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Microstructural analysis of rat ethanol and water drinking patterns using a modified operant self-administration model

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HIGHLIGHTS

- We evaluate drinking microstructure within a model of operant self-administration.
- Initial patterns of EtOH vs. water intake differ in operant self-administration.
- CIE vapor alters total consummatory/appetitive behavior and drinking microstructure.
- CB1 antagonist SR141716a reverses many of these CIE effects.

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ABSTRACT

Background: Ethanol drinking pattern has emerged as an important factor in the development, maintenance, and health consequences of alcohol use disorders in humans. The goal of these studies was to further our understanding of this important factor through refinement of an operant rodent model of ethanol consumption capable of drinking pattern microstructural analysis. We evaluated measures of total consumption, appetitive behavior, and drinking microstructure for ethanol and water at baseline and assessed alterations induced by two treatments previously shown to significantly alter gross ethanol appetitive and consummatory behaviors in opposing directions.

Methods: Male Long–Evans rats were trained on an FR1 operant paradigm which allowed for continuous liquid access until an 8 second pause in consumption resulted in termination of liquid access. Total appetitive and consummatory behaviors were assessed in addition to microstructural drinking pattern for both ethanol and water during a five day baseline drinking period, after chronic intermittent ethanol vapor exposure, and following administration of a cannabinoid receptor antagonist SR141716a.

Results: As in previous operant studies, ethanol vapor exposure resulted in increases in ethanol-directed responding, total consumption, and rate of intake. Further, striking differential alterations to ethanol and water bout size, duration, and lick pattern occurred consistent with alterations in hedonic evaluation. Vapor additionally specifically reduced the number of ethanol-directed lever presses which did not result in subsequent consumption. SR141716a administration reversed many of these effects.

Conclusions: The addition of microstructural analysis to operant self-administration by rodents provides a powerful and translational tool for the detection of specific alterations in ethanol drinking pattern which may enable insights into neural mechanisms underlying specific components of drug consumption.

1. Introduction

Operant self-administration has served for decades as a valuable tool in evaluating mechanisms underlying the drive to seek out and consume ethanol. Many operant studies examine components of ethanol directed behaviors through a focus on the total amount of ethanol consumed [1,2] and appetitive responses performed, such as lever presses or nose-pokes [3–5]. Though such measures remain critical components in alcohol research, the specific patterns of ethanol intake have also emerged as important factors. For example, individual drinking patterns are potent indicators for the development and maintenance of alcohol abuse-like behaviors in both humans [6–8] and non-human primate models [9]. In the most general terms, patterns of consumption across the short term, such as individual days or hours, consist of frequency of drinking events ("wanting" of ethanol) and amount consumed per each event ("liking" of ethanol) [10]. These patterns can be

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evaluated over weeks to months or years as in human studies; but, potential pharmacotherapies typically act to reduce long-term ethanol intake through alteration of one or both of these short-term components. The short-term consumption patterns during these individual drinking sessions thus underlie the long-term intake patterns which emerge during periods of abuse and relapse. That consumption pattern within individual drinking sessions can impact therapeutic efficacy of treatments for alcohol use disorders in humans [11] adds further importance to understanding the neural mechanisms which underlie this pattern. Thus, evaluation of short-term ethanol drinking patterns within rodent self-administration has reemerged as an area of active interest in basic research [12–21].

In rodents, total liquid intake is broken into temporally distinct clusters of licks, termed licking 'bouts.' Individual rats may consume identical volumes of liquid but differ significantly with respect to moment-by-moment patterns (microstructure) that are related to the "wanting" (bout number and time between individual bouts (interbout-interval)) and "liking" (bout size, bout duration, intrabout licking rate) of the substance [22]. This drinking microstructure can reflect the relative hedonic value of the liquid, its caloric value, prior experiences (either positive or negative) with the substance, post-ingestive feedback from the gut, and the physiological state of the animal [22–28]. For highly palatable, hedonically valuable solutions like sucrose, a monotonic relationship exists between concentration and bout size [29], which contrasts with the typical inverted U-shaped relationship found between increasing concentrations and total intake volume [30]. An inverse relationship in bout size occurs in consumption of aversive substances like quinine (bitter taste) [27]. Additionally, even the rate and pattern of individual licks within a bout of consumption reflect this complex hedonic evaluation or relative "liking" of the substance. For example, the induction of conditioned taste aversion results in increasing amounts of a slower, 'hesitant' licking behavior characterized by long pauses between licks [26]. Drinking microstructure has most frequently been assessed during limited access (10–120 min), but importantly these relationships have been demonstrated over periods of up to 23 h access [31]. Thus, the analysis of drinking microstructure provides unique insights into specific components of consumption related to both "wanting" and the shifting hedonic values or "liking" for a substance. Further, as sucrose-directed licking microstructure has been shown to undergo distinct alterations following administration of a D2 or D1 antagonist [32], analysis of this pattern may also eventually contribute to understanding the relative contribution of distinct neurobiological mechanisms to specific components of ethanol intake.

Previous work has demonstrated treatment specific effects on ethanol bout frequency and structure using non-operant models of self-administration and operant models that procedurally separate appetitive (lever press) and consummatory behaviors [17–19,21,33–40]. However, concurrent evaluation of ethanol and water drinking microstructure at the millisecond level paired with an ongoing seeking component has not been performed. The concurrent analysis of total intake, appetitive behavior, and consummatory microstructure is of value in the full examination of neural mechanisms regulating interactions between ethanol seeking, evaluation, and consumption. A fixed ratio operant self-administration session is composed of a series of distinct periods of consumption, each unambiguously initiated by an appetitive response. As these consummatory periods are typically restricted by experimental design to a limited access period/amount of liquid following a response, the precise pattern of consumption as it relates to each seeking action cannot be evaluated within a typical operant session. We sought to address this limitation through modification of a classic FR1 operant procedure to allow for uninterrupted liquid access until a pause in licking behavior (selected based upon previous microstructural work within rats [25]) terminated the consummatory bout. The intake pattern was assessed for both ethanol and water during initial introduction to ethanol self-administration and following pharmacological manipulations previously demonstrated to significantly alter total ethanol consumption in opposing directions. As ethanol and water drinking patterns have been previously reported as most obviously distinct in the first 4–6 h of self-administration [12], we further sought to evaluate if the pattern of appetitive and consummatory behaviors changed over time through use of a prolonged 6 h session. Our microstructural analysis of ethanol drinking reveals unique outcomes associated with both chronic ethanol exposure and endocannabinoid receptor modulation.

2. Methods

2.1. Animals

Adult male Long–Evans rats weighing 200 g at arrival (7–8 weeks old) were purchased from a commercial supplier (Harlan Laboratories, Indianapolis, IN). A total of 16 animals were used. Animals were housed in groups of 4 on a reverse light/dark cycle (lights off at 9 am) under standard conditions. Animals were habituated to housing environment for one week before initiation of behavioral testing and were handled and weighed daily throughout the study. Rodent chow and tap water remained available ad libitum in the animal home cage for the duration of the study except when in the operant chamber. 10–12 g of chow was available within the chamber during operant sessions. All procedures were approved by the Wake Forest Institutional Animal Care and Use Committee and were consistent with the NIH Guide for the Care and Use of Animals. No rats were excluded from study.

2.2. Operant self-administration

Operant self-administration occurred as daily six-hour long sessions, seven days per week, initiating between 09 and 11 am. The operant self-administration chambers (Med Associates, St. Albans, VT) were housed within sound-attenuating chambers (Med Associates, St. Albans, VT) as previously described [41]. Each chamber contained a house light which remained on for the duration of the session. Operant chambers contained two levers located on opposing side walls; each lever was associated with one sipper bottle, the access point for which was located 2.25 in. away from the lever on the same side of the chamber. Successful completion of a lever press resulted in retraction of both levers and the associated sipper tube lowering into the chamber. The opposing sipper tube was therefore inaccessible until bout termination. Following an 8 second pause in sipper tube contacts the sipper tube was then retracted and both levers reinserted into the chamber. Commercially available software (MedPC, Med Associates) recorded data and controlled levers and sipper tube access.

Following the completion of an operant session, each chamber interior was cleaned with 70% ethanol, while waste trays, food bowls, and sipper bottles were cleaned with dish soap and hot water. To assess fluid intake, bottles were weighed before and following each operant session and g/kg ethanol consumption calculated based on each animal's weight prior to the session. As sessions were run daily, no tail bloods were collected for blood ethanol concentration determination in order to avoid disruption of subsequent animal behavior.

2.2.1. Training

Animals were initially trained on an FR1 schedule for 16 second access to 10% sucrose in water. Following a two day learning period with this solution, a modified sucrose substitution procedure [42] was utilized over a 2 week period. Briefly, ethanol was gradually increased from 0% to 10% while sucrose was decreased until animals reliably pressed for access to a 10% ethanol solution in water. Early within the second week, the protocol was altered from a 16 second access to the 8 second bout-termination criterion discussed below. Additionally, one sucrose/ethanol bottle was replaced with a bottle containing solely tap water at this time. Following introduction of the water tube, 10% ethanol and water sipper tubes alternated sides daily to prevent side bias from influencing results.