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Ethanol Self-Administration in Mice under a Second-Order Schedule

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Abstract

Long Fixed-Interval (FI) schedules, particularly second-order schedules, can engender substantial responding before drug or ethanol delivery that is uninfluenced by the direct effects of the drug or ethanol. Thus, these schedules can be used to study the effects of medications upon drug- or ethanol-seeking, uninfluenced by the direct effects of the self-administered drug or ethanol. Long FI second-order schedules are frequently used in primates and occasionally in rats. Under second-order schedules, completion of one response requirement, e.g., a Fixed Ratio 10 (FR10:S), produces a brief stimulus presentation, e.g., a 1-sec 80-dB 4-kHz tone, and this FR10:S serves as the response unit under another schedule, e.g., a FI 1800-sec. Thus, the first FR10 completed after 1800 sec would result in delivery both of the tone and of reinforcement, e.g., 10×0.01 mL 16% (w/v) ethanol. To examine if such schedules could be effectively used in mice, which have advantages in neurobiological and genetic studies, we trained eight C57BL/6J mice to respond under the schedule just described. This schedule maintained substantial responding. The temporal pattern of behavior was typical of an FI schedule with responding accelerating across the interval. We also examined the effects of acute and chronic administration of fluvoxamine on this responding, and these were modest. Finally, we examined responding when alcohol and/or tone deliveries were withheld, and found that extinction occurred most rapidly when both were withheld. This work demonstrates that long FI schedules of ethanol delivery may be useful in studying ethanol seeking in mice.

Keywords

conditioned reinforcement; selective serotonin reuptake inhibitor; relapse; reinstatement; alcoholism; alcohol

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Introduction

In order to study the neurobiology of addiction and problem drinking, self-administration procedures in which responding changes rapidly as conditions change have been developed. For instance, procedures that allow the assessment of a dose-response curve within a single session (e.g., Zernig, Lewis, & Woods, 1997) or the maximum amount of work that a given dose of drug might maintain within a single session have been developed (e.g., Gilpin & Koob, 2010; Roberts, Loh, & Vickers, 1989). These procedures are quite useful, and many have been extended to the mouse (e.g., Elmer, Pieper, Hamilton, & Wise, 2010), allowing the use of sophisticated neurobiological and genetic tools that are most readily available in the mouse.

As useful as such procedures have been, they have important limitations. These rapid procedures almost always involve repeated drug or alcohol delivery within an experimental session, with responding for the drug or alcohol occurring between deliveries of drug or alcohol. Thus, incidental drug accumulation may impinge upon discriminative and reinforcement processes across the session. Additionally, the self-administered drug may itself elicit behavior that directly affects responding to obtain further drug. Both possibilities complicate the interpretation of the effects of pharmacological, biological, or environmental manipulations on responding that lead to drug or alcohol delivery (see Herling & Woods, 1980). In particular, as Ettenberg (2004) has noted, repeated drug administrations during a session may interfere with identification of processes important to the onset and initiation of drug taking.

Responding that precedes drug or alcohol delivery is of particular interest because behavior that leads to the acquisition of (or opportunity to acquire) drug or alcohol necessarily precedes consumption, and so, this initial seeking behavior represents the starting point in a cascade of events terminating with drug consumption. Thus, drinking or drug taking can be thought of as a chain of behaviors that consists of at least two elements, seeking and consumption. For instance, going to the bar or liquor store precedes drinking. The alcohol-seeking behavior that makes up this acquisition phase may itself consist of a number of sub-chains of behaviors, and earlier elements in the chain of behaviors leading to drinking are generally thought to be more effective places to intervene to reduce drinking, and it is the persistence of these earlier elements of the chain that put the problem drinker at continued risk for problem drinking.

Samson, among others, recognized the importance of distinguishing between behaviors that result in access to the opportunity to drink or take drugs (seeking) and the behaviors that result in drug or alcohol consumption (consummatory; see Samson & Czachowski, 2002). Samson, Denning, and Czachowski (2003), for example, required rats to complete 5 to 10 responses to gain access to a sipper tube filled with 10% (v/v) alcohol. The advantage of the procedure is that seeking behavior is uncontaminated by direct effects of the self-administered drug because only one consumption period is allowed per session. A number of studies using this approach support the concept that seeking and consumption of alcohol are governed by distinct processes (e.g., Samson, Sharpe, & Denning, 1999), and highlight

the value of such procedures. At the same time, this procedure is limited in that the “seeking” component is very short, resulting in a limited sample of behavior.

Typically, these samples of seeking behavior are usually 30 or fewer than 30 responses. Larger samples of seeking behavior have been maintained in monkeys responding under second-order schedules, particularly schedules that involve long fixed-interval schedules of reinforcement as their primary schedule (e.g., Goldberg, Morse, & Goldberg, 1976). Under such a procedure, every time the monkey completed a fixed number of responses, e.g., 10, a brief stimulus was presented, usually a light (FR10:S), and the first FR10:S completed following 60 minutes resulted in drug delivery (FI 60 m (FR10:S)). Such schedules can maintain robust responding that is persistent and can increase or decrease in response to various manipulations. Additionally, the brief stimulus presentations may play a role in the maintenance of behavior akin to the role theorized to be played by alcohol- or drug-paired stimuli in problematic drinking or addiction (see Markou et al., 1993). For these reasons, we examined if such a procedure could be developed in the mouse because of the valuable neurobiological and genetic tools that are more easily available in the mouse.

We trained C57BL/6J mice to respond for ethanol under an FI 1800-sec (FR10:S) schedule. This schedule is similar to ones that maintain robust and persistent behavior in primates. Further, C57BL/6J mice readily and avidly learn to drink and work for ethanol solutions (Elmer, Meisch, & George, 1986). Once reliable responding was obtained, we examined the effects of acute and chronic fluvoxamine administration on this responding. Fluvoxamine is a specific serotonin reuptake inhibitor (Wong, Bymaster, Reid, & Threlkeld, 1983). We have examined the acute effects of this drug upon responding for ethanol under a variety of schedules in the rat (e.g., Ginsburg, Koek, Javors, & Lamb, 2005; Ginsburg & Lamb, 2006a; Ginsburg & Lamb, 2014; Lamb & Järbe, 2001) and progressive-ratio responding in mice (Lamb & Daws, 2013), and we and others have studied the chronic effects of fluvoxamine or other specific serotonin reuptake inhibitors on responding for ethanol in rats and mice (Ginsburg & Lamb, 2006b; Gulley, McNamara, Barbera, Ritz, & George, 1995). Thus, we had a substantial body of work to which we could compare any effects we might see in the mouse. Finally, we examined the effects of removing contingent ethanol and/or brief stimulus deliveries upon responding maintained by this schedule. This allowed us to examine the persistence of this behavior and the role of the brief stimulus.

Materials and methods

Mice

Eight male C57BL/6J mice purchased from Jackson Laboratories were used in these experiments. Housing was in a temperature- and humidity-controlled vivarium, under a 12-h light/dark cycle (lights on at 6:00 AM). Water was freely available except during experimental sessions. Food was limited to 2.5 g rodent chow provided each day following experimental sessions. In general, food restriction increases longevity, keeps mice at similar weights, and makes development of ethanol self-administration easier. All procedures conducted on the mice were approved by the local institutional animal care and use committee and were in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Apparatus

Experiments were conducted in 8 mouse operant chambers (Med Associates, St. Albans, VT). Located on either side of the front wall of each chamber were two head-entry detectors with associated dipper mechanisms and yellow LED lights located at the rear of the head-entry detector. The head-entry detector on the left was used in these experiments. The dippers used could hold a volume of 0.01 mL. In addition, chambers had a house light and speaker. Operant chambers were housed inside large fan-equipped sound-attenuating chambers. A white noise generator in the room provided additional masking noise.

Procedure

Mice were initially trained to break the photo beam inside the head-entry detector to earn a dipper presentation of a 20% (w/v) sucrose solution when the house light and the LED in the head-entry detector were lit. The 5-sec dipper presentation was accompanied by turning off the house light and the LED and presentation of an 80-dB, 4000-kHz tone. Initially, session length was 1 h. Over time, session length was reduced to 30 min, 10 beam breaks were required for presentation of the dipper, and the solution was changed from 20% sucrose to 16% (w/v) ethanol by gradually reducing the sucrose concentration and increasing the ethanol concentration (Samson, Tolliver, Pfeffer, Sadeghi, & Haraguchi, 1988). After responding was maintained under this FR10 schedule of ethanol presentations (which took a median of 35 sessions with a range of 27 to 37 sessions), the schedule was changed to an FI (FR10:S) schedule of ethanol presentation, in which each FR10 completion resulted in a 5-sec presentation of the 80-dB, 4-kHz tone, and the completion of an FR10 after the elapse of the FI resulted in a 5-sec tone and dipper presentations. Initially, the FI was 60 sec and completion of the schedule requirement resulted in a single dipper presentation with this schedule repeating itself until the 30-min session had expired. Over five sessions the FI length was increased to 1800 sec and completion of the schedule requirements resulted in ten 5-sec tone-dipper presentations each separated by 2-sec periods in which the dipper was lowered to be refilled.

Ethanol

Ethanol solutions were made by diluting 190 proof ethanol (Decon Labs, King of Prussia, PA) with tap water to make up a 16% (w/v) solution.

Fluvoxamine

Fluvoxamine was administered intraperitoneally 30 min before experimental sessions and was dissolved in saline to a concentration such that the volume administered was 1 mL/100 g body weight.

Design

Baseline data are reported from the week before the acute effects of fluvoxamine were determined, which followed about 9 months of training under the final schedule. The acute effects of each fluvoxamine dose and saline were determined once in each mouse on either a Tuesday or Friday with dose order counterbalanced across mice. Injections of saline were also given on Thursdays. After examining the acute effects of fluvoxamine, we examined

the effects of daily administrations of 10 mg/kg and then 30 mg/kg administered before experimental sessions (only injections were given on most weekends). Each dose was administered for 2 weeks. Following drug testing, the effects of canceling contingent brief stimulus presentations, removing the ethanol solution, or both were determined in counter-balanced order with each condition in effect for 2 weeks with a 2-week return to baseline between each manipulation. Following these manipulations, a prospectively determined 2-week period without any manipulation was assessed for comparison. Finally, the effects of extended removal of contingent brief stimulus deliveries and the ethanol solution were examined. Once day-to-day responding appeared to be at stable low levels, the effects of again contingently delivering the brief stimulus were examined over five sessions.

Analysis

Fluvoxamine—A single point for each subject for each dose and for the saline vehicle given on either Tuesday or Friday along with two Thursday control injections of saline were determined with the following exception: one mouse was mistakenly given 30 mg/kg fluvoxamine twice. This, also, resulted in the collection of three Thursday control injections of saline. Response rate is presented and analyzed as percent of the Thursday control rate of responding. Mean effects on response rate and quarter-life (the percent of the FI required to make one-quarter of the total responses) (Herrnstein & Morse, 1957) are presented in the figures. The data were analyzed using a repeated-measures ANOVA with dose and order as factors. Sphericity was accounted for by using the Box correction. As the interaction between dose and order was not significant in either ANOVA, this interaction was dropped from the analysis. In the subsequent analysis without the interaction, the order factor was not significant and so ANOVA results including only fluvoxamine dose as a factor are presented.

Mean response rate and quarter-life on the days preceding and following, as well as during, daily administration of 10 and then 30 mg/kg fluvoxamine are presented. Mean values during these periods were compared using ANOVA, and sphericity was accounted for by using the Box correction. As the effects of condition were significant, further comparisons were made using contrasts controlling for the false discovery rate using the method of Benjamani & Liu (1999).

Extinction—Mean rates of responding and quarter-life across days before and following, as well as during, removal of contingent brief stimulus presentations and ethanol deliveries as well as the control comparison are presented. These data were analyzed by repeated-measures ANOVAs comparing responding across conditions in the last 5 days of each manipulation. The model used included day of the week, but not its interactions, and presence or absence of the brief stimulus and ethanol delivery were treated as separate factors. When these analyses indicated significant effects, follow-up analyses consisting of repeated-measures ANOVAs comparing responding in the 5 days before the manipulation to the last 5 days of the manipulation, and pair-wise contrasts, were conducted. The False discovery rate for multiple comparisons for the pair-wise contrasts was controlled using the method of Benjamani and Liu (1999). Sphericity was accounted for using the Box correction. The appropriateness of the control comparison condition, which was always

tested last, was examined in two ways. First, whether order affected responding in the 5 days preceding all four manipulations was examined. Order affected neither rate [$F(3,145) = 0.45$, $p = 0.50$ Box correction] nor quarter-life [$F(3,145) = 2.54$, $p = 0.12$ Box correction]. Second, we examined if rate of responding or quarter-life in the 5 days before the control manipulation or the last 5 days of the control manipulation differed. Neither response rate [$F(1,67) = 2.77$, $p = 0.10$ Box correction] nor quarter-life [$F(1,67) = 3.93$, $p = 0.051$ Box correction] differed. Thus, our primary analysis compared responding during the last 5 days of each manipulation, but included a confirmatory analysis comparing responding in the 5 days preceding each manipulation to the last 5 days of that manipulation.

Data from the extended extinction conditions are presented similarly. These data were analyzed in the following manner. First, responding in the week before extinction was compared to responding in the week before the return of the brief stimulus. There was no difference in response rate between these two periods [$F(1,52) = 3.58$, $p = 0.06$ Box correction], which was the result of one mouse responding at a higher rate during the extinction period. Removal of this mouse resulted in those periods being significantly different [$F(1,45) = 15.3$, $p = 0.0003$ Box correction]. Because of this, we compared responding in the week before, week of, and week following reinstatement of the brief stimulus with and without this mouse. As these results were essentially identical, we report only the results including this mouse. To make this comparison, we conducted a repeated-measures ANOVA on the weekly mean rate of responding. One caveat to note is that one mouse did not respond on 3 of the 4 days that the brief stimulus was programmed to be presented. However, excluding this mouse only resulted in a trend level difference in response rate with rate of responding going in the opposite direction of what would be predicted.

Results

Training and baseline responding

After transitioning to the final schedule, mice were trained for approximately 9 months. Response rate increased over the first 2–3 months of training and quarter-life increased over the first 4–6 months (data not shown). In the last 3 months of training, responding was relatively stable with little change in the rate or pattern of responding.

As expected from studies with ethanol and other reinforcers in various species, high levels of responding were garnered by a second-order schedule in non-intoxicated mice. During the week before testing, as can be seen in Fig. 1A, most mice responded at between 0.100 and 0.150 responses/sec on average. Further, as can be seen in Fig. 1B, most of this responding was concentrated in the latter half of the interval. This is illustrated in the average cumulative response curves for each individual mouse in Fig. 1C. As can be seen, most mice averaged more than 200 responses during the interval and most of these responses occurred in the last half of the interval, a pattern of responding typical of FI schedules (Ferster & Skinner, 1957).

As is also apparent, there were substantial differences in overall response rate among mice. We examined how this might relate to their earlier responding under an FR10 schedule.

There was no relationship between the two measures. The median number of dipper presentations under the FR10 ranged from 12 to 20.5 for individual mice. The mouse with the highest number of dipper presentations under the FR10 had the fourth-highest response rate under the second-order schedule, while the mouse with the lowest number of dipper presentations ranked fifth. Conversely, the mouse with the highest response rate under the second-order schedule ranked seventh in the number of dipper presentations earned under the FR10 schedule, while the mouse with the lowest response rate under the second-order schedule ranked fifth in the number of dipper presentations earned under the FR10. The extensive training under the second-order schedule may in part account for this, as mice had 174 training sessions between the end of FR10 training and second-order data to which it is compared.

Acute effects of fluvoxamine

As can be seen in Fig. 2, acute administration of fluvoxamine did not have robust, reliable effects on responding for ethanol. This is confirmed by a repeated-measures ANOVA in which the effects of fluvoxamine dose as a factor are non-significant [$F(4,28) = 1.27$, $p = 0.30$; Fig. 2A]. Acute administration of fluvoxamine also did not have reliable effects on the FI quarter-life [$F(4,28) = 1.35$, $p = 0.28$; Fig. 2B).

Chronic effects of fluvoxamine

Chronic fluvoxamine did not generally have robust, reliable, interpretable effects upon responding. These are shown in Fig. 3 (rate is shown in Panel A and quarter-life is shown in Panel B). The changes in rate and pattern across time were significant [rate: $F(3,21) = 14.54$, $p = 0.007$]. The first thing to note is that the rate and pattern of responding changed from the period preceding chronic fluvoxamine administration to the period following chronic fluvoxamine administration. In particular, the mean response rate increased from 0.099 (SD = 0.027) to 0.133 (0.035) responses/sec from before to after chronic fluvoxamine administration ($p = 0.001$), and the mean quarter-life went from 44.1% (4.4) to 58.4% (4.5) of the interval [$F(3,21) = 22.64$, $p = 0.002$; contrast $p < 0.001$]. While these changes may reflect fluvoxamine-induced changes, these changes may also simply reflect time-related increases in responding and the efficiency of responding, and this needs to be taken into account in the interpretation of any changes in responding seen during chronic fluvoxamine administration.

Responding during chronic administration of 10 mg/kg/day fluvoxamine increased relative to the period before chronic administration (0.126 [0.036] vs. 0.099 responses/sec; $p = 0.004$), but was unchanged compared to responding following chronic fluvoxamine administration ($p = 0.456$). Response rate during chronic administration of 30 mg/kg/day fluvoxamine was unchanged relative to the period before chronic administration (0.085 [0.020] vs. 0.099 responses/second; $p = 0.11$), and was decreased compared to responding that followed chronic fluvoxamine administration (0.133 responses/second; $p < 0.001$).

The effects on quarter-life were somewhat more consistent. During chronic administration of 10 mg/kg/day fluvoxamine, quarter-life was decreased both relative to the period before chronic administration (37.3% [4.4] vs. 44.1% [10.2]) of the interval; $p = 0.021$), and

following chronic administration ($p < 0.001$). However, quarter-life was increased during chronic administration of 30 mg/kg/day fluvoxamine relative to the period preceding chronic administration (58.4% [4.5] vs. 44.1% of the interval; $p < 0.008$), but decreased relative to the period following chronic administration (58.4% of the interval; $p = 0.033$). These effects of chronic fluvoxamine are difficult to interpret due to the changing baseline, but chronic administration of 10 mg/kg fluvoxamine did seem to produce a consistent decrease in quarter-life.

Effects of stopping brief stimulus presentations and/or ethanol deliveries

Ending brief stimulus presentations, alcohol deliveries, or both had only small effects on behavior once it was established. These effects were most consistent when both were removed. Fig. 4 shows response rate preceding, during, and following (removal of (A) both ethanol deliveries and brief stimulus presentations, (B) just ethanol deliveries, (C) just the brief stimulus presentations or (D) neither for 2 weeks (10 sessions). Fig. 5 similarly presents the data for quarter-life. These effects were modest, but for removal of both brief stimulus presentations and ethanol deliveries, these effects were consistent: response-rate was decreased and quarter-life was increased. There was some indication that response rate was also decreased following the removal of either brief stimulus presentations or ethanol deliveries alone. However, quarter-life was not affected by removal of only the brief stimulus presentations or ethanol deliveries.

These conclusions are based upon the following results. For response rate, the interaction between the factors for brief stimulus presentations and ethanol deliveries was significant [$F(1,145) = 5.49$, $p = 0.021$ Box correction]. This resulted from response rate being higher when both the brief stimulus was presented and ethanol was delivered for 2 weeks (Fig. 4D) than following removal of the brief stimulus presentations alone (marginal means of 0.127 vs. 0.088 responses/sec for the contrast, $p = 0.007$; Fig. 4C), or removal of ethanol deliveries alone (0.103 responses/sec, $p < 0.001$; Fig. 4B) or removal of both (0.092 responses/sec, $p = 0.001$; Fig. 4A). The rate of responding following removal of the brief stimulus presentations alone, ethanol deliveries alone, or both did not differ from each other ($p > 0.08$). Only the conclusion that removal of both the brief stimulus and ethanol presentations led to a reduction in response rate was supported by follow-up ANOVAs comparing response rate in the week before the manipulation to the rate in the last week of the manipulation [$F(1,67) = 7.18$, $p = 0.0093$ Box correction, marginal means of 0.112 vs. 0.092 responses/sec; removal of ethanol alone: $F(1,67) = 1.84$, $p = 0.18$ Box correction, 0.098 vs. 0.088 responses/sec; removal of brief stimulus alone: $F(1,67) = 3.77$, $p = 0.056$ Box correction, 0.117 vs. 0.103 responses/sec].

For quarter-life, the interaction between brief stimulus and ethanol presentations was significant [$F(1,145) = 11.66$, $p = 0.0008$ Box correction]. This was the result of the quarter-life following the manipulation involving removal of both the brief stimulus presentations and ethanol delivery (marginal mean of 65.2% of FI) being greater than the quarter-life for the control condition (57.3%; contrast $p = 0.002$; compare Fig. 5A & D) and either removal of the brief stimulus presentations alone (55.2%; $p < 0.001$; Fig. 5C) or removal of ethanol deliveries alone (55.4%; $p < 0.001$; Fig. 5B). Quarter-life when the brief stimulus was

presented and ethanol delivered, or following removal of brief stimulus presentations alone or removal of ethanol delivery alone did not differ ($p > 0.41$). The conclusion that quarter-life was increased when both the brief stimulus presentations and the ethanol presentations were removed was confirmed by an ANOVA comparing responding in the week before this manipulation to responding in the last week of this manipulation [$F(1,67) = 12.55, p = 0.007$ Box correction; marginal means of 60.3 vs. 65.2%], as was the conclusion that removal of the brief stimulus presentations [$F(1,67) = 3.60, p = 0.062$ Box correction; 59.3 vs. 55.3%] or ethanol deliveries [$F(1,67) = 2.24, p = 0.14$ Box correction; 59.3 vs. 55.4%] alone did not change quarter-life.

Reinstatement of brief stimulus presentations following extended removal of both brief stimulus presentations and ethanol deliveries had no effect on behavior. As can be seen in Fig. 6, responding declined when the brief stimulus was not presented and ethanol was not delivered, but again delivering the brief stimulus did not increase response rate compared to responding before or after this manipulation (marginal means of 0.103 vs. 0.074 vs. 0.69 responses/sec; $F(2,14) = 2.54, p = 0.16$ Box correction – please see methods for a further discussion of these analyses).

In summary, responding declined slowly in extinction, and termination of both ethanol and brief stimulus deliveries appeared necessary for this to happen in the period of time examined. Reinstatement of contingent brief stimulus presentations after responding was at lower levels had minimal effects on the overall rate of responding.

Discussion

In most mice, 200 or more responses were maintained under the second-order FI 1800-sec (FR10:S) schedule ethanol delivery. This behavior was appropriate to the FI schedule contingencies with about three-quarters of the total responding occurring in the last half of the interval (Ferster & Skinner, 1957). This pattern of responding is similar to that seen in other species and indicates that second-order schedules of drug self-administration in mice may be useful assays for studying the effects of medications on drug-seeking behavior and for studying behaviors related to drug-seeking behavior (see Schindler, Panlilio, & Goldberg, 2002). The behavior was slow to extinguish and required eliminating contingent delivery of both the brief stimulus and ethanol presentations. This indicated some level of control by brief stimulus presentations. However, reinstituting contingent brief-stimulus presentations did not rapidly reinstate responding, indicating that this control was limited.

Long Fixed-Interval schedules of drug self-administration can result in substantial behavior before drug delivery (e.g., Gomez & Meisch, 2004; Kaminski, Goodwin, Wand, & Weerts, 2008), though not necessarily more overall behavior than that obtained under simple FR schedules (compare responding of these mice initially under an FR schedule reported in the text to responding under second-order schedule in Fig. 1C). This behavior provides a useful baseline for studying medication effects on drug seeking and is of interest in its own right. In the typical drug self-administration procedure, other than the first few responses, responding is influenced by the direct effects of the self-administered drug in addition to the pre-existing propensity of the animal to respond. Samson and Czachowski (2002) have

distinguished between this responding that precedes drug consumption (seeking) and responding that is linked to consumption (consummatory). In their procedure, however, the rat was only required to make 10 responses before gaining access to an ethanol solution, and the effects of medications on this responding were examined when the responding was in extinction (Samson et al., 2003), to gain a larger sample of behavior and one that had more range to vary. Long Fixed-Interval schedules arrange a larger sample of behavior on which the effects of medications can be assessed, and have been successfully used in rhesus monkeys, rats, and baboons (for review see Schindler et al., 2002).

The seeking behavior that precedes drug consumption is of particular interest as drug abuse often consists of an acquisition and a consumption phase. This seeking phase is a desirable place to intervene to reduce drug use, as the initial links of a chain are generally thought to be more weakly maintained (see Kelleher & Gollub, 1962), and thus, more easily disrupted. This seeking behavior also provides a measure of the motivation to take the drug independent of the direct effects of drug delivery. This differs from most currently used procedures in which contingent drug deliveries and the measure of motivation are intermingled such that responding is influenced both by the upcoming contingent drug delivery and the direct effects of the past drug deliveries. Procedures such as the one used here could be used to study the effects of other manipulations on drug-seeking behavior (see Schindler et al., 2002 for further discussion of these issues).

In the experiments reported here, we used such a baseline to study the effects of a medication, fluvoxamine, and the effects of removing and reinstating contingent brief stimulus presentations. We examined the effects of both acute and repeated administration of fluvoxamine on responding for ethanol. In both cases, the effects of fluvoxamine were inconsistent and minimal. Two explanations for this lack of effect come readily to mind. First, insufficient doses of fluvoxamine might have been administered to adequately affect the target system, serotonin reuptake. We do not believe this to be the case. A dose of 30 mg/kg of fluvoxamine likely blocks most serotonin transporters (Hirano et al., 2005) and reduces progressive ratio responding for ethanol in mice (Lamb & Daws, 2013). The second is that fluvoxamine has relatively little effect on ethanol seeking, a finding that would be consistent with its limited utility in treating problem drinking (e.g., Chick, Aschauer, Hornik, & Investigators' Group, 2004). This is interesting, as previous studies in rats in which responding for alcohol was intermingled with ethanol deliveries have shown reductions in responding for ethanol following fluvoxamine administration (Ginsburg et al., 2005; Lamb & Järbe, 2001). These reductions in ethanol-maintained responding are selective with ethanol-maintained responding being decreased at lower fluvoxamine doses than food-maintained responding. An interesting exception to this selectivity is concurrent schedules of ethanol- and food-maintained behavior. In this case, fluvoxamine decreases food-maintained behavior at lower doses or doses similar to those that are needed to decrease ethanol-maintained behavior (Ginsburg & Lamb, 2006a; Ginsburg & Lamb, 2014). It may be that both the responding that comes before ethanol delivery on a long-FI schedule and responding for ethanol on a concurrent schedule represent forms of ethanol-seeking behavior, and this ethanol-seeking behavior is relatively insensitive to the effects of fluvoxamine. Whether these differences reflect species differences or differences between consummatory and seeking behavior is an interesting area for future investigation.

benefited from additional controls. This may be especially true for the effects of chronic fluvoxamine in which the changing baseline from before to after treatment made these results difficult to interpret. A final limitation that relates to this is the small reduction in responding seen in the various extinction conditions. We are confident that the behavior under the second-order schedule was a consequence of this behavior having been reinforced given that the resulting pattern of behavior would be unlikely to occur otherwise. We view the resistance of this behavior to extinction as a strength of the procedure more than as a weakness, as it is the resistance of addictive behavior to change that is a hallmark of addiction.

In summary, long Fixed-Interval schedules with or without brief stimulus presentations provide a means of studying medication effects on drug seeking and drug consumption independently (e.g., Gomez & Meisch, 2004; Kaminski et al., 2008), and to study how environmental factors can increase or decrease these behaviors. The present work shows that such procedures can be instituted in mice, a species in which genetic factors can be more readily manipulated. This study also suggests that the comparison of second-order responding controlled by a localized light to responding controlled by a diffuse tone may provide a way of studying the hypothesized role(s) of elicited approach behavior or sign tracking in the development and maintenance of problematic substance use (Flagel et al., 2009; Tomie, 1996).

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Mice are a valuable tool for studying ethanol neurobiology.

Ethanol-seeking and consumption may be under separate neurobiological control.

Most procedures with mice do not separate seeking and consumption.

Ethanol self-administration in mice was maintained under a FI 1800 sec (FR10:S).

This schedule maintained many responses before ethanol delivery and provides a means of studying ethanol seeking in mice.

Fig. 1.

Fig. 1 illustrates the baseline rates and patterns responding under an FI 1800-sec (FR10 response:S) schedule of ethanol delivery. Panel A shows response rate for each of 5 days, as well as the mean value, and Panel B shows quarter-life for each of these 5 days, as well as the mean value. Panel C shows average cumulative responses across the Fixed Interval for each mouse for these five sessions (Y-axis cumulative responses; X-axis sequential time-bins).

Fig. 2.

Fig. 2 illustrates the effects of acute fluvoxamine administration (intraperitoneally 30 min before the session) on the rates and patterns of responding under a FI 1800-sec (FR10 response:S) schedule of ethanol delivery. The two panels show the effects of fluvoxamine (X-axis, log scale) on A) mean response rate (response/sec) and B) mean quarter-life (percent of interval in which the first 25% of the total responses occurred) for all eight mice. The error bars represent the standard error of the mean.

Fig. 3.

Fig. 3 illustrates the effects of chronic fluvoxamine on mean A) rate of responding and B) quarter-life (bottom panel) across days (open unconnected points) and the mean rate during each phase (filled points). The error bars represent the standard error of the mean.

Fig. 4.

Fig. 4 illustrates the effects of A) stopping both contingent brief stimulus and ethanol deliveries, B) only contingent brief stimulus deliveries, C) only ethanol deliveries or D) stopping neither on mean response rate across sessions (error bars represent standard errors of the mean). Lines break between Friday and Monday sessions. Filled points represent sessions before and after the manipulations when both ethanol and the brief stimulus were delivered contingently.

Fig. 5.

Fig. 5 illustrates the effects of A) stopping both contingent brief stimulus and ethanol deliveries, B) only contingent brief stimulus deliveries, C) only ethanol deliveries or D) stopping neither on mean quarter-life across sessions (error bars represent standard errors of the mean). Lines break between Friday and Monday sessions. Filled points represent sessions before and after the manipulations when both ethanol and the brief stimulus were delivered contingently.

Fig. 6.

Fig. 6 shows the effects of prolonged termination of contingent brief stimulus and ethanol deliveries and the reinstitution of brief stimulus deliveries on the mean response rate of mice under a FI 1800-sec (FR10:S) schedule of ethanol delivery. Open points represent daily means and filled points represent weekly means. Breaks in lines for the open points represent weekends. Bars mark changes in conditions. The error bars represent the standard error of the mean.