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Incubation of ethanol reinstatement depends on test conditions and how ethanol consumption is reduced

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Abstract

In reinstatement studies (a common preclinical procedure for studying relapse), incubation occurs (longer abstinence periods result in more responding). This finding is discordant with the clinical literature. Identifying determinants of incubation could aid in interpreting reinstatement and identifying processes involved in relapse. Reinstated responding was examined in rats trained to respond for ethanol and food under a multiple concurrent schedule (Component 1: ethanol FR5, food FR150; Component 2: ethanol FR5, food FR5—alternating across the 30-min session). Ethanol consumption was then reduced for 1 or 16 sessions either by suspending training (rats remained in home cage) or by providing alternative reinforcement (only Component 2 stimuli and contingencies were presented throughout the session). In the next session, stimuli associated with Component 1 were presented and responses recorded but ethanol and food were never delivered. Two test conditions were studied: fixed-ratio completion either produced ethanol- or food-associated stimuli (signaled) or had no programmed consequence (unsignaled). Incubation of ethanol responding was observed only after suspended training during signaled test sessions. Incubation of food responding was also observed after suspended training. These results are most consistent with incubation resulting from a degradation of feedback functions limiting extinction responding, rather than an increased motivation.

Keywords

Incubation; Relapse; Reinstatement; Alcoholism; Alcohol; Ethanol

1. Introduction

Recovery from addiction and problematic drinking is threatened by relapse. Pre-clinically, relapse is most often studied using the reinstatement procedure (De Wit and Stewart, 1981; Stretch and Gerber, 1973). In the reinstatement procedure, animals are trained to respond for drug delivery. Once responding is established, animals are placed in extinction or otherwise prevented from responding (e.g. by being removed from the environment where drug was available). Re-exposure to the drug, stimuli associated with drug-delivery, or stressors result in reinstatement of responding (Crombag et al., 2008; Marchant et al., 2013). The amount of

responding that occurs is thought to reflect the propensity to relapse, and model craving (Epstein et al., 2006; Marchant et al., 2013). Thus, treatments decreasing reinstated responding are thought to hold promise for preventing relapse (presumably by reducing the motivation to seek a drug) while manipulations increasing responding are thought to increase relapse risk.

Reinstatement has provided a means of studying processes involved in relapse, and for identifying potential treatments to prevent relapse (Marchant et al., 2013). However, reinstatement has been criticized for discrepancies with the clinical phenomenon of relapse (Katz and Higgins, 2003). In particular, longer periods of abstinence often result in increased reinstated responding. This incubation of reinstated responding is discordant with the clinical literature in which longer periods of abstinence generally reduce the risk for relapse (Gilpin et al., 1997; Gossop et al., 1990). Survival curves show a rapid return to substance use early in treatment, but a decreasing likelihood of relapse with increasing lengths of abstinence (e.g. Hunt et al., 1971). Thus, incubation poses a challenge to the interpretation that the amount of reinstated responding reflects the likelihood of relapse.

Incubation has been demonstrated for many drugs including cocaine, heroin, ethanol, and nicotine, as well as for sucrose (Epstein et al., 2006). Despite the broad generality of incubation across many drugs and situations, the behavioral mechanisms responsible for incubation remain poorly understood. Identifying the experimental parameters responsible for incubation could improve our interpretation of it and treatments that affect it.

Conditions present during reinstatement tests might influence the expression of incubation. While drug or food is never delivered during the reinstatement test, in some cases responses result in presentation of the stimuli paired with their delivery. In other cases, responses have no programmed consequence. Incubation of cocaine reinstatement has been observed after periods of suspended access in both these situations (Grimm et al., 2001; Lu et al., 2004). However, the generality of these observations remains unclear.

The way responding is reduced prior to reinstatement testing could also affect incubation. Incubation has been demonstrated after suspended training (Abdolahi et al., 2010; Bienkowski et al., 2004; Grimm et al., 2001; Li and Frantz, 2010; Lu et al., 2004; Zhou et al., 2009). In contrast, reducing responding with extinction in the experimental context attenuates incubation of cue-induced cocaine reinstatement (Di Ciano and Everitt, 2002; Kelamangalath and Wagner, 2009). Further, incubation of reinstatement for ethanol or saccharin seeking does not occur when responding is reduced by reinforcing a different response with food delivery (Ginsburg and Lamb, 2013a, 2013b).

Here, we examine whether the expression of incubation depends on the way in which ethanol self-administration is reduced (either by removing the rat from the operant environment or by providing alternative reinforcement within the operant environment). Further, we examine whether presentation of stimuli contingent on fixed-ratio (FR) completion (and previously associated with ethanol delivery) during extinction affects incubation. The results indicate that experimental conditions profoundly affect the expression and extent of incubation of cue-induced reinstatement. Specifically, incubation of

reinstated ethanol responding occurs when ethanol self-administration is reduced by suspending training, but not when it is reduced by providing alternative reinforcement, and then, only when FR completion was signaled during the test session.

2. Materials and methods

2.1. Materials

2.1.1. Subjects—Male Lewis rats ($n = 11$, Harlan, Inc Indianapolis, IN) served as subjects. Rats were fed 12–15 g of rat chow per day and had ad libitum access to water in their home cage. These rats had already engaged in studies characterizing the persistence of responding for ethanol during test sessions in which an ethanol-associated stimulus was presented after varying periods of reduced ethanol use due to alternative reinforcement (Ginsburg and Lamb, 2013a, 2013b, 2013c), and data from those studies are included here (alternative reinforcement for 1 or 16 sessions with presentation of stimuli upon FR completion (signaled) test sessions; Fig. 2 lower panel, open symbols; Fig. 1, lower right panel; Fig. 3, open symbols).

2.1.2. Apparatus—Experiments were conducted in commercially available operant test chambers (ENV-008, Med Associates, St. Albans, VT). On one wall, two levers were arranged horizontally, one on each side of the wall. Stimulus lights were located above each lever. Equidistant between the levers was a receptacle providing access to 45 mg pellets (Bio-Serve, Frenchtown, NJ) via a pellet dispenser and to a solution via a 0.1-ml retractable dipper. An additional stimulus light was located on the wall opposite the levers and receptacle, and a house light was located above the chamber. Pink noise was generated in the procedure room to mask ambient noise. Chambers also had a speaker connected to a tone generator (ANL- 926, Med Associates, St. Albans, VT) that produced tones, which served as stimuli for some rats. Stimuli presentation and reinforcement delivery as well as data collection were accomplished by software written using a commercially available programming language (Med-PC, Med Associates, St. Albans, VT).

2.1.3. Ethanol—Ethanol (95%) was obtained from Decon Labs, Inc. (King of Prussia, PA) and mixed with filtered tap water to obtain a 10% (w/v) solution. Solutions were made fresh daily, and allowed to reach room temperature before being provided to the rats.

2.2. Methods

2.2.1. Training—Training of these rats has been described elsewhere (Ginsburg and Lamb, 2013a, 2013b). Briefly, rats were first trained to respond for ethanol with a widely used sucrose fading technique (Samson, 1986) during a 30 min session each day. Depending on the subject (and detailed in Table 1), either a light (above the left lever) or tone (16 kHz) indicated that responses on the left lever would result in delivery of 0.1 mL of an 8% sucrose solution. Upon delivery, the stimulus (light or tone) was turned off, and replaced with another stimulus (a light on the opposite wall of the chamber or white noise). After 30-s, this light or tone was turned off, the dipper was returned to an inaccessible position, and the stimulus that signaled the lever was active was again presented. Over a few sessions, the response requirement was increased to 5 (FR5) and then ethanol was added to the solution

(10% w/v). Gradually, the sucrose was removed from the solution until rats were responding under an FR5 maintained by delivery of 0.1 mL 10% (w/v) ethanol.

Subsequently, rats were trained to respond for food in a separate 30-min session. In these sessions, a distinct stimulus (either a light above the right lever or 8 kHz tone; see Table 1) signaled that responses on the right lever would provide a 45 mg food pellet. Upon delivery, the stimulus (light or tone) was turned off, and a distinct paired stimulus was presented (house light above the chamber or 0.1 kHz tone). After 30-sec, the paired stimulus was turned off and the stimulus that signaled the lever was active was again presented. Over the next few sessions, the response requirement was increased to 5 (FR5), then rats were introduced to the multiple concurrent schedule.

2.2.2. Multiple concurrent schedule—Under the multiple concurrent schedule, responses on either lever were reinforced during each component, and components alternated between the two stimuli and associated contingencies. When the light above the left lever was illuminated or the 16 kHz tone was present, the ethanol contingency was FR5 and the food contingency was FR150. When the light above the right lever was illuminated or the 8 kHz tone was present, the ethanol contingency remained FR5, but the food contingency was also FR5. Completion of the FR was based on total responses (no reset) and no changeover delay was imposed; however the counter on both levers reset after completion of an FR on either lever. The order of components was randomized within each block of two components. Training under these conditions continued until each rat responded >80% on the ethanol lever when the food FR was 150 and >80% on the food lever when the food FR was 5. By the time rats were tested for the first time, they had been responding for ethanol alone in water for an average 193 days (range: 85–246).

2.2.3. Baseline responding for ethanol—Once rats were trained to respond under the multiple concurrent procedure, they were exposed to a 30-min session each weekday in which only the ethanol FR5 and Food FR150 contingencies and associated stimuli were present for at least 5 consecutive sessions. This established a baseline level of responding for ethanol during 30-min sessions where responding for food remained very low (see Fig. 1, filled symbols). Details of the amount of ethanol earned by each subject are provided in Table 1.

2.2.4. Reduced responding for ethanol—Subsequently, responding for ethanol was reduced, either by suspending training (i.e. rats remained in their home cage and were not allowed access to the operant chamber) or by providing alternative reinforcement (i.e. rats were exposed to 30-min sessions in which the contingencies were Food FR5, ethanol FR5 and presented with the appropriate stimuli). These conditions persisted for either 1 or 16 sessions. This resulted in periods where responding for ethanol either was eliminated (in the suspended training situations) or was very low (see Fig. 1, open symbols and Table 1).

2.2.5. Reinstatement test—After each intervention period, rats were tested for reinstatement of responding on the ethanol-associated lever in two different test conditions (unsigaled or sigaled FR completion). In both reinstatement test conditions, rats were placed in the operant chamber on the test day and the left stimulus light or the 16 kHz tone

was presented, which signaled a food FR150 and ethanol FR5. In the unsignaled condition, responses on either lever had no programmed consequence. In the signaled stimulus condition, completion of the FR (150 for food or 5 for ethanol) resulted in presentation of the stimulus paired with reinforcement delivery, but no food or ethanol was delivered.

For the alternative reinforcement, signaled test session condition, the order of 1 or 16 session interventions was counterbalanced across all 11 rats. For the other conditions, the 16 session intervention always preceded the 1 session condition in a given rat. Thus, the 1st and 2nd tests followed the alternative reinforcement intervention in a signaled test session (1 or 16 sessions of the intervention was counterbalanced across subjects). Subsequently, the order of testing was generally: 3. suspended training unsignaled test (16 session intervention); 4. alternative reinforcement, unsignaled test (16 session intervention); 5. alternative reinforcement, unsignaled test (1 session intervention); 6. suspended training signaled test (16 session); 7. suspended training unsignaled test (1 session intervention); 8. suspended training signaled test (1 session intervention). The specific conditions under which every rat was tested are shown in Table 1.

2.2.6. Measures—The total number of responses on the ethanol-associated lever during the test session was the main measure of interest. In addition, the number of responses on the food lever before the first five ethanol responses were completed in signaled test sessions and the total number of responses on the food-associated lever in all test sessions were measured

2.2.7. Analysis—Each measure was analyzed with a repeated measures ANOVA with intervention period (1 or 16 sessions), intervention (suspended training or alternative reinforcement), and test condition (no contingencies, contingent stimuli) as factors. Analyses were accomplished by performing a linear mixed-effects regression with intervention, its duration and test condition as factors and subject as the repeated measure. The linear model was then analyzed with the ‘anova’ function with type III sum of squares in the *R* statistical programming language (*R* Foundation for Statistical Computing, 2008). Epsilon values for Mauchly’s test were greater than one, so *p*-values were not corrected for sphericity. Effects with $p < 0.05$ were considered significant. Significant main effects and interactions were further analyzed using *t*-tests, corrected for multiple comparisons using the method of Benjamini and Hochberg (1995).

3. Results

As shown in Table 1 and Fig. 1, rats earned very few ethanol deliveries during the alternative reinforcement intervention period. Further, rats responded on the ethanol-associated lever far fewer times during these intervention periods than during the test sessions. Rats were unable to respond or earn ethanol during the suspended training intervention.

Consistent with previous reports, we observed incubation of responding for ethanol when animals were prevented from drinking by suspending training, but only in signaled test sessions (Fig. 2, open symbols, top left panel). As shown in Table 1, four of the six (67%)

rats tested after both intervention periods in unsignaled test sessions exhibited incubation and the other two rats responded an equal number of times under both conditions. In contrast, five of the nine (55%) rats tested after both intervention periods in unsignaled test sessions exhibited incubation and the other four rats responded less after 16 sessions of suspended training than after 1 session (Table 1; Fig. 2, closed symbols). This difference between the conditions was due to lower levels of responding in the signaled versus unsignaled test session following a single session of suspended training (Table 1; Fig. 2; open versus closed symbols above 1 session, top left panel): an effect that was seen in all 7 rats that responded under both conditions. Responding after 16 sessions of suspended access was similar in both test conditions. Incubation was also not observed when rats' drinking was reduced by providing alternative reinforcement (Fig. 2, bottom left panel). As shown in Table 1, only two of thirteen (15%) or three of eight (38%) of rats tested under both alternative reinforcement intervention periods exhibited incubation. Parallel analyses were performed using only the subset of 5 rats that participated in tests of every condition (RE18, RE19, RE20, RE22, and RE24), and results were similar, though the p -value for incubation of reinstated ethanol responding (suspended training, unsignaled test session) fell slightly below the standard threshold for significance ($p < 0.06$).

An ANOVA on ethanol-associated lever responses revealed an interaction between abstinence period (1 versus 16 sessions) and intervention (suspended training versus alternative reinforcement, $F[1,64] = 8.7$, $p < 0.05$), and a main effect of the test session condition (signaled versus unsignaled, $F[1,64] = 7.8$, $p < 0.05$). Responding after 16 sessions of intervention (both alternative reinforcement and suspended training) differed significantly from responding after 1 session, but only after suspended access in signaled test sessions. No other comparison between 1 versus 16 sessions of intervention was significant after correction for multiple comparisons.

One advantage of the present procedure is that we can also assess responding on an alternative, previously-reinforced lever (food-associated) during the reinstatement test. This is only relevant for the suspended access condition, where responding for food was prevented in parallel with responding for ethanol. In the alternative reinforcement condition, rats continued to have access to and to respond for food throughout the intervention period, so in this condition, responding for food continued during the 1 or 16 sessions of reduced responding for ethanol. Thus, this condition was eliminated from this analysis. We observed incubation of responding for food following suspended access (Fig. 2, top right panel). An ANOVA on food-associated lever responses after suspended training revealed a main effect of the length of the intervention ($F[1,24] = 8.6$, $p < 0.05$). No effects of the test condition (signaled versus unsignaled) or of an interaction between length of the intervention and test condition were present. However, one should note that while there were programmed differences in responding for food between the two test conditions, these differences were never experienced by the rats, i.e., during these test sessions, no rat made 150 responses on the food-associated lever that resulted in presentation of the food-paired stimulus. Thus, the outcome of total responses on the food lever was effectively the same for the two test session conditions.

We were also able to assess the number of responses on the food lever before completion of the first FR on the ethanol lever in the signaled test sessions (where the paired stimulus was presented after each FR completion). As shown in Fig. 3, rats tended to complete fewer than 5 responses on the food lever before completing the first FR on the ethanol lever after a single suspended training session (filled symbol). However, after 16 suspended training sessions, rats responded significantly more on the food lever prior to completing the first FR on the ethanol lever ($p < 0.02$). This same pattern (increased responding for food prior to completion of the first ethanol FR) was present after the alternative reinforcement intervention, but was exaggerated ($p < 0.003$). The difference between the number of responses on the food lever after 16 sessions of suspended training or alternative reinforcement was significant ($p < 0.03$). Indeed, an ANOVA showed an interaction between intervention (suspended training, alternative reinforcement) and sessions (1, 16), $F[1,35] = 4.4$, $p < 0.05$. Main effects of intervention and session were also significant ($F[1,35] = 6.2$ and 21.9 , $p < 0.02$ and 0.00004 , respectively).

4. Discussion

Here we demonstrate that experimental conditions can profoundly affect the expression and extent of incubation of cue-induced reinstatement and conclude that incubation is more likely to reflect changes in discriminative control over responding than changes in motivation. In previous work, we saw no incubation of ethanol reinstatement after using the intervention of alternative reinforcement to reduce responding for ethanol (Ginsburg and Lamb, 2013a, 2013b). However, we did see increases in responding for food prior to completion of the first five responses for ethanol during the reinstatement test. Here, we extend these studies to examine whether the intervention we used and/or conditions present in the test session might have influenced the expression of incubation. The results suggest that two factors are involved in the expression of incubation: a decline in the ability of stimuli that signal FR completion to attenuate responding in extinction after extended suspended training, and degraded control over responding by discriminative stimuli after a recent history of alternative behavior within the operant context.

We observed incubation of ethanol reinstatement in signaled test sessions (where FR completion was signaled) after 16 versus 1 session of suspended training. This is similar to conditions others have used to observe the effect. Agabio et al. (2000) showed an increase in rats' ethanol intake after 15 versus 1 days of suspended access. Our results are also consistent with those reported by Grimm et al. (2001) where incubation of responding for cocaine was observed in signaled test sessions after 15 versus 1 session of suspended training.

However, we did not observe incubation after the same suspended training interventions in unsignaled test sessions (where FR completion was unsignaled). The lack of incubation was attributable to relatively high levels of responding in the unsignaled test session after 1 suspended training session (compared to responding after the same intervention during the signaled test session).

This suggests the feedback function of presenting the stimulus paired with FR completion (and ethanol delivery) generally seems to limit extinction responding. This is apparent in Fig. 2 where responding in the signaled test sessions tended to be greater than responding in unsignaled test sessions. We saw two exceptions. First, there was little difference in food responses during signaled and unsignaled test sessions after suspended training. This is likely because the two conditions were effectively the same (in neither case were enough responses on the food lever completed to produce the paired stimulus presentation). Second, there was little difference between total ethanol responses during signaled and unsignaled test sessions after 16 sessions of suspended training. We hypothesize this is due to a lack of recent experience with the feedback function of the signal. Thus, we conclude that a lack of recent experience can degrade the effectiveness of this feedback function.

This raises a question about why 16 alternative reinforcement sessions did not also result in the apparent degradation of the feedback function (i.e. ethanol responding remained attenuated in signaled test sessions after this intervention). We hypothesize that this is due to either generalization of the feedback function provided by responding for food during the alternative reinforcement intervention, or, perhaps more likely, reflects a failure to discriminate the different signals contingent on ethanol versus food FR completion (which were qualitatively very similar).

It should also be noted that Bienkowski et al. (2004) report incubation of responding after 1 versus 28 sessions of suspended training, however the effect was observed in unsignaled test sessions. This difference from our results may be because presentation of the signal provided no additional information to the rats in the earlier study; the context of the experimental chamber was sufficient to guide responding for ethanol (by design). In the present study, due to the more complex multiple concurrent schedule, the paired stimulus presentations likely provided additional information about whether the response was appropriate under the prevailing conditions.

There is precedent for presentation of a stimulus change contingent on fixed-ratio completion attenuating responding in extinction (Weissman and Crossman, 1966). In that study, pigeons exposed to a stimulus change after ratio completion in extinction had longer post-ratio pauses than those with no stimulus changes during extinction, consistent with the stimulus change functioning as a discriminative stimulus. However, differences in total response output during extinction were not different between groups. Others have also suggested that stimuli paired with reinforcement can come to serve as discriminative stimuli (Dinsmoor, 2004). Such stimuli are more likely to come to serve as discriminative stimuli when they provide nonredundant information about the likelihood of a subsequent response being reinforced, particularly in situations where the contingencies frequently change (Boutros et al., 2011).

In the present study, the multiple concurrent schedule represents such a situation. Thus, it is highly likely that the paired stimuli came to serve as a discriminative stimulus, particularly for the ethanol response. Eliminating these contingent stimulus presentations in turn eliminated the feedback function they served during extinction, and resulted in an increase in responding. The similarity of responding in signaled and unsignaled test sessions after 16

sessions of suspended training indicates that the feedback function of the paired stimulus presentations was diminished.

One finding from the present study would seem at odds with our hypothesis that incubation results from a degradation in the feedback function signaling the absence of ethanol delivery: incubation of food responding with suspended access regardless of the test condition. This is even more remarkable given that the rats' never experienced the food-paired stimulus presentation during test sessions; the situation where we did not see incubation of ethanol responding due to high levels of responding after a single intervention session. In this case, we believe that it is still a degradation of stimulus control that results in incubation. However, it is the degradation of control by stimuli signaling the unfavorable reinforcement contingencies for food. These stimuli kept food-associated responding at low levels following one session of suspended access, but were less effective following 16-sessions of suspended access.

In addition to changes in the feedback function of the paired stimulus, the control exerted by the programmed discriminative stimuli also appears to be affected by recent experience. Previously we saw that more recent experience in the alternative reinforcement condition reduced the initial effectiveness of the stimulus that occasioned ethanol responding (Ginsburg and Lamb, 2013c). The data presented here are consistent with those results, as the longer intervention period diminished the ability of the stimulus that occasioned responding for ethanol to attenuate responding for food.

Degraded control over responding by the discriminative stimulus may also explain why we saw incubation for food responding. Previously we showed that alternative reinforcement can decrease discriminative control by the stimulus that signals a high food FR over response allocation (Ginsburg and Lamb, 2013b). It appears a similar degradation occurs after a period of suspended training, as responding for food increased after 16 versus 1 suspended training session.

The increase in the number of food responses before completion of the first ethanol FR over longer intervention periods is also consistent with this hypothesis. After a single session of suspended training, rats typically responded less than 5 times on the food lever before completing 5 responses on the ethanol lever. This was similar to the pattern of responding after a single session of alternative reinforcement, where rats were exposed to the lower food FR and associated stimuli. However, responding on the food lever before the first ethanol FR was completed was significantly increased after 16 suspended training sessions. Responding on the food lever was even further increased after 16 sessions of alternative reinforcement (where rats were exposed to the lower food FR and associated stimuli). These data are consistent with general phenomenon of decreased stimulus control of (inhibited) responding for food over time (Ginsburg and Lamb, 2013b).

Considered together, the results suggest that attention to discriminative stimuli declines over time since the last exposure, and that this might occur differently, depending on the experience during that time. When subjects are returned to the context, but presented with alternative stimuli and reinforcement, attention to the programmed discriminative stimulus

diminishes. When subjects are removed from the context entirely for that time, there appears to be less decline in attention to the programmed discriminative stimulus. However, attention to the feedback function of conditioned reinforcement seems to be more profoundly decreased. While these hypotheses are intriguing, clearly they require testing in future studies.

There are some important limitations of the present study to consider. Only 2 periods of abstinence were studied. Observed increases in responding could decrease after longer periods, similar to results reported by Bienkowski et al. (2004). Also, the history of these animals could have affected the results. Often, incubation is observed between groups. Here we observe incubation in the same rats exposed to different periods of abstinence at different times. It is possible that the more extensive behavioral history decreased our ability to see incubation. However, we observed incubation in the situation with the most extensive history, as the 1-session suspended training intervention with the signaled test session was the last test performed. Thus, we would expect similar outcomes in between-subjects designs. Indeed, others have shown similar levels of incubation of responding for ethanol after 28 days (versus 1) with no ethanol access, and others have shown incubation of ethanol intake after 15 days of ethanol deprivation in between-subjects designs (Agabio et al., 2000; Bienkowski et al., 2004). The extent to which presentation of stimuli that signal FR completion might impact such results in between-subjects designs remains unclear. Finally, the order of test conditions was not counter balanced across subjects. Thus, for a given condition, the 16 session intervention often preceded the 1 session condition. However, in many cases responding in test sessions after 1 intervention session was similar across conditions, regardless of when the condition was assessed. Thus, while we cannot entirely rule out the influence of the test order, it does not appear to explain the observed effects.

Despite these limitations, one distinct advantage of the present procedure is that we were able to assess responding on another, previously reinforced lever simultaneous with ethanol responding. This is particularly interesting in the case where access to ethanol and food (during operant sessions) was suspended. The results clearly show that incubation occurs for both behaviors in parallel. This is consistent with other reports demonstrating that incubation is not specific to a particular drug or even type of reinforcer (Crombag et al., 2008; Marchant et al., 2013). In fact, the present results suggest that withholding access can produce incubation for all behaviors that were simultaneously prevented.

Two lines of evidence indicate that incubation is more likely to reflect changes in stimulus control rather than changes in motivation. First, higher levels of responding in unsignaled versus signaled tests after suspended training is inconsistent with an explanation based on changes in motivation. Based on decades of work (e.g. Kelleher and Gollub, 1962), the stimuli that signal FR completion (and are paired with ethanol delivery) should serve as conditioned reinforcers, and thus would be expected to increase motivation for ethanol. However, responding in the presence of these stimuli was generally lower than responding in their absence. Second, we observed an increase, no change, and a decline in responding for ethanol across similar interventions, so it is hard to conceive of how motivation could fully account for these differences.

The present study demonstrates that the conditions under which responding is reduced and reinstatement is assessed can influence the expression of incubation. These results suggest that incubation is a product of both the subject's history and the environment in which reinstatement is assessed. These results are also most consistent with incubation being a result of a degradation of stimulus feedback functions that limit responding rather than an increased motivation for drug or propensity for relapse.

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Fig. 1.

Responses per session for each condition. Filled symbols represent sessions with ethanol FR5, food FR150, and the stimuli associated with these contingencies. Open symbols represent sessions with ethanol FR5, food FR5, and the stimuli associated with these contingencies. Grey symbols represent test sessions where food or ethanol was not provided. The top row shows data from signaled test sessions where completion of an FR was accompanied by presentation of the appropriate paired stimulus while the bottom row shows unsignaled test sessions. The left column shows data when the intervention was suspended training and the right column shows data when the intervention was alternative reinforcement. Each point represents the mean and standard error. The number of animals represented for each condition can be found in Table 1.

Fig. 2.

Total responses on the ethanol-associated lever (left panels) or food-associated lever (right panel) following 1 or 16 sessions of suspended access (top) or alternative reinforcement (bottom). Ethanol and food were never available during this session. Closed circles represent unsignaled test sessions (where responding had no programmed consequences) and open circles represent signaled test sessions (where paired-stimuli associated with ethanol delivery followed 5 responses and paired-stimuli associated with food delivery followed 150 responses). Points represent the mean $\pm$ SEM. Data for responding after 1 or 16 sessions of alternative reinforcement in signaled test sessions (lower panel, open symbols) were previously reported (Ginsburg and Lamb, 2013a, 2013b). * indicates a significant difference from the number of responses after 1 session under matched conditions. ($p < 0.05$). ‡

indicates a significant difference from the number of food responses after 1 session, collapsed across both signaled and unsignaled test session conditions.

Fig. 3. Number of responses on the food lever before the first ethanol FR was completed during signaled test sessions. The dotted line represents the number of ethanol responses necessary to complete the first FR (5). Points represent the mean and standard error. The number of animals represented in each condition can be found in Table 1.

Ethanol-associated lever responding and ethanol earned during the intervention period and test session for each subject.

Table 1

	Paired Stimulus Presented Alternative Reinforcement*				Suspended Training		No Paired Stimulus Presented Alternative Reinforcement				Suspended Training	
	Intervention period		Test Session		Test Session		Intervention period		Test Session		Test Session	
	Ethanol deliveries ^a	1	16	1	16	1	Ethanol deliveries ^a	1	16	1	Ethanol responses ^b	1
Intervention period	1	16	1	16	1	16	1	16	1	16	1	16
RE9	0	0	2	1	25	25	125	3	2	18	8	71
RE13	0	0	0	1	50	50	75	1	2	17	15	52
RE14	0	0	10	2	25	25	25	1	5	6	23	43
RE16	1	0	11	2	25	25	50					
RE17	0	0	8	2	75	75	25	0	0	0	36	66
RE18	0	0	0	5	25	25	16	2	0	14	2	35
RE19	0	0	0	1	50	50	25	0	1	1	5	27
RE20	0	0	0	0	25	25	5	0	0	1	2	104
RE22	0	0	0	3	25	25	25	0	0	16	11	99
RE23	13	1	67	4	50	50	50	1	8		135	114
RE24	0	1	2	7	20	20	25	1	0	12	7	86
Total number of subjects tested			11	11	7	10	10	10	8	10	10	9

Italics indicate tests conducted in the same rat that show incubation from 1 to 16 session interventions.

Indicates results that were previously reported in Ginsburg and Lamb, 2013a,b).

^a Average number of ethanol deliveries earned per session during the intervention period.

^b Average number of responses on the ethanol-associated lever per session during the intervention period.

^c Number of responses on the ethanol-associated lever during the test session.