EVALUATION OF ETHANOL’S EFFECTS ON THE BIOPHYSICAL CHARACTERISTICS OF LICKING

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Alcohol use disorders are a public health issue related to adverse effects for individuals and society. A low level of response, or decreased sensitivity, to alcohol has been identified as a heritable risk factor for development of alcohol use disorders. One method for researching level of response to alcohol is through the use of rodent models, which are developed to mimic human conditions while eliminating barriers to conducting research with people. Current rodent models used to evaluate effects of ethanol on motor performance have been criticized for not being well matched to human tasks that measure level of change in body sway after alcohol consumption. This study looks at oromotor behavior as a potential alternative to gross motor performance in hopes of increasing correspondence between human and rodent measures of intoxication. To evaluate rodent oromotor performance a force transducer lickometer is used to measure several dimensions of licking behavior after administration of different concentrations of ethanol solution via gavage. Results show that force of licking is not sensitive to dose of ethanol. The total number of licks per session show dose related decreases and licking rhythm, evaluated by the length and distribution of interlick intervals, either increased or decreased for three of the four subjects. Recommendations are made for procedural modifications in order to reduce variability in data and further investigate oromotor performance and level of response to alcohol.
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By

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INTRODUCTION

Alcohol-use disorders significantly contribute to public health issues that threaten the quality and longevity of life for individuals, their families, and society. The World Health Organization (WHO, 2014) reported that 3.3 million people die each year due to harmful alcohol use. Harmful use of alcohol was identified as a causal factor in over 200 disease and injury conditions, as well as associated with risk for developing a range of behavioral and mental disorders. Health problems include alcohol dependence, liver cirrhosis, cancer, cardiovascular diseases, incidences of infectious diseases, and injuries resulting from violence and traffic accidents. Alcohol dependence is one of the most prevalent major psychiatric conditions and is associated with considerable morbidity and mortality (Schuckit, 2002). Untreated complications from excessive alcohol consumption can incur health care costs at least 100 percent higher for alcoholics than non-alcoholics (Johnson & Ware, 1998). Thus, alcoholism is a social problem that continues to call for research that will increase understanding and give rise to effective methods for prevention and treatment.

A variety of biological, environmental, and cultural factors contribute to risk for alcoholism (Hasin et al., 2003). Although the volume of alcohol consumed and drinking patterns are pinpointed as determinants for the impact alcohol has on individuals and society, additional environmental factors contribute to risk for development of alcohol-related problems. The WHO (2014) identifies economic development, culture, availability of alcohol, and alcohol policies as additional factors contributing to risk. No one dominant risk factor has been identified; rather, an accumulation of factors contributes to individual vulnerability and the magnitude of population level alcohol-related problems.
The role of biological, psychological, and environmental factors have been investigated for their contribution to risk for development of alcohol use disorders. Genetic risk factors are estimated to account for 60% of the variance, with environmental factors contributing to the remaining 40% (Schuckit, 2002). Alcoholism has been identified to run in families since the 19th century (Goodwin, 1985). Twin and adoption studies dominated early years in alcoholism research. Goodwin (1985) and Newlin and Thomson (1990) summarized results from twin and adoption studies. Twin studies compared groups of identical and fraternal twins, the rationale being that if identical twins were more often concordant for alcoholism than fraternal twins then results would support a genetic influence for alcoholism. A review of results from twin studies provided mixed findings for the influence of genetics in development of alcohol problems. Adoption studies compared half siblings with full siblings of alcoholics, with the rationale that if full siblings were more often alcoholics than half siblings there would be support for genetic influence. A review of findings from adoption studies did not clearly support the influence of genetics in development of alcoholism.

Further evaluation of results from adoption studies looked at alcoholism in offspring of biological parents with alcoholism compared with foster parents. Evaluation of genetic and environmental components resulted in identifying alcoholism in sons to be highly correlated with having a biological father with alcoholism; no correlation was found with alcoholism in surrogate fathers. Sons of alcoholics were three or four times more likely to be alcoholic than sons of non-alcoholics, independent of being raised by alcoholic biological parents or nonalcoholic adoptive parents (Goodwin, 1985). A link was also identified between alcoholism in biological mothers and alcoholism in adopted-out daughters. Goodwin (1985) concluded that
family history of alcoholism was the strongest predictor of future alcoholism in adolescents; in both sexes having a family history of alcoholism increased risk four or fivefold. Newlin and Thomson (1990) also concluded that results suggested a strong hereditary component to alcoholism.

In a continued search for genetic influences for alcoholism, the relationship between genetic markers and phenotypes are evaluated. Specific phenotypic and genetic markers are pinpointed in order to study how phenotypes and candidate genes could contribute to drinking and development of alcohol-related problems. The probability that multiple genes influence a phenotype complicates the identification of genetic influences for alcohol use disorders (Schuckit, 2002).

Another complication in identifying relationships between markers is that alcohol use disorders (e.g. alcohol abuse or dependence) are broad phenotypes lacking precise definitions. Hasin et al. (2003) presented findings from a 2002 symposium charged with summarizing known validity of the Diagnostic and Statistical Manual of Mental Disorders (4th ed.) (DSM-IV) and the International Classification of Diseases and Related Health Problems (10th ed.) (ICD-10) diagnostic criteria for alcohol dependence and abuse since 1994. A review of test-retest reliability studies showed that alcohol dependence had excellent reliability and alcohol abuse, defined by social problems (DSM-IV) or hazardous use (DSM-IV and ICD-10), had much lower reliability. The symposium attributed low reliability to separating abuse as a residual category and suggested an alternative hierarchal relationship, from abuse to dependence. Hasin et al. (2003) concluded that generic diagnostic criteria need to be operationally defined, and in the absence of highly sensitive and specific biological tests less precise behaviorally-oriented
criteria sets would continue to be used. A potential alternative position is that behavioral markers themselves may be improved, and do not necessarily have to be less precise than biological markers.

When the DSM was updated, the two DSM-IV disorders were combined into one disorder, alcohol use disorder (AUD), with sub-classifications of mild, moderate, and severe (National Institute on Alcohol Abuse and Alcoholism, 2013). The severity of AUD classification is based on an increasing number of criteria met, but criteria continue to use language open to interpretation. An important criterion from the DSM-IV and –V is the consumption of alcohol in larger amounts or over longer periods of time than intended. The criterion exemplifies the need for specificity in defining thresholds for problematic and risky amounts of alcohol consumption and lengths of time for consumption.

Sher, Grekin, and Williams (2005) presented the suggestion that less complex behavioral phenotypes foundational to alcohol use disorders might have more utility for studying effects of individual genes. Schuckit (2002) searched for central themes among possible markers and identified several potential domains of risk factors; among those the level of response was speculated to reflect the final phenotypic path through which many different markers operate and could be the characteristic to pull together other findings. An individual’s level of response (LR) to alcohol refers to how an individual is affected after consuming alcohol. Previous research by Schuckit (1985) and Schuckit and Gold (1988) has indicated that individuals who have a first- or second-degree relatives who were alcoholic, referred to as family history positive (FHP), were less responsive to alcohol than individuals that had no first- or second-degree relatives who were alcoholic, family history negative (FHN).
Demonstration of a low level of response to alcohol has been identified as a predictor for individuals being at risk for diagnosis of alcohol dependence or developing alcohol-related problems (Crabbe, Bell, & Ehlers, 2010). In short, a low LR describes a decreased sensitivity to alcohol (Schuckit, 1985) or a need for higher doses of ethanol to produce an effect (Schuckit, 2002). This recognized biobehavioral marker, and heritable phenotype, for risk of alcoholism has been defined as requiring a higher number of standard drinks to reach behavioral markers of intoxication (Ray, Hart, & Chin, 2011), reflecting an innate tolerance to alcohol in certain individuals. Approximately 40% of the estimated 700 children of alcoholics included in the majority of studies have demonstrated a low level of response to alcohol (Schuckit, 2002). Longitudinal and follow-up studies have confirmed low LR as a significant predictor of later alcohol abuse or dependence (Schuckit, 1998).

A low level of response has been identified as a risk factor for heavy drinking, which in turn puts individuals at risk for developing alcohol use disorders. Peele (1985) proposed that offspring of alcoholics having a lowered sensitivity to blood alcohol levels may not be as aware of onset of intoxication or they may have a greater tolerance to alcohol. Schuckit (2002) proposed that individuals with a low LR to alcohol may have an enhanced probability of heavy drinking, which may encourage formation of peer groups with heavy drinking habits, and lead to acquisition of tolerance. Schuckit (1994) describes a multifactorial genetic model for alcoholism where genetic factors interact with environmental factors resulting in an increased probability that an individual will drink heavily and regularly. This model proposes that individuals inherit genetic factors that, when combined with an environment that supports heavy-drinking, result in increased risk for consumption of large quantities of alcohol,
subsequently elevating risk for development of psychological and/or physical dependence. In order to study theories regarding low LR, the mechanisms involved, and the development of alcohol use disorders, several preparations have been utilized.

The administration of an alcohol challenge is one approach to studying LR in humans. For the alcohol challenge, alcohol is given to participants and measures are taken for changes in magnitude of response to alcohol at particular blood alcohol concentrations. Measures of changes in motor performance, hormone levels, and electrophysiological activity have been used to determine a participant’s level of response (Schuckit, 2002). One strategy for evaluation of motor performance is to measure changes in body sway, or static ataxia. Body sway measures are obtained through the use of a stabilometer platform or with a rope-and-pulley system with magnetic sensors attached to a harness that participants wear (Newlin and Thomson, 1990). A low body sway score indicates postural stability. Using the rope-and-pulley system, Schuckit (1994) found significant differences in body sway scores between groups of men that did and did not subsequently develop alcohol dependence. Men who did develop alcohol dependence had lower sway scores than men who did not develop alcohol dependence. Significantly lower sway scores were seen at peak blood alcohol level approximately one hour after consumption of alcohol and 1 and 2 hours after peak effect.

The alcohol challenge is also used with a Subjective High Assessment Scale (SHAS) to measure subjective feelings of intoxication (Ray, MacKillop, Leventhal, & Hutchison, 2009; Schuckit, Tipp, Smith, Wiesbeck, & Kalmijn, 1997; Wilhelmsen et al., 2003). Participants use a rating scale to report levels of discomfort, high, clumsiness, confusion, slurred speech, dizziness, nausea, drunkenness, sleepiness, disorientation to time, drug/alcohol effects,
problems concentrating, and floating feelings after alcohol consumption (Schuckit et al., 1997). Ray et al. (2009) stated that the most compelling evidence supporting subjective responses to alcohol as a predictor for alcohol use and misuse came from a longitudinal study by Schuckit and Smith (1996) and Schuckit, Smith, Anderson, and Brown (2004). The longitudinal study’s results showed that individuals who demonstrated a low level of response to alcohol on the SHAS were significantly more likely to develop alcoholism at 8- and 20-year follow-ups.

The alcohol challenge method involves high cost, time-consumption, and a limited participant pool due to legal drinking age restrictions with healthy nonalcoholic individuals. These practical limitations lead to the development and use of the Self-Rating of the Effects of Alcohol (SRE) as an alternative method for obtaining measures for level of response through self-report with young participants prior to onset of alcoholism (Schuckit et al., 2007). When using the SRE, participants report the number of drinks needed to experience certain effects: initial effects of intoxication, slurred speech or dizziness/slurred speech, stumbling gait, and passing out. Ray et al. (2011) tested the predictive validity of the SRE related to alcohol problems assessed with the Alcohol Use Disorders Identification Test. Researchers concluded that the SRE was a valid and reliable measure of subjective responses to alcohol among college-age drinkers. Schuckit et al. (2007) obtained SRE scores for 95 participants between the ages of 18 and 35 and evaluated its predictability for alcohol intake and problems 5 years later. Schuckit et al. (2007) concluded that the SRE-based LR scores were consistent with their ability to predict alcohol-related outcomes over the next 5 years. Although the SRE has predictive utility, it is an indirect measure and ineffective for experimental investigation of variables that may influence behavior related to the development of alcohol use disorders.
Another approach to studying LR that obviates barriers associated with human research is through the use of animal models. Animal models are used in research aimed at learning about human behavior, disease, and disorders. Froehlich (2010) defines an animal model of disease as an experimental preparation developed to study a condition found in humans. Froehlich (2010) explains that animal models are used under the assumption that there is homology, or analogy, between physiological and behavioral characteristics across species. Despite the potential utility of preclinical models for understanding LR to alcohol, little progress has been made with current approaches.

Crabbe et al. (2010) reviewed animal models for LR across four domains identified in humans: behavioral measures of subjective intoxications, body sway, endocrine responses, and stimulant, autonomic, and electrophysiological responses. The goal of the review was to evaluate the four domains and their potential as targets for continued development of more consilient phenotypes across species, specifically humans and rodents. Crabbe et al. (2010) identified weaknesses in consilience across each of the different domains; I will focus on animal models of body sway in humans as a measure of motor performance, or postural stability, which the authors note as a promising domain with considerable resources for increasing consilience.

Various rodent motor performance tasks have been employed in efforts to identify a rodent model for body sway in humans. Some examples of tasks given to rodents after administration of ethanol are balancing on a beam or remaining on a moving dowel with increasing rotation speed. During such tasks, measures are taken for the number of instances of rodent foot slips or the duration of time the rodent remains on the apparatus. Multistrain
comparisons of selectively bred rodents for alcohol related traits (e.g., alcohol-preference, alcohol-accepting, alcohol-drinking) provided inconsistent results across behavioral assays; rodents would appear to be intoxicated on one measure and not another (Crabbe et al., 2010).

Crabbe et al. (2010) reported weak parallels between human body sway and rodent measures of motor performance. Crabbe, Metten, Cameron, and Wahlsten (2005) identified a shortcoming in rodent tasks as the involvement of many neural systems in task completion. For a given motor task, vision, balance, proprioceptive feedback and gait coordination, locomotion, muscle strength, and attention may be required, and these specific physiological and behavioral systems do not appear to tap into a common construct such as “body sway”. Crabbe et al. (2010) offered an analysis of postural steadiness in humans, previously given by Horak (2006), as a task involving proprioceptive sensory information, biomechanical stimuli generated by gravity, cognitive processes affected by movement, and intentions regarding movement and spatial orientation, among other factors. The complexity of tasks used to measure postural steadiness leaves open the important question of which aspects resulting in body sway are affected by alcohol, as well as related neural systems. Crabbe et al. (2010) supported the study of phenotypes across species because of the inherent utility of animal studies to explore neurobiological mechanisms including genetic sources of influence, but suggested that refined measures are needed to more directly compare LR between humans and laboratory animals. Identification of a strong measure across species would foster research in the area of alcoholism, potentiate developments in the area of alcoholism prevention, assist in the specification of neurological pathways sensitive to alcohol, and lend to discovery of genes related to susceptibility and protection for developing alcohol use disorders (Li, 2000).
Thus, it appears one barrier in establishing consilience between rodent and human measures is identifying equivalent questions across species; it is not clear different measures of movement and posture are asking equivalent questions across species. An unexplored marker for level of intoxication in humans is alcohol-induced impairments in orolingual function. Slurred speech has been used as an observable marker of intoxication in alcohol research with humans (Sobell & Sobell, 1972; Wall et al., 1999) and in clinical settings (Bendtsen, Hultberg, Carlsson, & Jones, 1999; Holt et al., 1980). The Self-Rating of the Effects (SRE) of Alcohol (Schuckit, Smith, & Tipp, 1997; Wall et al., 1999; Ray et al, 2011) and the Subjective High Assessment Scale (SHAS) include slurred speech as a phenotype for assessing level of response to alcohol in humans. Although speech cannot be modeled in rodents, measures of oromotor performance in the form of licking can be obtained through the use of a lickometer. Licking may be a less complex phenotype, compared to phenotypes currently targeted in rodent models, and may lend to constructing a more consilient rodent assay for human measures of alcohol related behavior and level of response.

Oromotor activity is involved in speech and mastication. Speech uses many of the jaw, facial, and tongue muscles active during mastication, swallowing, and respiration. Lund & Kolta (2006) proposed that brainstem central pattern generator (CPG) neuronal circuits involved in mastication are also employed in orofacial movements during speech. CPGs are comprised of networks of neurons that include information required to sequence and activate motor neurons at particular intensities to generate motor patterns (Marder & Bucher, 2001; Barlow & Estep, 2006). Vertebrates and invertebrates share CPGs as a common type of neural control structure involved in the production of patterns of motor behavior. Various categories of CPGs
are implicated in the coordination of reflexes, such as swallowing or coughing, to rhythmic movements exhibited during respiration (Grillner, 2003).

Grillner (2003) wrote that many features of the organization of basic motor function are similar throughout evolution. Wainwright (2002) evaluated the evolution of feeding motor patterns in vertebrates and deduced that under the same conditions closely related species show little or no differences in motor patterns. He states that mammals from different Orders display common chewing motor patterns and very little variation between species can be ascribed to evolutionary modifications. Although feeding motor patterns are highly conserved, Wainwright (2002) comments on variability in motor patterns due to features of the material they are consuming (e.g., prey type, size, position, or toughness).

Travers, Dinardo, and Karimnamazi (1997) proposed ingestion of fluids by licking as a more straightforward approach than mastication to studying rhythmic oromotor activity. They explain that ingestion of fluids via licking employs many of the components of the motor pattern used to consume solid food, but does not require mechanical repositioning of food during consumption. Licking involves repetitive tongue and jaw movements controlled by a network of brainstem neurons forming a CPG (Lin, Pierce, Light, & Hayar, 2013). A rhythmic pattern is exhibited during licking in the repetitive alternation of tongue protrusion and retraction. Stellar and Hill (1952) studied drinking behavior of rats at different amounts of water deprivation. They found that rats drink at a constant rate of 6-7 tongue laps per second, regardless of level of water deprivation. The predictable rate of 6-7 licks per second is the maximum rate of licking a rat can sustain and 90-95% of rat licks occur within this frequency (Stellar & Hill, 1952).
Rat licking typically occurs in bouts of licks separated by either short or long pauses between licks (Davis, 1989; Houpt & Frankmann, 1996). Any adjustments in the rate of licking are typically due to increasing the length of pauses between licks (Houpt & Frankmann, 1996). Davis (1989) reported that 90% or more of interlick intervals (ILIs) fall within the range of 0-0.2 seconds, with an average of approximately 0.16 second. Lick rhythm can be assessed through the distribution of ILIs and further analyzed through the construction of ILI histograms (Lin et al., 2013), and so pattern is easily quantified. Lin et al. (2013) noted that the consistency in the frequency of licking is a result of keeping conditions constant, as opposed to a rigid output of a central rhythm generator. Environmental manipulations identified to slow licking patterns include increasing the distance between the rat and the water source (Weijnen, 1998), palatability of the solution (Lin et al., 2013), and drug administration (Fowler & Wang, 1998). Fowler & Wang (1998) administered haloperidol to rats and found dose dependent alterations in tongue movement dynamics and rhythm of licking.

Thus, licking emerges as a quantifiable and sensitive measure for investigating alcohol intoxication. The present study investigates the effects of ethanol on licking behavior in rats. Should licking be a sensitive measure for dose-dependent changes, it could provide a rodent model useful in research for level of response to alcohol and factors that contribute to the development of alcohol use disorders.
METHOD

Subjects and Housing

Four experimentally naïve Long Evans rats were used. Rats weighed approximately 450-550 g throughout the experiment. All animals were housed individually in standard plastic cages on a reverse 12-hour light-dark schedule, where lights were turned on at 9:00 PM and off at 9:00 AM. Food was freely available in home cages. Room temperature was maintained at 68°-78° F and relative humidity at 30-70%. All procedures for the study were approved by the Institutional Animal Care and Use Committee at the University of North Texas.

Apparatus and Measurement

Two identical chambers were used for all behavioral training and testing. Each chamber was individually enclosed in an ice chest. White noise at approximately 65 dB was played in the background to mask extraneous sounds. Each chamber had a stainless steel grid floor that allowed for waste collection in a removable tray containing bedding. A 5-cm strip of the floor narrow edge composed of solid steel formed a drinking platform. A 1.2-cm hole was cut centrally into the platform and allowed access to a lick disk. The lick disk was a hollow 1-cm aluminum disk mounted atop an isometric force transducer (Model 31a, Sensotec, Columbus, OH). The lick disk was located 5 mm below the hole in the drinking platform. Fluid could be made available on the lick disk for consumption via tubing connected to a peristaltic pump (BRS Drew’s Doser, Golden Valley, MN). Fluid was delivered to the disk at a rate of 1.2 ml/min. Figure 1 is picture of the apparatus and components.

When the rat extends its tongue down through the hole and contacts the force transducer lickometer, water is pumped onto the center of the disk. The force threshold for lick
detection was 2-g force. The first lick delivered 20-ul of water, after that every five licks delivered an additional 20-ul of water. The rate of water delivery was selected through pilot experiments. At this rate, rats could drink water about as fast as the pump filled the disk. Rats could drink for several minutes continuously without spillage.

The force transducer is an instrument that allows for direct and continuous real-time measurement and quantification for dimension of the licking response. Data were recorded in real time through the use of a PC computer. A data acquisition card (USB 6009, National Instruments, Austin, TX) was used to read data from the transducer. Data were sampled at 100 samples/s. Notterman and Mintz (1965) stated that force emissions constitute basic motor output. Several quantitative variables were recorded for each lick over the 15-minute session. The computer program recorded daily files for each session and included total number of licks per 15-minute session, peak force, time integral of force, and ILIs. Peak force is a measure of the maximum force emitted while the rat’s tongue is in contact with the lickometer during each lick. Time integral of force is a measure of the force exertion emitted throughout the duration of the response. An ILI is a measure of the time elapsed between two successive licks. ILIs were used as a measure for licking rhythm. Figure 2 is a 1-s sample of baseline licking from L1, which illustrates the dimensions of licking recorded in daily files.

Procedures

*Initial training.* Rats were weighed and handled for approximately 5 minutes per day for 12 days prior to beginning training for alcohol administration procedures.

*Ethanol administration.* Intragastric gavage procedures were used for alcohol administration and involved inserting a 26-g gavage needle down the rat’s esophagus and
injecting ethanol solution into the rat’s stomach. To train animals to comply with gavage procedures and minimize stress the animal may experience, lab personnel began by exposing rats to manual restraint over 17 days. Initial exposure was implemented by briefly holding the rat by the scruff of its neck. Ultimately lab personnel held the rat while securing its head with their fingers. After a secure and safe hold was established, rats were slowly exposed to oral gavage procedures over the next month. Rats were initially exposed to the procedure with only a dry needle and then water was introduced from 1-1.5 ml.

*Water deprivation.* Daily water consumption was monitored under 24-hour free access conditions in their home cages for approximately 3.5 months. Water bottles were then removed for 22.5 hours and subsequently weighed before and after 90 and then 30 minutes of free access to determine how much time was required for subjects to maintain a healthy status of water consumption. Those values were used to inform deprivation conditions for the study. For the study, rats were kept at approximate 22.5-hr water deprivation prior to each experimental session. Sessions were conducted Monday through Friday between 9:00 AM and 11:00 AM. During the 15-min session and for 30 minutes following sessions rats had unrestricted access to water. After sessions on Fridays, the rats were given unrestricted access to water over the weekend. Water restriction resumed on Sundays at approximately noon to ensure deprivation conditions were in place for the next session.

*Lick training.* Rats were exposed to the chamber for 4 days with drops of water around the hole in the chamber floor for rats to learn to lick water from a force-sensing disk. By the fifth day all rats licked the lickometer at approximately 1000 licks per session.
Baseline. Baseline measures were obtained over the next 17-30 sessions depending on
stability of data for each individual subject. Stability was determined through visual inspection
of line graphs depicting total licks and average peak force across sessions.

Ethanol’s effects. To explore the relationship between ethanol and measures of licking
dose-response functions were determined. To obtain a dose-response function, measures of
the response are obtained after administration of different doses of drug on separate sessions.
On Mondays and Wednesdays sessions were run in the absence of any drug administration
procedures. Each Monday ethanol solutions (10%, 20%, 30%, 40%, 50%, or 60% v/v) were
prepared from ethanol mixed with tap water, and these concentrations corresponded to doses
of 0.5-, 1.0-, 1.5-, 2-, 2.5-, and 3-g/kg ethanol given at a constant volume of 5 ml/kg. At the
outset of the study, it was not clear which doses would be relevant for the study, hence a wide
range of doses was used. After initial determinations, it became clear that the range of effects
were captured by ethanol given at 1, 2, and 3 g/kg; thus, the remaining doses were no longer
administered. The data from these initial pilot determinations are not included in the final
analysis.

Drug administration procedures were implemented on Tuesdays, Thursdays, and
Fridays. Initial dose order was randomly determined and then the function was re-determined
by repeating the doses in the reverse order. On Tuesdays and Fridays rats were administered a
single dose of ethanol solution via gavage 30 minutes prior to session. The 30-minute pretreat
time allowed for drug absorption. Each Thursday rats were exposed to drug administration
procedures 30 minutes prior to session, but ethanol was not administered. Thursdays were
procedural control days and only water was delivered via gavage. A second control of 0 was
interspersed on Tuesdays and Fridays; these additional control points did not differ from the
values obtained on Thursdays and have been aggregated with those data for the analyses
presented below.
RESULTS

Dose-response functions were constructed to illustrate changes in average peak force, average time integral of force, and total number of licks per session. Dose-response functions for average peak force (Figure 3) and average time integral of force (Figure 4) are relatively flat for each subject. In contrast to the complete lack of effect of ethanol on force and effort of licking, total number of licks decreased in three out of four rats. Still, it is important to note that there was quite a bit of variability in control points, and the dose-related decreases are average effects (Figure 5). There was a consistent reduction in average total licks from control to 1 g/kg across all subjects, differences between subjects occurred at higher doses. L1 and L2 had relatively no change in average total licks from 1 to 2 g/kg and to a lesser degree for L2 than L1 a decrease is visible from 2 to 3 g/kg. For L3 and L4 there was a decrease from 1 to 2 g/kg and an increase from 2 to 3 g/kg.

Individual functions and replicates for total licks per session are displayed in Figure 6. Multiple control points within each individual function and replicate were averaged together. L1 progressed through six functions during the study. For function one, total licks decreased slightly from control to 1 g/kg and to a greater extent from 1 to 2 g/kg. During the second, third, and fourth exposure to 2 g/kg there was an increase in total licks. On the fifth exposure to 2 g/kg only 5 licks occurred and responding remained low at 3 g/kg. For the last function a slight decrease in total licks occurred as dose increased from 1-3 g/kg.

L2 progressed through four functions during the study. Total licks are variable at each dose across functions, with the least variability at 3 g/kg. For the first function there was a decrease between control and 2 g/kg, both data points have a higher number of licks than all
but one subsequent session. Function two illustrates an increase from control and 2 g/kg, followed by and a decrease of 1219 licks at 3 g/kg. Replicate two illustrates a decrease from control and 2 g/kg, followed by and an increase of 823 licks at 3 g/kg. During the last function, a large decrease of 894 licks occurred from control to 1 g/kg followed by a slight increase across 1-3 g/kg, ranging from 277 at 1 g/kg to 509 at 3 g/kg.

L3 completed three functions during the study. During function 1, decreases in the number of licks are seen as dose increased from 1-3 g/kg, total licks were 968, 272, and 13 respectively. During replicate 1, licks decreased from 1809 licks at 1 g/kg to 1 lick at 2 g/kg and remained low at 3 g/kg. During function 2, licks decreased from control to 1 g/kg and 1 to 2 g/kg, total licks increased by 900 licks from 2 to 3 g/kg.

All three functions for L4 show a different pattern across doses, but each function ends with a high number of licks at a 3 g/kg. Function one shows a small increase from control to 1 g/kg and a large decrease from 1 to 2 g/kg. Replicate one shows a decrease from control to 1 g/kg and an increase from 1 to 2 g/kg. Function two shows a decrease from control to 1 g/kg and from 1 to 2 g/kg. Between 2 and 3 g/kg, function one had an increase from 1-1189 licks, replicate one decreased by 178 licks (from 1025 to 847 licks), and for function two there was an increase of 704 licks.

Licking rhythm was evaluated through visual analysis of ILI frequency histograms. Histograms were divided into twelve bins, bin one includes ILIs <110 ms, there are ten bins separated by 10-ms increments between 110 and 200 ms, and the last bin includes ILIs >200 ms. The bins between 110 and 200 ms were broken into smaller bins because this is the range in which most ILIs occur within a bout of licking, bins greater than 200 ms generally reflect
intervals between, rather than within, bouts of licking. Figure 7 displays cumulative frequency histograms for average ILI distributions across conditions. Histograms reveal some slowing of licking rhythm by lines shifting to the right due to a decrease of ILIs primarily between 120 and 160 ms. For 3 of the subjects an increase in licking rhythm is indicated by an increase in percentage of the shortest interval bins, <110 ms and 110 ms, for at least one dose condition. Figure 8 displays frequency histograms that further illustrate change in percentage of ILIs that fall in the shortest (within-bout) and longest (between-bout) intervals, as well as the scarcity of change for ILIs separating within- and between-bout intervals. For L1, L2, and L4 there was a low percentage of ILIs and minimal change across doses in the 190- and 200-ms bins, for L3 this occurred across 160-200-ms bins.

For L1, the largest percentage of average ILIs across each condition fell in the >200-ms bin which reflects intervals occurring between bouts of licking. Average percentage for each condition was 33% for control, 45% for 1 g/kg, 30% for 2 g/kg, and 55% for 3 g/kg. For the percentage of ILIs ranging from <110 through 200 ms, representing within-bout intervals, averages for control and 1-g/kg conditions show the majority of ILIs fall in bins 130-160 ms, peaking at 150 ms. At 2 and 3 g/kg there was a large increase in the percentage of ILIs in the shortest bins, with 17% of ILIs occurring at <100 ms for 2 g/kg and 19% were 110 ms for 3 g/kg.

The largest percentage of ILIs for L2’s averages across each condition fell in the >200 ms bin, with 3 g/kg having the highest percentage by 12%. For percentage of ILIs ranging from <110 through 200 ms, averages for control and 1-g/kg conditions show the majority of ILIs in 120-160-ms bins, peaking at 130-140 ms. Average ILIs for 2 g/kg increased to 10% at <100 ms and peaked at 12% at 130 ms, the majority fell between 120-140 ms. Averages at 3 g/kg had 7% of
ILIs at <100 ms and 13% at 150 ms. The majority of within-bout ILIs clustered around 130-160 ms, and 40% of ILIs can be categorized as between-bout intervals.

The largest percentage of L3’s control and 1-g/kg average ILIs fell in 110 to 140-ms bins, peaking at 120-130 ms. Most ILIs for 2-g/kg averages were between <110 and 140 ms, with the largest percentage of 33% in the <110 ms bin. 3 g/kg within-bout averages were predominantly <110 ms and were distributed across to the 130-ms bin. The greatest portion, 48% of ILIs, was between-bout intervals at 3 g/kg.

Averages for all L4’s conditions showed the majority of ILIs under 200 ms occurring between 130-170 ms. Peaks for average ILIs under 200 ms were: 18% at 140 ms for control, 20% at 140 and 150 ms for 1 g/kg, 16% at 150 for 2 g/kg, and 16% at 160 ms for 3 g/kg. Although little variation in within-bout ILI distribution is seen across doses 44% of ILIs for 2-g/kg averages lasted >200 ms, at least 26% higher than the other 3 conditions.

Overall, inconsistent effects were seen across subjects. For L1 and L3 at dose of 2 and 3 g/kg increased the proportion of very short intervals, <110 and 110 ms. L2’s data show a slowing of licking. L4’s data show little effect of ethanol on licking rhythm; a change in ILI distribution is only seen at averages for 2 g/kg and a significant portion of the shift was due to the increase in between-bout intervals.
DISCUSSION

This study examined the effects of different doses of ethanol on several dimensions of licking behavior of rats that have not been studied previously. Results showed relatively no effect of ethanol on peak force (Figure 3) or time integral of force (Figure 4). Average total licks show a dose-related decrease for three out of four rats (Figure 5), although variability can be seen in control points and across individual functions and replicates (Figure 6). Notterman and Mintz (1965) reported finding independence between measures of response output and force. For ILLI frequency distributions (Figures 7 and 8) there were inconsistent effects across rats, but at selected doses there are hints that ethanol increased the proportion of very short intervals for L1 and L3, of a slowing of licking for L2, and little effect for L4.

Future studies on the effects of ethanol on licking could benefit from procedural alterations to decrease variability. Possible factors that could contribute to variability include water deprivation and ethanol administration procedures. At our facility, our protocol was approved for 22.5-hour water deprivation for only 5 days per week, it is possible that implementing water deprivation procedures 7 days per week could improve stability of control points for total licks per session, although stability across sessions with low variability in total licks was observed during the study. Illness was another factor that may account for the lowest control points for three of the subjects seen in Figure 5. Under advisement of our facility veterinarian each rat was temporarily removed from the study due to being ill in close temporal proximity to sessions for the lowest control points.

In addition, modifications related to the ethanol administration procedures could lead to more consistent behavior. Livy, Parnell, and West (2003) used a 2-hour food deprivation
procedure prior to gavage procedures in order to hold additional experimental parameters constant that could affect blood ethanol concentrations. Access to food prior to ethanol administration would not have an effect on variability in control days when only water was administered via gavage, but could have contributed to variability on dose days. There are also alternatives to using water deprivation and gavage procedures altogether, such as using food deprivation procedures with sucrose as the licking solution or using intraperitoneal injection as the route of administration.

Another procedure that could be changed is the timing for ethanol pretreat and duration of time in the chamber. This study used a 30-minute pretreat followed by a 15-minute session. Livy et al. (2003) tested blood ethanol concentration (BEC) in 40-day old Sprague-Dawley rats through tail blood samples collected at different intervals over 450 minutes post ethanol delivery. The authors found that when a single dose of 3.8 g/kg of a 21% ethanol solution was administered via gavage, rats showed a rapid rise in BEC over 30 minutes to a relatively low concentration with a subsequent gradual rise to peak levels at 60 minutes, and then a gradual decline to close to zero over the next 390 minutes. Crabbe et al. (2010) reported that with humans there is a stimulatory phase on the ascending limb and unpleasant feelings are experienced during the descending limb. L1 and L3 demonstrated an increase in the shortest ILIs at higher doses, which could be interpreted as a stimulatory effect, and this effect was not seen at all for L4 and to a lesser degree for L2. The apparent stimulation for L1 and L3 could be due to individual differences in metabolism. Future studies could conduct full time-course assessments of licking and test subjects’ BEC. Crabbe et al. (2010) add that evidence links low LR with aversive aspects of alcohol and subsequent development of AD; individuals
less sensitive to aversive aspects of alcohol continue to drink. It could be worthwhile to reduce session duration and place the subjects back in the chamber at a later time for a second session.

One other important aspect of future studies will be to assess the relationship between alcohol’s effects on oromotor function and alcohol consumption. Should oromotor function show to be reliably affected by alcohol, rats could be evaluated for alcohol preference and that preference correlated with determinations of oromotor impairment. A commonly studied trait for genetic relationship with low level of response is the two-bottle preference test (Crabbe et al., 2010). During the two-bottle preference test the rat is given concurrent free access to water and ethanol solution (usually 10%), the amount of solution drunk from each bottle is measured daily and used to evaluate preference.

In the end, this study was a successful initial investigation into the effects of alcohol on rodent licking. Although the data were not as clean as one might have hoped, the data show evidence that licking is sensitive to ethanol. Additionally, this study identified that dimensions of the licking response do not covary; total licks and rhythm of licking call for further investigation, whereas peak force and time integral of force did not show sensitivity to ethanol. This study also identified that doses of 1, 2, and 3 g/kg capture changes in total licks and interlick intervals without eliminating responding all together. Overall, this study assisted in identifying methodological strengths and weaknesses that will inform future studies that aim to further evaluate oromotor behavior and its potential role in alcohol research.
Figure 1. Apparatus. Figure 1 displays apparatus components: (a) chamber (b) water jar (c) peristaltic pump (d) clamp holding transducer in position (e) drinking platform with hole (f) lick disk (g) tubing for water delivery (h) lick disk in position under hole in platform.
Figure 2. Sample force-time recording and dimensions of licking. Figure 2 illustrates a 1-second sample of licking from L1 during baseline. Seven licks occurred during the time sample and labels are included for the dimensions of peak force (g), time integral of force (g-ms), and interlick intervals. Regularity can be seen in licking rhythm by the consistent spacing between licks (ILIs).
Figure 3. Dose-response functions for average peak force. Figure 3 shows the average peak force across doses in filled circles. Data points for average peak force across individual functions and replicates are displayed in the background to illustrate the range of average peak force at each dose. The y-axis is the average peak force in grams and the x-axis is the dose of ethanol (control, 1, 2, and 3) in g/kg.
Figure 4. Dose-response functions for average time integral of force. Figure 4 shows the average time integral of force across doses in filled circles. Data points for time integral of force across individual functions and replicates are displayed in the background to illustrate the range of average time integral of force at each dose. The y-axis is the time integral of force in grams-second and the x-axis is the dose of ethanol (control, 1, 2, and 3) in g/kg.
Figure 5. Dose-response functions for average total number of licks per 15-minute session.

Figure 5 shows the average total licks per session across doses in filled circles. Data points for total licks per session across individual functions and replicates are displayed in the background to illustrate the range of total licks at each dose. The y-axis is the average total licks per session and the x-axis is the dose of ethanol in g/kg (control, 1, 2, and 3).
Figure 6. Dose-response functions for total number of licks per 15-minute session. Figure 6 shows the total licks per session across doses. A distinct marker shape and line connects data points within each function or replicate. The y-axis is the total licks per session and the x-axis is the dose of ethanol (control, 1, 2, and 3) in g/kg.
Figure 7. Cumulative frequency histograms for average interlick intervals (ILIs). Figure 7 shows the average ILI distribution across doses, each dose (control, 1, 2, and 3 g/kg) has a distinct line. The y-axis is the average cumulative percentage and the x-axis shows ILIs bins separated by 10 milliseconds intervals. The first bin includes all ILIs under 110 milliseconds and the last bin includes all ILIs greater than 200 milliseconds.
Figure 8. Frequency histograms for average interlick intervals (ILI). Figure 8 shows the average ILI distribution across doses, each dose (control, 1, 2, and 3 g/kg) has distinct shading. The y-axis is the average percentage and the x-axis shows ILIs bins separated by 10-millisecond intervals. The first bin includes all ILIs under 110 milliseconds and the last bin includes all ILIs greater than 200 milliseconds. Within-bout ILIs are reflected by bins less than 200 ms and between-bout intervals at greater than 200 ms.
REFERENCES


