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Demonstrating the P300 Psychophysiological Response in Rats During an Auditory Stimulus Detection Task Using Subcutaneous Electrodes

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DEMONSTRATING THE P300 PSYCHOPHYSIOLOGICAL RESPONSE IN RATS DURING AN AUDITORY STIMULUS DETECTION TASK USING SUBCUTANEOUS ELECTRODES

by

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William and Amanda; where would a girl be without her siblings? William, when things get stressful, I know I can always count on you to make my day lighter and happier, and in that way you have always been my saving grace. Amanda, I know you've always looked up to me as an academic mentor: from helping you learn how to read, to helping you with school assignments, and even helping you learn how to drive. In many ways, you were my first student, and it all paid off when you once asked me a question

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Abstract

Meyer, Abby Elizabeth. Ph.D. The University of Memphis. August, 2015. Demonstrating the P300 Psychophysiological Response in Rats during an Auditory Stimulus Detection Task using Subcutaneous Electrodes. Major Professor: Helen J. K. Sable, Ph.D.

The P300 is a psychophysiological response that occurs 300-500 ms after the onset of a novel stimulus. This signal has been recorded in humans using ERP methods, but the recording method for non-human mammals has been surgically intrusive. While beneficial to determining specific brain regions that elicit the P300, a survey of current research indicates that targeting specific areas for recording does not yield substantial benefits over more global measures of neurophysiological activity. We tested rats in an auditory oddball task to demonstrate if a less-intrusive means of recording the P300, using subcutaneous electrodes, can be achieved in non-human mammals. We expected to find a robust P300, as the rats were tested in an active task wherein the oddball target stimulus was rare and meaningful. Furthermore, we expected that the amplitude of the P300 would be dose-dependently reduced following IP administration of ethanol (0.75 and 1.5 g/kg) versus vehicle (IP saline). When the mean amplitude for the target tone was compared to the mean amplitude for the standard tone (latency 300-400 ms) for all trials, the P300 to the target tone that was identified was significantly higher than that for the standard tone. Furthermore, while not statistically significant using parametric analyses, large effect sizes (f > .40) indicated the P300 to the target was larger in females than in males and that the amplitude of the P300 was reduced by acute ethanol administration. Analysis of the behavioral results for the oddball task indicated the rats did not demonstrate very accurate performance. When comparing hits (i.e., correct

responses to the target) versus correct rejections (i.e., no response to the standard tone), a substantial number of trials had to be eliminated from the ERP analysis due to premature responding before either tone was presented. As such, the resulting waveforms contained too much noise to deduce typical auditory ERP waveforms, thus preventing direct examination of P300 amplitude for only the correct behavioral response trials. Overall, these results indicate that, despite poor behavioral performance, the target tone elicited a P300 and was thus more salient than the standard tone.

Keywords: P300, auditory discrimination task, subcutaneous electrodes

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Chapter 1: Introduction to the P300

The fields of psychology and physiology are consistently expanding while also becoming more intertwined. One example of this expansion and consolidation is in the field of psychophysiology, which seeks to understand and explain psychological concepts, such as attention, learning, and memory, through the use of neurophysiological techniques and technology, such as measuring brain activity via electroencephalography (EEG) and event-related brain potentials (ERPs). As such, researchers in this field propose that neurological processes can be measured and understood through changes in the currents of electrical activity in the brain. The P300 is one common psychophysiological component that is studied in regard to attentional processes.

The P300

History and general characteristics. ERPs consist of positive and negative deflections in EEG activity that occur at specific ranges of time, (typically measured in ms) following the presentation of a meaningful external stimulus (Ehlers, Somes, Lopez, & Robledo, 1998; Finn, 1999; Polich & Bondurant, 1997; Yamaguchi, Globus, & Knight, 1993). The P300 is a positive deflection in neural activity that is most commonly recorded from scalp electrodes and can be elicited during auditory or visual stimulus discrimination tasks (Cohen & Polich, 1997). Numerous investigations of the P300 have noted the signal's potential for enabling researchers to further understand and explain how the brain processes information (Duncan-Johnson, 1981). Changes in the characteristics of this signal (such as amplitude and latency) during auditory and visual tasks are considered to be indicators of a subject's understanding and recognition of the stimulus (Hill & Shen, 2002). Specifically, the amplitude indicates how much neural

activation is being generated in response to a stimulus, whereas the latency indicates the time needed for processing the stimulus information (Duncan et al., 2009; Polich, 2007). These components can serve as a quantitative and temporal measure of neural activity underlying attention allocation and immediate memory operations (Cohen & Polich, 1997).

The P300 signal is a robust peak in positive deflection in voltage that occurs, on average, around 10-20 μV above baseline in humans (Polich & Bondurant, 1997), and is predominately found along the centro-parietal (Cz-Pz) midline (Duncan et al., 2009; Lindín, Zurrón, & Díaz, 2004; see Figure 1, left).

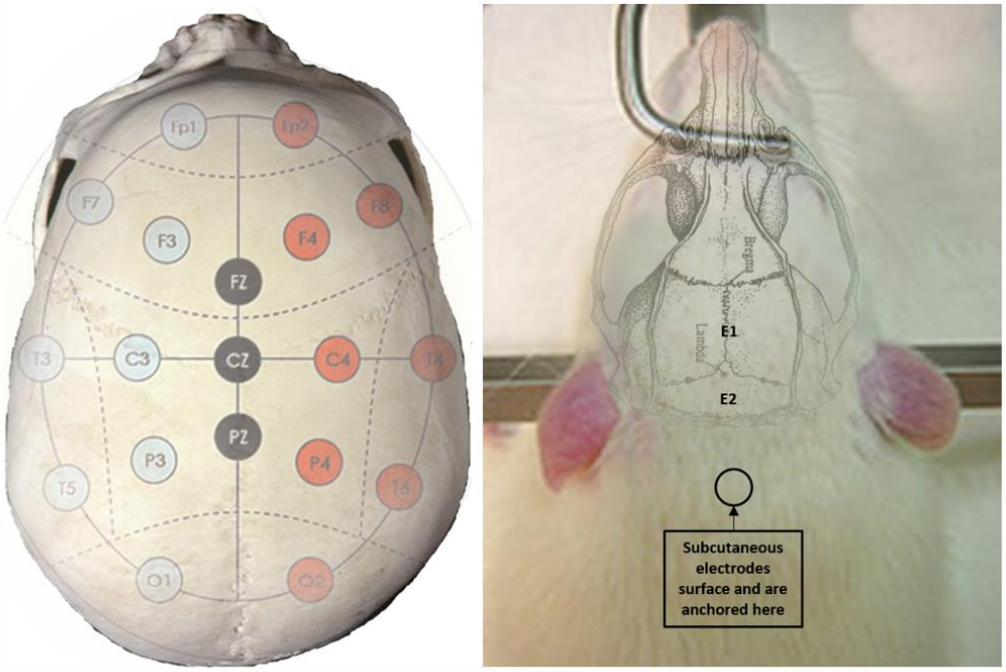

Figure 1. International 10-20 Array and Subcutaneous Electrode Placements in Rats. The locations of EEG recording in humans (on left) are traditionally noted in terms of the labels Fz, Cz, and Pz. As can be seen to the right of this side by side comparison, the placement of our recording electrode (E1) posterior to bregma is akin to the Pz location in humans. We have also included the implantation of a reference electrode (E2) posterior to lambda (midline between the ears). The electrode leads will exit the skin and will be anchored together through a small injection site at the nape of the neck.

The P300 response peaks around 300 ms after presentation of a rare and meaningful stimulus (Duncan et al., 2009; Euser et al., 2012; Steinhauer & Hill, 1993; van der Stelt, Geesken, Gunning, Snel, & Kok, 1998). Sutton, Braren, Zubin, and John (1965) discovered the P300 while studying the human brain's response to auditory stimulus presentations that varied in regard to certainty of presentation. They reported that the most robust positive increase and difference in psychophysiological recordings occurred at roughly 300 ms (hence, P300) after presentation of rare stimuli. They also reported that a negative deflection occurred in response to the presentation of rare stimuli around 110 ms (thereafter known as the N100, which is another ERP component often studied in the field of psychophysiology). Unlike the P300, the N100 occurs after the presentation of any stimulus and does not require attention (Sutton et al., 1965). The amplitudes of the P300 and N100 signals remain relatively stable over time and, as such, are regarded as standard measures of typical psychophysiological activity. It is important to note that the P300 that is generated by rare and unexpected stimuli is thought to be triggered by the cognitive evaluation of the *characteristics of the stimulus* more so than the *qualities of the subject* processing the stimuli (van der Stelt et al., 1998). In essence, this means that following the presentation of a rare stimulus, the P300 is believed to be generated by external, rather than internal factors.

It is also important to consider the methods by which the P300 is studied in various experimental models, including humans and non-human mammals. The behavior and activity of the human brain, as recorded as specific ERP components, is often studied in a non-invasive way with electrodes adhered into place on the outside of the scalp during recordings (Duncan et al., 2009). Rodent and primate studies most typically

involve more invasive means of implanting electrodes into and/or through the skull to record ERPs (Arthur & Starr, 1984; Breen & Morzorait, 1996; Criado & Ehlers, 2010; Ehlers, 1989; Ehlers, Chaplin, Lumeng, & Li, 1991; Ehlers, Desikan, & Wills, 2014; Ehlers, Kaneko, Robledo, & Lopez, 1994; Ehlers, Kaneko, Wall, & Chaplin, 1992; Ehlers & Somes, 2002; Ehlers et al., 1998b, 1999a; Ehlers, Somes, Lumeng, & Li, 1999b; Ehlers, Wall, & Chaplin, 1991b; Jodo, Takeuchi, & Kayama, 1995; Glover, Onofrj, Ghilardi, & Bodis-Wollner, 1986; Katner, Slawecki, & Ehlers, 2002; Robledo, Lumeng, Li & Ehlers, 1994; Shinba, 1997; Slawecki, Betancourt, Li, & Ehlers, 2000; Slawecki Grahame, Roth, Katner, & Ehlers, 2003; Slawecki, Thorsell, & Ehlers, 2005; Slawecki, Walpole, Purdy, & Ehlers, 2000; Umbricht et al., 2004; Yamaguchi et al., 1993). Another difference between experimental P300 models is that human studies measuring the P300 most often employ an active oddball stimulus discrimination task wherein participants are asked to make active responses to presentations of rare (i.e., infrequently presented) versus standard (i.e., frequently presented) stimuli (Lindín et al., 2004). In contrast, P300 experiments with animal models often employ a passive task, wherein the stimuli are presented to the awake and conscious subject but no active response is required. This difference calls into question whether the subject in a passive task understands the meaningfulness of the rare stimulus. Often, a passive task is used in order to minimize movement artifacts that can cloud the ERP data, despite the fact that it is known that the P300 is not a product of motor movements (Arthur & Starr, 1984). One objective of the current experiment was to alleviate the above-mentioned discrepancies between human and animal P300 studies by measuring the P300 in rodents equipped with

minimally invasive subcutaneous electrodes while they were engaged in an *active* oddball auditory stimulus discrimination task.

P3a versus P3b. Continued study of the P300 has led to the notion that this response is comprised of distinct subcomponents, namely the P3a and P3b (Polich, 2007). Duncan et al. (2009) noted that the P3a is distinct from the P3b (often termed the P300) in that the P3a has a shorter latency. Furthermore, the P3a is believed to occur when a stimulus is presented that is supposed to be ignored and is largest along more anterior regions of the scalp, including the fronto-central midline (i.e., Fz and Cz, see Figure 1, left) – regions that are often associated with the recognition of a novel stimulus (Shinba, 1997). The P3b, on the other hand, occurs when a rare stimulus is presented and attended to and is generated by posterior regions that are associated with the anticipation of the presentation of a novel stimulus (Hill & Shen, 2002; Shinba, 1997). The peak of the P3a typically occurs between 200 and 500 ms after the presentation of a rare and meaningful stimulus and is often seen as an early component of the much larger P3b signal that is typically elicited 350 – 600 ms after rare stimulus presentation. Often the P3a is found following a rare and meaningful novel stimulus that suddenly interrupts an ongoing set of stimuli (Holguin, Porjesz, Chorlian, Polich, & Begleiter 1999). For example, in a three tone task, the rare but non-target tone elicits a P3a, while the rare but target tone elicits a P3b (Polich, 2007). The existence and potential significance and meaningfulness of the distinct P3a and P3b responses in rats have not been well established. Shinba (1997) found that the positive peaks that occurred in rats 300 ms after stimulus presentation, during both active and passive stimulus discrimination tasks, was akin to the P3a and P3b responses found in humans. However, it is unclear if the same factors (such as

anticipation) that elicit these responses in humans are also present in animal models. So, while the distinct P3a and P3b components are readily evident in human studies (especially those that use a three tone stimulus discrimination task), these components have not been fully operationalized in a rat model. Thus, analyzing these responses separately can make it more difficult to find significant results, and does not (at present) provide an advantage over discerning one overall P300. Furthermore, due to the relatively novel electrode placement approach used in this study, in addition to the use of an active *two*-tone stimulus discrimination task (rather than a three tone task), no a priori hypotheses about the amplitude or latency of the P3a versus these measures for the overall P300 was made.

P300 in Human Studies

Sex differences. The P300 has been most extensively studied in humans, particularly in undergraduates that exhibit normal or typical functioning without cognitive or psychological impairments (Bauer, Costa, & Hesselbrock, 2001; Bennington & Polich, 1999; Cohen & Polich, 1997; Katayama & Polich, 1996; Lindín et al., 2004; Magliero, Bashore, Coles, & Donchin, 1984; Polich, 1986b, 1987; Polich & Bondurant, 1997; Polich, Burns, & Bloom, 1988; Polich & Margala, 1997; Ravden & Polich, 1999). Studies of the P300 often include only male participants (Cohen, Wang, Porjesz, & Begleiter, 1995; Ehlers, Garcia-Andrade, Wall, Sobel, & Phillips, 1998; Elmasian, Neville, Woods, Schuckit, & Bloom, 1982; Hill, Steinhauer, & Locke, 1995b; Holguin et al., 1999) and rarely include only female participants (Hill & Steinhauer, 1993b; Suresh et al., 2003). The most comprehensive and informative studies, of course, include both male and female participants (Ceballos, Nixon, & Tivis, 2003; Hill, Locke, & Steinhauer,

1999; Justus, Finn, & Steinmetz, 2001; Steiner, Barry, & Gonsalvez, 2014), but more studies are needed to systematically identify sex differences in ERPs between males and females (Euser et al., 2012; Finn, 1999). Most studies that have included both male and female participants either did not find or report a sex difference in the amplitude and/or latency of the P300 (Bauer et al., 2001; Bennington & Polich, 1999; Ceballos et al., 2003; Cohen & Polich, 1997; Hill, Muka, Steinhauer, & Locke, 1995, 1999; Katayama & Polich, 1996; Lindín et al., 2004; Polich et al., 1988; Sinha, Bernardy, & Parsons, 1992; Steiner et al., 2014; Steinhauer & Hill, 1993; Suresh et al., 2003; van der Stelt et al., 1998). Few studies have reported significant differences in the P300 between males and females (Hill & Steinhauer, 1993a; Justus et al., 2001) with males typically exhibiting smaller P300 amplitudes than females.

Age differences. The P300 is correlated with cognitive ability, as demonstrated by shorter latencies in individuals that exhibit a high level of performance on cognitive tasks, as well as in individuals that are in their cognitive prime (young adulthood to middle adulthood). Longer latencies are found in individuals that are cognitively premature, such as children, and in individuals that experience cognitive decline, such as older adults (Polich, 2007). An identifiable P300 occurs in children and elderly adults, but the amplitude is reduced compared to more cognitively adept populations, arguably due to deficits in the memory systems (Polich & Bondurant, 1997). Specifically, Hill and Steinhauer (1993a) found that children younger than 12 had lower P300 amplitudes and longer latencies than older children, demonstrating the impact of neurological immaturity. Steinhauer and Hill (1993) found similar results wherein children aged 8-12 years had smaller P300 and N100 amplitudes and longer P300 latencies than children

aged 13-18 years. In order to elicit and study robust P300s, the age of the subjects should be taken into consideration, with the subjects preferably being in their cognitive prime.

Alcoholism and alcohol intake. Many human studies have been conducted to investigate differences in the P300 in alcoholic versus non-alcoholic populations (Ceballos et al., 2003; Cohen et al., 1995; Hill & Steinhauer, 1993b; Hill et al., 1995b; Holguin et al., 1999; Justus et al., 2001; Sinha et al., 1992; Suresh et al., 2003), as well as in social drinkers (Teo & Ferguson, 1986). Overall, several studies have shown that men and women that have struggled with alcoholism and disordered drinking behaviors (Bauer et al., 2001; Cohen et al., 1995; Ehlers, Garcia-Andrade et al., 1998; Hill et al., 1995b, 1999; Hill & Steinhauer, 1993b; Holguin et al., 1999; Justus et al., 2001; Suresh et al., 2003; Teo & Ferguson, 1986) had significantly reduced P300 amplitudes compared to typically functioning men and women that did not exhibit any disordered drinking behaviors or alcoholism (Cohen & Polich, 1997; Magliero et al., 1984; Katayama & Polich, 1996; Lindín et al., 2004; Polich, 1987; Polich & Bondurant, 1997; Polich & Margala, 1997; Sommer, Leuthold, & Hermanutz, 1993; Steiner et al., 2014). Individuals who exhibit a high preference for ethanol, but who do not have a personal history or family history of alcoholism, demonstrate a reduction in P300 amplitude during an acute ethanol challenge using moderate to high doses of ethanol compared to participants who do not demonstrate a high ethanol preference (Campbell & Lowick, 1987; Elmasian et al., 1982; Lukas, Mendelson, Kouri, Bolduc, & Amass, 1990; Teo & Ferguson, 1986). However, not every study finds this reduction in P300 amplitude (Erwin, Linnoila, Hartwell, Erwin, Guthrie, 1986; Sommer et al., 1993) or latency (Colrain, et al., 1993; Fowler & Adams, 1993) in acute ethanol challenges. The differences among these

findings could be due to several reasons, including the doses of ethanol used (the studies above used a range of doses from to 0.3 to 0.94 g/kg ethanol), the point during the intoxication period at which measurements were taken, ethanol preference/aversion within the sample, and consideration of risk factors for disordered drinking behavior such as drinking history and family history of alcoholism (Bijl, de Bruin, Kenemans, Verbaten, & Böcker, 2005).

P300 in Rodent Studies

Studies that investigate the P300 in rats allow for increased rigor when investigating certain factors that impact its amplitude and latency, including acute administration of various doses of ethanol. A survey of the current literature shows that, when rats demonstrate neither an ethanol preference nor a potential genetic predisposition to alcoholism, then an acute ethanol challenge does not produce a significant change in the P300. Ehlers et al. (1992b) conducted EEG recordings in Wistar rats during a passive task with an acute ethanol challenge and did not find significant differences in P300 amplitude following a 0.75 g/kg dose of ethanol. Similarly, Ehlers et al. (2014) found that acute IP injections of 1.5 and 3.0 g/kg ethanol in outbred Wistar rats given 15 min prior to EEG recording increased the *latency* of the P300, but did not reduce amplitude, in both the Fz and Pz regions. Further, they found this effect was more profound in adult compared to adolescent rats. The lack of an ethanol effect on P300 *amplitude* could be due to the fact that the P300 was divided and analyzed into the separate P3a and P3b components, which, as previously discussed, can make it harder to find significant results. The lack of results on P300 amplitude found by Ehlers et al. (2014) may have also been due to the fact that they used a passive auditory discrimination task instead of

an active one. Passive tasks have been shown to lead to a smaller P300 amplitude than active tasks (Bennington & Polich, 1999; Ehlers et al., 1994; Shinba, 1997), which is likely due to the fact that passive tasks do not require attention and are thus less likely to elicit a robust P300. Of course, the dose of ethanol administered and the strain of rat used are factors that must be considered in rodent studies as well. In addition to using a passive auditory discrimination task, Slawecki et al. (2005) reported that a 0.75 g/kg dose of ethanol administered to Wistar rats via IP injection *15* min prior to testing produced no significant effect on the amplitude or latency of the N100 or P300. In an earlier study, Slawecki et al. (2000b) administered IP injections of ethanol (0.0, 0.25, 0.5, and 1.0 g/kg) to Wistar rats *5* min prior to EEG recordings and found significant dose-dependent reductions of cortical EEG activity as well as reductions of N100 amplitude.

As was discussed for human studies, Lee et al. (1990) provides important evidence that the point during the intoxication period at which psychophysiological measurements are taken is also an important consideration. Lee et al. (1990) administered three doses of ethanol (0.5, 2.5, and 5.0 g/kg) to Wistar rats via IP injection and measured their effects on the auditory brain stem response (ABR), which reflects stimulus-evoked neural activity before the input reaches the cortex. While not specifically measuring the N100 or P300, they noted their results were affected by the time point during the blood alcohol curve at which the measures were assessed, suggesting ERPs like the N100 and P300 would likely be similarly affected.

Overall, differences in results among the rodent studies discussed above can likely be attributed to some of the same factors previously discussed for human studies – differences in the alcohol doses used and the time of psychophysiological assessment. In

addition, P300 studies in rodents demonstrate that the type of task used appears to be a factor, with passive tasks yielding less robust results.

P300 in Non-Human Primate Studies

A review of the current body of literature regarding animal models in which the P300 was measured reveals a very limited number of studies involving monkeys. Only a handful of studies have been conducted wherein the P300 was investigated in the pure sense, without the use of concurrent administration of other substances (Arthur & Starr, 1984; Glover et al., 1986). Arthur and Starr (1984) tested monkeys in an active auditory oddball discrimination task similar to those used in human studies, and found that the amplitude of the P300, but not the N100, increased as presentations of the rare tone were reduced from 50% to 30% and ultimately to 10%. Glover et al. (1986) tested cynomolgus monkeys using a conditioning paradigm in which presentations of the rare tone (at probabilities of 50%, 30%, and 10%) coincided with electrical stimulation to the right lateral malleolus. They found that decreasing the probability of the rare tone increased the amplitude of the P300.

From other studies that have examined the effects of administration of various substances on the P300 in monkeys, important information about the characteristics of the P300 can be obtained by examining the results taken at baseline (prior to administration of a xenobiotic, supplement, or some other substance) and following placebo administration. For example, it was found that a P300 was reliably recorded in Rhesus monkeys during a passive auditory oddball task (Abe, Sawada, Horiuchi, & Yoshimura, 1999) and in Squirrel monkeys during a visual oddball task (Pineda & Swick, 1992), both during baseline measures and after administration of a placebo. There were

no studies found during our extensive literature search using non-human primates (with or without a personal history/family history of alcohol dependence) that examined the effects of alcohol on P300 amplitude and latency.

The reasons behind the dearth of studies that have been conducted with non-human primates have not been reported. Studies with monkeys provide ERP results that are nearly identical to those found in human studies (Arthur & Starr, 1984), and as such monkeys are the most relatable animal model available. Despite this fact, they are not widely included as an experimental model in P300 research, possibly due to the expense associated with this animal model but also possibly because of a limited ability to maintain functional electrodes in awake monkeys. In the P300 monkey studies that currently exist, systematic examination of the effects of sex and age on the N100 and P300 in non-human primates is non-existent. Non-human primates are the closest genetic relatives to humans, but without a broader scope of non-human primate research to draw upon for comparisons between these models, rodent models remain the most translational in regards to the P300 at this point in time.

Chapter 2: Measuring the P300

Common Problems when Measuring the P300

Preliminary studies of the P300 termed this response as such because it was found to occur approximately 300 ms after the onset of a stimulus. However, newer technology and methodology have revealed that the P300 can occur over a wider latency range (Duncan-Johnson, 1981). As a result, there is no exact or uniform latency window for classifying a positive spike in electrical activity as a distinct P300. This is also true in regards to determining the latency window of other ERP components, such as the N100. Absent consistent guidelines, researchers often determine latency windows for these signals based on the waveforms generated from their ERP data. This data-driven approach to designating the latency range for N100 and P300 can potentially lead to misguided conclusions when making comparisons between studies that have designated different latency windows. In addition to this basic definitional problem of P300 latency, researchers also note that there is little agreement in the field as to the definitions for terms relating to the analyses used (Cohen & Gulbinaite, 2014; Cohen & Polich, 1997; Oscar-Berman, 1987). Various analytical terms that can be applied to a variety of fields of research, such as ANOVA and correlation, are unambiguous and precise. However, terms that are specific to psychophysiological research, such as *synchronization* and *time-frequency response*, are more ambiguous and are often specific to the laboratory conducting the study or to the type of equipment used. Further, researchers disagree as to whether or not EEG and ERP techniques can accurately assess factors such as *activation* due to the lack of a universally accepted definition of what "activation" actually is and how it should best be applied to more complex, multi-dimensional studies of EEG and

ERP activity. As noted by Cohen and Gulbinaite (2014), this lack of consensus causes ambiguity and confusion, which makes it difficult to compare the results of different experiments and research models thereby impeding progress in the field.

Several other problems can arise when measuring the P300. Researchers often find disparate results in their studies, particularly those involving an ethanol challenge, while investigating factors that influence the amplitude of the P300. As noted by Bauer (2001), researchers need to consider the dose of ethanol used, as well as the time points at which EEG activity is being recorded (i.e., either while blood alcohol levels [BAL] are increasing, such as at the beginning of the ethanol challenge and testing session, or when they are decreasing, such as toward the end of the ethanol challenge and testing session) as this can lead to problems when comparing results between studies. The impact of time, BAL, and one's level of risk for developing alcoholism on EEG activity has been studied by Cohen, Porjesz, and Begleiter (1993). They found that males that were at a high risk for alcoholism, compared to those who were at a low risk for alcoholism, demonstrated a sensitization to alcohol via an increase in slow alpha activity as BAL rose. They also demonstrated an increase in tolerance to the negative effects of alcohol via a faster slow alpha recovery of baseline voltage levels as BAL fell. As such, it is important to consider the dosage of ethanol administered, as well as the specific phase of BAL (ascending versus descending), while recording EEG and ERP components in a subject, particularly when making comparisons between studies that incorporate an ethanol challenge.

The nature of the task is also an important factor to consider when making comparisons between studies of the P300 (Steinhauer & Hill, 1993). An oddball *auditory*

stimulus discrimination paradigm is used most often to study the P300, as this has been found to elicit a stronger P300 than comparable oddball *visual* stimulus tasks. The auditory task can be active (wherein an active response is needed) or passive (wherein no response is needed). Oscar-Berman (1987) suggested that the reduction in P300 amplitude found during alcohol challenge studies is related solely to alcohol, as results are the same regardless of whether the task is visual or auditory, and that the reduction is not related to the difficulty of the task. In more contemporary studies, the passive oddball task has not been found to evoke a strong or consistent P300 and an active oddball task seems to be the better choice. The use of one task over another can be problematic for cross-study comparisons because the amplitude of the P300 associated with a passive task is usually much lower. However, the amplitude of the P300 can be increased during a passive task when a single rare tone is presented within a long train of standard tones. For example, Bennington and Polich (1999) conducted a passive auditory task that included sets, or trains, of ten tones; the first six tones were always standard, and only one of the remaining four tones was rare. In this passive design, the rare tone elicited a strong P300, similar to what is typically found in an active task. Unless a modification like this is made, it is difficult to compare the findings of a passive task to an active task due to the substantial differences in P300 amplitude. A later review by Lindín et al. (2004) also serves to shed light on the contrasting results that have been recently found in regard to factors that influence the P300. They noted that the discrepancies seen may be predominantly due to the fact that the experimental designs varied significantly from one study to another. In addition to the active versus passive nature of the task, there are also differences in the intensity of the stimuli being

presented, the inter-stimulus intervals (ISIs), and the designation of the latency range of the P300. With this many differences between studies, it is often difficult and largely inappropriate to make cross-study comparisons.

In addition to the influence of the nature of the task used in studies investigating the P300, it is also important to consider the demographics of the participants that are studied, as mistakes can be made when drawing comparisons between different groups. It has been well established that the reasons for the differences in results between P300 studies could be due to misguided comparisons between studies that use different age groups and different ratios of men to women (Steinhauer & Hill, 1993). For example, most human studies include participants in their cognitive prime (i.e., early 20s). Of the studies conducted on prepubescent age groups, only a handful include both boys and girls. Additionally, it is important to consider that, in human studies, auditory two-tone tasks that involve either keeping a mental count (of the number of rare stimuli presentations) or making a response (when the rare stimulus is presented) have largely been used to elicit the P300. However, this method can be troublesome for certain populations such as children who are cognitively immature, or the elderly and those with disease states associated with cognitive decline (e.g., alcoholism, Alzheimer's).

When studying individuals who are not in their cognitive prime, difficulties in obtaining a large number of artifact-free trials, as statistical problems often arise when potentially noisy and artifact-riddled data are removed from a data set **(**Polich, 1986b). In an effort to achieve cleaner data with cognitively impaired populations, a simpler version of the auditory oddball task can be used wherein one tone is played at random times throughout periods of silence instead of requiring participants to discriminate between

presentations of two distinct tones (Polich & Margala, 1997). Additionally, various populations, such as children and the elderly, who are unable to undergo long experimental sessions, need to be able to be tested with shorter testing sessions. Polich and Bondurant (1997) report that using shorter stimulus-sequence testing sessions yields the same results as longer stimulus-sequence testing sessions, indicating that the same variables that influence the size of the P300 in longer stimulus-sequence testing sessions similarly affect the P300 in shorter stimulus-sequence testing sessions. Because these shorter sessions have been found to elicit a robust P300, this approach can be ideal when testing subjects that have attentional or cognitive difficulties. The studies described above illustrate the various factors that affect P300 results. These factors need to be considered when designing P300 studies in order to account for potential negative influences associated with task demand and participant demographics.

Changes in Stimulus Parameters

Sutton et al. (1965) were the first to demonstrate that the P300 signal can be altered by changing the probability at which the target stimuli are presented; the more rare the target stimulus is, the larger the P300 amplitude. This has been shown in numerous other studies, including an early experiment conducted by Arthur and Starr (1984) wherein a rare tone was presented at probability levels of 10%, 30%, and 50%. They found that the amplitude of the P300 and other ERP components were largest at the lower probability levels. This same conclusion was also drawn by Polich (1987), Steinhauer and Hill (1993), Ehlers et al. (1994), Katayama and Polich (1996), Ehlers and Somes (2002), and Glover et al. (1986). In regard to the effect of probability level on the latency of the P300, most researchers found that the P300 latency is reduced when the

probability of the rare tone is reduced (Polich & Margala, 1997). However, other studies have found that the latency does not seem to be influenced by the probability of the rare, target tone (Katayama & Polich, 1996; Polich, 1986a; Polich, 1987), while others found that decreasing the probability of the rare stimulus actually served to increase the latency of the P300 signal (Cohen & Polich, 1997; Steinhauer & Hill, 1993). These studies have consistently demonstrated that the 20% probability level for the rare tone produces the most robust P300 amplitude.

The time span between presentations of stimuli, otherwise known as the ISI, is another parameter that has been shown to influence the characteristics of the P300 signal. Polich and his colleagues, who have extensively studied this aspect, have obtained important evidence about the influence of the ISI on the P300. In one study, they compared a 5 s ISI to a 2 s ISI and found that the longer ISI led to greater P300 amplitude (Polich, 1987). In a follow-up study, they reported that the P300 amplitude for a 6 s ISI was similar to that seen following a 2 s ISI (Polich & Bondurant, 1997), but later results by Polich (2007) again found that reducing the ISI led to a reduction in the P300 amplitude. A short span of time between trials, such as a 2 s ISI, produced smaller P300 amplitudes than longer spans of time between trials, such as a 6 s ISI. The time spent between trials is as important as the number of times the rare, target stimulus is presented. Presenting the rare, target stimulus continuously results in desensitization to the importance and meaningfulness of the stimulus, and thus leads to a reduction in P300 amplitude, whereas presenting the rare, target stimulus adequately spaced amongst several presentations of the standard, non-target stimulus results in a robust P300 (Lindín et al., 2004; Steiner et al., 2014). In the current study, a rare or standard stimulus was

presented every 6 s (20% targets) throughout the length of the session, in an attempt to yield a robust and reliable P300.

Latency Ranges of N100 and P300

The latency of a stimulus-evoked ERP component is typically defined as the time between the onset of the stimulus and a peak deflection in voltage (either positive or negative). A review of the current literature indicates that there is not a consistent or uniform latency range for N100 and P300 responses, as evidenced by the wide range of data-driven latencies denoted throughout various experiments. This range of latency has been shown to be dependent on the experimental model used and also the type of task (visual versus auditory) that was used. In regard to the N100 signal in mice, early latency ranges are often considered, especially the 10-150 ms window (Ehlers & Somes, 2002; Slawecki et al., 2003). For rats, similar windows of time are often considered for N100 amplitude analysis, including 25-100 ms (Ehlers et al., 1991b), 41-80 ms (Sambeth et al., 2003), 50-100 ms (Katner et al., 2002; Slawecki et al., 2005), 50-120 ms (Slawecki et al., 2000a), and finally 50-150 ms (Ehlers et al., 1999a, 1999b). The 50-120 ms latency window has also been considered for analysis of N100 in monkeys (Ehlers, 1989). For human populations the latency window increases even more to include 75-150 ms (van der Stelt et al., 1998), 80-120 ms (Polich, 1986b), 80-136 ms (Hill et al., 1995a; Hill & Steinhauer, 1993b; Steinhauer & Hill, 1993), 80-180 ms (Ravden & Polich, 1999), and 90-150 ms (Sambeth et al., 2003). Given the breadth of latency ranges used, and with consideration of the animal model that was implemented in the current study, we used a data-driven approach and looked at the ERP waveforms to select the appropriate latency range for the N100 only if a negative peak in the appropriate latency range was present.

The selection of a latency range for the P300 follows the same pattern as that seen with the N100, wherein the latency used in mouse studies is typically earlier than those used in rat, monkey, and human studies. In regard to the P300 in mice, relatively early latency ranges are often considered, especially the 200-300 ms (Slawecki et al., 2003) and 200-400 ms (Ehlers & Somes, 2002) windows. For rats, a wide array of later windows are often considered for P300 analysis, including 200-400 ms (Katner et al., 2002), 250-325 ms (Ehlers et al., 1999a), 250-350 ms (Slawecki et al., 2005), 250-400 ms (Ehlers et al., 1991b), 250-500 ms (Sambeth et al., 2003), 250-600 ms (Jodo et al., 1995), 265.7-462.7 ms (Shinba, 1997), 273-350 ms (Slawecki et al., 2000a), 300-400 ms (Ehlers et al., 1994), and 300-450 ms (Ehlers et al., 1999b). The 200-325 ms (Ehlers, 1989) and the 298-330 ms (Glover et al, 1986) latency windows have typically been considered for analysis of P300 in monkeys. For human populations, the selections of the P300 latency window varies substantially to include 220-360 ms (Polich, 1986b), 250-375 ms (Ceballos et al., 2003), 250-400 ms (Cohen & Polich, 1997; Katayama & Polich, 1996), 250-500 ms (Justus et al., 2001; Lindín et al., 2004); 250-650 ms (Bauer et al., 2001), 256-416 ms (Hill & Steinhauer, 1993a), 264-424 ms (Hill et al., 1995a, 1999; Hill & Steinhauer, 1993b; Steinhauer & Hill, 1993), 300-500 ms (Cohen & Polich, 1997), 300-600 ms (Bennington & Polich, 1999; Ravden & Polich, 1999), 300-700 ms (Rangaswamy et al., 2007), 300-800 ms (van der Stelt et al., 1998), 320-450 (Sambeth et al., 2003), and 325-550 ms (Holguin et al., 1999). Given the breadth of latency ranges used, and with consideration of the animal model that was incorporated in the current study, we again implemented a data-driven approach and examined the resulting ERP waveforms to

select the appropriate latency range for the P300 only if a positive peak within the appropriate latency range was present.

Brain Regions of Interest

The P300 signal has been extensively recorded from various cortical regions, including the frontal and the parietal cortices, where identifiable P300 and other ERP components like the N100 have been recorded in mice (Criado & Ehlers, 2009; Ehlers & Somes, 2002), rats (Ehlers, Chaplin, et al., 1991; Ehlers, Wall, et al., 1991; Ehlers, Kaneko, et al., 1992, Ehlers et al., 1994; Ehlers, Somes, et al., 1998, Ehlers, Somes, Li, et al., 1999, Ehlers, Somes, Lumeng, et al., 1999; Katner et al., 2002; Slawecki, Betancourt, et al., 2000), non-human primates (Arthur & Starr, 1984; Ehlers, 1989), and humans (Cohen & Polich, 1997; Cohen et al., 1993; Cohen et al., 1995; Elmasian et al., 1982; Hill et al., 1995a, 1999; Hill & Shen, 2002; Hill & Steinhauer, 1993a, 1993b; Holguin et al., 1999; Justus et al., 2001; Katayama & Polich, 1996; Lindín et al., 2004; Magliero et al., 1984; Polich, 1987; Polich & Bondurant, 1997; Polich & Margala, 1997; Ravden & Polich, 1999; Steiner et al., 2014; Steinhauer & Hill, 1993; Suresh et al., 2003; van der Stelt et al., 1998).

Many of these human studies have investigated a large number of electrode sites across the international 10-20 array (see Figure 1, left), and the majority of these studies found that the P300 is strongest from the Pz region. Only one study from the current literature review found no difference in ERP signal strength across Fz, Cz, and Pz (Bennington & Polich, 1999). As such, there is substantial compelling evidence to target the Pz region for recording the P300.

Chapter 3: The Current State of P300 Research

Experimental Task

Visual tasks. One way to elicit a P300 is to present rare visual oddball stimuli interspersed among standard visual stimuli. To date, only humans have been studied in experiments where a visual task is used to elicit a P300. Active visual stimulus discrimination tasks, wherein a subject is required to actively engage with the information being presented, are most commonly used in human P300 experiments (Bauer et al., 2001; Begleiter, Porjesz, Bihari, & Kissin, 1984; Ceballos et al., 2003; Sommer et al., 1993), including a task that requires participants to actively discern between figures of plain ovals versus rudimentary figures of faces (Hill, Muka, et al., 1995; Hill et al., 1999; Hill & Shen, 2002; Hill & Steinhauer, 1993a, 1993b). This task is useful for humans, specifically, because the visual stimuli are figures that are familiar. Additionally, this task can be adjusted in difficulty level in order to be applied to a wide variety of age groups and cognitive ability levels. Other active visual tasks that can easily be applied to various ages and cognition levels include: the detection of lines that are either vertical or askew (Holguin et al., 1999), visual presentations of horizontal lines and checkerboard patterns (Ravden & Polich, 1999), detection of presentations of an X versus an O (Justus et al., 2001; van der Stelt et al., 1998), and detection of a target word presented either alone or with conflicting, visual noise (Magliero et al., 1984).

Overall, individuals who exhibit a high risk for alcoholism show a reducedP300 amplitude in these active visual discrimination tasks, and these signals are much stronger than those elicited by passive visual tasks (Bennington & Polich, 1999; Cohen & Polich, 1997). Cohen and Polich (1997) compared visual and auditory tasks and determined that

the P300 amplitude was larger and the latency was shorter for the auditory task. Based on this evidence, the experimental design used in the current study involved an active auditory discrimination task.

Auditory tasks. Active auditory stimulus discrimination tasks are occasionally used in P300 research involving rats (Ehlers et al., 1994, 1998b; Grupe, Grunnet, Laursen, & Bastlund, 2014; Jodo et al., 1995; Sambeth et al., 2003; Shinba, 1997) and are often used in research with humans (Bennington & Polich, 1999; Cohen et al., 1995; Elmasian et al., 1982; Sambeth et al., 2003; Teo & Ferguson, 1986). A common active auditory task consists of the participants being asked to keep a continuously updating mental tally of the number of presentations of the rare tone (Hill, Muka, et al., 1995, Hill, Steinhauer, et al., 1995; Hill et al., 1999; Hill & Steinhauer, 1993b; Steinhauer & Hill, 1993). Several other active auditory human tasks involve making a response: either by pressing a button (Katayama & Polich, 1996; Lindín et al., 2004; Sambeth et al., 2003; Steiner et al., 2014; Suresh et al., 2003) or raising a finger when the rare tone is detected (Polich, 1986b; Polich & Bondurant, 1997; Polich & Margala, 1997).

Most relevant to the experimental design used in this study are the findings from studies conducted by Hattori, Onoda, and Sakata (2010) and Shinba (1997), wherein rats were tested on both active and passive auditory tasks. During the active task, the rats had to lever press within 2 s of hearing the rare tone to receive a food reward. They found a much larger P300 amplitude and longer P300 latency during the active task, at Fz, Pz, and in hippocampus, and a much smaller peak during the passive task, especially at Fz and in the amygdala. The amplitude of the N100 signal was largest during the active task at Fz.

The findings from Sambeth et al.'s (2003) study are also relevant to the current study. A significantly larger P300 amplitude was found in their rats for the rare tone versus the standard tone in a task that required the rats to make an active response by visiting the food magazine after presentation of the rare tone in order to earn a reinforcer. They did not find any differences in N100 amplitude and latency or P300 latency.

The results of Hattori et al. (2010), Sambeth et al. (2003), and Shinba's (1997) active reward-based study contribute a substantial amount of information to psychophysiology, as the majority of researchers that use animal models of ERPs rely upon a passive auditory task (Cohen et al., 1995; Ehlers, Chaplin, et al., 1991, 2014; Yamaguchi et al., 1993; Umbricht et al., 2004). Also, most commonly, ERP researchers use a passive three-tone oddball task wherein a standard tone and a rare tone are used along with bursts of white noise (Criado & Ehlers, 2010; Slawecki, Betancourt et al., 2000; Slawecki, Walpole et al., 2000; Slawecki et al., 2003, 2005). Passive auditory tasks can also be implemented with human participants (Umbricht et al., 2004), but the preferred tasks to use with humans are active in nature, as active auditory tasks have been shown to result in a larger and more robust P300 than passive tasks (Bennington & Polich, 1999; Ehlers et al., 1994; Shinba, 1997).

The benefits and drawbacks of conducting an active versus a passive task are discussed at length by Ehlers and Somes (2002). A number of valid points are made as to why one would chose to use a passive oddball paradigm, including the fact that a passive task does not require extensive training and can easily be used in humans that have limited attentional processes. There is clearly a utility in using a passive task; however, because attention has been argued to be necessary to elicit a P300 (Cohen & Polich,

1997; Hill & Shen, 2002), requiring some type of active response to ensure the participant/subject is attending to the stimuli seems to be an important experimental control. Requiring an active response also serves as an indicator of performance on the discrimination task by indicating how well the subject is attending to the stimulus presentations. In the current study, an active reward-based auditory discrimination task was chosen in order to ensure that the rats were attending to and understanding the meaningfulness of the rare tone.

Two-tone human auditory P300 discrimination tasks often employ a rare tone that is a of a higher frequency and decibel level than the standard tone (Bennington & Polich, 1999; Cohen & Polich, 1997; Hill, Muka et al., 1995; Hill & Steinhauer, 1993b; Lindín et al., 2004; Polich, 1986b; Polich & Margala, 1997), wherein the rare tone ranges from 1.2 kHz, 60 dB, and 40 ms to 2 kHz, 85 dB, 50 ms and the standard tone ranges from 800 Hz, 60 dB, and 40 ms to 1 kHz, 85 dB, and 50 ms. Two-tone tasks are also commonly used in rat studies (Criado & Ehlers, 2010; Ehlers, Wall et al., 1991; Ehlers, Kaneko et al., 1992; Ehlers, Somes, et al., 1998; Ehlers, Somes, Li, et al., 1999; Ehlers, Somes, Lumeng, et al., 1999; Gao, Zheng, Han, Tang, & Sun, 2009; Grupe et al., 2014; Jodo et al., 1995; Katner et al., 2002; Sambeth et al., 2003; Shinba, 1997; Slawecki, Betancourt et al., 2000), wherein the rare tone ranges from 1 kHz, 60 dB, and 10 ms to 10 kHz, 85 dB, and 800 ms.

After an extensive literature search, only one study was found that used a two-tone discrimination paradigm with tones that were different lengths (300 ms vs. 75 ms) instead of different frequencies (Elmasian et al., 1982). By using this novel approach to elicit a P300 in humans, the amplitude of the P300 was found to be significantly reduced

in all subjects following an ethanol challenge, with the greatest P300 amplitude reduction seen in those with a family history of alcohol dependence. One goal of the current study is to expand upon and replicate this experimental design in a rodent model to investigate the changes in the P300 in response to an acute ethanol challenge. We also expect to find a robust P300 due to the active nature of the task. In addition to the use of a reward-based, two-tone auditory discrimination task, this study will also attempt to establish a novel means of performing less invasive EEG recordings in a rodent model.

Surgical Procedures

Skull-invasive surgeries. The methods used to record EEG/ERP activity in humans is very disparate from the way in which recordings are typically performed with animals. In the former experimental design, the researcher attaches electrodes to the outside of the participant's scalp in an entirely non-invasive way. This is not the case for mouse and rat experimental models, wherein invasive surgery is all-to-commonly conducted to place skull screw recording electrodes directly into the exposed and cleaned skull, typically at Fz, Pz, and Cz according to the international 10-20 system (see Figure 1, right; Arthur & Starr, 1984; Cohen et al., 1995; Criado & Ehlers, 2010; Hattori et al., 2010; Ehlers, 1989; Ehlers, Chaplin et al., 1991; Ehlers, Wall et al., 1991; Ehlers, Chaplin et al., 1992; Ehlers, Somes et al., 1998; Ehlers, Somes, Li et al., 1999; Ehlers, Kaneko et al., 1999; Ehlers et al., 1994, 2014; Ehlers & Somes, 2002; Jodo et al., 1995; Katner et al., 2002; Sambeth et al., 2003; Shinba, 1997; Slawecki Betancourt, et al., 2000; Walpole, et al., 2000; Slawecki et al., 2003, 2005; Umbricht et al., 2004; Yamaguchi et al., 1993).

Studies using this invasive means of recording EEG (Ehlers et al., 1992a; Katner et al., 2002; Slawecki et al., 2003) and ERPs (Arthur & Starr, 1984; Ehlers, Chaplin et al.,

1991; Ehlers et al., 1994; Ehlers, Somes, Li et al., 1999; Ehlers & Somes, 2002; Grupe et al., 2014; Jodo et al., 1995; Sambeth et al., 2003) have typically found robust and reliable results. However, despite the precision of this implantation procedure, some of the studies report weak and conflicting findings (Arthur & Starr, 1984; Ehlers et al., 2014; Ehlers, Kaneko et al., 1992; Ehlers, Somes, Lumeng et al., 1991; Katner et al., 2002; Slawecki, Betancourt et al., 2000; Slawecki et al., 2005; Yamaguchi et al., 1993) that call into question the utility of using such an invasive surgery. The lack of strong results may be an outcome of targeting specific brain regions, which dilutes the intensity of the more global response (Arthur & Starr, 1984; Ehlers et al., 2014; Katner et al., 2002; Yamaguchi et al., 1993), using a passive instead of active response task (Ehlers, Kaneko, et al., 1992), or a combination of these factors (Ehlers, Wall, et al., 1991; Slawecki, Betancourt et al., 2000; Slawecki et al., 2005). The discrepancy in findings is actually surprising, given that the nature of the surgery and the placement of the skull screw electrodes is relatively consistent across studies.

Brain-invasive surgeries. A wide variety of studies have shown that P300-like responses are generated by subcortical regions. As such, surgeries have been conducted to place electrodes directly within the hippocampus (Ehlers, Chaplin et al.,1991; Grupe et al., 2014; Shinba, Andow, Shinozaki, Ozawa, & Yamamoto, 1996), the amygdala (Criado & Ehlers, 2010; Katner et al., 2002; Slawecki, Betancourt et al., 2000), both of these regions at the same time (Hattori et al., 2010; Ehlers et al., 1994; Ehlers, Kaneko et al., 1992, Ehlers, Somes et al., 1998, Ehlers, Somes, Li et al., 1999, Ehlers, Somes, Lumeng et al., 1999; Ehlers, Wall et al., 1991; Robledo et al., 1994), or other subcortical regions (Breen & Morzorait, 1996; Ehlers, Somes, et al., 1998; Jodo, et al., 1995;

Robledo et al., 1994). Many of these studies have been conducted using strains of rats that have been bred though multiple generations based on either a high preference for drinking ethanol (alcohol preferring, or P, rats) or a low preference for drinking ethanol (alcohol non-preferring, or NP, rats) and have found significant differences between these strains on the amplitude and latency of the P300 (Breen & Morzorait, 1996; Criado & Ehlers, 2010; Ehlers, Chaplin et al., 1991; Ehlers, Somes, Li et al., 1999). Similarly, robust ERPs have been found using this surgical procedure with a second line of selectively bred rats that exhibit high levels of alcohol drinking (HAD1) and low levels of alcohol drinking (LAD1; Katner et al., 2002). Wistar rats have also been used to demonstrate ERPs in stimulus discrimination tasks following a brain invasive surgery (Ehlers et al., 1991b, 1994). Much like the findings from the skull invasive surgeries reviewed above, results obtained following these brain invasive surgeries were sometimes contradictory (Ehlers, Somes et al., 1998; Jodo et al., 1995). Specifically, Katner et al. (2002) found no difference between HAD1 and LAD1 rat strains in the power of any of the standard EEG frequency bands, measured from the amygdala. Additionally, no differences were found between the lines in the latencies or amplitudes of the N100 or P300 in the amygdala. Null findings may also have resulted in these subcortical recordings because a passive task was used (Ehlers, Wall, et al., 1991; Ehlers, Kaneko, et al., 1992).

In addition to the inconsistent results derived from these skull and brain invasive surgeries, it is also important to make note of the impact this type of surgery has on the basic health and well-being of the animal. Numerous researchers reported that their subjects required recovery periods that spanned several days (Hattori et al., 2010; Shinba,

1997), a week (Slawecki et al., 2003; Yamaguchi et al., 1993), 9 to 10 days (Ehlers & Somes, 2002; Grupe et al., 2014; Jodo et al., 1995), 2 to 3 weeks (Ehlers, Wall et al., 1991; Sambeth et al., 2003; Slawecki et al., 2000a, 2000b, 2005), and even as long as one month (Ehlers, 1989). Furthermore, Ehlers et al. (1999b) reported that they lost nine P rats and one NP rat as a direct result of the impact of surgery. Katner et al. (2002) also reported that they had to eliminate two HAD2 rats at the beginning of their study and another three LAD2 rats prior to the ethanol administration part of the study due to damage to the electrode apparatus. Because the mortality rate in these research models is not commonly reported, it is difficult to determine how many are actually lost due to this invasive procedure.

Overall, there does not seem to be a substantial benefit or compelling evidence to undertake more invasive surgery. The physical impact of invasive surgery on the rat is substantial and the findings are not robust enough to warrant the necessity of such a procedure. As such, we believe using much less invasive subcutaneous electrodes to record neurophysiological activity in the rat brain during an active task will yield a robust P300 while promoting two of the "3 R's" (reduce and refine) associated with animal research (Russell & Burch, 1992). Specifically, a less invasive method should reduce animal discomfort and attrition, thereby minimizing the total number of animals necessary to complete the study. As previously mentioned, the recovery time for the subcutaneous electrodes is substantially less, which will minimize pain and suffering. This refinement has the additional distinct benefit of promoting improved animal welfare.

Minimally-invasive surgeries. To date, only one study has used a minimally-invasive means of placing electrodes in lieu of relying on invasively placed skull screw or

indwelling subcortical electrodes. Gao et al. (2009) were the first to report using subcutaneous electrode implantations that did not involve an invasive surgery. Their study incorporated a passive auditory oddball task and recorded the N100 and P300 from rats that exhibited depressive-like symptoms and stress. Despite the innovation of incorporating a subcutaneous technique, their study was methodologically flawed in a few ways. First, the brain regions they investigated were not the widely recognized regions reviewed above. Instead of targeting the more common Fz and Pz, they placed the needle electrodes in the more caudal fonticulus minor of median structure, as well as a reference on the tip of the nose and a ground electrode in the tail. Secondly, the rats were anesthetized during the study and were completely unresponsive. Not surprisingly, a P300 was not found. Recall the P300 requires attentional processes, so sedated rats were unable to attend to the auditory signals being presented. Using subcutaneous needle electrodes placed along the Cz-Pz region of the rat homolog to the international 10-20 system would likely provide a more robust and minimally-invasive means of recording both the N100 and P300 and should be done in rats that are awake and that are ideally performing an active task during testing.

Chapter 4: Rationale and Hypotheses of Current Study

Active Task with a Minimally-Invasive Surgery

Overall, there does not seem to be a benefit to recording EEG and ERP data from electrodes implanted invasively into brain regions such as the hippocampus and the amygdala as a means of studying global features of the P300. There also does not seem to be an overarching benefit of implanting screw electrodes into the skull because reliable EEG and ERP recordings have been obtained using less invasive means. Human studies largely rely upon scalp electrodes for EEG and ERP recordings, and an analogous animal model of this measure should employ a technique that is as similar as possible. Along this same line, the human studies reviewed previously most often employed an active stimulus discrimination task to elicit a P300, while the majority of animal studies employed a passive task (Jodo et al., 1995). Our goal is to build upon existing studies in the current literature to develop an animal model to measure the P300 in an active task using minimally-invasive electrodes.

The current study was designed to build upon research conducted by Gao et al. (2009) who used minimally-invasive electrodes in an attempt to measure the N100 and P300 in rats. However, their study was flawed and they failed to find differences in ERP signaling because they used a passive task in anesthetized rats and their electrode locations were atypical for measuring these ERP components. The current study built upon their methods by using subcutaneous electrodes to record the P300 signal from typical scalp locations associated with this signal in rats that were engaged in an active auditory stimulus discrimination task.

Several studies have shed light on the fact that invasive surgeries to target specific brain regions are not necessarily essential for recording robust ERP signals, as it can be difficult to pin-point where a P300 has been generated when using EEG and ERP recording methods. Duncan et al. (2009) specifically noted that the P300 is generated by multiple regions that function simultaneously rather than specific subcortical regions in isolation. It has been found that the P300 can be successfully recorded from cortical just as well as subcortical regions (Ehlers et al., 1994). Specifically, Ehlers, Somes, et al. (1991) stated that the P300 has been recorded from multiple subcortical regions, suggesting that the signals are generated by these regions. However, targeting these areas for ERP recordings does not appear to serve a benefit greater than recording the P300 from cortical regions. Taken one step further, we hoped to demonstrate that electrodes placed subcutaneously provide the same information.

Research Considerations

Because we used a novel means of recording a well-established ERP component, it was necessary to consider several factors that may potentially influence the results. For example, the use of a reward-based active auditory discrimination task was judged to be informative and was expected to yield a robust P300, but it is cautioned that the rats must be sufficiently trained on the goals of the task. Specifically, Jodo et al. (1995) found that the rats showed a P300 to correct responses to the rare, target tone as well as to correct withholding of responses to the standard tone. This similarity disappeared after the rats were well trained on the task, but it was still important to ensure that the rats fully understood the nature of the task before we performed the surgery to implant electrodes in order to make sure this type of confound was not present in the data. Along the same

line, sufficient training was necessary to produce the most robust P300 possible; Takeuchi et al. (2000) found that the amplitude of the P300 and N100 increased as accuracy levels of lever pressing to the target tone increased. Changing the reinforcement paradigms can also influence the amplitude of ERP components. Specifically, the N100, but not the P300, has been shown to decrease when reinforcement is no longer provided in an active task (Ehlers, Somes, et al., 1998). As such, ensuring that the rats fully understand the task demands for earning a reinforcer, and decisions regarding the removal of a reinforcer, need to be taken into consideration for active ERP studies.

The selection of the latency windows for the ERP components of interest should also be considered very carefully. As discussed earlier, there is no agreed-upon window in which all researchers consider a specific ERP component. Additionally, one must consider the time span over which the neuroelectrical information travel in order to be detected by the electrodes from the various regions from which they are generated. Due to the large number of generators of the ERP components, and the divergent time points at which the signals reach the recording electrodes, one should also consider using a broader latency range in order to fully capture the incoming signals (Ehlers, Wall, et al., 1991).

One final consideration that should be made is in regard to discerning the various ERP components is to understand that the signal can vary depending upon the nature of the evoking stimulus. According to Donchin, Ritter, and McCallum (1978), ERPs that are always elicited by stimuli and events that occur beyond the organism's physical nervous system are considered to be exogenous. Conversely, ERPs that are elicited with

or without external stimulation are considered to be endogenous. This was further supported by Umbricht et al. (2004), who stated that if an ERP is evoked by a sensory event that occurs in the animal's external environment, then they are considered to be sensory/exogenous. Also, if the ERP components occur because of an internal response to a stimulus, such as a response that is often made following a presentation of a rare tone, then they are considered to be cognitive/endogenous. Donchin et al. (1978) emphasized the importance of this distinction, specifically because when variance and noise in ERP data are not directly due to variance in the presented stimuli (i.e., occurring externally), then the resulting ERPs are considered to be endogenous (i.e., resultant of an internal, cognitive nature). Also of considerable note is the fact that endogenous ERPs are considered to be associated with a subject's decision making processes and intended responses (Donchin et al., 1978); both of which occur internally. With this in mind, it becomes clear that when conducting an active stimulus discrimination task involving rodent models the resulting ERP responses should be considered to be exogenous.

Numerous considerations also must be taken into account when using an acute ethanol challenge to alter the P300. First and foremost, divergent findings have resulted from acute ethanol challenges wherein some studies show that high doses of ethanol lead to a significant reduction in P300 amplitude and increase in P300 latency while others do not report these effects (Bauer, 2001; Colrain et al., 1993; Elmasian et al., 1982; Erwin et al., 1986; Fowler & Adams, 1993; Teo & Ferguson, 1986; Sommer et al., 1993). The differences in these findings can be clarified when one considers other factors, such as the dose of ethanol used and the point at which ERPs are recorded, both of which influence the effect that ethanol administration has on the P300 in humans (Bauer, 2001)

and rats (Lee et al., 1990). If an ethanol challenge is used while engaged in an active task, considerations must be made in regard to the subjects' motor skills while undergoing detoxification. According to the mild generalized dysfunction hypothesis, alcoholics are prone to developing impaired performance on neuropsychological tests that involve motor skills (Tivis, Beatty, Nixon, & Parsons, 1995). Likewise, the intoxication levels in rodent models should be monitored closely during a study involving an acute ethanol challenge to ensure the subject is still capable of performing the active responses.

The main goal of this investigation was to determine if the P300 could be recorded in a rodent model using minimally-invasive subcutaneous electrodes in lieu of the invasive surgical procedures that have been commonly used to record this component. The P300 is a distinct ERP that occurs approximately 300 ms after the onset of a novel stimulus and is studied as a psychophysiological marker for drug dependence. The current study implemented a series of ethanol challenges using two different ethanol doses to determine the impact on the amplitude and the latency of the P300 in Wistar rats during an active auditory stimulus discrimination task.

The use of the current experimental paradigm will help to establish a scientifically rigorous model for studying ERPs in rodents that promotes reduction and refinement. The P300 has been recorded in humans using minimally invasive methods, but the recording method for non-human mammals has been, to date, intrusive; the scalp is opened, recording screws and electrodes are typically surgically implanted into the skull or subcortical regions, and sutures are used to close the wound. This intrusive approach is beneficial in order to determine specific brain areas that might elicit the P300. However, a survey of current research indicates that targeting specific areas for recording does not

yield substantial benefit over more global cortical measures of the P300. As such, this study used a less invasive means of recording the P300 by using subcutaneous electrodes that could easily be implanted under the scalp via a needle rather than an invasive surgery (see Figure 1, right).

This experiment was thus a proof-of-concept study using male and female Wistar rats to determine the success of this minimally-invasive recording procedure. We hypothesized that a robust P300 would be found using this minimally-invasive method, and that the chosen ethanol doses (0, 0.75, and 1.5 g/kg IP) would dose-dependently attenuate the amplitude of the P300 response.

Chapter 5: Methods

Subjects

Six male and five female Wistar rats (Harlan; Indianapolis, IN) were delivered to the laboratory at around post-natal day (PND) 45, at which time they were weighed and handled daily with food and water available *ad libitum*. For each phase of the experiment, rats were housed in same-sex pairs in standard rat shoe-box caging. At 50 days old, all rats were placed on an IACUC-approved food restriction schedule, designed to maintain the rats at 85% of their free-feeding weight (Harlan Teklad 2018), which persisted until the end of the experiment. All rats were kept on a regular 12-hr light/dark cycle (lights on at 0730 hr) and were weighed, tested, and fed at the same times each day during the lights-on phase.

Apparatus

All operant behavioral programs were controlled via a PC equipped with Med–PC IV software (Med Associates; St. Albans, VT). The testing chambers were both sound-attenuating and ventilated. On one wall of the chambers were two retractable response levers, each 7 cm above the floor of the chamber and 5.7 cm from the midline of the wall. Two cue lights were also present in the operant chambers, 5.7 cm above the response levers. A house light was situated on the wall opposite the levers. A food magazine was situated between the response levers. A speaker in the operant conditioning chamber presented both the rare and standard tones throughout testing. The Med-PC system generated TTL pulses that were synchronized with the onset of each stimulus and sent this information to the DataWave SciWorks Express software program (DataWave Technologies Corporation, Loveland, CO). These TTL pulses were transmitted through a

TTL cable connected from the Med Associates interface to a digital input on the TTL relay interface (Breakout Box, model SG-726-TTL). This relay interface was connected to the DataWave interface (16-Bit High-Speed Multifunction DAQ Device model #USB-1608G, manufactured by Measurement Computing; Norton, MA), which was connected to a computer running the SciWorks Express software. This setup was necessary to establish a means of electrical communication between the two systems in order to time-lock the psychophysiological recordings to the individual behavioral oddball test trials occurring within the Med-Associates operant testing chambers. All electrophysiological measures were amplified on Grass 8-16 E amplifiers.

Ethanol Solutions

Studies have demonstrated that high doses of ethanol can lead to psychophysiological deficits as well as motor deficits (Ehlers et al., 2014; Freund, 1969; Hunter, Boast, Walker, & Zornetzer, 1973). Because motor deficits would have been problematic for this study, we chose to use lower doses (0.75 and 1.5 g/kg) expected to induce reductions to the P300 without profound motor impairment. The 0.75 and 1.5 g/kg doses when given intraperitoneally (IP) in Wistar rats have been shown to produce BALs of approximately 75 and 140 mg%, respectively, and these elevated BALs persisted 60 min after IP injection (Walker & Ehlers, 2009). Stock solution of 200 proof ethanol was diluted with 0.9% physiological saline to yield a 15% (w/v) concentration. The doses of 0.75 g/kg and 1.5 g/kg BW were achieved by adjusting the volume administered to each rat. The 0 g/kg dose (saline control) used a volume equivalent to the highest ethanol dose.

Procedure

At 60 days old, rats began training and testing in the operant chambers. All behavioral testing was conducted during the light part of the rat sleep/wake cycle between 0700 and 1600 hrs. Operant testing occurred 7 days/week.

Autoshaping and fixed ratio training. During autoshaping, both levers were made available to the rat, and every press on either lever resulted in the delivery of a 45 mg, banana-flavored, purified, dustless precision reward pellet (Bio Serv; Frenchtown, New Jersey) into the food magazine. If no lever press occurred within 3 min, the cue lights were illuminated for 15 s, followed by the delivery of a free reward pellet to entice the rat to explore the operant box and to press the levers. This process was repeated every 3 m if no lever pressing occurred. The rats were given a maximum of 1 hr to earn 100 pellets, including free pellets, and remained on autoshaping until 100 lever presses occurred in a session and zero free pellets were dispensed.

Following autoshaping, rats began fixed ratio 1 (FR1), FR3, and FR5 training. Each lever press on the active and available lever in the FR1 task resulted in the delivery of a food pellet into the food magazine. On the FR3 and FR5 tasks, a pellet was delivered only after 3 and 5 lever presses were made, respectively. When a reinforceable lever press was made on the FR tasks, a 250 ms, 2 kHz, 85 dB auditory tone sounded and a food reward was delivered; this tone was later used as the rare tone in the auditory oddball task. This addition of the rare tone to the FR schedules trained the rats to form an association between the rare tone and the delivery of a food pellet and also served to help the rats understand that not every lever press resulted in delivery of a food reward.

Signal detection auditory oddball task. Once 100 reinforcers were earned under the FR5 response contingency, signal discrimination training began wherein rats learned to lever press for a food reward only after detecting the rare, target (250 ms, 2 kHz, 85 dB) auditory target. A standard, non-target (500 ms, 2 kHz, 85 dB) auditory signal was used as the non-reinforced stimulus. At the start of each trial, the right response lever was extended and the cue light above the lever was illuminated in order to signal the start of a new trial. After 1000 ms the presentation of either the standard tone or the rare tone occurred. The percentage of trials with presentations of the rare tone gradually decreased with increasing testing sessions. Rats first underwent training in which each session had 60% standard stimulus presentations with 40% rare stimulus presentations (60-40). These were followed by sessions with 70% standard and 30% rare stimulus presentations (70-30), and ultimately 80% standard, 20% rare stimulus presentations. To allow for a larger number of training trials, the 60-40 and 70-30 sessions were 100 min long. The 20-80 sessions were only 50 min long.

During the 60-40 and 70-30 training sessions, if a response was made on the lever within 2,000 ms after the *rare* tone was presented (i.e., a "hit") then this resulted in delivery of a food pellet, termination of the cue light, and inactivation of the response lever. The cue light remained off until 5,000 ms elapsed from onset of the previous *tone* at which point a new trial began. This delay was necessary to allow the rats to have time to collect and eat their food reward and to maintain a consistent trial length of 6,000 ms. If no lever press response was made within 2,000 ms after the presentation of the *rare* tone, then the lever was retracted, the trial was counted as an error (i.e., a "miss"), the cue light was turned off and a new trial presented 5,000 ms after onset of the previous tone.

If a response was withheld for a full 2,000 ms after the presentation of a *standard* tone (i.e., a "correct rejection") then the cue light was turned off, and a new trial presented 5,000 ms after onset of the previous tone. If a lever press response was made after presentation of the standard tone (i.e., a "false alarm") or if no lever press occurred within 2,000 ms of the target tone (i.e., a "miss") then the cue light and house light were turned off, the trial was counted as an error, and a time out (with the house light extinguished) of 18 s occurred before a new trial began. In the event of an incorrect response (i.e., a miss or false alarm) the program initiated a correction trial, which repeated until a correct response was made. These correction trials were necessary to determine if a rat was lever pressing to each and every stimulus (only to be rewarded on some trials), or if the rat understood that only the rare tone signaled reinforcer availability. The 20-80 task did not include correction trials or an extended time-out for incorrect trials to ensure that every trial was the same length of 6,000 ms. Continuous EEG data were collected during the 20-80 task. The elimination of the correction trials and time-out periods were necessary to ensure an accurate time-lock between the Med-Associates and DataWave interfaces and allow for the presentation of a large number (i.e., 500) of individuals trials. Likewise, because psychophysiological recordings occurred during the 20-80 task it was necessary to ensure the entire testing period was completed while the BAL of each rat was elevated (Lee et al., 1990).

Great care was taken to ensure that there was no distracting environmental noise from people or equipment while the rats were testing on the auditory stimulus discrimination tasks. Attentional processes and resources were paramount to completing the task successfully. More importantly, it has been found that, when distractions are

present during testing, the auditory stimuli will not elicit the P300 that would have been found in an otherwise distraction-free environment (Duncan et al., 2009). Testing on each stimulus discrimination program continued until the rats reached a high level of accuracy for responses to the rare trials (at least 80%) and an acceptable level of accuracy to the standard trials (at least 20%) across a minimum of seven consecutive sessions. The majority of the rats tested in this study surpassed these criteria for performance.

Surgery. In order to maintain high levels of performance, the rats were only tested on the 20-80 oddball task for one day before undergoing surgery. Because the 20-80 did not have correction trials or time-outs for incorrect responses, there was a concern that keeping them on the 20-80 program for an extended period could potentially lead to a decrease in performance. After a day of testing on the 20-80 program, each rat underwent surgery to implant 7 mm subcutaneous needle electrodes (Rhythmlink International LLC; Columbia, SC) using aseptic procedures. Needle electrodes were chosen based upon the need for an electrode with enough structure and form to stay in place, at Pz, during daily testing sessions. Mayaud et al. (2013) found that there was no difference in relative electrode performance when using traditional disc electrodes, which adhere to the scalp and are traditionally used in human EEG studies, compared to subcutaneous needle electrodes.

Sedation was accomplished via a mix of oxygen and Isothesia isofluorane (1%) using a Surgivet Classic T^3 Isofluorane delivery system (Dublin, Ohio). A single recording electrode was placed on the midline at Pz, 3 mm posterior to bregma. A reference electrode was placed posterior to lambda, at roughly -6 mm (see Figure 1, right). Correct placement of the electrodes was determined by radiographs (DuoView;

Kennesaw, GA) taken immediately after the surgical procedure while the rats remained anesthetized (see Figure 2, left). The amount of radioactive exposure to the rats was recorded and was found to be minimal. This process was necessary to ensure correct placement of the electrodes prior to EEG recordings, as it indicated precise placement of

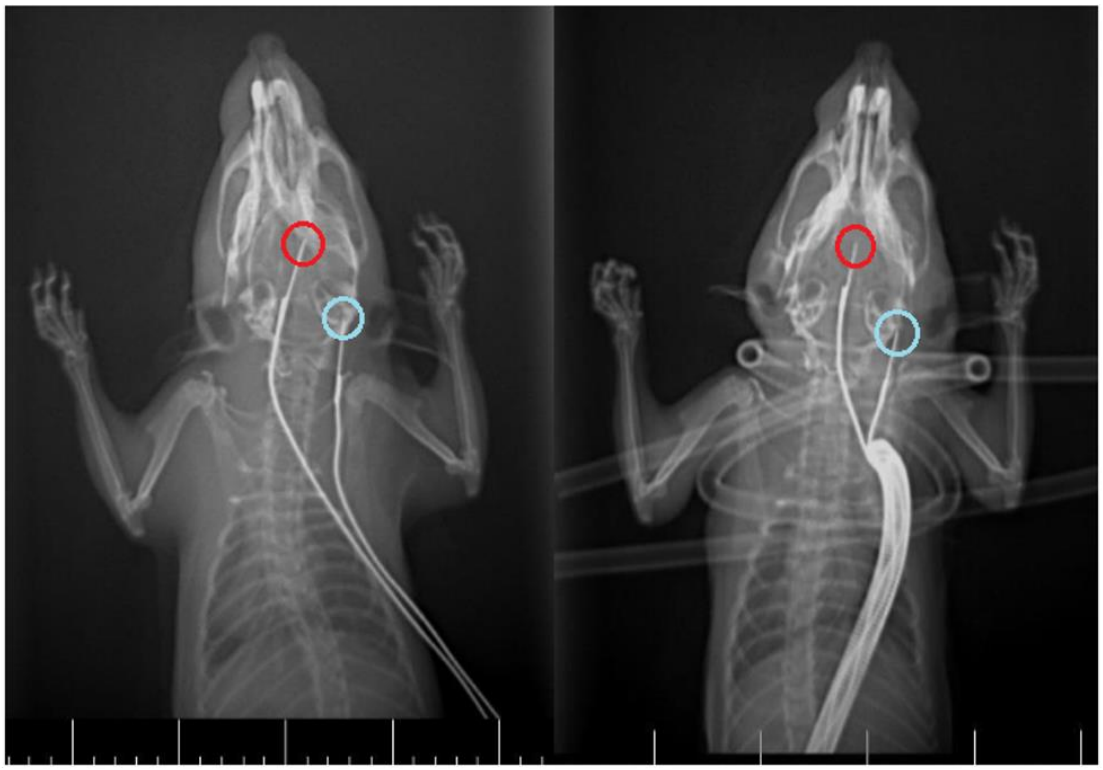

Figure 2. Radiographs of Electrode Placements. The use of needle electrodes allowed for daily ERP measurements with little movement of the electrodes from their recording position at the time of surgery (left) to the time the electrodes were removed (right).

the recording and reference electrodes. If the radiographs revealed incorrect placement of the electrodes, the incorrect electrode was removed, replaced, and an additional radiograph was taken to ensure proper placement.

The subcutaneous electrode lead wires exited the scalp at the injection sites and were secured in place with sutures and adhesive material to prevent movement. An access harness (Instech Solomon; Plymouth Meeting, PA) was used in order to stabilize and protect the electrode lead wires. When the surgery was complete, rats were given subcutaneous injections of baytril (an antibiotic, 2.5 mg/kg) and carprofen (an anti-inflammatory, 2.5 mg/kg), and were allowed 18 hrs for recovery. After recovery, the rats resumed testing on the 20-80 auditory oddball task.

EEG Acquisition

Within each 20-80 testing session, the rats experienced a total of 500 trials (400 standard tone trials, 100 rare tone trials). EEGs were recorded from the recording electrode at Pz, which was referenced to another electrode placed posterior to lambda (see Figure 1, right). The incoming EEG activity was digitized at a sampling rate of 50 kHz at a high-pass setting of 0.1 Hz and a low pass of 35 Hz, first through the Grass 8-16 E amplifiers and then again, at these same settings, through the SciWorks Express software to reduce systematic hardware artifacts in the data.

Ethanol Challenges

For all rats, the first session of the 20-80 oddball task after surgery was preceded by IP injection of saline given 15 min before testing and continuous EEG recording began. Over the following 2 days, each rat was given either a 0.75 g/kg or a 1.5 g/kg IP dose of ethanol. Each dose was given only once and the order of presentation was counterbalanced. Fifteen min after ethanol administration and prior to continuous EEG recording, the intoxication level of each rat was assessed. We used a modified version of the same intoxication scale used by Ehlers et al. (2014), who modified this scale from

Freund (1969) and Hunter et al. (1973). The level of physiological intoxication for each rat is rated on a scale of 0 (no intoxication) to 4 (hazardous level of intoxication marked by immobility). The ratings for this intoxication scale can be found in Table 1. According to Ehlers et al.'s (2014) results, the 1.5 g/kg dose led to an average intoxication score of 2.71 in adult males 15 min after injection while the 3 g/kg dose led to an average intoxication score of 3.71 in adult males 15 min after injection. An intoxication score that is 3 or higher indicates motor deficits and impairment in gait.

Table 1. Ehlers et al. (2014) Neurobehavioral Assessment of Ethanol Intoxication in Rats

Rating	Neurobehavioral Profile
0	Rat does not show any signs of intoxication
1	Rat is subdued and has decreased muscle tone when handled
2	Rat shows mild ataxia that is presented with a rapid gait
3	Rat shows major impairment in gait and often falls to one side
4	Rat is immobile and does not have any muscle tone when handled

Because this was an active task, it was important that intoxication levels not exceed the point at which motor problems would develop. As such, we decided to use doses of 0.75 and 1.5 g/kg in an attempt to induce intoxication without impairing motor function.

Euthanasia and Final Verification of Electrode Placement

At the conclusion of the study, rats were euthanized via overexposure to CO_2, at which point a final radiograph was taken to confirm that the electrodes remained in proper position during testing (see Figure 2, right).

Chapter 6: Data Analysis

A list of all of the dependent measures that were analyzed has been included in Table 2. All data analyses were conducted via SPSS for Windows, version 22.

Table 2. Dependent Variables used in ERP Data Analyses

Type of Measure	Dependent variable(s)
Behavioral	Percent correct responses Response latency to target tone
Psychophysiological	Amplitude of the N100 signal Amplitude of the P300 signal
Intoxication level	Score on ethanol intoxication index

20-80 Behavioral Data

To determine the percent correct responding to each tone and trial type (rare and standard), we used the following equations (abbreviations presented in Table 3):

$$\%\ \text{correct to rare, target tone} = \frac{\text{CORT}}{(\text{CORT} + \text{INCT})}$$

$$\%\ \text{correct to standard, non-target tone} = \frac{\text{CORS}}{(\text{INCS} + \text{CORS})}$$

Table 3. List of Behavioral Measures on Auditory Stimulus Discrimination Program

Abbreviation	Behavioral Measure
CORT	Number of correct responses to the rare tone
INCS	Number of incorrect responses to the standard tone
INCT	Number of incorrect inhibitions of responding to the rare tone
CORS	Number of correct inhibitions of responding to the standard tone

As ERP measures were determined during the 20-80 oddball task, only these data are presented here. The average response latency to the target tone (minus trials with premature responses that occurred within 100 ms of tone presentation) as well as the percent correct to the rare targets (i.e., hits) and the percent correct to the standards (i.e., correct rejections), were each analyzed separately using a 2 (sex) x 3 (ethanol dose) x 2 (tone) mixed ANOVA where sex and tone were between-subject factors and ethanol dose was a repeated-measures factor.

ERP Data Analyses

For each day of psychophysiological recordings, the data sets were saved as text files and loaded into MATLAB (MathWorks; Natick, MA) in order to compile the large data sets into averages. ERP data from each individual 20-80 oddball trial was sorted into one of four response bins (hits, misses, correct rejections, and false alarms) using MATLAB (MathWorks; Natick, MA) based on trial-by-trial information recorded by the Med-PC IV software for each 20-80 testing session. Using MATLAB, the data was

down-sampled by a factor of 100; each point in the new dataset (~500 Hz) was the average of 100 consecutive points in the old dataset. The down-sampled data were then segmented, screened for artifacts, and sorted by MATLAB. If an epoch contained 50 consecutive data points (~100 ms) across which the amplitude varied less than 1 μV (basically a flat line), activity that exceeded 500 μV (plus or minus, relative to the pre-stimulus baseline), or adjacent data points that differed by more than 100 μV, it was not included in the data averages.

Averages were taken for all trials that did not contain artifacts. However, the extreme amount of noise and artifacts in the raw data led to the elimination of a substantial number of trials from our analyses. The high sampling rate that was needed for this study also made it necessary to filter the data twice; first through Sciworks and then again through MATLAB. Despite efforts to increase the fidelity of the EEG signals, we still contended with quite a lot of noise. The noise was found to be relatively high frequency, but it was so large and unsystematic that the digital filtering did not remove all of it. Data epochs were 600 ms in duration, including a 100 ms baseline period prior to the onset of each tone. All data that was calculated and plotted into waveforms were baseline corrected by subtracting baseline EEG activity from the values recorded after stimulus presentation. All ERP data across all trials without artifact were first averaged into a single overall waveform and then the trials were separated into two separate waveforms for target versus standard trials (regardless of behavioral response). Lastly, the ERP data were sorted according to the response type (i.e., hits, misses, correct rejections, and false alarms). Based on visual inspection of the overall waveform, the N100 was denoted as the most negative deflection in voltage that occurred between 60

and 100 ms after tone presentation, and the P300 was denoted as the most positive peak in voltage that occurred between 300 and 400 ms after tone presentation.

The first set of analyses was conducted on the overall waveform, which represented the average of all artifact-free trials (ranging from 285-474 trials, mean = 398) to confirm the presence of the N100 and P300 responses. Two separate single-sample *t*-tests were conducted to compare the mean amplitude of the N100 (latency 60-100 ms) and the mean amplitude of the P300 (latency 300-400 ms), respectively, to the known value of 0 μV, which represents a lack of EEG activity.

For the second set of analyses, the ERP waveforms were separated into rare tone trials (including hits and misses) and standard tone trials (including false alarms and correct rejections). The number of target tone trials included in this set of analyses ranged from 65-97 trials (mean = 84). The number of standard tone trials included in this set of analyses ranged from 268-384 trials (mean = 334). As was done on the overall ERP waveform, the mean N100 and P300 amplitude for the rare and again for the standard tone trials were analyzed using two separate one sample *t*-tests (H_0: $\mu = 0$). In addition, the mean amplitudes for each ERP component of the target and tone were compared to each other, using a paired-samples *t*-test. Lastly, a 2 x 3 mixed ANOVA was conducted on the mean amplitudes of the N100 and P300 to the target tones in order to examine the main effects of sex and ethanol dose as well as the interaction of these factors on these ERP components.

For the third and final set of analyses, ERP waveforms were created for rare tone trial hits (hits only; range = 11-92 trials, mean = 58) and correct rejection standard tone trials (correct rejections only; range = 15-365 trials, mean = 164), both of which excluded

any trials in which a premature behavioral response was made (i.e., responses occurring less than 100 ms after presentation of either tone).

20-80 ERPs: Ethanol Challenge Data

Additional analyses were conducted to evaluate the effects of acute ethanol injection. Recall that prior to collecting ERP data, an ethanol challenge was administered via IP injection at doses of 0.0, 0.75, and 1.5 g/kg body weight and the intoxication level of each rat was assessed by two trained raters using a scale of 0 (no intoxication) to 4 (severe intoxication), 15 min after IP injection. Interrater reliability was calculated using Cohen's Kappa to determine consistency in intoxication ratings between raters, based on the level of ethanol used. The interpretation of the relative strength of the Kappa scores has been reported to be poor agreement if less than 0, slight agreement if 0 – 0.20, fair agreement if 0.21 – 0.40, moderate agreement if 0.41 – 0.60, substantial agreement if 0.61 – 0.80, and almost perfect to perfect agreement if 0.81 – 1.00 (Landis & Koch, 1977). The two ratings at each time point were averaged and these intoxication ratings were analyzed using a 2 (sex) x 3 (ethanol dose) mixed ANOVA where sex was a between-subjects factor and ethanol dose was a repeated-measures factor.

Chapter 7: Results

20-80 Oddball Behavioral Performance

An initial review of performance measures on the 20-80 oddball task revealed that the rats had a tendency to lever press prematurely (i.e., before either tone was presented). The premature latency of responses indicated that behavioral responses were often made prior to perceptual processing of the auditory stimuli. In order to ensure that the behavioral data reflected actual responses to the tones, and not premature responses that occurred before processing of the tone could occur, all trials that had a latency of response less than 100 ms were eliminated from analysis. A 2 (sex) x 3 (ethanol dose) mixed ANOVA revealed no significant main effect for sex, $F(1,9) = 4.355$, $p = .067$, nor a significant interaction between sex and ethanol dose, $F(1,18) = 0.012$, $p = .917$, on the average response latency of target tone hits. However, there was a significant main effect of ethanol dose, $F(2,18) = 4.56$, $p = .025$, wherein the 0.75 g/kg ethanol dose led to a significantly faster response latency compared to saline, $p = .002$. The latency of response to the target tone following 1.5 g/kg dose of ethanol was reduced, but this finding was not significant, $p = .103$ (see Figure 3).

Analysis of the percent correct using a 2 (sex) x 3 (ethanol dose) x 2 (tone) mixed ANOVA revealed that the main effect of sex, $F(1,9) = 0.50$, $p = .497$, and the main effect of ethanol dose, $F(2,18) = 1.819$, $p = .191$, were not significant. However, the main effect of tone was significant, $F(1,9) = 6.538$, $p = .031$, wherein the percent of correct response inhibitions to the standard tone was greater than the percent hits to the target tone (M = 61.982, SEM = 4.454 and M = 39.171, SEM = 4.330, respectively). The same analysis revealed no significant interactions. These results are presented in Table 4.

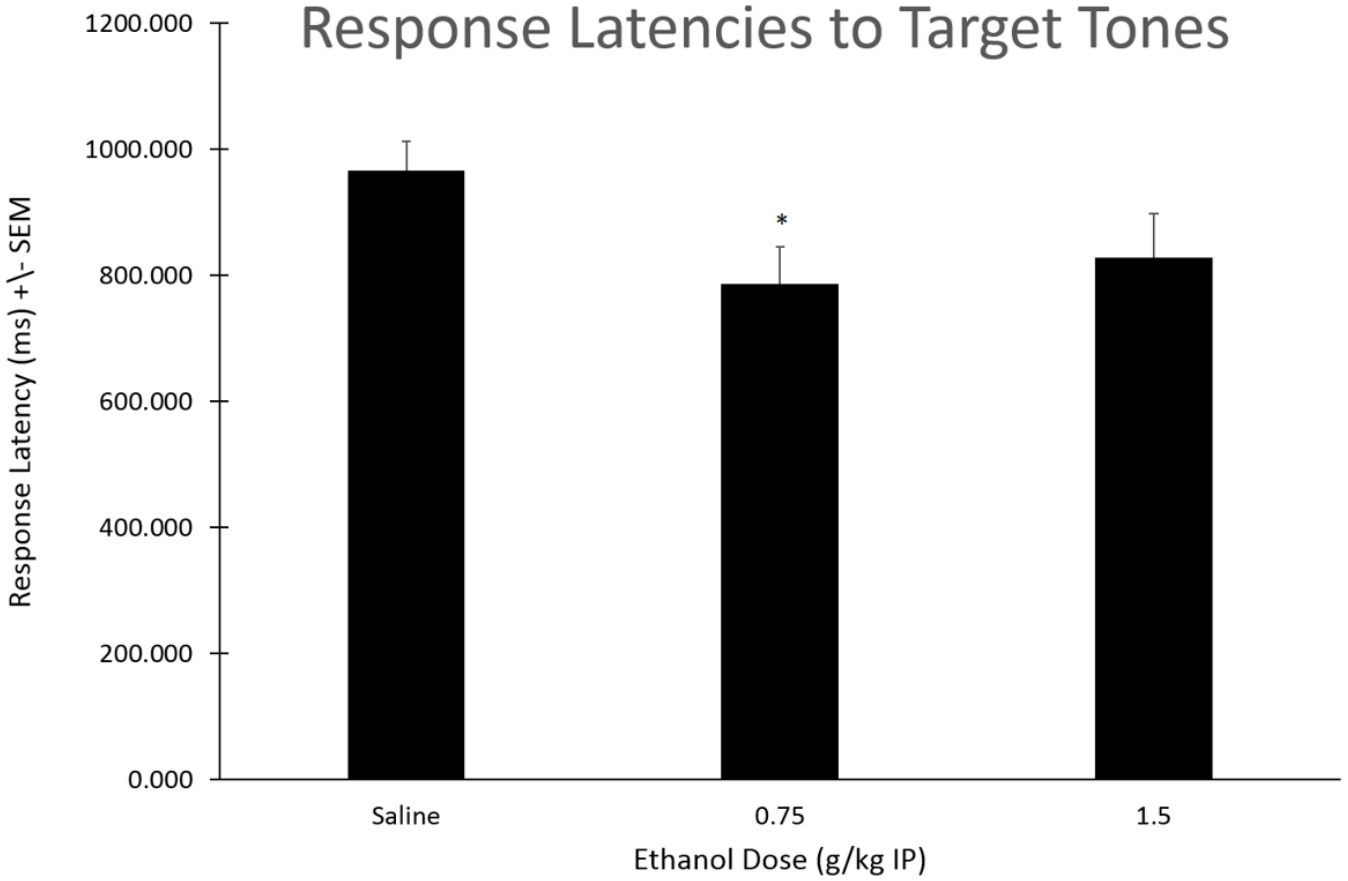

Figure 3. Effect of Ethanol on Average Response Latency to Target Tone. There was a significant main effect of ethanol dose on the average response latency to correctly respond to presentations of the target tone, wherein the 0.75 g/kg dose of ethanol led to a significantly faster response time compared to the vehicle saline dose. *p = .002 (n = 11)

ERP Measures Across all Trials

For the first set of analyses, all ERP data were analyzed collectively, collapsed across all factors, in order to determine if an N100 and P300 were present.

N100 and P300. Visual inspection of the data revealed no clear N100 but an apparent P300 that was largest in the 300-400 ms latency range (see Figure 4). However, a single sample *t*-test revealed that the average amplitude of the P300 (latency 300-400 ms) was not significantly different from 0, $t(10) = 2.228$, $p = .190$.

Table 4. Performance on 20-80 Oddball Task following Acute Ethanol Challenge

	Percent Correct to Rare, Target Tone	Percent Correct to Standard, Non-target Tone
Males		
Ethanol Dose		
0.0 (saline)	58.83	39.50
0.75 g/kg	53.33	44.95
1.5 g/kg	61.24	39.40
Females		
Ethanol Dose		
0.0 (saline)	55.88	41.80
0.75 g/kg	68.40	31.96
1.5 g/kg	48.48	52.90

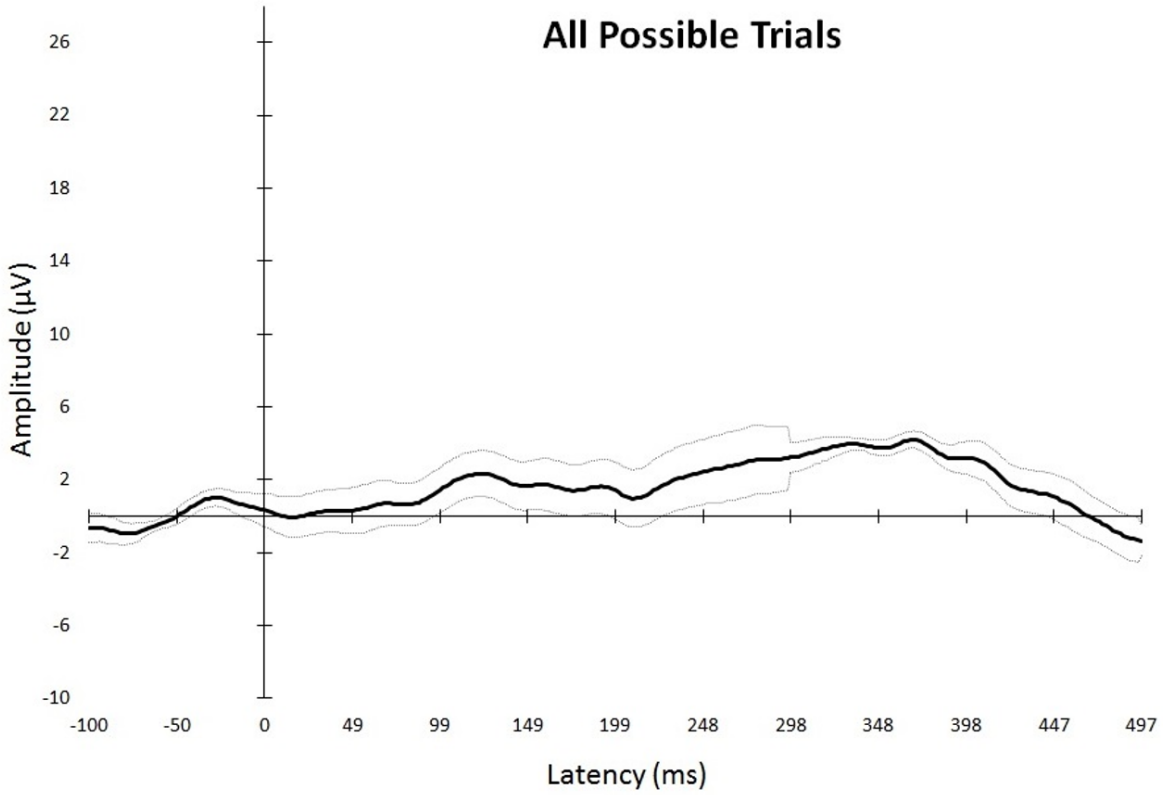

Figure 4. Average ERP Activity Collapsed across all Trials (means ± SEMs). The negative occurring approximately 60-100 ms was chosen to represent the latency range for the N100, while the positive deflection from about 300-400 ms was selected as the latency for the P300 (n = 11)

ERP Measures for Target versus Standard Trials

Knowing that the P300 is more likely for rare, target tones than for standard tones, the second set of analyses separated the ERP waveforms based on the tone presented (target vs. standard) for all trials, regardless of behavioral response.

N100 and P300. Visual inspection of the data revealed no clear N100 or P300 ERP to the standard tone. However, visual inspection suggested the possibility of a P300 to the target tone. A single sample *t*-test revealed that the mean amplitude of the N100 to the target tone (latency 60-100 ms) was not significantly different from 0, $t(10) = 0.358$, $p = 0.728$. Similar analysis of the average P300 (latency 300-400 ms), however, did reveal

a significant difference, $t(10) = 3.07$, $p = .012$ (see Figure 5). A paired-samples t-test revealed the mean amplitude of the target P300 was significantly higher than the mean within the same latency range following presentations of the standard tone, $t(10) = 4.117$, $p = .002$.

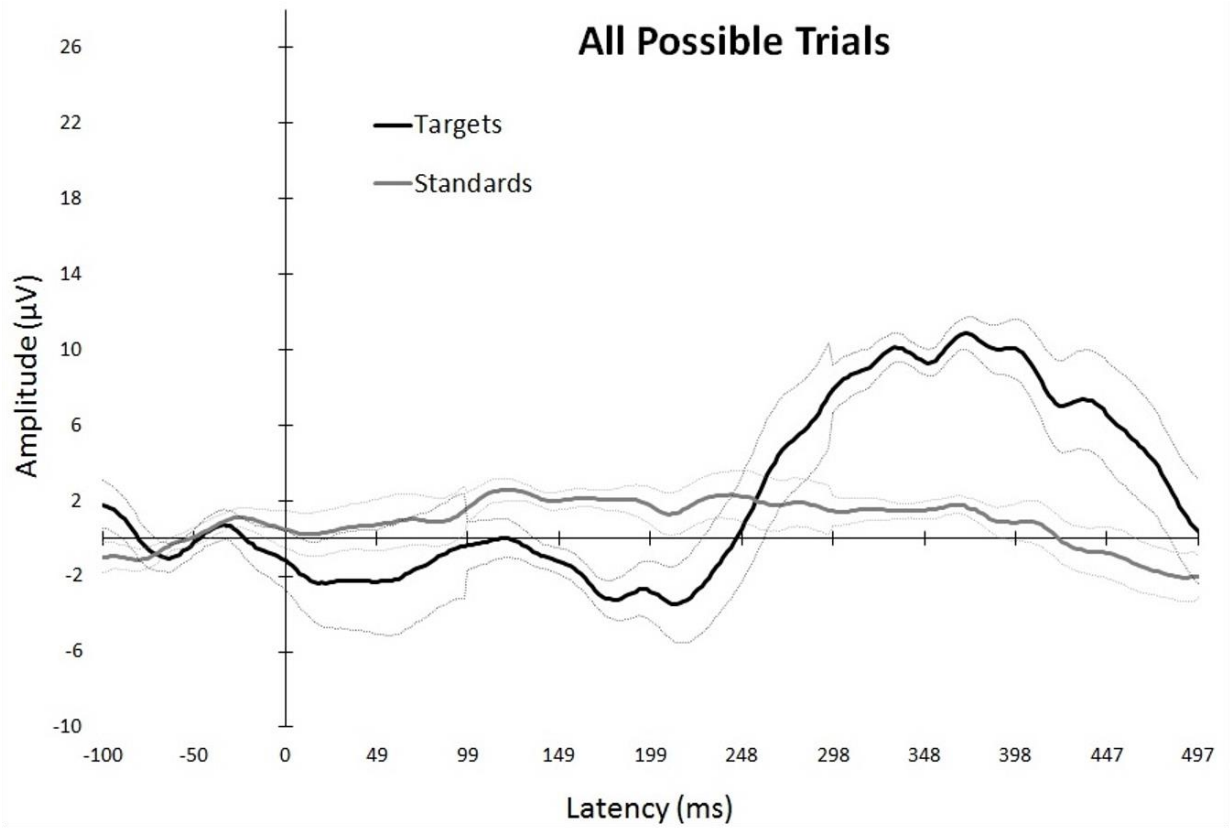

Figure 5. Average ERP Activity for Target and Standard Tone Trials (means ± SEM). A N100 response was not found following presentations of the standard and target tones, likely due to the posterior placement of the recording electrode. A significant P300 was found for the target tone, the amplitude of which was significantly higher than that of the standard tone demonstrating that the rats were able to cognitively discern between the two tones with the target being more meaningful ($n = 11$)

Evaluation of sex and ethanol effects. Visual investigation of the waveforms led to an expectation for the P300 amplitude to be larger for females than in males (Figure 6). Likewise, the P300 amplitude appeared to be larger for saline than after either

ethanol dose (Figure 7). However, a 2 (sex) x 3 (ethanol dose) mixed ANOVA revealed no significant main effect of sex, $F(1,9) = 2.381$, $p = .157$, dose, $F(2,18) = 1.564$, $p = .26$, or an interaction between these two factors, $F(2,18) = 0.160$, $p = .853$. Given the small sample size, analyses of effect size are included. Using Cohen's *f*, large effect sizes for sex and dose ($f = 0.514$ and 0.417, respectively) were found. Only a small effect size for the sex x dose interaction was found ($f = 0.132$). The mean P300 amplitudes from 300-400 ms for the males and females for the target tone are presented in Figure 8. Females appeared to have a higher mean amplitude than males. Likewise, the mean P300 amplitude from 300-400 ms across the three ethanol doses are presented in Figure 9. Ethanol appeared to attenuate the P300 amplitude elicited by the target tone.

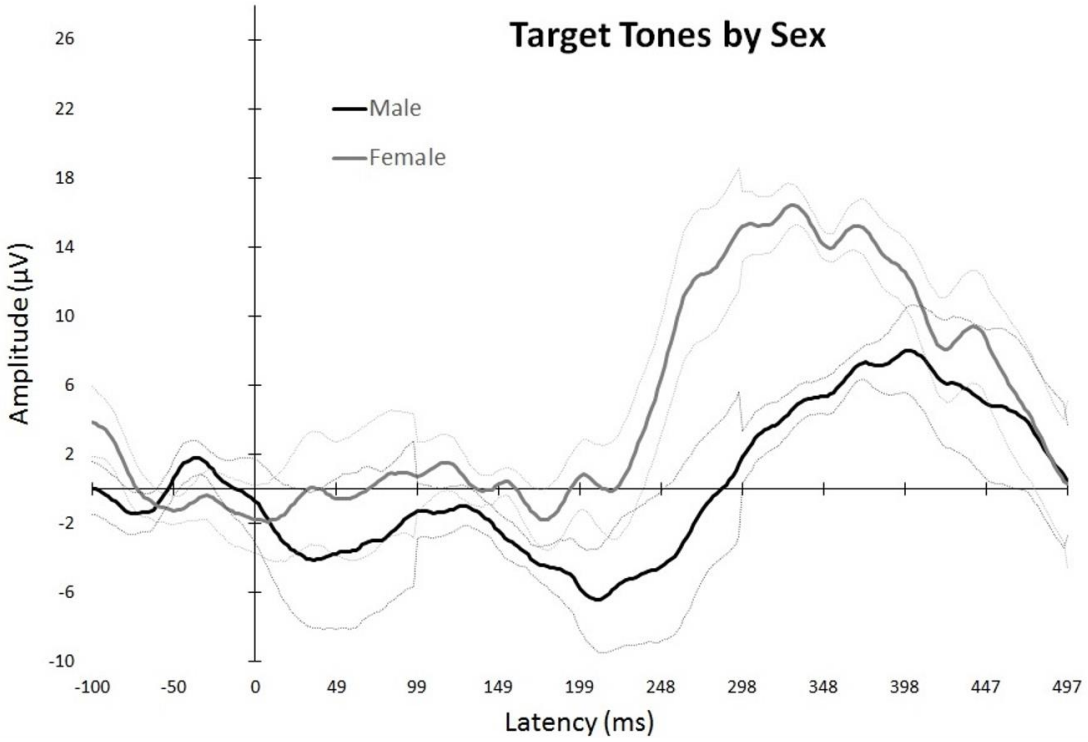

Figure 6. The Effect of Sex on ERP Activity Following Presentations of the Target Tone. There was no main effect of sex on P300 amplitude, however a large effect size was found ($f = .514$). Females ($n = 5$) appeared have a higher P300 amplitude than males ($n = 6$).

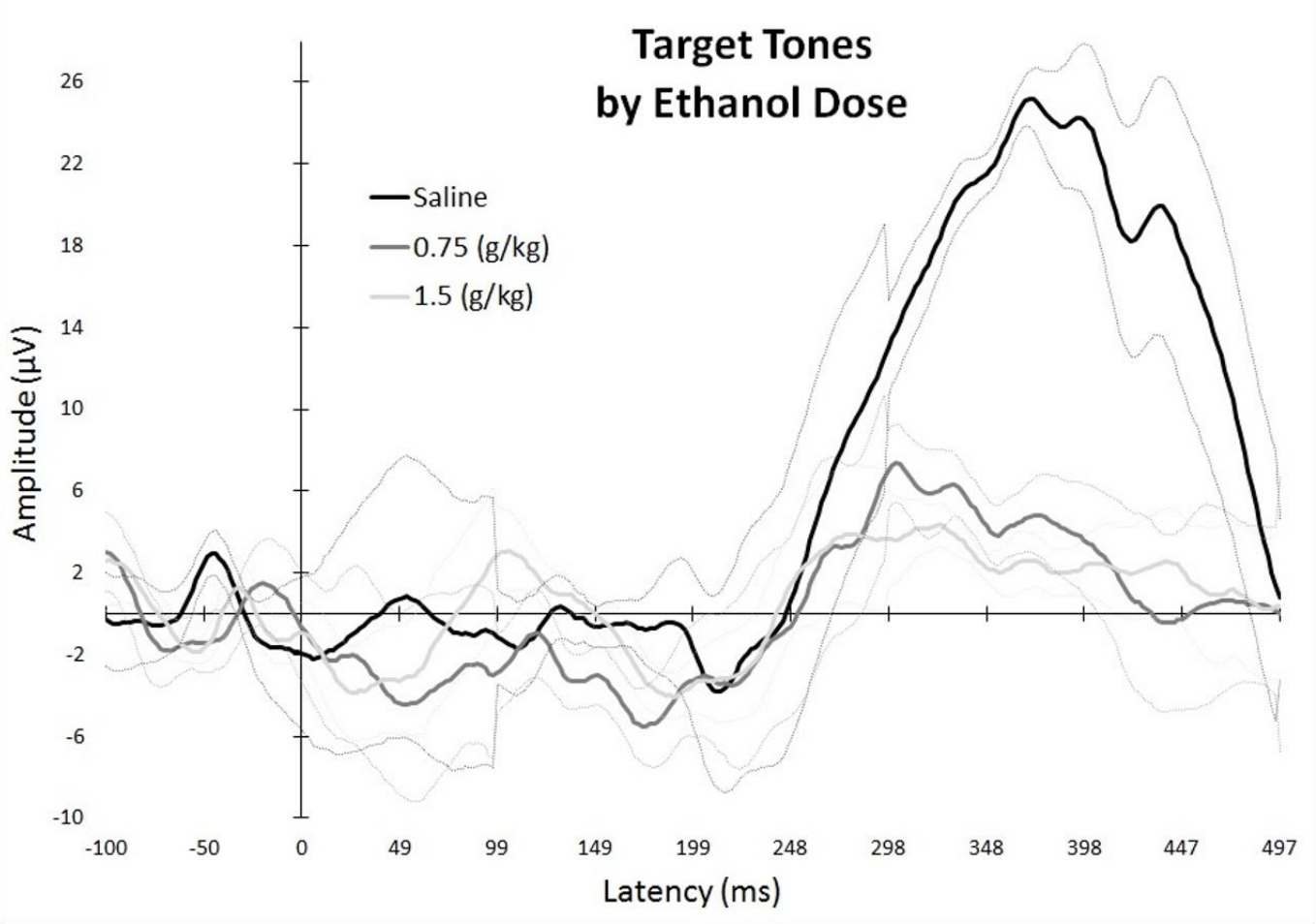

Figure 7. The Effect of Ethanol on ERP Activity Following Presentations of the Target Tone (means ± SEM). There was no main effect of ethanol on P300 amplitude, however a large effect size (f = .417) was found. Ethanol appeared to attenuate the amplitude of the target P300 (n = 11).

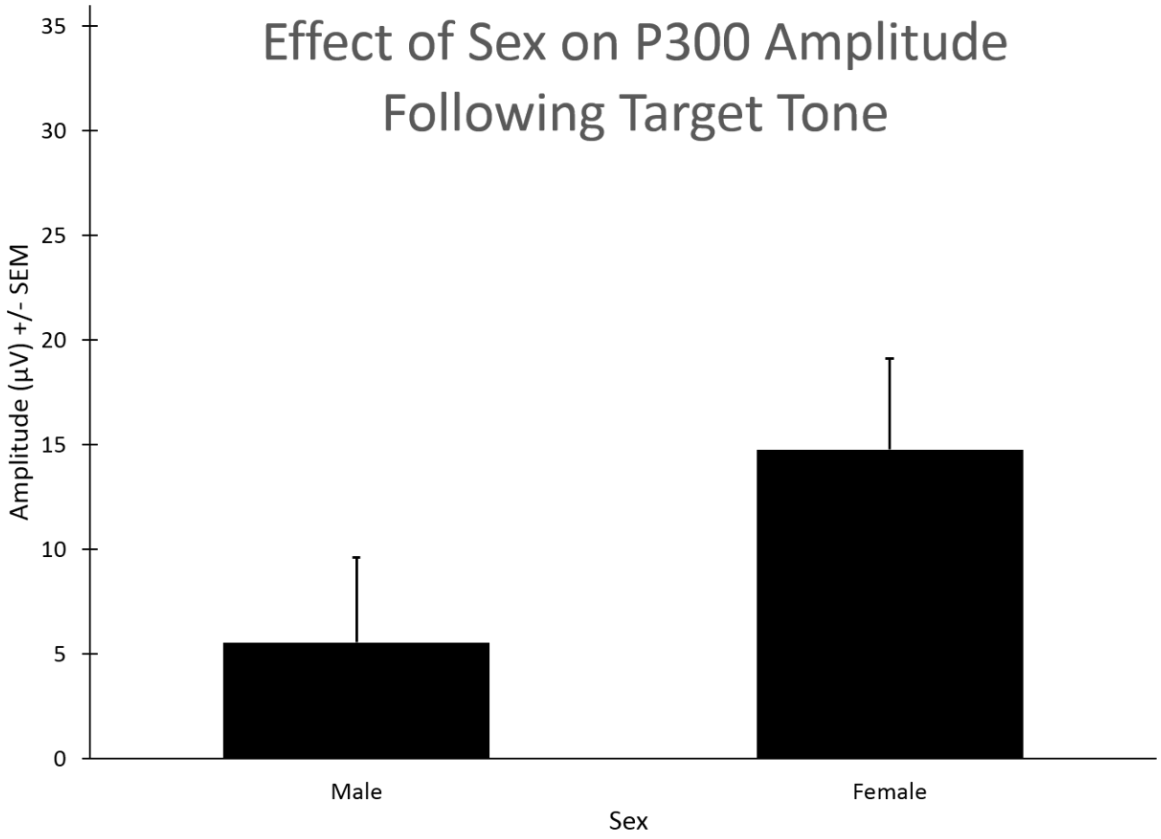

Figure 8. Effect of Sex on Average Target P300 Measured between 300-400 ms. Target P300 amplitude was larger for females (n = 5) compared to males (n = 6), but this effect was not statistically significant even though a large effect size was present (f = .514).

To evaluate the degree of intoxication following IP administration of ethanol, an ethanol intoxication scale was used. The interrater reliability of the ratings was Kappa = 454. ($p < 0.001$), 95% CI (0.285, 0.6222), which is considered to be moderate agreement. The average ratings are presented in Table 5. Notably, no intoxication ratings exceeded 3, which would suggest motor impairment. Analysis of average intoxication ratings using a 2 (sex) x 3 (ethanol dose) mixed ANOVA revealed that the main effect of sex and the sex x ethanol dose interaction was not significant, $F(1,9) = 0.146$, $p = 0.712$, and $F(2,18) = 2.878$, $p = .082$, respectively. There was an expected main effect of ethanol dose on

intoxication, $F(1,18) = 144.102$, $p < 0.001$, wherein the 0.75 g/kg dose led to greater levels of intoxication relative to saline and the 0.75 and 1.5 doses did not differ.

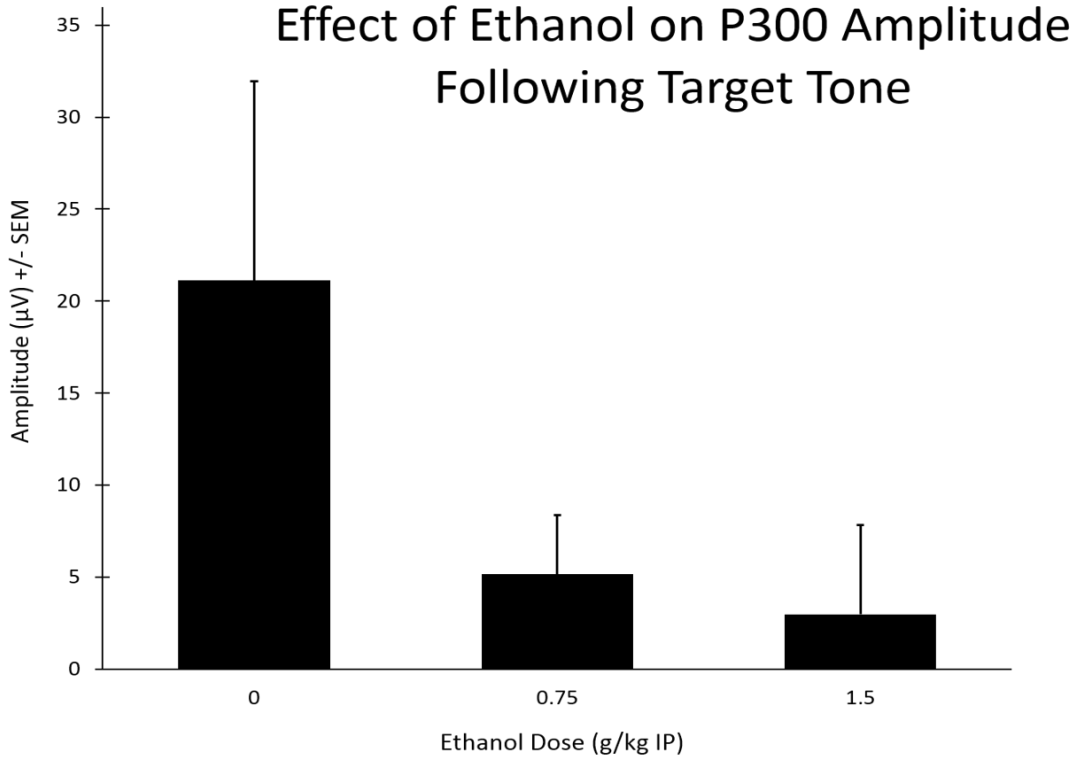

Figure 9: Effect of Ethanol on Average Target P300 Measured between 300-400 ms. Target P300 amplitude was attenuated by both doses of ethanol, but the main effect of ethanol dose was not statistically significant even though a large effect size was present ($f = .417$; $n = 11$).

Table 5. Average Ratings of Intoxication (±SEM) Using Ehlers et al. (2014) Neurobehavioral Assessment of Ethanol Intoxication in Rats

Ethanol Dose (IP)	Males	Females
0 g/kg (saline)	0 (± 0.0)	0 (± 0.0)
0.75 g/kg	1.69 (± 0.18)	2.07 (± 0.25)
1.5 g/kg	1.88 (± 0.15)	1.68 (± 0.18)

ERP Measures for Hits and Correct Rejections

For the third and final set of analyses, average ERP responses to correct lever press responses to target tones were compared to average ERP responses to correct inhibition of responses to the standard tone.

N100 and P300. Visual inspection of the data revealed little evidence for the presence of either an N100 response in the 60-100 ms latency range or a P300 response in the 300-400 ms latency range when the data were separated by trial and response type (see Figure 10). Because there was no clear visual evidence of significant findings, separate analyses for the effects of sex and dose were not conducted.

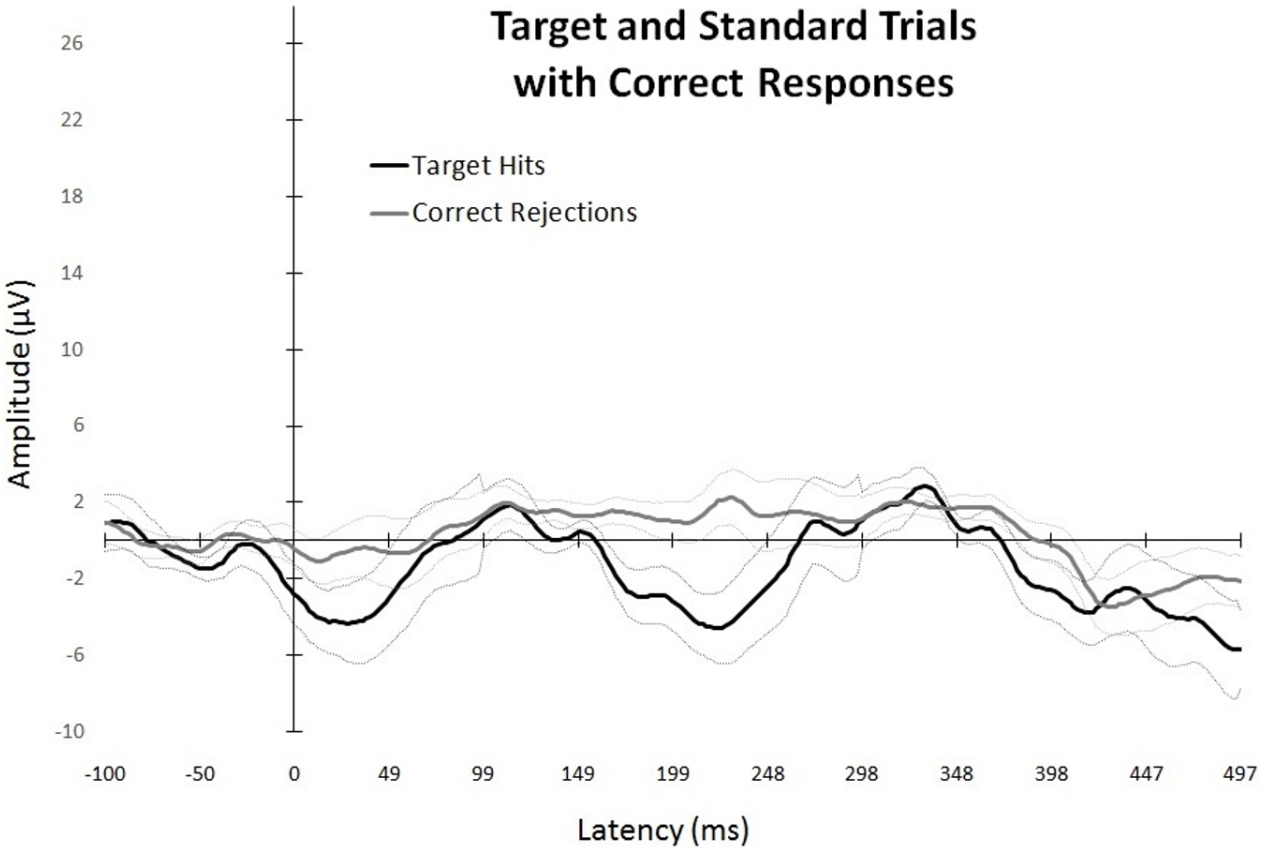

Figure 10. Average ERP Activity for Hits and Correct Rejections (means ± SEMs). When only the trials that were hits and correct rejections and were not premature were used to generate the ERP waveforms, neither a robust N100 nor P300 was demonstrated. This outcome is difficult to evaluate as the number of trials used to establish the hit and correct rejection waveforms was much smaller than the number used to compare all target to all standard trials (regardless of behavioral response; (n = 11).

Chapter 8: Discussion

Summary of Results and Relation to Previous Research

Averaging across all trials (regardless of sex, tone type, and ethanol dose), in an attempt to demonstrate the existence of the N100 and P300 responses, did not reveal these ERP components. The absence of the N100 response was likely due to the data filtering methods implemented in this study, wherein high-frequency noise was often eliminated and considered to be artifacts. Only the relatively large, low-frequency P300 seemed to maintain some level of visibility through the noise. Also, because a P300 was not expected to be observed following standard tone trials, and because averaging data across target and standard trials diminishes the potential for a P300 (Jodo et al., 1995), the decision was made to evaluate the ERP waveforms separately for the target trials regardless of the rat's behavioral response. As demonstrated in Figure 5, it is possible to measure the P300 using subcutaneous needle electrodes in rats during an active task, but a discrimination must be made between target and standard tones. The difference in P300 amplitude for target versus standard trials suggests that rats found the target tone meaningful even though the behavioral data indicated this information did not have a profound effect on their operant responding. Thus, the presence of a large P300 amplitude to the target tone suggests an association between the target tone and food delivery, even though the rats' behavior did not appear to be influenced by this association.

The omnibus analysis of the N100 and P300 amplitude for all target trials did not reveal a main effect of sex on these ERP components, but the effect size for sex on the P300 amplitude was large. Males showed a reduced P300 compared to females in

response to presentations of the target tone. These results are in line with previous research that has reported males exhibited reduced P300 amplitudes (Hill & Steinhauer, 1993a; Justus et al., 2001; Lindín et al., 2004; Steinhauer & Hill, 1993). However, there is little consensus in the literature regarding how sex affects the P300. Specifically, a number of studies have found no difference in the characteristics of the P300 between males and females (Bauer et al., 2001; Bennington & Polich, 1999; Ceballos et al., 2003; Cohen & Polich, 1997; Hill, Steinhauer, et al., 1995; Katayama & Polich, 1996; Polich et al., 1988; Sinha et al., 1992; Steiner et al., 2014; van der Stelt et al., 1998).

In this study, injections of ethanol did not impair 20-80 auditory oddball behavioral operant performance, as there was no main effect of ethanol dose on percent correct responses to the rare, target tone or the percent correct inhibition of responses to the standard, non-target tone. Nonetheless, a large effect size present in the ERP data suggested that acute ethanol administration decreased the amplitude of the P300 – an outcome previously shown in rats (Ehlers, Chaplin, et al., 1991; Ehlers, Kaneko, et al., 1992; Slawecki, Walpole, et al., 2000; Slawecki et al., 2005). Importantly, this effect does not seem to be due to some type of motor impairment following ethanol administration, as the intoxication assessment did not reveal motor impairments following either dose of ethanol. Given the fact that large effect sizes were found for the main effects of both sex and ethanol dose in this study, a future study should attempt to replicate these findings but use a sample size that would provide enough power to demonstrate significant differences.

Limitations Associated with Subcutaneous Electrodes

Given that the current experiment was a proof-of-concept study, a number of methodological challenges surfaced, and the solutions used are presented here in an attempt to conserve resources and make future replication as uncomplicated as possible. Researchers who wish to conduct similar studies in the future would do well to consider the challenges and solutions discussed below. While our goal was to make the procedure as minimally invasive as possible, several adaptations had to be made to protect the electrodes and lead wires. These adaptations did increase the invasiveness of the procedure, but, compared to surgical methods used to implant electrodes (described above), were still minimally invasive.

Electrode placement. One of the challenges encountered pertained to our initial plan to use subcutaneous *wire* electrodes. In a small group of pilot animals, we determined that implanting the wire electrodes under the skin was feasible, and accurate placement was confirmed via radiograph. However, after a few days, we found that the electrode lead wires became twisted and would easily break. We therefore better secured the electrode lead wires to the rat in order to avoid this problem. We then discovered that the adapter ports that connected to the electrode lead wires were not very secure and easily became disconnected during operant testing. To solve this problem, we permanently glued the electrode lead wire to the adapter port in order to maintain a strong connection. This created another problem of how to secure the length of the electrode adapter wire to the rat. This was done by using an access harness (Instech Solomon, Plymouth Meeting, PA) to secure the adapter wire, yet make it available during EEG recording.

Electrode integrity. After these pilot testing sessions using the wire electrodes with attached adapter wires were completed, final radiograph images were taken to determine if the placements of the subcutaneous wire electrodes were still accurate. The images revealed that the electrodes had significantly shifted out of place. To remedy this problem, we chose to instead use subcutaneous needle electrodes, because they are more rigid and have more structure than the wire electrodes. As previously mentioned, Mayaud et al. (2013) found no difference in electrode performance when using traditional disc electrodes versus subcutaneous needle electrodes to record from Cz in humans.

Electrode adapter wires. A number of problems were also noted during daily pilot testing on the 20-80 auditory oddball task. First, the rats would often pull on and chew through the electrode lead and/or adapter wires, severing their connections to the DataWave system. We therefore used a typical solution to this type of problem - a stainless steel spring tether (Braintree Scientific, Braintree, MA) connected to the harness to protect the electrode leads.

Behavioral programs. A potential confounding and limiting factor of our study pertains to the Med-PC behavioral programs used, as well as the sequence of the testing sessions. Specifically, the use of fixed-ratio programs during early lever-press training may have unintentionally over-trained the rats to respond on the lever as much as possible. This is counterintuitive to the goal of the current auditory 20-80 oddball task, wherein the rats were to make a response only to the rarely presented target tones because responding to the target was the only way they could earn a reinforcer. Additionally, the auditory 20-80 oddball stimulus-discrimination task that was used was quite difficult for the rats to learn. This was because we were limited to presenting rare and standard tones

that varied by *length* instead of varying by frequency and/or loudness, making the discrimination between the tones quite difficult for the rats to do. Future studies should include a rare tone that differs from the standard tone along multiple dimensions (latency, frequency, and loudness) in order to create a broader distinction between the target and standard tones. Likewise, the auditory oddball tasks used in this study only required one response lever; as such, the rats had to learn to associate this lever both with actively pressing (to the target) and inhibiting pressing (to the standard). A better solution would be to first train the rat to respond quickly on the lever for the target tone and introduce the standard that is never reinforced only after this behavior is well established. Using this strategy will ensure that a response on the lever is associated with food only when preceded by presentation of the target tone.

A final limitation that may have negatively influenced our data was the fact that we used a single cue light to signal when each trial began and when it ended. The cue light preceded tone presentation by 1,000 ms and response times to tone presentations sometimes appeared to correspond more closely to the activation of the cue light than to presentation of either tone. As such, rats were not clear on the nature of the task because they were often responding to the cue light instead of the tone. To reduce the impact of this confound we removed trials where the response latency was less than 100 ms. Future research studies of this nature should be designed to eliminate this problem by using extensive stimulus detection training to force the rats to learn the difference between the tones and press the response lever only *after* hearing the target tone.

Despite the number of challenges and limitations that were encountered throughout this study, we successfully demonstrated that a P300 ERP response can be

recorded using needle electrodes implanted subcutaneously under the scalp through a minimally-invasive surgical procedure. Even though the rats did not demonstrate accurate 20-80 oddball performance, our findings are in line with other studies demonstrating a significant increase in P300 amplitude following presentations of a rare, target tone (Jodo et al., 1995; Polich, 1986b, 1987; Polich & Bondurant, 1997; Polich & Margala, 1997; Shinba, 1997; Steinhauer & Hill, 1993; Suresh et al., 2003; Sutton et al., 1965). As previously mentioned, the apparent sex and ethanol dose effects on P300 amplitude will need to be replicated in a study using a larger sample size. Likewise, the significance of the location of electrode placement (in the Fz, Cz, and Pz regions) on evaluation of the N100 warrants further investigation.

Future Research

Alcoholism and the P300. The present experiment was designed to serve as a proof-of-concept study for establishing a new, less invasive way of measuring the P300 in rats during performance of an active auditory discrimination task. While this study provides important data about the effects of acute alcohol administration on the amplitude of the P300, future studies are needed to replicate the current results as well as explore the effects of alcohol on the amplitude and latency of the P300 in those with and without a genetic predisposition to alcoholism. Several investigations in both humans and rodents have demonstrated that participants that have a genetic predisposition to alcoholism (i.e., a family history of alcoholism or selective breeding for high ethanol preference or intake), show that this internal factor significantly influences the amplitude and latency of the P300 (Cohen et al., 1995; Duncan et al., 2009; Ehlers & Schuckit, 1990, 1991; Euser et al., 2012; Hill, Muka, et al., 1995; Hill & Steinhauer, 1993a, 1993b;

Holguin et al., 1999; Justus et al.,2001; Katner et al., 2002; Newlin & Thomson, 1990; Porjesz, Rangaswamy, Kamarajan, Jones, & Begleiter, 2005; Rangaswamy et al., 2007; Slawecki et al., 2003; Steinhauer & Hill, 1993; van der Stelt et al., 1998). However, the novel means of recording the P300 signal that was established in the current study has not been used in studies with alcoholic subjects.

Methodologically rigorous studies involving rodent models that show differences in genetic predisposition to alcoholism will serve to broaden our knowledge of how genetics may negatively impact the P300 signal. Along this line, a future study is planned to investigate the amplitude and latency of the P300 in alcohol-preferring (P) and non-preferring (NP) rats using the same subcutaneous electrodes used in this proof-of-concept study. Findings from studies using more invasive skull screw electrodes have determined that P300 amplitude is reduced in these strains both during baseline ERP measures and during an acute ethanol challenge (Ehlers, Chaplin, et al., 1991).

Conclusions

The goal of this project was to measure the P300 (and N100) using subcutaneous needle electrodes in rats performing an active auditory discrimination task. The EEG data that we obtained was atypical, due to a substantial amount of noise that was recorded alongside our EEG recordings. Due to the amount of noise present in the data, which was not indicative of true neuroelectrical activity, the data was substantially filtered to remove artifacts. Despite this, we were able to demonstrate a significant P300 following presentation of the rare, target tone. We also found a large effect size for ethanol, which appears to attenuate the amplitude of the P300 following presentation of the target tone. Females also appear to show a larger P300 amplitude to the target tone than males.

Overall, we successfully recorded the P300 in rats during an active auditory stimulus discrimination task using minimally-invasive subcutaneous needle electrodes. Future research will be necessary to evaluate further whether auditory oddball task accuracy has any influence on the characteristics of the P300.

References

Abe, K., Sawada, T., Horiuchi, M., & Yoshimura, K. (1999). Effects of S-8510, a benzodiazepine receptor partial inverse agonist, on event-related potentials (P300) in monkeys. *Psychopharmacology, 141*(1), 71-76. doi:10.1007/s002130050808

Arthur, D. L., & Starr, A. (1984). Task-relevant late positive component of the auditory event-related potential in monkeys resembles P300 in humans. *Science, 223*(4632), 186-188. doi:10.1126/science.6691145

Bauer, L.O. (2001). Electroencephalographic studies of substance use and abuse. In M. J. Kaufman (Ed.), *Brain imaging in substance abuse: Research, clinical, and forensic applications* (pp. 77– 112). New Jersey: Humana Press.

Bauer, L. O., Costa, L., & Hesselbrock, V. M. (2001). Effects of alcoholism, anxiety and depression on P300 in women: A pilot study. *Journal of Studies on Alcohol, 62*(5), 571-579. doi: 10.15288/jsa.2001.62.571

Begleiter, H., Porjesz, B., Bihari, B., & Kissin, B. (1984). Event-related brain potential in boys at risk for alcoholism. *Science, 225*(4669), 1493-1496. doi: 10.1126/science.6474187

Bennington, J. Y., & Polich, J. (1999). Comparison of P300 from passive and active tasks for auditory and visual stimuli. *International Journal of Psychophysiology, 34*(2), 171-177. doi:10.1016/S0167-8760(99)00070-7

Bijl, S., de Bruin, E. A., Kenemans, J. L., Verbaten, M. N., & Böcker, K. B. (2005). Effects of chronic alcohol consumption in a visual attention task and an auditory

oddball task: An event-related potential study. *Alcoholism: Clinical and Experimental Research, 29*(11), 2029-2038. doi:10.1097/01.alc.0000187163.52577.0d

Breen, T. E., & Morzorati, S. L. (1996). Innate differences in medial septal area burst firing neurons and the hippocampal theta rhythm during ambulation in selectively bred rat lines. *Brain Research, 714*(1-2), 156-164. doi:10.1016/0006-8993(95)01520-5

Campbell, K. B., & Lowick, B. M. (1987). Ethanol and event-related potentials: The influence of distractor stimuli. *Alcohol, 4*(4), 257-263. doi:10.1016/0741-8329(87)90021-8

Ceballos, N. A., Nixon, S. J., & Tivis, R. (2003). Substance abuse-related P300 differences in response to an implicit memory task. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 27*(1), 157-164. doi:10.1016/S0278-5846(02)00347-0

Cohen, H. L., Porjesz, B., & Begleiter, H. (1993). The effects of ethanol on EEG activity in males at risk for alcoholism. *Electroencephalography and Clinical Neurophysiology, 86*(6), 368-376. doi:10.1016/0013-4694(93)90132-F

Cohen, H. L., Wang, W., Porjesz, B., & Begleiter, H. (1995). Auditory P300 in young alcoholics: Regional response characteristics. *Alcoholism: Clinical and Experimental Research, 19*(2), 469-475. doi:10.1111/j.1530-0277.1995.tb01533.x

Cohen, J., & Polich, J. (1997). On the number of trials needed for P300. *International Journal of Psychophysiology, 25*(3), 249-255. doi:10.1016/S0167-8760(96)00743-X

Cohen, M. X., & Gulbinaite, R. (2014). Five methodological challenges in cognitive electrophysiology. *Neuroimage, 85*(Part 2), 702-710. doi:10.1016/j.neuroimage.2013.08.010

Colrain, I. M., Taylor, J., McLean, S., Buttery, R., Wise, G., & Montgomery, I. (1993). Dose dependent effects of alcohol on visual evoked potentials. *Psychopharmacology, 112*(2-3), 383-388. doi:10.1007/BF02244937

Criado, J. R., & Ehlers, C. L. (2009). Event-related oscillations as risk markers in genetic mouse models of high alcohol preference. *Neuroscience, 44*(4), 506-523. doi:10.1016/j.neuroscience.2009.06.039

Criado, J. R., & Ehlers, C. L. (2010). Event-related oscillations in the parietal cortex of adult alcohol-preferring (p) and alcohol-nonpreferring rats (np). *Alcohol, 44*(4), 335-342. doi:10.1016/j.alcohol.2010.02.004

Donchin, E., Ritter, W., & McCallum, C. (1978). Cognitive psychophysiology: The endogenous components of the ERP. In E. Callaway, P. Tueting, & S. Koslow (Eds.), *Brain event-related potentials in man* (pp. 349-441). New York: Academic Press.

Duncan, C. C., Barry, R. J., Connolly, J. F., Fischer, C., Michie, P. T., Näätänen, R., & ... Van Petten, C. (2009). Event-related potentials in clinical research: Guidelines for

eliciting, recording, and quantifying mismatch negativity, P300, and N400. *Clinical Neurophysiology, 120*(11), 1883-1908. doi:10.1016/j.clinph.2009.07.045

Duncan-Johnson, C.C. (1981). P300 latency: A new metric of information processing. *Psychophysiology, 18*(3), 207-215.

Ehlers, C. (1989). EEG and ERP responses to naloxone and ethanol in monkeys. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 13*(1-2), 217-228.

Ehlers, C. L., Chaplin, R. I., Lumeng, L., & Li, T. K. (1991). Electrophysiological response to ethanol in P and NP rats. *Alcoholism: Clinical and Experimental Research, 15*(4), 739-744. doi:10.1111/j.1530-0277.1991.tb00590.x

Ehlers, C. L., Chaplin, R. I., Wall, T. L., Lumeng, L., Li, T. K., Owens, M. J., & Nemeroff, C. B. (1992). Corticotropin releasing factor (CRF): Studies in alcohol preferring and non-preferring rats. *Psychopharmacology, 106*(3), 359-364. doi:10.1007/BF02245418

Ehlers, C. L., Desikan, A., & Wills, D. N. (2014). Event-related potential responses to the acute and chronic effects of alcohol in adolescent and adult Wistar rats. *Alcoholism: Clinical and Experimental Research, 38*(3), 749-759. doi:10.1111/acer.12299

Ehlers, C. L., Garcia-Andrade, C., Wall, T. L., Sobel, D. F., & Phillips, E. (1998). Determinants of P3 amplitude and response to alcohol in Native American

Mission Indians. *Neuropsychopharmacology, 18*(4), 282-292. doi:10.1016/S0893-133X(97)00160-7

Ehlers, C. L., Kaneko, W. M., Robledo, P., & Lopez A.L. (1994). Long-latency event-related potentials in rats: Effects of task and stimulus parameters. *Neuroscience, 62*(3), 759-769. doi:10.1016/0306-4522(94)90474-X

Ehlers, C. L., Kaneko, W. M., Wall, T. L., & Chaplin, R. I. (1992). Effects of dizocilpine (MK-801) and ethanol on the EEG and event-related potentials (ERPs) in rats. *Neuropharmacology, 31*(4), 369-378.

Ehlers, C. L., & Schuckit, M. A. (1990). EEG fast frequency activity in the sons of alcoholics. *Biological Psychiatry, 27*(6), 631-641. doi:10.1016/0006-3223(90)90531-6

Ehlers, C. L., & Schuckit, M. A. (1991). Evaluation of EEG alpha activity in sons of alcoholics. *Neuropsychopharmacology, 4*(3), 199-205.

Ehlers, C. L., & Somes, C. (2002). Long latency event-related potentials in mice: Effects of stimulus characteristics and strain. *Brain Research, 957*(1), 117-128. doi:10.1016/S0006-8993(02)03612-0

Ehlers, C. L., Somes, C., Li, T. K., Lumeng, L., Kinkead, B., Owens, M.J., & Nemeroff, C. B. (1999). Neurotensin studies in alcohol naïve, preferring, and non-preferring rats. *Neuroscience, 93*(1), 227-236. doi:10.1016/S0306-4522(99)00113-X

Ehlers, C. L., Somes, C., Lopez, A. L., & Robledo, P. (1998). Long latency event- related potentials in rats: Response of amygdala, nucleus accumbens, dorsal hippocampus and frontal cortex to changes in reward characteristics of conditioned stimuli. *Brain Research, 780*(1), 138-142. doi:10.1016/S0006-8993(97)01294-8

Ehlers, C. L., Somes, C., Lumeng, L., & Li, T. K. (1999). Electrophysiological response to neuropeptide Y (NPY): In alcohol-naive preferring and non-preferring rats. *Pharmacology, Biochemistry and Behavior, 63*(2), 291-299. doi:10.1016/S0091-3057(99)00012-X

Ehlers, C. L., Wall, T. L., & Chaplin, R. I. (1991). Long latency event-related potentials in rats: Effects of dopaminergic and serotonergic depletions. *Pharmacology, Biochemistry and Behavior, 38*(4), 789-793. doi:10.1016/0091-3057(91)90243-U

Elmasian, R., Neville, H., Woods, D., Schuckit, M., & Bloom, F. (1982). Event-related brain potentials are different in individuals at high and low risk for developing alcoholism. *Proceedings of the National Academy of Sciences, 79*(24), 7900-7903. doi: 10.1073/pnas.79.24.7900

Erwin, C. W., Linnoila, M., Hartwell, J., Erwin, A., & Guthrie, S. (1896). Effects of buspirone and diazepam, alone and in combination with alcohol, on skilled performance and evoked potentials. *Journal of Clinical Psychopharmacology, 6*(4), 199-209. doi: 10.1097/00004714-198608000-00002

Euser, A. S., Arends, L. R., Evans, B. E., Greaves-Lord, K., Huizink, A. C., & Franken, I. A. (2012). The P300 event-related brain potential as a neurobiological

endophenotype for substance use disorders: A meta-analytic investigation. *Neuroscience and Biobehavioral Reviews, 36*(1), 572-603. doi:10.1016/j.neubiorev.2011.09.002

Finn, P.R. (1999). Psychophysiology. In Oh, P.J., Tarter, R.E., & Ammerman, R.T. (Eds.), *Sourcebook on substance abuse: Etiology, epidemiology, assessment and treatment* (pp. 141-152). Boston: Allyn & Bacon.

Fowler, B., & Adams, J. (1993). Dissociation of the effects of alcohol and amphetamine on inert gas narcosis using reaction time and P300 latency. *Aviation, Space, and Environmental Medicine, 64*(6), 493-499.

Freund, G. (1969). Alcohol withdrawal syndrome in mice. *Archives of Neurology, 21*(3), 315-320. doi:10.1001/archneur.1969.00480150105013

Gao, D., Zheng, Z., Han, M., Tang, X., & Sun, X. (2009). Findings of P300-like and CNV-like potentials in rat model of depression following repeatedly forced swim stress. *International Journal of Psychophysiology, 72*(2), 160-165. doi:10.1016/j.ijpsycho.2008.12.002

Glover, A. A., Onofrj, M. C., Ghilardi, M. F., & Bodis-Wollner, I. (1986). P300-like potentials in the normal monkey using classical conditioning and an auditory 'oddball' paradigm. *Electroencephalography & Clinical Neurophysiology: Evoked Potentials, 65*(3), 231-235. doi:10.1016/0168-5597(86)90058-4

Grupe, M., Grunnet, M., Laursen, B., & Bastlund, J. F. (2014). Neuropharmacological modulation of the P3-like event-related potential in a rat two-tone auditory

discrimination task with modafinil and NS9283, a positive allosteric modulator of α4β2 nAChRs. *Neuropharmacology, 79,* 444-455. doi:10.1016/j.neuropharm.2013.12.008

Hattori, M., Onoda, K., & Sakata, S. (2010). Identification of rat P3-like processes in the anterior cingulate cortex and hippocampus. *Neuroscience Letters, 472*(1), 43-46. doi:10.1016/j.neulet.2010.01.052

Hill, S. Y., Locke, J., & Steinhauer, S. R. (1999). Absence of visual and auditory P300 reduction in nondepressed male and female alcoholics. *Biological Psychiatry, 46*(7), 982-989. doi:10.1016/S0006-3223(99)00054-2

Hill, S. Y., Muka, D., Steinhauer, S., & Locke, J. (1995). P300 amplitude decrements in children from families of alcoholic female probands. *Biological Psychiatry, 38*(9), 622-632. doi:10.1016/0006-3223(94)00384-7

Hill, S. Y., & Shen, S. (2002). Neurodevelopmental patterns of visual P3b in association with familial risk for alcohol dependence and childhood diagnosis. *Biological Psychiatry, 51*(8), 621-631. doi:10.1016/S0006-3223(01)01301-4

Hill, S. Y., & Steinhauer, S. R. (1993a). Assessment of prepubertal and postpubertal boys and girls at risk for developing alcoholism with P300 from a visual discrimination task. *Journal of Studies on Alcohol, 54*(3), 350-358. doi: http://dx.doi.org/10.15288/jsa.1993.54.350

Hill, S.Y., & Steinhauer, S. (1993b). Event-related potentials in women at risk for alcoholism. *Alcohol, 10*(5), 349-354. doi:10.1016/0741-8329(93)90019-K

Hill, S.Y., Steinhauer, S., & Locke, J. (1995). Event-related potentials in alcoholic men, their high-risk male relatives and low-risk male controls. *Alcoholism: Clinical and Experimental Research, 19*(3), 567-576. doi:10.1111/j.1530-0277.1995.tb01550.x

Holguin, S.R., Porjesz, B., Chorlian, D.B., Polich, J., & Begleiter, H. (1999). Visual P3a in male alcoholics and controls. *Alcoholism: Clinical and Experimental Research, 23*(4), 582-591. doi: 10.1111/j.1530-0277.1999.tb04158.x

Hunter, B. E., Boast, C. A., Walker, D. W., & Zornetzer, S. F. (1973). Alcohol withdrawal syndrome in rats: Neural and behavioral correlates. *Pharmacology, Biochemistry and Behavior, 1*(6), 719-725. doi:10.1016/0091-3057(73)90036-1

Jodo, E., Takeuchi, S., & Kayama, Y. (1995). P3b-like potential of rats recorded in an active discrimination task. *Electroencephalography & Clinical Neurophysiology: Evoked Potentials, 96*(6), 555-560. doi:10.1016/0013-4694(95)00067-9

Justus, A. N., Finn, P. R., & Steinmetz, J. E. (2001). P300, disinhibited personality, and early-onset alcohol problems. *Alcoholism: Clinical and Experimental Research, 25*(10), 1457-1466. doi:10.1111/j.1530-0277.2001.tb02147.x

Katayama, J., & Polich, J. (1996). P300, probability, and the three-tone paradigm. *Electroencephalography & Clinical Neurophysiology: Evoked Potentials, 100*(6), 555-562. doi:10.1016/S0168-5597(96)95171-0

Katner, S. N., Slawecki, C. J., & Ehlers, C. L. (2002). Neurophysiological profiles of replicate line 2 high-alcohol-drinking (HAD-2) and low-alcohol-drinking (LAD-

2) rats. *Alcoholism: Clinical and Experimental Research, 26*(11), 1669-1677. doi:10.1097/00000374-200211000-00009

Landis, J. R., & Koch, G. G. (1977). The measurement of observer agreement for categorical data. *Biometrics, 33*(1), 159-74. doi: 10.2307/2529310

Lee, J. A., Schoener, E. P., Nielsen, D. W., Kelly, A. R., Lin, W. N., & Berman, R. F. (1990). Alcohol and the auditory brain-stem response, brain temperature, and blood alcohol curves: explanation of a paradox. *Electroencephalography and Clinical Neurophysiology, 77*(5), 362-75. doi:10.1016/0168-5597(90)90058-L

Lindín, M., Zurrón, M., & Díaz, F. (2004). Changes in P300 amplitude during an active standard auditory oddball task. *Biological Psychology*, *66*(2), 153-167. doi:10.1016/j.biopsycho.2003.10.007

Lukas, S. E., Mendelson, J. H., Kouri, E., Bolduc, M., & Amass, L. (1990). Ethanol-induced alterations in EEG alpha activity and apparent source of the auditory P300 evoked response potential. *Alcohol, 7*(5), 471-477. doi:10.1016/0741-8329(90)90034-A

Magliero, A., Bashore, T., Coles, M.G.H., & Donchin, E. (1984). On the dependence of P300 latency on stimulus evaluation processes. *Psychophysiology, 21*(2), 171-186. doi: 10.1111/j.1469-8986.1984.tb00201.x

Mayaud, L., Congedo, M., Van Laghenhove, A., Orlikowski, D., Figère, M., Azabou, E., & Cheliout-Heraut, F. (2013). A comparison of recording modalities of P300

event- related potentials (ERP) for brain-computer interface (BCI) paradigm. *Clinical Neurophysiology, 43*(4), 217-27. doi: 10.1016/j.neucli.2013.06.002

Newlin, D. B., & Thomson, J. B. (1990). Alcohol challenge with sons of alcoholics: A critical review and analysis. *Psychological Bulletin, 108*(3), 383-402. doi:10.1037/0033-2909.108.3.383

Oscar-Berman, M. (1987). Alcohol-related ERP changes in cognition. *Alcohol, 4*(4), 289-292. doi:10.1016/0741-8329(87)90025-5

Pineda, J. A., & Swick, D. (1992). Visual P3-like potentials in squirrel monkey: Effects of a noradrenergic agonist. *Brain Research Bulletin, 28*(3), 485-491. doi:10.1016/0361-9230(92)90051-X

Polich, J. (1986a). Attention, probability, and task demands as determinants of P300 latency from auditory stimuli. *Electroencephalography and Clinical Neurophysiology, 63,* 251-259. doi:10.1016/0013-4694(86)90093-3

Polich, J. (1986b). P300 development from auditory stimuli. *Psychophysiology, 23,* 590-597. doi: 10.1111/j.1469-8986.1986.tb00677.x

Polich, J. (1987). Task difficulty, probability, and inter-stimulus interval as determinants of P300 from auditory stimuli. *Electroencephalography and Clinical Neurophysiology, 68,* 311-320. doi:10.1016/0168-5597(87)90052-9

Polich, J. (2007). Updating P300: An integrative theory of P3a and P3b. *Clinical Neurophysiology, 118*(10), 2128-2148. doi:10.1016/j.clinph.2007.04.019

Polich, J., & Bondurant, T. (1997). P300 sequence effects, probability, and interstimulus interval. *Physiology & Behavior, 61*(6), 843-849. doi:10.1016/S0031-9384(96)00564-1

Polich, J., Burns, T., & Bloom, F. E. (1988). P300 and the risk for alcoholism: Family history, task difficulty, and gender. *Alcoholism: Clinical and Experimental Research, 12*(2), 248-254. doi:10.1111/j.1530-0277.1988.tb00189.x

Polich, J., & Margala, C. (1997). P300 and probability: Comparison of oddball and single-stimulus paradigms. *International Journal of Psychophysiology, 25*(2), 169-176. doi:10.1016/S0167-8760(96)00742-8

Porjesz, B., R Rangaswamy M., Kamarajan, C., Jones, K. A., & Begleiter, H. (2005). The utility of neurophysiological markers in the study of alcoholism. *Clinical Neuropathology, 116*(5), 993-1018. doi:10.1016/j.clinph.2004.12.016

Rangaswamy, M., Jones, K. A., Porjesz, B., Chorlian, D. B., Padmanabhapillai, A., Kamarajan, C., Kupermand, S. Rohrbaughe, J. O'Connorc, S. J., Bauer, L. O. Schuckitf, M. A., Begleiter, H. (2007). Delta and theta oscillations as risk markers in adolescent offspring of alcoholics. *International Journal of Psychophysiology, 63*(1), 3-15. doi:10.1016/j.ijpsycho.2006.10.003

Ravden, D., & Polich, J. (1999). On P300 measurement stability: Habituation, intra-trial block variation, and ultradian rhythms. *Biological Psychology, 51*(1), 59-76. doi:10.1016/S0301-0511(99)00015-0

Robledo, P., Lumeng, L., Li, T., & Ehlers, C. L. (1994). Effects of MK 801 and diazepam on the EEG of P and NP rats. *Alcoholism: Clinical and Experimental Research, 18*(2), 363-368. doi:10.1111/j.1530-0277.1994.tb00026.x

Russell, W.M.S., & Burch, R. L. (1992). The Principles of Humane Experimental Technique (Special Edition). Altweb (Online service); Johns Hopkins Bloomberg School of Public Health.; Johns Hopkins Center for Alternatives to Animal Testing.; Universities Federation for Animal Welfare. http://altweb.jhsph.edu/pubs/books/humane_exp/het-toc

Sambeth, A., Maes, J. R., Van Luijtelaar, G., Molenkamp, I. S., Jongsma, M. A., & Van Rijn, C. M. (2003). Auditory event-related potentials in humans and rats: Effects of task manipulation. *Psychophysiology, 40*(1), 60-68. doi:10.1111/1469-8986.00007

Shinba, T. (1997). Event-related potentials of the rat during active and passive auditory oddball paradigms. E*lectroencephalography & Clinical Neurophysiology: Evoked Potentials, 104*(5), 447-452. doi:10.1016/S0168-5597(97)00047-6

Shinba, T., Andow, Y., Shinozaki, T., Ozawa, N., & Yamamoto, K. (1996). Event-related potentials in the dorsal hippocampus of rats during an auditory discrimination paradigm. *Electroencephalography & Clinical Neurophysiology: Evoked Potentials, 100*(6), 563-568. doi:10.1016/S0168-5597(96)95205-3

Sinha, R., Bernardy, N., & Parsons, O.A. (1992). Long-term test-retest reliability of event-related potentials in normals and alcoholics. *Biological Psychiatry, 32*(11), 992–1003. doi:10.1016/0006-3223(92)90060-D

Slawecki, C. J., Betancourt, M., Li, T., & Ehlers, C. L. (2000). Neurophysiological findings and drinking levels in high-alcohol drinking (HAD) and low-alcohol-drinking (LAD) rats. *Alcoholism: Clinical and Experimental Research, 24*(10), 1492-1499. doi:10.1097/00000374-200010000-00005

Slawecki, C. J., Grahame, N. J., Roth, J., Katner, S. N., & Ehlers, C. L. (2003). EEG and ERP profiles in the high alcohol preferring (HAP) and low alcohol preferring (LAP) mice: Relationship to ethanol preference. *Brain Research, 961*(2), 243-254. doi:10.1016/S0006-8993(02)03959-8

Slawecki, C. J., Thorsell, A., & Ehlers, C. L. (2005). Antagonism of neuropeptide YY1 receptors does not inhibit ethanol's effects on cortical EEG and ERPs in Wistar rats. *Journal of Studies on Alcohol, 66*(4), 559-566. doi: http://dx.doi.org/10.15288/jsa.2005.66.559

Slawecki, C. J., Walpole, T., Purdy, R. H., & Ehlers, C. L. (2000). Comparison of the neurophysiological effects of allopregnanolone and ethanol in rats. *Psychopharmacology, 149*(4), 351-359. doi:10.1007/s002139900365

Sommer, W., Leuthold, H., & Hermanutz, M. (1993). Covert effects of alcohol revealed by event-related potentials. *Perception & Psychophysics, 54*(1), 127-135. doi:10.3758/BF03206944

Steiner, G. Z., Barry, R. J., & Gonsalvez, C. J. (2014). Nontarget-to-nontarget interval determines the nontarget P300 in an auditory equiprobable Go/NoGo task. *International Journal of Psychophysiology, 92*(3), 113-121. doi:10.1016/j.ijpsycho.2014.03.010

Steinhauer, S. R., & Hill, S. Y. (1993). Auditory event-related potentials in children at high risk for alcoholism. *Journal of Studies on Alcohol, 54*(4), 408-421. doi: http://dx.doi.org/10.15288/jsa.1993.54.408

Suresh, S., Porjesz, B., Chorlian, D. B., Choi, K., Jones, K. A., Wang, K., Stimus, A., & Begleiter, H. (2003). Auditory P3 in female alcoholics. *Alcoholism: Clinical and Experimental Research, 27*(7), 1064-1074. doi:10.1097/01.ALC.0000075549.49800.A0

Sutton, S., Braren, M., Zubin, J., & John, E. R. (1965). Evoked-potential correlates of stimulus uncertainty. *Science, 26*(3700), 1187-1188. doi:10.1126/science.150.3700.1187

Takeuchi, S., Jodo, E., Suzuki, Y., Matsuki, T., Hoshino, K., Niwa, S., & Kayama, Y. (2000). ERP development in the rat in the course of learning two-tone discrimination task. *Neuroreport: For Rapid Communication of Neuroscience Research, 11*(2), 333-336. doi:10.1097/00001756-200002070-00022

Teo, R. K., & Ferguson, D. A. (1986). The acute effects of ethanol on auditory event-related potentials. *Psychopharmacology, 90*(2), 179-184. doi:10.1007/BF00181237

Tivis, R., Beatty, W.W., Nixon, S.J., & Parsons, O.A. (1995). Patterns of cognitive impairment among alcoholics: are there subtypes? *Alcoholism: Clinical and Experimental Research 19*(2), 496-500. doi:10.1111/j.1530-0277.1995.tb01537.x

Umbricht, D., Vyssotky, D., Latanov, A., Nitsch, R., Brambilla, R., D'Adamo, P., & Lipp, H-P. (2004). Midlatency auditory event-related potentials in mice: Comparison to midlatency auditory ERPs in humans. *Brain Research, 1019*(1-2), 189-200. doi:10.1016/j.brainres.2004.05.097

Van der Stelt, O., Geesken, R., Gunning, W. B., Snel, J., & Kok, A. (1998). P3 scalp topography to target and novel visual stimuli in children of alcoholics. *Alcohol, 15*(2), 119-136. doi:10.1016/S0741-8329(97)00106-7

Walker, B. M., & Ehlers, C. L. (2009). Age-related differences in the blood alcohol levels of Wistar rats. Pharmacology, Biochemistry And Behavior, 91(4), 560-565. doi:10.1016/j.pbb.2008.09.017

Yamaguchi, S., Globus, H., & Knight, R. T. (1993). P3-like potential in rats. *Electroencephalography & Clinical Neurophysiology: Evoked Potentials, 88*(2), 151-154. doi:10.1016/0168-5597(93)90066-X

Appendix A

THE UNIVERSITY OF MEMPHIS

IACUC PROTOCOL ACTION FORM

To:	Helen Sable
From	Institutional Animal Care and Use Committee
Subject	Animal Research Protocol
Date	April 14, 2015

The institutional Animal Care and Use Committee (IACUC) has taken the following action concerning your Animal Research Protocol No. **0766** Psychophysiological and Behavioral Measures in P and NP Rats during a Signal Detection Task

☒ Your protocol is approved for the following period:

From: April 14, 2015 To: April 13, 2018

☐ Your protocol is not approved for the following reasons (see attached memo).

☐ Your protocol is renewed without changes for the following period:

From: To:

☐ Your protocol is renewed with the changes described in your IACUC Animal Research Protocol Update/Amendment Memorandum dated for the following period:

From: To:

☐ Your protocol is not renewed and the animals have been properly disposed of as described in your IACUC Animal Research Protocol Update/Amendment Memorandum dated

[redacted]

Amy L. de Jongh Curry, PhD, Chair of the IACUC

[redacted]

Dr. Karyl Buddington, University Veterinarian and Director of the Animal Care Facilities