct rejection trials (±SE) (Figure 15A and B left). The rats responded correctly 86% (±10%) of the time in response to target tones. Their responses to targets were significantly different from responses to distractors according to the chi-squared test of hits/targets and false alarms/distractors with p values of less than 0.05. Therefore misses signified poor attention, while correct rejections signified good attention for response inhibition. Individual session peak amplitudes over the 5 rats are shown on the right. In both frontal (A) and parietal (B) areas, miss and correct rejection ERP components seemed similar (left). However miss trials in the parietal area (B) seem to show a trend toward having larger grand average ERPs (left) as well as individual session peak amplitudes (right). This trend was not significant when two-tailed t-tests were applied to the parietal grand average ERPs (p = 0.07) and individual session peak amplitudes (p = 0.09).
We also wanted to compare other types of trials to examine effects of attentional performance on ERPs, so we then compared trial types that both involved licking (hits and false alarms). Grand average traces (left) and individual session peak amplitudes (right) are plotted in Figure 16 (±SE). Hits signified good attention and perception, and the rats got a water reward. False alarms showed poor attention for response inhibition. In false alarm trials the rats licked and did not get a water reward. Hit trials in both brain areas show a trend toward larger P2 components than those evoked in false alarm trials in the grand averages (left) and individual session peak amplitudes (right).

**Figure 15**: Grand averages ERPs (left) and individual session peak amplitudes (right) over varied latencies for correct rejections (blue) and misses (red) (±SE). Grand averages from frontal (A) and parietal (B) cortex were calculated (left). Individual session peak amplitudes were calculated and averaged over sessions and rats (right). Shaded regions and error bars show 1 standard error. Correct rejections showed good attention for response inhibition, while misses showed poor attention. Neither of these trial types involved licking. ERPs were averaged over 9 sessions from 5 rats. Beeps occurred at 0 seconds. In the parietal area (B) misses showed a trend toward evoking larger amplitude P3 responses in ERP grand averages (left) as well as individual session peak amplitudes (right).
session peak amplitudes (right). The differences in the grand averages were significant for P2 using two-tailed t-tests in both areas ($p = 0.03$ in both areas), but they were not significant in individual session peak amplitudes ($p = 0.15$ for both areas). In parietal cortex (B) P3 shows a trend toward being larger in false alarm grand average ERPs (left), but this difference was not significant ($p = 0.22$). The P2 effect cannot be caused by water received on hit trials since the rats do not lick until the latency of N2 (Figure 14), while the P3 trend might be caused by receiving water since P3 occurs after N2.

**Figure 16:** Grand average ERPs (left) and individual session peak amplitudes (right) for hit trials (blue) vs. false alarm trials (red) (±SE). Grand averages (left) and individual session amplitudes (right) were calculated in both frontal (A) and parietal (B) brain areas. Shaded regions and error bars show 1 standard error. Hits showed good attention, while false alarms showed poor attention for response inhibition. Hits warranted a water reward. Both trial types involved licking. ERPs were averaged over 8 sessions from 4 rats. Beeps occurred at 0 seconds. Asterisks denote significant differences between trial types (two-tailed t-tests, $p < 0.05$). Hit trials show a trend toward evoking larger P2 components in grand average ERPs (left) as well as individual session amplitudes (right) in both brain areas. P3 in the parietal cortex (B) shows a trend toward being larger in grand average ERPs for false alarms (left).
We also completed comparisons of the remaining combinations of trial types to test for attentional effects in ERP components. Grand average ERPs (left) and individual session peak amplitudes (right) of hits and misses are plotted in Figure 17 (±SE) for both frontal (A) and parietal (B) areas. Hit trial ERP grand averages (left) showed a trend toward eliciting larger P2 components compared to misses. The trend was significant according to two-tailed t-tests in frontal ($p = 0.001$) and parietal cortex ($p = 0.005$). Hits also showed a trend towards evoking larger P2s in parietal individual session peak amplitudes (B right), but this trend was not significant ($p = 0.20$). Miss trials seem to elicit larger P3s in both the grand averages (left) and individual session amplitudes (right) in both brain areas. T-tests showed that this difference was significant in individual session peak amplitudes in the parietal cortex ($p = 0.04$), but not in frontal cortex ($p = 0.07$). The difference in P3 grand average ERPs was not significant (frontal $p = 0.32$, parietal $p = 0.37$).
Correct rejections and false alarm grand average ERPs (left) as well as individual session peak amplitudes (right) are also compared in Figure 18 in both frontal (A) and parietal (B) areas (±SE). False alarm trials show a trend toward larger N2 peaks in both brain areas compared to correct rejection trials in grand average ERPs (left). Two-tailed t-tests showed that this difference...
was significant in the frontal cortex \((p = 0.02)\), but not in the parietal cortex \((p = 0.69)\). However this trend was not evident in individual session amplitude data (right).

![Figure 18: Grand average ERPs (left) and individual session peak amplitudes (right) for correct rejections (blue) and false alarms (red) (±SE).](image)

Grand averages (left) and individual session peak amplitudes averaged over varied latencies (right) were calculated in both frontal (A) and parietal (B) brain areas. ERPs were averaged over 7 sessions from 4 rats. Shaded regions and error bars show values of 1 standard error. Beeps occurred at 0 seconds. Asterisks denote significant differences between trial types (two-tailed t-tests, \(p < 0.05\)). Correct rejections showed good attention for response inhibition, while false alarms showed poor attention for response inhibition. False alarms involved licking, which appears to increase the amplitude of N2 (see Figure 14). N2 peaks in both brain areas show trends toward being larger during false alarm trials in grand average ERPs (left). This trend was not evident in individual session amplitude data (right).

To statistically compare the relationship among trial types and ERP component amplitudes, we applied a repeated three-way ANOVA. The factors were PEAK (N1, N2, P2, and P3), brain AREA (frontal and parietal), and TRIAL TYPE (hits, misses, correct rejections, and
false alarms) with within-subjects levels of all three factors. We saw a significant effect of peak \((\text{dof} = 3, F = 16.4, p = 1 \times 10^{-4})\), showing that there was a significant difference between ERP peak amplitudes. There was no main significant effect of trial type \((p = 0.35)\) or area \((p = 0.69)\). There was a trend in the interaction effect between peak and trial type \((p = 0.08)\). While the interaction was not significant, based on the pairwise comparisons reviewed above (Figures 14-18) the ANOVA trend might suggest that increased P2 for hits, P3 for misses, N2 for lick trials, and N2 for false alarms might become significant with additional future recording sessions.

Overall it seems as though attentional and/or licking effects show trends toward altering some of the ERP peaks evoked during the go/no-go paradigm. P2s showed trends towards being increased in hit trials (Figures 16 and 17), while P3s showed trends toward increasing during miss trials (Figure 15 and 17). Lick trials showed trends towards increased N2 components (Figure 14), which may also explain the false alarm N2 increases seen in the comparison between correct rejections and false alarms (Figure 18).

III. Active and Passive Paradigm Comparisons

Comparisons between ERPs elicited during the passive and active paradigms may reveal differences in ERPs caused by different kinds of attention. The passive paradigm is believed to engage bottom-up attention, and the active paradigm engages top-down attention. Individual session peak amplitudes for each component were calculated for passive (A) and active (B) paradigms in both frontal (left) and parietal (right) areas (±SE) (Figure 19). The active condition did not produce a visible P3E and P3L (Figure 10), as seen in the passive paradigm (Figure 11). In general, the active go/no-go condition (Figure 19B) evoked larger ERPs than those in the passive oddball condition (Figure 19A). In the passive condition the largest amplitude ERPs resulted from oddball tones, with P2 in the parietal cortex up to almost 25 microvolts (A right). In the active condition hit trial ERP amplitudes were higher, with P2 up to 55 microvolts in the parietal cortex (B right).
ERP latencies were then compared across both brain areas in passive and active conditions (±SE) (Figure 20). All trial types for each individual ERP peak showed similar latencies within the windows defined (Table 2). N2 and P3 peaks in the active go/no-go task (Figure 20A) seem to occur at longer latencies than their respective peaks in the passive oddball paradigm (Figure 20B). N2 in the active paradigm occurred at about 400 milliseconds post beep compared to about 200 milliseconds in the passive paradigm. P3 in the active paradigm (B) had a latency of about 1.3 seconds in both areas, while P3L in the passive paradigm (A) occurred at about 400 milliseconds in both areas. P2 and N1 occurred at similar latencies between paradigms.

Figure 19: Passive (A) and active (B) paradigm ERP peak amplitudes (±SE). Averages in the passive paradigm (A) were obtained across 61 two-tone session pairs in 14 rats and 36 single-tone sessions in 9 rats in both frontal and parietal cortex. Averages from the active paradigm (B) were obtained from 7 sessions in 4 animals in both brain areas. Active task amplitudes (B) in general seemed to be higher than those in the passive task (A).
Figure 20: Passive (A) and active (B) paradigm ERP peak latencies (±SE). Averages in the passive paradigm (A) were obtained across 61 two-tone session pairs in 14 rats and 36 single-tone sessions in 9 rats. Averages from the active paradigm (B) were obtained from 7 sessions in 4 rats. N2 and P3 seemed to occur at longer latencies in the active paradigm.
Discussion

Medio-dorsal frontal and posterior parietal event related potentials (ERPs) were recorded during active and passive auditory attentional paradigms to examine the effects of top-down and bottom-up attentional processing on ERPs in rats. Analysis of passive oddball and active go/no-go data may help identify the functional significance of various peaks as well as identify species similarities and differences between rats and humans. In the passive paradigm we saw rare-tone enhancements in the two-tone sessions that were significantly greater than those seen in the single-tone sessions suggesting that these enhancements cannot be accounted for solely by stimulus specific adaptation (SSA) (Figure 21A). In the active paradigm lick trials produced greater N2 peaks compared to no-lick trials, and hit trials produced larger P2 peaks compared to false alarms and misses (Figure 21B). The active paradigm produced larger peak amplitudes in general and greater N2 and P3 peak latencies compared to the passive paradigm. While the active and passive tasks did evoke different ERP peaks, the functional significance of the peaks in comparison to human data is still unclear. These findings help extend the correspondence between human and rat sensory processing, while also beginning to identify species differences.
Figure 21: Schematic ERPs illustrate main findings from the passive (A) and active (B) tasks. The oddball effect from the two-tone sessions (left) was greater than the stimulus specific adaptation (SSA) effect from the single-tone sessions (right) (A). Therefore SSA cannot account for the rare-tone oddball effect enhancements seen in the two-tone sessions. In the active task lick trials evoked large N2 peaks compared to no-lick trials (B). Hit trials produced greater P2 peaks compared to misses and false alarms (B). Active peak amplitudes were greater in general compared to the passive case, and active N2 and P3 latencies were greater than those seen in the passive paradigm.
**I. Passive Oddball**

To constrain the mechanisms of enhanced responses to rare tones, ERPs were recorded while rats were exposed to single or two-tone passive auditory oddball paradigms. Low and high frequency oddball sessions were collected on the same or successive day in pairs in varying orders to control for potentially different obligatory responses to the different pitches. Comparisons were therefore only made between responses to the same pitch in different contexts.

**I.1. Rare tone enhancements in the two-tone paradigm**

Significant “oddball effect” enhancements were seen in N2, P3E, and P3L ERP components in both frontal and parietal areas (Figure 11). N1 in the frontal cortex as well as P2 in the parietal cortex also showed significantly larger oddball effect sizes. These findings are consistent with previous studies (Yamaguchi et al., 1993b, Bennington and Polich, 1999), although some studies only found significant effects in certain components if at all (Shinba, 1997, Hattori et al., 2010). Neither of these latter studies controlled for pitch differences between oddball and standard tones, so it is difficult to interpret their results. While we found both early and late P3 peaks in these components, it is unclear why other previous auditory passive studies have only found one or the other (Yamaguchi et al., 1993b, Shinba, 1997, Sambeth et al., 2003, Hattori et al., 2010). Latency variations among rats in grand average ERPs may have obscured effects. The fact that we were able to identify statistically significant rare-tone response enhancements by focusing on peak amplitude measurements from each session, rather than relying on direct subtraction of ERPs in different conditions, suggests that latency variations might obscure grand averages and supports the validity of our approach.

**I.2. Enhancements cannot be accounted for by Stimulus Specific Adaptation (SSA)**

Rare-tone enhancements in the two-tone paradigm were significantly larger than enhancements in the single-tone paradigm. Therefore enhancements to infrequent tones cannot be accounted for solely by SSA among the responding neurons between the periphery and the frontal or parietal recording sites. They may be considered to reflect “true deviance detection” involving an implicit comparison of the current stimulus to the context of recent past stimuli. All components except the parietal N1 and frontal P2 supported this trend, which suggests that the
non-SSA mechanism responsible for the augmented rare-tone response in the two-tone context may be distributed across latencies and between frontal and parietal cortex. It is also possible that the extra rare-tone enhancements in the two-tone context may be inherited by the frontal cortex from the parietal cortex. Either way the deviance detection effect was dominated by the parietal P3E (Figure 13).

The effects seen in the parietal and frontal areas should reflect response properties of neurons in upstream areas up to and including the recording sites. Cortical and sub-cortical SSA differ in that sub-cortical SSA has relatively broad specificity and requires short gaps between stimuli (less than 50 milliseconds) (Anderson et al., 2009), and cortical SSA has high specificity, long sensory memory, and relatively long duration (up to at least 2 seconds) (Taaseh et al., 2011). Adaptation of sub-cortical responses to repeated stimuli has been reported in the auditory thalamus (Anderson et al., 2009, Yu et al., 2009). In general however, it appears that SSA can account for all response enhancements seen at subcortical sites (Duque et al., 2012, Anderson and Malmierca, 2013, and personal communication Miguel Malmierca, Institute of Neuroscience of Castilla y Leon and University of Connecticut).

In the cortex, results are conflicting as to whether SSA can account for auditory oddball enhancements. Ulanovsky et al., (2003, 2004) observed single unit recordings from cat primary auditory cortex that did exhibit SSA that accounted for rare-tone enhancements (Ulanovsky et al., 2003, Ulanovsky et al., 2004a). They proposed that SSA was a mechanism of sensory memory and novelty detection. On the other hand, Taaseh et al (2011) observed that SSA alone could not account for rare-tone enhancements in their study of multi-unit and LFP activity in the auditory cortex of anesthetized rats (Taaseh et al., 2011).

Our results show that ERP responses in posterior parietal cortex and medio-dorsal frontal cortex exhibit novelty detection that cannot be accounted for solely by SSA. This effect was greatest in the P3E parietal peak. These responses could reflect synaptic inputs to parietal neurons as dendritic potentials, but they might also be influenced by population spiking activity nearby. Therefore there is a possibility of non-SSA novelty detection inherited from A1, but additional non-SSA enhancement may also be generated downstream of A1.
II. Go/No-Go

An active go/no-go auditory attentional paradigm was used to examine ERP components as possible neural correlates of top-down attention. LFPs were recorded in frontal and parietal cortex while rats completed the go/no-go task. The task required the rats to be trained to correctly respond to target tones to receive a water reward and to correctly ignore distractor tones.

II.1. Potential Licking Confounds

Our null hypothesis was that licking may have altered ERP components. Gustatory-reward learning has been shown to induce changes in rat neural responses (Gutierrez et al., 2010). Oromotor function in rats involves inputs from many areas including the frontal cortex, striatum, and amygdala with main inputs from the brain stem and reticular formation (Travers et al., 2000). Recordings that we are analyzing are from frontal and parietal cortex, so activity from this licking pathway has the potential to influence ERPs. A study by Guitierrez et al. (2010) found that with learning in a go/no-go task involving a taste reward, spiking synchrony between neurons from different brain areas in the gustatory cortex as well as the number of licking-coherent neurons increased. Eimer et al. (2005) showed that ERPs during response preparation and ERPs during instructed attentional shifts were relatively similar, except in a negative peak at 140 milliseconds (N140) and a positive peak at 90 milliseconds (N90). N140 and P90 were both enhanced during response preparation. Therefore it is plausible that licking could be reflected in cortical ERPs.

We initially analyzed average results from trials that involved licking compared with trials that did not involve licking to determine if licking itself may have altered ERP components rather than variations in attentional processing (Figure 14). In our results most of the licks occurred between 0.2 and 0.6 seconds (Figure 14C), which corresponded closely to the latency of N2 between 0.3 and 0.6 seconds in the active go/no-go paradigm (Figure 20). N2 showed a trend toward being larger in lick trials vs. no-lick trials in the grand averages of both brain areas (Figure 14A and B left). In contrast, P3 showed a trend toward being larger in no-lick trials in the individual session peak amplitudes, although these differences were not significant, as shown by two-tailed t-tests (frontal $p = 0.13$, parietal $p = 0.09$). We saw trends towards increases in P2 on hit trials in grand average ERPs (Figures 16 and 17 left), which occur before lick responses.
(Figure 14C) at a latency of about 100 milliseconds (Figure 20). While this P2 increase might reflect response preparation in P2 for hits since the latencies seen in our results of about 100 milliseconds is close to the response preparation increase seen by Eimer et al. (2005) at 90 milliseconds, we consider this interpretation to be unlikely because we do not see a P2 increase in false alarm trials that also involve licking (Figures 15 and 18). Therefore the P2 increase is more likely to be related to attention. Since licking has been shown to change neural responses in rats, licking might have altered N2 and/or P3 peaks in the ERPs. Because licking occurred during the latencies of N2 and the trends seen in the individual session amplitudes were not significant for P3 while they were significant in N2 grand averages, it appears be more likely that licking alters N2 specifically in these results.

However, other studies did not find differences in ERPs and spiking activity due to behavioral responses. A study specifically looking at ERPs in go/no-go responses in humans found that ERPs were not affected by behavioral responses (Pfefferbaum et al., 1985). Pfefferbaum et al. (1985) showed that in humans N2 and P3 evoked responses were larger to auditory ‘no-go’ stimuli compared to ‘go’ stimuli regardless of whether subjects responded by silently counting (no motor response) or by manually tapping with a finger. They concluded that behavioral responses to somatosensory stimulation do not change ERPs. Shinba et al. (1999) concluded that lever pressing in an auditory oddball paradigm was not correlated with changes in neural responses at a parietal surface electrode and a hippocampal depth electrode in terms of ERPs or spiking activity in rats (Shinba, 1999). They showed that reaction time was not correlated with activation peaks, and that the average peak of unit firing occurred before lever pressing. Another study also did not find changes in ERPs evoked during auditory active go/no-go paradigms specifically to behavioral lever pressing responses in humans or rats (Sambeth et al., 2003).

Our lick-evoked N2 increase (and possible lick-evoked decrease in P3) may conflict with these studies because of task and species differences. The rats were likely to have been more motivated than humans in go/no-go tasks because they were deprived of water and therefore fulfilling a need, which may have altered ERP responses. Additional processes such as swallowing and tasting might also alter activation during hit trials specifically, while motor planning might alter early peaks. Different areas of the brain are also utilized in my experiments and therefore processing in various areas may not be identical. Shinba et al. (1999) and Sambeth
et al. (2003) had their rats respond by pressing a lever for a food paste or pellet reward, so these specific behavioral responses might produce different neural responses than licking for water in our go/no-go task.

In the future it may be possible to subtract out the licking evoked signals to isolate the parts of the ERPs that we think are attention-related, analogous to Del Cul et al. (2007). They identified a N2 peak in their ERPs that was evoked solely by their “mask” visual stimulus and subtracted the signal from overall ERPs to isolate the activity evoked by their visual target stimuli (Del Cul et al., 2007).

II.2. Attentional P2 effects

P2 has been shown to be associated with attention in humans (Picton and Hillyard, 1974, Crowley and Colrain, 2004). P2 amplitudes in humans have been shown to be decreased in schizophrenia (O'Donnell et al., 2004, Lijffijt et al., 2009). This ERP peak is thought to reflect integrative cognitive processing (Crowley and Colrain, 2004) and has been hypothesized to be associated with filtering mechanisms involved in attentional allocation (Lijffijt et al., 2009). We found larger P2 peaks in both frontal and parietal areas in hit trials compared to misses (Figure 17) and false alarms (Figure 16). P2 has been shown to be increased in active auditory oddball detection tasks in rats (Shinba, 1997, Hattori et al., 2010), which seems to agree with our results since hit trials signify good attentional responses. It is therefore possible that P2 filtering mechanisms were successfully applied to evoke increased P2 amplitudes during hit trials. However, the ANOVA only showed a trend in the interaction between peak and trial type ($p = 0.08$), so a larger number of sessions and rats might increase statistical power enough to show significance if the effect is real.

Even though hit trials involved licking, our results do not support licking as the cause of the increase in P2. P2 was not increased in our lick vs. no lick comparisons (Figure 14). The latency of P2 also did not correlate with lick response times (Figure 14C), and P2 is therefore unlikely to be a correlate of licking for a water reward since licking comes later during the latency of N2. Even though P2 was not increased in correct rejection trials (Figures 15 and 18), which also represent good attention, good attention for withholding a response (correct rejections) might look different in ERPs from good attention for responding (hits).
II.3. Attentional N2 effects

N2 has been associated with attentional processing in humans. N2 is increased in visual oddball go/no-go paradigms to rare visual targets over parietal, temporal, and occipital areas in humans compared to frequent non-targets (Ritter et al., 1983, Folstein and Van Petten, 2008). However, at frontal and central areas N2 is larger for ‘no-go’ non-target stimuli (Pfefferbaum et al., 1985, Kiefer et al., 1998, Bruin and Wijers, 2002). In contrast, we found a trend towards an increase in N2 to false alarms compared to correct rejections in both frontal and parietal areas (Figure 18), in addition to licking trials overall (Figure 14). False alarms represent poor attentional response inhibition, when the rats licked after ‘no-go’ distractor tones. The most straightforward interpretation of our data is that the N2 enhancement on false alarms reflects the action of licking. However, if this N2 increase is not a correlate of licking, our results suggest that it might be a correlate of poor attentional response inhibition of ‘go’ responses to ‘no-go’ distractor stimuli in both frontal and parietal cortex. However, additional recording sessions in the future might make this clearer. Also the studies mentioned only showed data from correct responses, so including incorrect response data might have altered grand averages (Ritter et al., 1983, Pfefferbaum et al., 1985, Kiefer et al., 1998, Bruin and Wijers, 2002, Folstein and Van Petten, 2008). We also may have seen differences in results due to alterations in stimuli and response modes, as well as varied anatomy and size in rats compared with humans.

II.4. Attentional P3 effects

P3 has previously been associated with increases during good attentional responses analogous to our hit trials in rats (Shinba, 1997, Kiefer et al., 1998, Sambeth et al., 2003, Wronka et al., 2008, Hattori et al., 2010) as well as in humans (Sambeth et al., 2003, Wronka et al., 2008). However in other human studies P3 was shown to be increased in ‘no-go’ response-inhibition analogous to our correct rejection trials, compared to ‘go’ response trials (hits) (Pfefferbaum et al., 1985, Kiefer et al., 1998, Bruin and Wijers, 2002). Thus this literature suggests that P3 is enhanced for both good attention for response inhibition and good attention for responding. This appears to be in conflict with our results in that the effect we saw was to increased P3 amplitudes in incorrect ‘no-go’ responses (misses), which correspond to a lack of attention (Figure 17). Our results seem to suggest that poor attentional ‘no-go’ responses to ‘go’ target stimuli show increases in P3.
Even though lick response times occurred before the latencies of P3 (Figure 14C), trends toward P3 increases during no-lick trials might explain this inconsistency. If P3 is increased in miss trials because no-lick trials overall produce larger P3 peaks, we might not see the attentional P3 peaks assessed in previous studies. Subtracting out lick-evoked ERP responses might present a possible solution to this problem (Del Cul et al., 2007). Increasing sample size might also make results more clear.

II.5. Pitch effects

While the oddball and standard pitch frequencies were switched to control for pitch effects in the passive paradigms, target and distractor pitches in the active task were not, which may have affected our results. It did not seem as though pitch had a visible affect on ERPs since correct rejections (distractor pitch) and misses (target pitch) produced similar grand averages and individual session peak amplitudes (Figure 15). However it remains possible that attentional effects were cancelled out by pitch related effects in those similar ERPs. Therefore our results may have been affected by differential activity of neurons tuned to specific pitches. Both Shinba (1997) and Hattori et al. (2010) did not control for pitch in their passive oddball paradigm, and they did not find rare-tone enhancements seen in other studies. Pitch effects may have altered their results. This issue could be further investigated in our experiments by examining passive responses to equally probable stimuli of the different pitches. However the rigorous way to address this issue will be to train some animals with reversed target and distractor pitches or to retrain the animals the opposite way.

III. Active vs. Passive

The active and passive paradigms used here were thought to evoke different types of attention. Top-down attention was targeted in the go/no-go task, while bottom-up attention was targeted in the passive oddball task. ERPs from the two paradigms were compared to examine the effects of varying attentional types on various peaks.

III.1. Active task amplitude increases

Individual session peak amplitudes from the active and passive paradigms showed that the active paradigm produced larger ERP amplitudes in both frontal and parietal areas (Figure
An exception is that N1 peaks in the active paradigm seemed comparable to the passive case. However P2, N2, and P3 all showed amplitudes of over 20 microvolts in the active condition. In the passive oddball paradigm almost all of the peak amplitudes remained below 20 microvolts. This increase in amplitude during active compared to passive tasks agrees with those of other studies in both humans and rats (Shinba, 1997, Sambeth et al., 2003, Wronka et al., 2008, Hattori et al., 2010).

Active auditory oddball paradigms have been shown to evoke larger components than passive paradigms because peak amplitudes are thought to be influenced by strength of attentional focus in humans (Katayama and Polich, 1999, Wronka et al., 2008). P3 especially has been examined in this context. Increases in task difficulty, for example using more similar target and distractor tones that are harder to discriminate, increase P3 amplitudes to target tones (Katayama and Polich, 1999). In our experiments the rats probably care about the tones in the active condition because they hold promise of a water reward, while the rats probably want to ignore the passive tones. The tones in the passive condition do not promise any kind of reward, and might just represent an annoyance. Much more attention overall is required to complete the active task than the passive task, and therefore the active task seemed to produce larger ERP peaks in general.

III.2. Active N2 and P3 latency increases:

ERP peak latencies were also calculated, and N2 and P3 peaks seemed to occur at longer latencies in the active go/no-go condition (Figure 20). Our results agree with studies in humans that have shown that N2 and P3 latencies increase with task difficulty (Ritter et al., 1983, Katayama and Polich, 1999, Folstein and Van Petten, 2008, Wronka et al., 2008). The active task required the rat to respond to tones, and therefore was more difficult than the passive task that did not require training.

While this latency increase was expected, the active N2 and P3 leaks occurred at latencies that were longer than those seen in previous active and passive oddball studies (Shinba, 1997, Sambeth et al., 2003, Wronka et al., 2008, Hattori et al., 2010). The passive components occurred at latencies that agreed with those found in these studies. However N2 and P3 in these studies occurred before 300 and 700 milliseconds respectively, whereas they occurred at about 500 milliseconds and 1 second in our active results. The windows in each condition were defined
based on visual inspection of grand average ERPs over rats and sessions, and therefore the windows were not the same in the two conditions (Table 2). Later N2 and P3 windows were defined in the active condition, which corresponds to the longer latencies seen in our results. Even though the maximum and minimum peak amplitudes did seem to correspond correctly to the windows defined, incorrect window constraints might have produced varied results in active vs. passive comparisons.

Active task paradigms have been shown to produce early and late P3 subcomponents in humans (Wronka et al., 2008, 2012). This appears to conflict with our active results (Figure 10), even though we saw early and late P3s in the passive paradigm (Figure 11). We defined one large window from 300 milliseconds to 2 seconds at P3 in the active condition (Table 2), and even though we did not see 2 definite P3 peaks, the latencies of the responses varied from 500 milliseconds to 1.85 seconds (Figure 10). Alternatively previous studies have only found single P3 peaks in rats, which do agree with our findings from the active paradigm (Yamaguchi et al., 1993b, Shinba, 1997, Sambeth et al., 2003, Hattori et al., 2010).

IV. Human vs. Rat

Rats provide potential for examining mechanisms of human attention. Exploring the validity of using rats as a model for ERP attentional studies is therefore important. Some of our results show similarities to human findings, while others might point to species differences. The most frequently studied novelty-related enhancements in human ERPs have been of the human P3 (Linden, 2005). In the passive oddball paradigm used here, rare tones evoked larger ERP responses that cannot be accounted for solely by SSA, which was strongest at P3E (Figure 13). The peaks were at comparable latencies to those seen in other rat and human studies (Shinba, 1997, Sambeth et al., 2003, Wronka et al., 2008, Hattori et al., 2010). The active go/no-go paradigms also produced characteristic ERP peaks of N1, P2, N2, and P3 (Figure 14). P2 peaks in the active paradigm showed a trend towards being larger in hit trials that involved good attention compared to misses (Figure 17) and false alarms (Figure 16). P2 has been associated with allocating attention in humans (Lijffijt et al., 2009).

Our results might also distinguish differences between the rat and human species. Novelty-related enhancements are associated with an early frontal component in humans.
(Linden, 2005), while we saw that the P3E oddball enhancement was strongest in parietal cortex (Figure 13). Even though the passive results showed early and late P3 subcomponents (Figure 11), the active paradigm only produced a single late P3 peak (Figure 10). P3a and P3b subcomponents in humans are localized in frontal and parietal brain areas respectively and are thought to be involved with different aspects of attentional processing (Linden, 2005, Wronka et al., 2012). P3a has been suggested to be associated with bottom-up involuntary alerting processes, while P3b has been associated with indexing top-down voluntary attention (Linden, 2005). The P3 peak that we saw was later and possibly analogous to P3b that was expected to increase in the active condition, since the active condition was thought to evoke top-down attention. It is therefore possible that the active task did not produce an early P3 peak analogous to P3a. We also did not see an increase in P3 with trial types utilizing good attention for responses and good response inhibition compared to bad attentional trial types that involved incorrect responses. Instead we saw trends towards increases in P3 for miss trials (Figure 15 and 17), as well as a larger N2 in false alarms (Figure 18) and lick trials overall (Figure 14).

Overall our results help extend the correspondence between human and rat sensory processing, while also beginning to identify species differences.

V. Conclusions and Future Directions

Passive rare tone enhancements were identified in characteristic ERP components that cannot be accounted for by SSA in rats, thereby setting the groundwork for finding mechanisms of non-SSA deviance detection. In the active go/no-go paradigm we have identified possible attentional and lick-related elements in rat evoked ERPs. The N2 peak showed a trend toward being greater in lick trials, and P2 showed a trend toward being greater in hit trials. Against our expectation, miss trials may produce a larger P3.

While the findings from the active go/no-go paradigm are a little unclear, additional sessions from new rats might give the results more statistical power. Controlling for pitch in the active paradigm might also make results more clear. Another technical improvement would be to subtract average baseline voltage from active ERPs as we did in the passive data analysis. Future data also hopefully will not require subjective manual artifact sorting, or a better automatic filtering system will be created to filter out artifacts and noise.

Future directions might include comparisons of single-unit spiking activity to ERPs or
direct comparisons of rat and human ERPs. We collect spikes with our depth electrodes, so the lab has a means for gathering spike data. Active and passive auditory experiments could be carried out with slight alterations in an EEG setup in humans to directly compare amplitudes and latencies of ERPs with those of rats. Alternatively, ERP studies can be conducted in rat models of neurological disorders that show symptomatic attentional defects, such as autism, schizophrenia, and ADHD. Pharmacological studies using drugs that block dopamine (e.g. haloperidol) or norepinephrine (e.g. clonidine) receptors may also reveal the importance of these neurotransmitter pathways in the generation of ERP components during passive bottom-up attentional processing and while rats are using top-down attention to actively detect and discriminate stimuli.
Literature Cited


