EFFECTS OF CONTEXT SHIFT ON TOLERANCE TO ALCOHOL AND PEAK-INTERVAL BEHAVIOR IN RATS

A Thesis
by
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FOREWORD

This thesis is written in accordance with the style of the *Publication Manual of the American Psychological Association (6 Edition)* as required by the Department of Psychology at Appalachian State University.

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Effects of Context Shift on Tolerance to Alcohol and Peak-Interval Behavior in Rats

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Abstract

The current study used 64 rats to examine the effect of context shift on tolerance to alcohol, assessed by performance on a peak-interval task. Past research has extensively studied chronic alcohol intake and the mechanisms that underlie development of tolerance to alcohol in both humans and animals (McCusker & Brown, 1990; White, Roberts, & Best, 2002). Specifically, prior research has examined the effect of context-specific tolerance to alcohol on animal's motor functioning and body temperature (Siegel & Sdao-Jarvie, 1986), with the general pattern of findings suggesting that tolerance is maintained only in a particular context previously associated with a drug. The current study extended previous findings on context-specific drug tolerance by examining whether a context change has an effect on tolerance to alcohol, assessed through examining an animal's internal clock. A peak-interval task was used to demonstrate the accuracy and precision with which animals time their responses. The subjects trained in an alcohol-related context were predicted to show tolerance to alcohol in the same context as indicated by a stable response curve on a peak-interval task. However, the response curve was predicted to shift to the left, indicating an increased number of premature responses for the subjects when moved to a different environment. The results indicated that the animals that received alcohol overestimated time in a novel environment compared to those receiving alcohol in the familiar context (and to control groups that received water). However, the amount of alcohol consumed was not associated with an increase in the number of premature responses, and, contrary to the original prediction, gender was found to have no effect on the amount of alcohol that the subjects drank. The limitations of the study and the directions for future research are also addressed.

Keywords: alcohol, tolerance, context shift, peak-interval

Effects of Context Shift on Tolerance to Alcohol and Peak-Interval Behavior in Rats

An extensively studied phenomena associated with chronic alcohol intake is the development of tolerance to alcohol and increased dependence on the drug in both humans and animals (Fillmore, Ostling, Martin, & Kelly, 2009; Gilpin, Richardson, Lumeng, & Koob, 2008; McCusker & Brown, 1990). Development of tolerance to a certain drug occurs when a repeated administration of the same quantity of a drug produces only a marginal effect on the body (Siegel & MacRae, 1984). The normal functioning in individuals who develop tolerance to a drug appears to be unimpaired at first; however, Siegel (1984) has pointed out that the majority of overdose deaths take place not when a higher level of drug is administered to the body, but when the tolerance to the usual dose of a drug fails unexpectedly.

Tolerance to alcohol develops when the same amount of alcohol has a small effect on the body and cognitive functioning, compared to the original effect of alcohol. Commonly, alcohol poisoning and black-outs associated with alcohol occur in individuals who have developed robust tolerance to alcohol, rather than in people with relatively little exposure to alcohol. The research has focused on identifying the mechanisms that underlie the development of drug tolerance, as well as the circumstances that are most likely to contribute to the tolerance failure.

The preponderance of past research suggests that mechanisms that underlie development of drug tolerance are closely linked to Pavlovian conditioning (Cunningham, Losli, & Risinger, 1992; Kesner & Cook, 1983; Siegel, 1984; Siegel, 2005). In a basic Pavlovian paradigm, a conditioned stimulus (CS) is repeatedly paired with an unconditioned stimulus (US), and eventually a conditioned response (CR) is elicited solely by the

presentation of a CS. When alcohol (US) is administered to the body, a natural compensatory reaction that an organism has to alcohol represents the unconditioned response (UR). The repeated alcohol presentation is accompanied by a number of environmental cues (CSs), which gradually become associated with alcohol as the frequency of alcohol intake in that environment increases. The compensatory response to the effects of the drug on the body eventually becomes a CR to the repeated pairings of the CS and the US together in time. Essentially, contextual cues (CS) present during drug administration reduce the effect of alcohol on the body because the CS comes to evoke compensatory responses from the body on its own (Siegel, 2001).

The decrease in effects of alcohol on the body due to contextual cues present during alcohol administration has been known as "context-specific tolerance to alcohol" (Siegel, 1976). Past research has suggested that effects of tolerance are specific to the place where alcohol intake takes place; thus, failure to tolerate a regular dose of alcohol occurs when alcohol is consumed in a context not previously associated with this particular drug. These contextual effects have been demonstrated in prior research with both human and animal subjects. For example, Seeley, Hawkins, Ramsay, and Wilkinson (1996) examined the effects of alcohol on plasma level of corticosterone in rats. Specifically, the context associated with alcohol injections was varied between the groups. The results showed that rats with a high tolerance to the alcohol-related context showed a severe tolerance disruption to injected alcohol in a saline-related context, as indicated by a significant increase in their corticosterone levels. Furthermore, through experimental manipulation, McCusker and Brown (1990) showed that human males who expected to receive alcohol in a particular

context were significantly less impaired on tasks of cognitive and motor performance than males who received alcohol in an unexpected context.

Behavioral research has also examined context-specific tolerance to ataxic and hypothermic effects of alcohol. The general finding of motor activity research suggests that alcohol does not significantly impair motor performance and motor coordination if the behavioral activities are performed in a context associated with alcohol. For example, Duncan, Alici, and Woodward (2000) examined the effects of alcohol on spontaneous motor activity in male rats. In this experiment, the researchers administered saline or two different quantities of ethanol to rats in two distinct contexts. The results indicated that the motor activity of rats was significantly more disrupted when they received alcohol in the context previously paired with saline. Similar results have been obtained by White, Roberts, and Best (2002), who utilized a tilting plane apparatus to assess tolerance to the ataxic effects of alcohol in rats. Specifically, three groups of rats (paired, unpaired, and control) were given alcohol or saline injections in either a testing room or a colony room, and they were later tested for disruptions in motor coordination. The researchers found that the group that received testing in the injection room (i.e., paired group) showed less deficits in motor performance compared to the groups that had no previous association between the testing room and alcohol administration. Alcohol acts as a depressant that slows down normal motor functioning when administered to an organism. It is possible that the familiar environmental cues help the subjects to relax and calm down, thus they appear to be tolerant to the effects of alcohol, and their functioning seems to be unimpaired. However, the ability to function normally persists only in this specific situation.

There is also evidence to suggest that disruption of tolerance can occur not just when alcohol is presented in a new physical context, but also when a new stimulus is introduced in the environment. Siegel and Sdao-Jarvie (1986) described this effect as "external inhibition", referring to the weakening of CR when a novel stimulus is introduced in the same context. These researchers investigated whether tolerance to the hypothermic effects of alcohol would be disrupted in rats when a new stimulus was introduced in the environment. The rats with a high degree of tolerance to alcohol, as indicated by their normal body temperature, were presented with a flashing strobe light after the ethanol injection. The body temperature of highly tolerant rats dropped significantly when this new stimulus was introduced in the environment. Furthermore, the body temperature of the rats did not return to its normal level (i.e., tolerance was not recovered) when the flashing light was withdrawn from the environment, indicating that external inhibitors can have long-term effects on disruption of tolerance.

A different explanation to the hypothermic effects of alcohol has been proposed by Peris and Cunningham (1987). They have argued that a novel stimulus, such as a flashing light, does not act as an external inhibitor, but as a major stressor at the time of alcohol injection. In other words, the rats display a drop in temperature because they are stressed by a new stimulus at the time of alcohol administration. This hypothesis was partially supported by Cunningham and Bischof (1987), who observed an increase in hypothermia when alcohol was associated with handling, probing, and a bright flashing light. However, these results did not generalize to the pairing between alcohol and a footshock, and the rats actually showed a smaller drop in body temperature compared to the rats that had been exposed to other stress stimuli. These results can be explained in terms of the depressant function that alcohol

serves. It is possible that a footshock elicits a higher degree of stress from an animal than other stimuli do. Alcohol may compensate for some of the stress associated with a footshock and, thus, reduce the normal body temperature by a nonsignificant amount.

There are certain limitations associated with past research on context-specific tolerance to alcohol consumption. The majority of aforementioned research has focused on examining tolerance specifically to the ataxic or hypothermic effects of alcohol. The evidence that motor activity is disrupted by alcohol presentation in a new context may be confounded by the fact that the stimuli associated with the new context may act as stressors for an animal and elicit a disruption in motor functioning on their own. Similarly, the alcohol-induced hypothermia may also be due to the presence of external stimuli (e.g., flashing light, loud sound) that are able to disrupt normal bodily functioning independently of alcohol.

Furthermore, the contextual change has been examined over the course of multiple trials and different days. However, of interest to the current study is the effect of an immediate (within-trial) context switch on tolerance to alcohol. The evidence regarding within-trial context shifts is scant and is based primarily on speculations, rather than experimental evidence. A review paper by Linnoila, Stapleton, Lister, Guthrie, and Eckardt (1986) summarized the possible factors that may contribute to an increased risk of being in a motor vehicle accident, as well as the frequency of the accidents. Specifically, the authors speculated that an individual that appears to be tolerant to alcohol in one setting (e.g., a bar) may lose tolerance as soon as he or she moves to a context where alcohol has not been regularly consumed (e.g., a car). However, the inability to test this hypothesis through

experimental manipulation makes it impossible to draw statistical conclusions regarding this prediction.

The current study examined the effects of an immediate (within-session) context shift on context-specific tolerance to alcohol by using a procedure that allows for the assessment of accuracy and precision with which animals time their responses. The peak-interval procedure was developed by Roberts (1981), who examined ways to isolate an animal's internal clock after noticing that certain animals were very precise at discriminating time. The internal clock mechanism assesses the degree to which subjects are able to inhibit their premature responses and their ability to wait for the proper time to make a response. The use of a peak-interval task makes it possible to directly assess context-specific tolerance to alcohol by evaluating the ability of each subject to inhibit early responses under the influence of a drug.

Timing is an important mechanism that allows animals to anticipate the occurrence of an event and to make an appropriate response required by this event (Brunner, Kacelnik, & Gibbon, 1992). In general, the behavior in a certain situation is contingent upon the memory for the similar situation that has happened in the past. It has been argued that the timing mechanism has developed in order to allow for the comparison between different events based on the amount of time that is left to make a response in an adaptive manner (Balsam, Sanchez-Castillo, Taylor, Van Volkinburg, & Ward, 2009). Interval timing can also be viewed in terms of Pavlovian conditioning because, essentially, an animal learns an association between a CS and the amount of time that is left before a US occurs. Past research has demonstrated that when the interval between a CS (e.g., tone) and a US remains constant throughout a session, subjects rapidly learn to anticipate the next presentation of the

US by expecting it to occur after a certain amount of time from the onset of the CS (Balsam, Drew, & Yang, 2002; Drew, Zupan, Cooke, Couvillon, & Balsam, 2005).

A number of properties of interval timing have been identified by previous research. The studies on temporal conditioning suggest that animals are able to extrapolate a significant amount of information when repeatedly presented with a CS-US association. The animals are able to learn multiple interval combinations, such as the time between the onset of the CS and the presentation of the US, the time between two US presentations, and the time between the end of the CS and the upcoming US (Balsam, Drew, & Gallistel, 2010; Kehoe & Napier, 1991). Additionally, the information about the length of the CS-US interval within a trial is encoded rapidly from the beginning of the trial, enabling animals to time their subsequent responses more accurately (Balsam, Fairhurst, & Gallistel, 2006).

It is possible to identify two theories that seek to explain mechanisms that underlie interval timing. A temporal information processing model has been proposed by Church (1984) to account for the ability of subjects to estimate the time until reinforcement onset. There are three primary parts to this model: clock, memory, and a decision process. In other words, when a subject encounters an event for the first time, the timing mechanism is activated, and it stores a memory regarding the duration of this event into the working memory. Upon the ending of the event, the memory for the duration of this event is transferred into the long-term memory. The subject later compares a similar event he or she encounters to the memory about the duration of the original event, which allows for the selection of an appropriate behavioral response that fits into the original time frame.

Another theory has been proposed by Balsam and Gallistel (2009), who argued that timing is based upon information provided by a discriminative stimulus (e.g., a tone)

regarding the temporal distance to the US. In other words, a given stimulus can be either informative or noninformative in signaling how much time is left to make an appropriate behavioral response in a particular situation. Balsam and Gallistel (2009) further argued that in this *informativeness* model, acquisition of a CS-US association is dependent upon the CS lessening ambiguity about the time of arrival of the next US.

In order to assess the degree to which animals have learned to time their responses in anticipation of a reward, a procedure called a *peak-interval task* was introduced by Roberts (1981). A basic peak procedure consists of two trial types: Fixed-Interval (FI) Trials and probe (i.e., peak-interval) trials. During the first stage of training, subjects are trained on a FI schedule of reinforcement, where a discriminative stimulus (e.g., tone, noise) signals that the first response will be reinforced after a fixed amount of time has passed since the onset of that stimulus. During the second stage of training, probe trials are introduced into training. During a probe trial, the discriminative stimulus comes on and stays on for a long duration of time (90 – 110 s); however, these peak trials are not reinforced. Balsam et al. (2009) emphasize an important distinction between these two trials. Specifically, during the FI trials the response rate increases until the reward is obtained, but during the probe trials the mean rate of responding is maximized at the time when subjects expect to receive a reward, and then decreases gradually to baseline rates of responding after the expected time of reward has passed. The dependent variables that are assessed during analysis of the nonreinforced trials are the location of the peak (accuracy) and the precision (variability) with which subjects make time estimates.

Although past research has emphasized the robustness of responses during a peak-interval task across different species, a number of studies have identified factors that can

interfere with accurate and precise responses. In general, certain factors such as low motivation and low attention span of the subjects, as well as high levels of distraction in a testing context can interfere with the accurate timing of events (Champagne & Fortin, 2008; Fortin & Couture, 2002; Galtress & Kirkpatrick, 2009). Furthermore, past research has also looked at the effects of commonly abused drugs on timing mechanisms, which is pertinent to the current study.

Previous research looked at common drugs of abuse and their impact on the accuracy of time estimation. For example, Matell, King, and Meck (2004) examined the effects of intermittent and continuous cocaine administration on time perception during a tri-peak procedure among rats. An acute administration of cocaine shifted the response curve of subjects to the left, indicating that speed of internal clock increased after an acute administration of a stimulant. In other words, animals perceived the time to go by faster after receiving a cocaine injection. Furthermore, chronic cocaine injections over the course of two weeks produced a gradual shift in the peak times to the left, consistent with an acute effect of cocaine.

Similar results were obtained by Matell, Bateson, and Meck (2006), who examined the effects of methamphetamine on the internal clock of rats. The authors found that animals consistently overestimated the time of the reinforcement arrival during a peak-interval task after five continuous methamphetamine injections. Similarly, Taylor, Horvitz, and Balsam (2007) studied the effects of amphetamines on the rate of responding during a peak procedure. Consistent with previous findings, the researchers showed that subjects who received four straight days of amphetamine injections perceived a reinforcer to arrive earlier compared to the control group.

Although cocaine and methamphetamine are stimulants, similar results have been obtained by the studies that have looked at the effects of alcohol (i.e., a depressant) on performance on tasks that assess timing mechanisms. However, the evidence regarding the effects of alcohol on timing mechanisms is rather scant and comes primarily from studies that have either used a differential reinforcement of low response rate (DRL) schedule or a regular FI schedule (i.e., FI 30 s). On a DRL schedule, the onset of a discriminative stimulus indicates the time that subjects have to wait before a response can be reinforced. Any premature responses result in cancelation of a reward, resulting in subjects having to wait until the next discriminative stimulus to have another opportunity to earn reinforcement. For example, Sanders and Pilley (1973) maintained rats on a DRL 1 hr schedule, while giving rats injections of ethanol in varying doses. The researchers found that alcohol administered in high doses significantly impaired the rats' performance on the timing task, leading to a high number of early responses (i.e., a leftward shift in the timing of responses). McDonough, Gill, and Nielson (1975) found similar results using FI schedule of reinforcement to assess effects of chronic alcohol consumption on timing. The data indicated that subjects that were chronically consuming alcohol had a lower rate of responding, with the majority of the responses occurring earlier in the procedure compared to the control animals.

Overall, previous research suggests several possible mechanisms that can account for the disruption of timing. For example, the drugs of abuse may disrupt the inhibition of responding, thus increasing the number of spontaneous responses during timing tasks. It is also possible that the internal clock of the subjects speeds up when a drug is administered to the body, which can result in the overestimation of time and an increased number of premature responses on timing tasks. Furthermore, different physiological and behavioral

mechanisms can account for the failure to inhibit the responses compared to the overestimation of time. However, the current study does not seek to address the underlying differences between these mechanisms, but rather uses a timing task to assess whether a contextual shift disrupts tolerance to alcohol.

The present experiment is conceptually similar to the past research on context-specific tolerance to alcohol. However, the current study is designed to obtain evidence regarding the effects of tolerance to alcohol on a within-trial (i.e., immediate), rather than a between-trial, context shift. Furthermore, the peak-interval task will be used to assess the degree of tolerance disruption in a novel context compared to the original context. The peak procedure will allow for a more direct assessment of tolerance disruption because it does not require additional stressful stimuli to be present in the environment. Peak-interval task also works across multiple contexts, which makes it possible to examine tolerance directly without subjecting animals to any additional tasks. Finally, using the peak procedure makes it possible to limit context exposure to the two basic types of operant chambers, which significantly reduces possible distractors associated with other contextual settings.

It is hypothesized that the subjects will develop tolerance to alcohol in a specific context, thus their pattern of responding on a lever-pressing task will resemble an upward slope around the time the reinforcer is scheduled to arrive. In other words, an animal with a high degree of tolerance to alcohol in a particular context (Context A) should be able to accurately estimate the time of reinforcer arrival in that same context. It is further predicted that the animals will show a disrupted pattern of responding on the peak procedure task, as indicated by an increased number of early responses when tested in a novel context (Context B) not associated with alcohol.

Present Experiment

The present experiment will investigate whether a within-trial context shift will result in disruption of tolerance to alcohol, as indicated by a leftward shift in a response curve on the peak-interval task in a novel context (Context B). During the first stage of the experiment, rats will be trained to self-administer alcohol (or water) in their home cages. This will be done in order to establish an appropriate level of tolerance to alcohol across all subjects.

During the second stage of the experiment, subjects will continue receiving alcohol (or water) in their home cages and will additionally receive operant training in Context A. Specifically, Groups Alcohol – Context Shift (Alc-CS) and Alcohol – No Context Shift (Alc-NS) will be given daily access to alcohol followed by operant training on an FI 30 s schedule of reinforcement in Context A. This will be done in order to establish an association between alcohol administration and performance on a lever-pressing task in a particular context. Subjects in Groups Control – Context Shift (Con-CS) and Control – No Context Shift (Con-NS) will receive equivalent operant training in Context A; however, these animals will be receiving water in their home cages instead of alcohol.

Upon completion of FI 30 s operant training, the effect of an immediate context shift on tolerance to alcohol will be assessed by using a peak-interval procedure. If tolerance to alcohol is associated with a particular context, then Groups Alc-CS and Alc-NS should gradually build tolerance to Context A, which will be indicated by the similar pattern of their response curves compared to Groups Con-CS and Con-NS. Furthermore, context-specific tolerance to alcohol should be disrupted for Group Alc-CS if they experience a within-session context shift. Subjects in Group Alc-NS are expected to maintain their tolerance level

after they are taken out of Context A and immediately put back into Context A. The purpose of including Groups Con-CS and Con-NS is to demonstrate the effects of context shift on peak-interval behavior in the absence of alcohol.

Method

Subjects

The subjects were 32 male and 32 female, experimentally naïve, Long-Evans rats that were obtained from the Appalachian State University Animal Breeding Colony. All subjects were between 80 and 120 days old, and housed (2 – 4 subjects per cage) in a vivarium maintained on a 14 hr light and 10 hr dark cycle. The daily experimental procedures occurred approximately 3 hr after the beginning of the light phase. A progressive food and water deprivation schedule was administered to all subjects a week prior to the beginning of the study. In this schedule, water was gradually reduced to 30 min per day during the course of the study (approximately one month). Food was available ad lib throughout the study. The approval for this and the subsequent experiment was obtained from the Appalachian State University IACUC on September 15, 2011 (Appendix A). Subjects were randomly assigned to one of four groups (Alc-CS, Alc-NS, Con-CS, Con-NS; $n = 16$), counterbalanced within groups for sex.

Apparatus

The apparatus consisted of eight operant conditioning chambers (Med Associates Inc., St. Albans, VT) with the interior of the chambers measuring 30.5 x 24.1 x 21.0 cm (length x width x height). The front walls, back walls, and the ceiling were constructed of clear Plexiglas; whereas, the side walls were constructed of stainless steel panels. The right wall of the chamber was divided into three equal sections. A stimulus light was located near

the top of the interior of the chamber, with 2.4 cm from the ceiling to the center of the light. The stimulus light measured 3.8 x 3.8 cm (square). This light protruded 1 cm from the side of the wall that had tapered sides. The response lever was positioned directly below the light. The lever was 4.8 cm wide, while protruding 1.9 cm from the wall. The lever was positioned 7.1 cm from the floor and 1.5 cm from the back wall to the closest edge. The middle section of the right-side wall also contained the liquid dipper. The opening to the dipper measured 5.0 x 5.2 cm (width x height) and was 3.2 cm deep. The volume of the dipper cup was .04 ml and delivered water for animals when raised. There was a Sonalert speaker (Med Associates Inc., St. Albans, VT) mounted behind the front section of the right side wall. This speaker delivered a 2000-Hz tone, 8 dB (C Scale) above the background noise level. The left-side wall of the chamber was also divided into three equal sections. The middle section contained a house light, measuring approximately 1.5 cm in diameter, 19 cm above the grid floor, left-right centered on the wall. An additional speaker, emitting a white noise stimulus (10 – 25000 Hz flat response) approximately 8 dB (C Scale) above the background noise, was mounted behind the section of the left wall closest to the rear of the chamber.

The floor in each chamber was constructed of 19 stainless steel rods, which were 4.8 mm in diameter and spaced 1.6 cm apart (center to center). In four of the chambers, the rods were spaced horizontally; whereas, in the other four chambers, the rods were staggered in a vertical pattern. Each experimental chamber was separately housed in an isolation chamber (Model ENV-018, Med Associates Inc., St. Albans, VT), which attenuated light and sound exposure. The enclosure measured 55.9 x 55.9 x 35.6 cm (width x height x depth), and was equipped with a ventilation fan. Background noise levels (approximately 74 dB, C Scale) were primarily delivered by these ventilation fans.

The chambers were manipulated to create two different physical contexts. Context A consisted of a standard operant chamber with the houselight turned on during each of the experimental sessions. Four of the chambers had the level grid floors, while the remaining four chambers contained the staggered grid floors. Context B was created by providing training for each subject in an experimental chamber that had a different grid floor (either level or staggered) from that used in Context A. Context B also consisted of an open ceiling and black and white overhead transparencies on the side walls of the chamber. An incandescent light bulb (Model 1820, Eiko Ltd., Shawnee, KS) served as a source of illumination for Context B. These physical contexts were counterbalanced for type within the groups.

Procedure

Context pre-exposure phase. This part of the experiment was conducted prior to any alcohol self-administration training with each subject receiving a 30 min exposure to each context. This phase was designed in order to familiarize subjects with both Contexts A and B before they began to learn an association between the effects of alcohol consumption and a specific context. An abrupt context shift can be stressful for the subjects, and it is possible that their rate of response can drop while adaptation to this new environment takes place. This stage of the experiment was designed to diminish any confounds associated with a sudden contextual shift.

Alcohol self-administration phase. The initial part of the experiment was 15 days in length, and consisted of 15 daily 30 min alcohol self-administration sessions. Ethanol was administered orally using a variation of Samson's sucrose-fading procedure (Samson, 1986).

This experimental phase served to establish a consistent level of alcohol self-administration as well as to increase the tolerance level to alcohol within these subjects.

On Day 1, and throughout the rest of the experiment, one bottle with an alcohol-sweetened solution was available for the total of 30 min per day. The alcohol-sweetened solution consisted of 5% ethanol (vol/vol) and 3% sucrose. On Days 2 through 5, the ethanol concentration was increased to 10% (vol/vol) and the sucrose concentration was maintained at 3% (vol/vol). On Days 6 through 10, the ethanol concentration was increased to 15% (vol/vol) and the sucrose level was 3%. On Days 11 through 15, and throughout the rest of the study, the ethanol and the sucrose concentrations were at 20% (vol/vol) and 3%, respectively. During this stage of the experiment, the subjects in Groups Alc-CS and Alc-NS received alcohol, and the subjects in Groups Con-CS and Con-NS received regular tap water. The amount of alcohol or water consumed by each subject was measured by subtracting the weight of the bottle post alcohol (or water) intake from the original bottle weight using a standard scale. The weight of the bottle was measured in grams, and the amount of alcohol or water consumed by each subject was recorded following each administration session.

FI training phase. During the second stage of the experiment, all subjects were trained to lever press for water in the operant chamber. Two experimental groups (Groups Alc-CS and Alc-NS) continued receiving alcohol in the home cage, and the control groups (Groups Con-CS and Con-NS) received water. Beginning on Day 16, the subjects were placed into individual cages where they received either alcohol-sweetened solution (20% ethanol [vol/vol] to 3% sucrose) or water for 30 min. All subjects stayed in their individual cages for 30 more min to either allow the alcohol to take effect, or to equate exposure to the context. The subjects then underwent preliminary training in the operant chamber to establish

consistent lever-pressing. On Day 16, all subjects received water contingent upon each lever press, and also received reinforcement on a variable-time 2 min schedule in order to condition an association between the sound of the liquid dipper and the availability of water. All training sessions that took place in the operant chamber were 60 min in duration. On Days 17 through 19, the same general procedure was in place, but the subjects were only reinforced upon each lever press. Days 20 through 29 consisted of the same procedures, except that lever pressing was reinforced on FI 30 s schedule, in which the first response after 30 s since the beginning of the trial was reinforced. The beginning of the 30 s interval was signaled by the onset of the white noise stimulus, which remained on until reinforcement was obtained (contingent upon the first response after the 30 s interval). Eight different inter-trial intervals (ITIs) with a mean ITI of 150 s were used.

Testing phase. On Days 30, 32, and 34 all subjects were tested on the FI 30 s schedule. The FI trials were interspersed with non-reinforced probe trials (90 – 110 s in duration) during which the white noise stimulus was presented for 90 – 110 s with no reinforcement available. This testing session was 60 min in duration and was used to assess the accuracy and precision with which animals that have an established level of tolerance to alcohol time their responses. In a typical probe trial, lever pressing increases near the time at which reinforcement is normally available (i.e., 30 s) and then declines soon after the animal fails to obtain the reinforcer. The time at which lever pressing peaks and the distribution of lever pressing during probe trials were the primary dependent variables used to assess the effects of alcohol on timing behavior. Furthermore, the probe trial days were interspersed with FI 30 s trial days, such that on Days 31 and 33 the subjects received the regular FI 30 s training. During the days that included probe trials, 30 min into the session, the animals from

Groups Alc-CS and Con-CS were taken out of one chamber (Context A) and placed into a different chamber (Context B); whereas, the animals from Groups Alc-NS and Con-NS were placed back into the original chamber (A) for the remainder of the session to assess any effect of handling on alcohol tolerance.

Design and Analysis

The current study used a mixed model analysis of variance (ANOVA) to assess whether tolerance to alcohol would be affected by a within-session contextual shift. The independent variables included: the type of drink consumed (water vs. alcohol-sweetened solution) and whether the subjects underwent a contextual shift or not.

At first, the daily FI 30 s trials were analyzed by combining responses for each subject across trials within each day of training, and then assessing whether the mean response time and the variance of responding changed across 10 days of training depending on whether subjects consumed alcohol or water. Specifically, a 2 x 10 mixed model ANOVA was used, where drink-type served as a between-subject variable and the timing of responses was assessed across the 10 FI training days. In other words, each FI 30 day served as a within-subject variable, which allowed the researchers to track changes in the mean response time as subjects began to develop alcohol tolerance.

The data from the probe trials were pooled across three test days, and the average peak time per subject, as well as the variability of responses per subject, served as dependent measures in the study. The probe trials were analyzed using a mixed model ANOVA, with drink type and context shift serving as independent variables, and the pre-shift versus post-shift session serving as the repeated-measures variable.

It was hypothesized that the experimental group Alc-CS would be statistically different from the other three groups following a within-session context shift. However, groups Alc-NS, Con-CS, and Con-NS were predicted to remain statistically similar. Specifically, the subjects in group Alc-CS were predicted to be significantly more impaired after the context shift, compared to the other three groups, as evidenced by a leftward shift in their response curve and a greater variability in their responses. In other words, the mean response time was expected to decrease and the variance was expected to increase for the Alc-CS group.

A correlational analysis was used to assess the relationship between the amount of alcohol consumed and the mean response time. It was hypothesized that the amount of alcohol consumed would have a significant effect on the mean response time and the variance of responses for the groups that consumed alcohol. Specifically, the greater amount of alcohol intake was predicted to result in a lower mean response time for both groups, regardless of the context shift. It was also predicted that males would consume more alcohol than females, which was tested using an independent samples *t*-test.

Results

Descriptive Statistics

The means and standard deviations for alcohol and water consumption are reported in grams. The basic descriptive analysis showed that the average alcohol consumption was 11.28 g (*SD* = 1.80) during the first test day, 12.38 g (*SD* = 1.76) during the second test day, and 10.75 g (*SD* = 1.67) during the third test day for Alc-CS and Alc-NS groups. Groups Con-CS and Con-NS consumed, on average, 11.31 g (*SD* = 1.87) on Day One, 11.06 g (*SD* = 1.78) on Day Two, and 11.30 g (*SD* = 1.67) of water on the last testing day. Subject 18 (Con-

CS) and subject 51 (Con-CS) were excluded from the analysis because they did not perform successfully on the FI 30 task (the total number of responses during the session were less than 10 responses).

Alcohol Consumption in Males and Females

A 2 x 2 factorial ANOVA was performed to assess the differences in the amount of alcohol or water consumed between male and female subjects. It was hypothesized that the males would have higher alcohol and water consumption than the females. Contrary to this prediction, there was no main effect of gender on the amount of drink consumed, $F(1, 60) = 0.58$, $p = .448$, $\eta_p^2 = .01$, and no main effect of drink type on the amount of alcohol or water consumed, $F(1, 60) = 0.77$, $p = .384$, $\eta_p^2 = .01$. Additionally, no significant interaction effect was observed between the gender and the type of drink consumed, $F(1, 60) = 0.06$, $p = .809$, $\eta_p^2 < .01$, indicating that males did not differ from females in the amount of alcohol or water consumption.

Amount of Alcohol Consumed and Response Time

The data were further analyzed to assess the relationship between the amount of alcohol or water consumption and the average response time across three test days. The results indicated that the group that received alcohol showed a negative relationship between the amount of alcohol consumed on Day 1 and the pre-shift mean response time, $r(30) = -.21$, $p = .252$, a slight positive relationship between the amount of alcohol consumed on Day 2 and the pre-shift mean response time, $r(30) = .11$, $p = 539$, and a slight negative correlation between the amount of alcohol consumed on Day 3 and pre-shift mean response time, $r(30) = -.11$, $p = .565$.

Additionally, the correlational analysis revealed a negative relationship between the amount of alcohol consumed on Day 1 and the post-shift mean response time, $r(30) = -.17$, $p = .363$, a slight positive relationship between the amount of alcohol consumed on Day 2 and the post-shift mean response time, $r(30) = .11$, $p = .543$, and a small positive relationship between the amount of alcohol consumed on Day 3 and the post-shift mean response time, $r(30) = .11$, $p = .551$. Since none of the correlation coefficients for the amount of alcohol consumed and the pre- and post-shift mean response times approached significance and due to a small sample size, no additional regression analysis was conducted on these variables.

FI 30 Trials

The data from the 10 FI 30 trials (i.e., trials that took place prior to the beginning of testing) were used to assess the change in tolerance to alcohol across the 10 training days through a mixed ANOVA. The fixed variable was the drink type (alcohol vs. water), and the change in the mean response time was assessed across the 10 FI 30 days. The means and standard deviations for FI 30 trials were reported in seconds. There was a significant main effect of 10 training days on the average response time, $F(9, 414) = 5.42$, $p < .001$, $\eta_p^2 = .11$. There was also a significant main effect of drink type on the accuracy of timing, $F(1, 46) = 5.46$, $p = .024$, $\eta_p^2 = .11$. The subjects that drank alcohol demonstrated a significantly earlier response time ($M = 25.38$ s, $SD = 4.56$) compared to subjects that drank water ($M = 31.28$ s, $SD = 9.06$). There was no significant interaction between the training days and drink type, indicating that the timing of responses did not change for subjects who drank alcohol compared to subjects who drank water across on each training day, $F(9, 414) = 1.10$, $p = .360$, $\eta_p^2 = .02$ (Figure 1).

The main effect comparisons with Bonferroni adjustment indicated, $p < .05$, that subjects showed a significantly later response time on Day 1 (M = 35.34 s, SD = 20.52) compared to Day 5 (M = 23.24 s, SD = 6.57), Day 8 (M = 22.24 s, SD = 4.07), and Day 10 (M = 24.18 s, SD = 10.17). The subjects also had significantly earlier responses on Day 5 compared to Day 7 (M = 28.26 s, SD = 9.52), on Day 8 compared to Day 7, and on Day 8 compared to Day 9 (M = 34.09 s, SD = 22.48).

Probe Trials

The data from the probe trials were collapsed across the three test days in order to assess the effect of context shift on tolerance to alcohol. A mixed ANOVA was conducted with two between-subject variables and one within-subject variable, such that the between-subject variables were the drink type (alcohol or water) and context shift (Context Shift or No Shift), and the within-subject variable was the pre-post context shift. The average response time (i.e., accuracy of responding) and the response variance (i.e., precision of responding) were assessed prior to the context shift and after the context shift.

All the results from the probe trials are reported in seconds. There was no significant main effect of the repeated measures variable (pre-post context shift) on the mean response time, $F(1, 58) = 3.26$, $p = .076$, $\eta_p^2 = .05$, indicating no change in the average response time after the context shift compared to prior to the shift across all groups. However, a significant interaction effect was detected between the pre-post shift variable and the context shift on the mean response time, $F(1, 58) = 4.28$, $p = .043$, $\eta_p^2 = .07$. Specifically, the subjects that received context shift showed faster response during the second 30 min of the session (M = 48.66, SD = 4.91) compared to the subjects that remained in the same context

(M = 50.25, SD = 5.12). There were no significant interaction effects between the pre-post shift variable and the drink type, $F(1, 58) = 0.96$, $p = .332$, $\eta_p^2 = .02$, or between the pre-post shift variable, drink type, and the context shift, $F(1, 58) = 0.27$, $p = .605$, $\eta_p^2 = .01$.

Furthermore, there was no main effect of the context shift variable, $F(1, 58) = 0.11$, $p = .746$, $\eta_p^2 < .01$, or the drink type variable, $F(1, 58) = 0.10$, $p = .754$, $\eta_p^2 < .01$, on the mean response time. However, a significant interaction effect was observed between the context shift variable and the drink type variable, $F(1, 58) = 11.99$, $p = .001$, $\eta_p^2 = .17$. The animals that received alcohol and were shifted into the new context responded significantly earlier (M = 47.46 s, SD = 3.04) compared to the animals that received alcohol and remained in the original context (M = 50.82 s, SD = 4.31). The control subjects that received water and did not receive the context shift responded significantly earlier (M = 47.47 s, SD = 3.54) than the subjects that received water and were moved to the novel context (M = 50.25 s, SD = 2.76).

A 2 x 2 factorial ANOVA was conducted in order to examine the difference between the groups specifically during the second 30 min of the test session (post-shift) and to assess the effect of context shift on response time. Context shift and drink type served as between-subject factors. There was no effect of drink type, $F(1, 58) = 0.05$, $p = .821$, $\eta_p^2 < .01$, or context shift, $F(1, 58) = 1.44$, $p = .235$, $\eta_p^2 = .03$, on the average response time post-shift. However, as hypothesized, there was a significant interaction effect between the drink type and the context shift, $F(1, 58) = 7.59$, $p = .008$, $\eta_p^2 = .12$. Consistent with the original prediction, the alcohol group that received a context shift responded earlier (M = 46.96 s, SD = 4.43) compared to the alcohol group that remained in the same context (M = 51.79 s, SD = 5.63), the water group that received the context shift (M = 50.60 s, SD = 4.86), and the

water group that stayed in the original context (M = 48.70 s, SD = 4.18). Figure 2 demonstrates the mean differences between the groups in the response time post-shift compared to pre-shift, and also shows the individual change in response time for each group from pre-shift to post-shift.

Furthermore, a 2 x 2 x 2 (drink type x context shift x time) mixed ANOVA with the same between-subject and within-subject factors described above was conducted to assess the effect of context shift on the precision of responding using the response variance as a dependent variable. There was a significant main effect of the pre-post shift variable, $F(1, 58) = 9.66$, $p = .003$, $\eta_p^2 = .14$, indicating that the subjects showed less variance in responding prior to the shift (M = 750.58, SD = 145.18) than after the shift (M = 1051.81, SD = 788.53). The variance of responses was not affected by any other variables in the experiment.

Discussion

The following experiment was designed to investigate the effect of an immediate (i.e., within-session) context shift on tolerance to alcohol in rats. The differences in the impact of context shift were assessed using a peak procedure task, which was originally designed in order to investigate the accuracy and precision with which animals are able to discriminate time and anticipate the arrival of a reinforcement (Roberts, 1981).

It was hypothesized that the subjects that received alcohol would be more affected by the context switch relative to the alcohol group that remained in the same physical context and compared to the control subjects that received water. Specifically, it was predicted that the group that received alcohol would show an increase in the number of premature responses and a decrease in the average response time on a peak procedure task. The

obtained results were consistent with the proposed hypothesis. The subjects that received alcohol showed a higher number of early responses in a novel context compared to the control group that received water and was switched to a novel environment and the alcohol group that was returned to the original environment. Additionally, the group that received alcohol and was moved to the new context was the only group that showed an increase in the number of early responses, indicating that these subjects overestimated time during the second part of the experimental session compared to the other three groups.

However, it was also predicted that the other three groups would remain similar in their response times after the context switch. The obtained results did not support this hypothesis because the subjects that received alcohol and remained in the same context showed a slower response time compared to the water control animals that remained in the same context. These results are surprising considering the fact that these subjects served as control animals that received only water and remained in the same environment for the entire duration of the session. These water control animals also showed an increase in their average response time during the second part of the session compared to the beginning of the session. This increase in the number of later responses may be due to satiation with water by the end of the session, which can decrease the motivation to obtain additional rewards and drive the rate of response down.

Additional predictions were also made regarding the variability in responses between the subjects, such that the group that received alcohol was hypothesized to show less precision in their responses after the context shift compared to the rest of the groups. Although the groups did show more variability in their responses during the second part of the session compared to the first part of the session, there were no differences present

between specific groups during the second part of the experimental session. It is possible that during the later stage of the session all subjects showed a decrease in motivation and general fatigue, thus all the groups showed a higher number of arbitrary responses not associated with the reinforcement by the end of the session.

Furthermore, it was hypothesized that males would consume significantly more alcohol compared to females, but the obtained results did not support this prediction. There were no significant differences between male and female subjects in the amount of alcohol consumed, and females actually drank slightly more alcohol compared to their male counterparts. However, these results may be due to a small sample size which can be sensitive to variability in the alcohol consumption.

Further analysis also showed no relationship between the amount of alcohol consumed and the average response time on the probe trials. Specifically, the subjects that consumed more alcohol did not show a greater number of premature responses during the probe trials when compared to the subjects that consumed less alcohol, regardless of whether they were moved to a novel context or remained in the same context. It is possible that the individual tolerance level may account for the lack of association between the amount of alcohol consumed and the average response time. The subjects that consumed more alcohol could show an actual preference to alcohol compared to water, thus they developed tolerance to alcohol faster, and their average response time during the probe trials was not as affected at the higher level of alcohol.

In general, the results of the experiment were consistent with the prior research literature that suggests that the timing processes can be disrupted by drugs of abuse. For example, Matell et al. (2004, 2006) demonstrated that the internal clock speed increased for

the subjects that received cocaine and methamphetamines, leading to premature responses on a peak-interval task. Although prior literature on the effects of alcohol on timing processes has been rather scarce, the obtained results are consistent with previous findings regarding the general effects of different drugs on anticipation and discrimination of time.

Additionally, this experiment also demonstrated that the physical context associated with alcohol consumption plays an important part in the accurate timing of responses on an operant task. Although the animals learn to inhibit the premature responses under the influence of alcohol in a familiar environment, an abrupt switch in physical environment disrupts this inhibitory association leading to an earlier pattern of responses in an unfamiliar environment. Since the animals that received alcohol in a familiar context and the animals that received water in a novel environment showed no impairment in their ability to time their operant responses and no disruption of inhibition, it is possible to argue that alcohol consumption explains a unique amount of variance in the interval timing over and above a simple context shift. These results are consistent with the proposition made by Linnoila et al. (1986). These researchers speculated that an abrupt switch in physical locations in an intoxicated state (e.g., going from a bar to a car after consuming alcohol) can result in an unexpected loss of tolerance and contribute to the frequency of motor accidents among different populations. The current study provided some statistical evidence for this prediction and may have implications for both context-specific tolerance research and timing research.

However, it is also important to address limitations that are associated with the present experiment. One of the limitations is the relatively small sample size that was used in this study. Furthermore, the between-group comparisons could be associated with a particularly low power to obtain significant results due to a small number of subjects in each

group (n = 16), which was further exacerbated by the loss of some subjects from the analysis. Additionally, small sample size is more sensitive to outliers and fluctuations in the data, thus one subject has a better chance of influencing the data in a particular direction compared to a larger sample. Even though it may be justifiable to remove the cases that influence the data from the analysis, it is not desirable to eliminate more data points from an already small sample size and further reduce the power to obtain statistically significant results. For example, the analysis showed the correlation coefficients greater than .2 and .3 not reaching the significance level. Given the sample size, there was not enough statistical power to pull the analysis to the statistically significant level. Future research needs to address this issue by replicating the study with a larger sample size in order to account for the variance in the data due to outliers and other influential cases.

Another potential limitation is the method by which alcohol consumption was measured in the experiment. Although self-administration of alcohol made it possible to mimic a more natural environment in which alcohol is consumed (as opposed to injecting subjects with alcohol), this method also made it more difficult to track the exact alcohol consumption and the blood alcohol levels in individual subjects. Although precautions were taken to prevent unintentional leakage of alcohol from the bottles and to make certain that the weight of water bottles was measured consistently across subjects, it is possible that measurement error weakened the relationship between consumption and the effects of alcohol on timing behavior. Thus, factors such as accidental leakage from a bottle and inconsistencies in scale measurements could be eliminated by using injection and blood sample tests in order to obtain objective measures of administration and blood alcohol levels among subjects.

The present experiment provided additional support for the context-specific tolerance to alcohol and also extended the research on the immediate context shift, rather than a between-session shift, on alcohol tolerance. The timing of operant responses was also assessed in this experiment by using a peak procedure task designed to evaluate the accuracy and precision with which subjects discriminate between time intervals. The timing of responses was significantly reduced for subjects consuming alcohol in a novel context when compared to the subjects that received water and the subjects that only consumed alcohol in a familiar context. In terms of human alcohol research, people who have developed a tolerance to alcohol in a particular place may experience substantial behavioral impairment when they change settings. For example, an individual demonstrating robust tolerance to alcohol in a familiar context may suddenly experience an enhanced effect from that earlier alcohol administration when his or her context is changed (e.g., when moving from a familiar to a novel situation in a short time period). Future studies need to examine these effects in larger sample sizes as well as use a more objective measure of blood alcohol concentration levels to ensure comparable levels of tolerance to alcohol among subjects.

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Figure 1. Average response time in seconds on each daily Fixed Interval 30 s (FI 30 s) session for the Groups Alcohol – Context Shift (Alc-CS) and Alcohol – No Context Shift (Alc-NS) that received alcohol and Groups Control – Context Shift (Con-CS) and Control – No Context Shift (Con-NS) that consumed water. No significant interaction effect, $p < .05$, was detected between the type of drink consumed and the ten FI 30 sessions on the average response time.

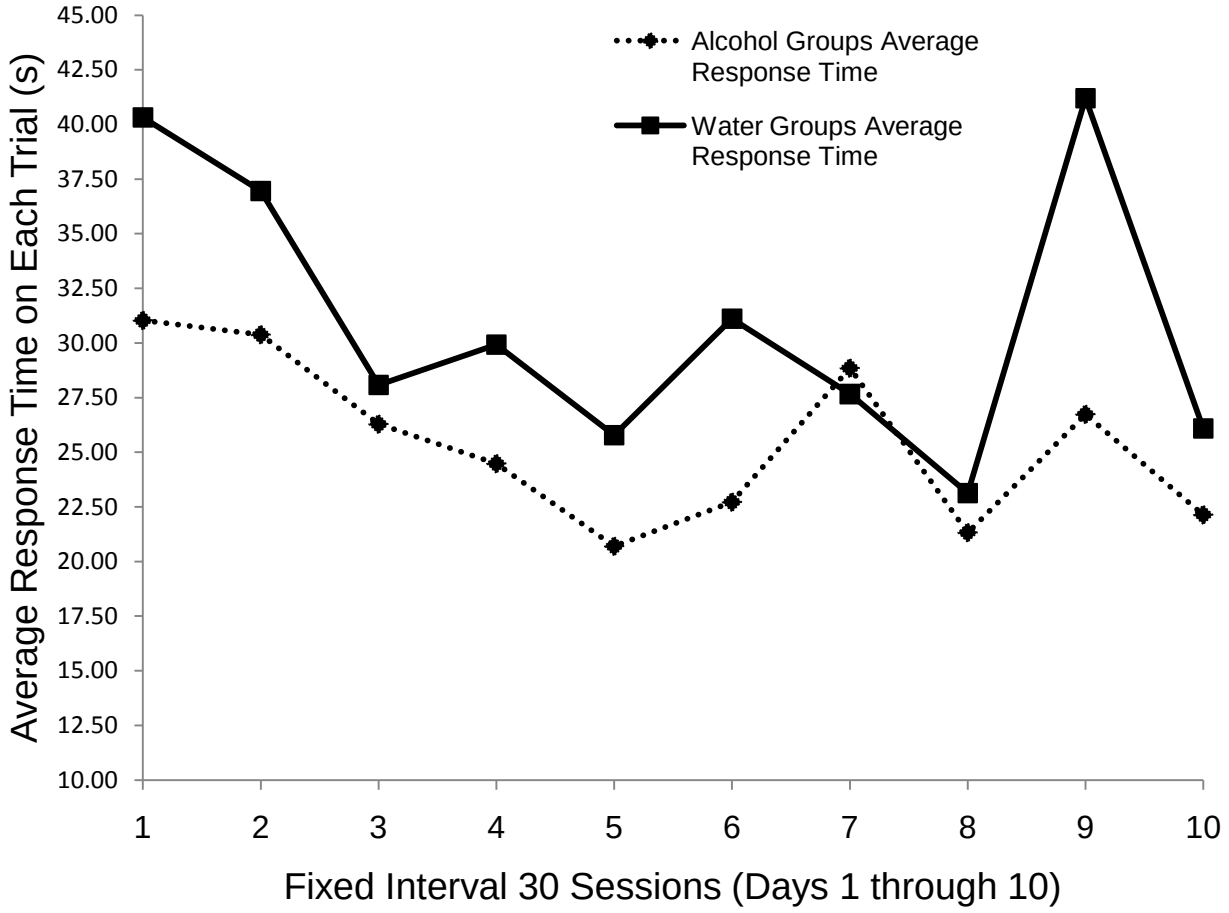
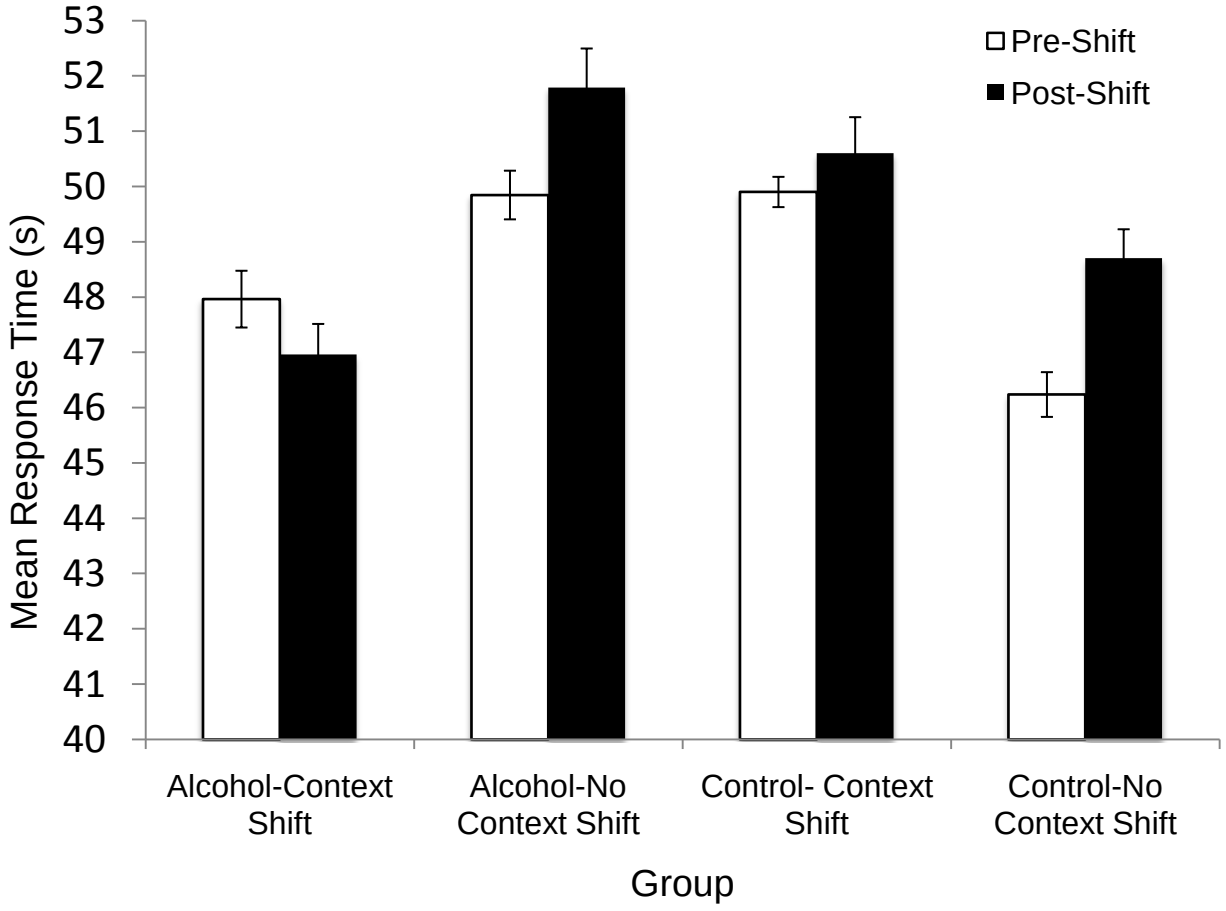


Figure 2. Average response time in seconds before and after the contextual shift for Groups Alcohol – Context Shift (Alc-CS) and Alcohol – No Context Shift (Alc-NS) that received alcohol and Groups Control – Context Shift (Con-CS) and Control – No Context Shift (Con-NS) that received water. Group Alc-CS responded significantly earlier post-shift, $p < .05$, compared to Alc-NS and to Con-CS.



Appendix A

Appalachian
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TO: Dr. James C. Denniston
Department of Psychology

FROM: Dr. Edelma Huntley, Institutional Official
Institutional Animal Care and Use Committee

DATE: September 15, 2011

SUBJECT: Institutional Animal Care and Use Committee
Request for Animal Subjects Research

REFERENCE: Effect of tolerance to alcohol on peak-interval behavior and context shift in rats

IACUC Reference #12-01

Initial Approval Date – September 15, 2011
End of Approval Period – September 14, 2014

The above referenced protocol has been approved by the IACUC for a period of three years.

Best wishes with your research.

ED/rst

VITA

Alexandra Kulikova was born in Moscow, Russia, where she attended elementary school and graduated from Moscow State School #41 in June 2005. The following autumn, she entered Wake Forest University to study Mathematical Economics and Psychology, and in May 2010 she was awarded the Bachelor of Science degree. In the fall of 2010, she accepted a research assistantship in Experimental Psychology at Appalachian State University. She was awarded the Master of Arts degree in May 2012. In September, 2012, Miss Kulikova will commence work toward her doctorate degree in Neuroscience at Wake Forest University.