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Original Investigation

Over-expectation generated in a complex appetitive goal-tracking task is capable of inducing memory reconsolidation

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Abstract

Rationale: Discrepancies in an expected outcome have been demonstrated to result in modification of behaviour in both appetitive and aversive conditioning settings.

Objectives: In this study, we sought to establish whether over-expectation generated from compound conditioning with two previously rewarded stimuli was able to induce memory destabilisation and subsequent reconsolidation in a pavlovian conditioned approach setting.

Results: It was shown that four days, but not one day, of over-expectation training was required to induce memory reconsolidation, and this was disrupted by application of the NMDAR antagonist MK-801 prior to over-expectation training, but not by MK-801 application 6 hours post training.

Conclusions: These data provide evidence that the memories underlying pavlovian conditioned approach do undergo reconsolidation and that such reconsolidation can be triggered by over-expectation. Therefore, the updating of appetitive CS-US associations underpinning conditioned responding in manners other than extinction training is likely achieved through memory reconsolidation.

Keywords: Over-expectation; Memory reconsolidation; Appetitive; Pavlovian conditioning; Goal tracking.

Introduction

Animals learn to respond to cues in a particular manner within environments if these stimuli are predictive of a certain salient outcome or consequence. The salience of the outcome is important in directing an animal's attention and forming an association between the stimulus and outcome. These associations result in relatively long-lasting behavioural adaptations; however, changes within an environment can lead to a previously good predictive cue becoming less relevant and thus the behavioural association may require modification to maintain appropriate responding. Thus, if long-term memories remain in a stable state despite environmental changes affecting relevance, newer behaviours must be learned and these may compete with existing memories. Instead of the formation of a new memory, the updating of an existing association is an efficient way of maintaining memory relevance. Reconsolidation of memories allows the updating of a stable memory trace (Lee 2009).

Despite memory reconsolidation having been observed across a variety of species and memory settings (Nader and Hardt 2009), Blaiss and Janak (2007) reported that an appetitive Pavlovian conditioned approach memory was not affected by either the psychostimulant amphetamine or the protein synthesis inhibitor anisomycin administered systemically at the time of memory reactivation. This failure to observe reconsolidation of the underlying memory occurred regardless of changes to the extent of training and stimulus re-exposure at memory reactivation. However, reactivation of the underlying pavlovian memory was attempted only by exposing rats to unreinforced presentations of the CS+ and CS-, or to reinforced presentations of the CS+.

The short extinction procedure used by Blaiss and Janak (2007) is a standard method of memory reactivation (Debiec et al. 2006; Duvarci and Nader 2004; Flavell et al. 2011; Lee and Everitt 2008; Lee et al. 2006b; Milekic and Alberini 2002; Milton et al. 2008; Nader et al. 2000; Przybylski and Sara 1997) that destabilises the memory and triggers subsequent memory reconsolidation. In other settings, a training trial is also sufficient successfully to reactivate a memory (Lee 2008; Milekic et al. 2006). However, these procedures do not encompass the full range of settings under which memories are updated in order to maintain their relevance. Indeed memories can be weakened in an over-expectation procedure in a manner that is qualitatively different from extinction (Witnauer and Miller 2009). Moreover, pavlovian over-expectation induces a behaviourally-relevant prediction error signal (Takahashi et al. 2009) that we have hypothesised may be important for triggering memory destabilisation (Lee 2009). Therefore, the present study sought to establish whether weakening the value of a specific CS via a Pavlovian over-expectation task (Lattal and Nakajima 1998; Rescorla 1970) is able to evoke memory destabilisation and thus, reconsolidation of the memory.

In the present complex Pavlovian goal-tracking task, rats are first trained to respond to two auditory cues (A1, rewarded; A2, non-rewarded) and one visual cue (V1, rewarded) that independently predict reward. Subsequently, the two rewarded cues are presented together, as an audiovisual compound (A1V1). The visual cue continued to be rewarded as during training, and the second auditory cue remained non-rewarded. When responding for the individual cues is assessed again later, rats exhibit reduced responding to the cue A1. Reduced responding is proposed to be underpinned by the violation of summed expectations for reward during compound training, causing a discrepancy between actual and expected outcomes. Thus, the rat expects to be delivered twice the number of rewards when both rewarded cues are presented in compound. However, the rat actually receives one reward, less than expected, thus generating a negative prediction error, shown by single unit recording to originate in the VTA in a similar over-expectation task (Takahashi et al., 2009). The generation of a prediction error has been theoretically posited to be a functional requirement for memory destabilisation to then allow reconsolidation processes to be engaged (Lee, 2009). If decreased conditioned responding is due to updated CS-US representations via memory reconsolidation, the destabilised memory is predicted to be disrupted by systemic administration of MK-801, this has previously been demonstrated to impair appetitive pavlovian memories (Lee and Everitt 2008; Milton et al. 2011; Sadler et al. 2007).

Method

Subjects

The subjects were 52 experimentally naive adult male Lister Hooded rats (supplied by Harlan OLAC, UK), weighing 250–300 g. They were housed in groups of four in holding rooms maintained at 21°C on a standard light cycle (12 h light/dark cycle; lights on at 7:00 A.M.). Food was restricted to 15 g/d; but water was available *ad libitum* throughout the experiment. Training and testing were conducted between 9:00 A.M. and 12:00 P.M. One rat was excluded from analysis in Experiment 2 for failing to acquire the discriminations during training. All procedures were conducted in accordance with the United Kingdom 1986 Animals (Scientific Procedures) Act (Project License PPL 40/3205).

Drugs

Rats were administered (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine maleate (MK-801, Sigma, UK) dissolved in saline (0.1 mg/ml) at a dose of 0.1 mg/kg by intraperitoneal (i.p) injection. Saline served as vehicle control.

Apparatus

Pavlovian conditioned approach training was performed in eight identical, standard operant chambers (30 cm wide, 21 cm high and 24 cm deep; supplied by Med Associates, St Albans, VT) contained in sound attenuating boxes and arranged in a four-by-two array in a testing room. Each chamber consisted of 3 walls and a ceiling, with the door serving as the fourth wall. The ceiling, door and back wall were made from clear Perspex and the left and right walls were made from stainless steel. The floor of each chamber was constructed of 19 stainless steel rods (4.8 mm in diameter, spaced 16 mm apart). Each chamber was illuminated by a 3W houselight located at the top centre of the left wall. The right walls of the chambers were fitted with a recessed magazine into which sucrose pellets (45 mg; Testdiet, Lancaster, NH) were delivered via a pellet dispenser. Two flat panel retractable levers were located to the left and right of the magazine; these remained retracted throughout the experiment. Above each lever was a 2 cm diameter panel light, illumination of these lights served as the visual stimulus in this experiment. The magazine entries were detected by an infra-red sensor. Auditory stimuli consisted of a 2 kHz pure tone delivered from a speaker located in the left wall and a 2 Hz train of clicks (80dB) generated by a relay clicker also located in the left wall. A computer equipped with MED-PC software (version IV; Med Associates Inc.) controlled the chambers and recorded the data.

Procedure

Table 1 illustrates the experimental design and reward contingencies during the different stages of the experiment.

--- TABLE 1 HERE ---

Behavioural training

Pavlovian training was carried out over 10 days (1 session per day) during which two discriminable auditory stimuli (A1 or A2, clicker or tone) and visual stimulus (V1, stimulus light illumination), were presented 8 times each for 30 seconds per session in a blocked design counterbalanced between sessions. The visual stimulus and one auditory stimulus (counterbalanced across rats) were reinforced with the delivery of 3 reward pellets on each presentation, whereas the other auditory stimulus was never reinforced. Each stimulus presentation was separated by a 60 second period in which no cues were presented, separated into 30 second inter-trial interval (ITI) and 30 second PreCS.

Compound conditioning

Following training, there was a) 1 or b) 4 days of compound conditioning, in which the visual stimulus and the rewarded auditory stimulus were presented together and were reinforced with 1

pellet. The visual stimulus was also presented alone and rewarded with 3 pellets, whereas the previously rewarded auditory stimulus was never presented alone. This elicits a negative prediction error for the rewarded auditory stimulus that weakens its predictive strength. In the reactivation groups, the amnesic drug MK-801 (0.1 mg/kg i.p.) or vehicle (saline) was administered 30 mins prior to the reactivation procedure. In the delayed treatment group compound conditioning was carried out for 4 days; however MK-801 (0.1 mg/kg i.p.) or vehicle (saline) were administered 6 hours post-compound training. This is because that a true non-reactivation control is not appropriate in this design; the impact of the compound training with MK-801 or saline administered outside of the “reconsolidation window” thus serves as a control for the effect of MK-801 on memory updating.

Test

At test, 24 hours following the completion of compound training, goal-tracking activity was measured by magazine entry responses during non-reinforced presentations of the auditory stimuli. Thus the clicker, light or tone were presented 10 times for 30 seconds, each trial separated by a 60 second stimulus free period, separated into 30 second inter-trial interval (ITI) and 30 second PreCS.

Analysis

Discrimination performance was measured by response ratios calculated by magazine responding during CS presentation / (CS + PreCS responding). Thus, response ratios greater than 0.5 indicate increased responding to the CS compared to baseline responding, whereas a ratio of 0.5 indicates no difference from PreCS responding and therefore no discriminative performance. Importantly, it is discriminative performance (i.e. greater response ratios to the rewarded CS than to a non-rewarded CS) that is truly indicative of acquired appetitive pavlovian approach behaviour. In addition, analyses of PreCS data at test were conducted to indicate whether there were any differences between groups to indicate whether MK-801 was generally affecting performance. Rats were excluded from analysis if they demonstrated greater responding to the neutral stimulus than rewarded stimuli on the final day of training, indicating that they had failed to learn the discriminations.

Data were analysed by repeated measures ANOVA using SPSS (IBM, version 20). Planned comparisons between A1 and V1 to test for over-expectation were performed to establish whether responding to A1 (compound element subject to over-expectation) was suppressed in comparison to V1 (compound element not subject to over-expectation) at test.

Results

One day compound training

Acquisition of Pavlovian discriminations

Sixteen experimentally naive rats were used in this experiment. Figure 1a shows the mean rate of magazine entry responding during the course of acquisition. Inspection of this figure shows that rats acquired a discriminated Pavlovian conditioned approach response to the reinforced auditory stimulus (A1) and visual stimulus (V1) but not the non-reinforced stimulus (A2). This was confirmed by a within subjects ANOVA with within factors of training session (1-10) and CS (A1, A2, V1) and between subjects factor of drug (MK-801, saline). Rats successfully acquired discriminated approach over training sessions (Session: $F_{(9, 126)} = 27.725, p < 0.001$; CS: $F_{(2, 28)} = 69.623, p < 0.001$; Session x CS: $F_{(18, 252)} = 6.943, p < 0.001$). Importantly, there was no difference in acquisition between saline and MK-801 rats (session x CS x drug: $F_{(18, 252)} < 1$; CS x drug: $F_{(2, 28)} = 1.559, p = 0.228$; drug: $F_{(1, 14)} = 1.376, p = 0.26$).

Compound training

Figure 1b shows the mean rate of magazine entry responding to the auditory, visual and compound stimuli during the one day of compound training. Inspection of this figure shows that saline and MK-801 treated rats showed equivalent levels of approach to A1V1 and V1, and less approach to A2. This was confirmed by a mixed ANOVA with within factors of CS (A1V1, A2, V1) and between subjects factor of drug (MK-801, saline), which revealed a significant main effect of CS ($F_{(2, 28)} = 133.79, p < 0.001$) and no significant main effect of drug ($F_{(1, 14)} < 1$). A significant interaction between the drug x CS was observed ($F_{(2, 28)} = 5.78, p < 0.01$). Simple effects analysis of the drug x CS interaction revealed significant effects of CS in both the MK-801 ($F_{(2, 13)} = 61.965, p < 0.001$) and saline ($F_{(2, 13)} = 113.527, p < 0.001$) groups. No significant effect of drug group was observed during A1V1 ($F_{(1, 14)} = 4.151, p = 0.061$), V1 ($F_{(1, 14)} = 1.765, p = 0.205$) and A2 ($F_{(1, 14)} = 4.507, p = 0.052$), however it appears that the interaction was driven by slightly quantitatively increased responding to A2 and suppressed responding to A1V1 in the MK-801 group compared to saline injected rats.

Test performance

Figure 1c shows the mean rate of magazine entry responding to the auditory stimuli during the extinction test trials in rats administered saline or MK-801 prior to compound training sessions. Inspection of this figure shows that rats administered saline or MK-801 maintained discriminated Pavlovian conditioned approach to A1 and V1, and less approach to A2. This was confirmed statistically by a mixed ANOVA with within factors of CS (A1, V1, A2) and between subjects factor of drug (MK-801, saline). There was no difference in responding between saline and MK-801 treated rats (drug x CS: $F_{(1, 14)} < 1$; drug: $F_{(1, 14)} < 1$), and responding discriminated between the cues ($F_{(1, 14)} = 8.751, p < 0.01$).

Planned comparison between performance during A1 and V1 presentations indicated no effect of MK-801 (drug x CS: $F_{(1, 14)} < 1$; drug: $F_{(1, 14)} < 1$), and no discrimination between the cues ($F_{(1, 14)} < 1$). Therefore there were no differences between A1 and V1 responding, indicating that one day of compound training did not result in decreased responding to A1.

In addition, no differences were observed in PreCS responding between drug conditions (data not shown). This was confirmed by a mixed ANOVA with within factors of CS (A1, V1, A2) and between subjects factor of drug (MK-801, saline). MK-801 had no effect on PreCS responding (drug x CS: $F_{(2, 28)} = 1.437$, $p = 0.255$; drug: $F_{(1, 14)} < 1$), and PreCS responding did not differ across CS ($F_{(2, 28)} < 1$).

----- FIGURE 1 HERE -----

Four days compound training

Acquisition of Pavlovian discriminations

Twenty experimentally naive rats were used in this experiment. One rat was excluded from analysis due to failing to acquire the discriminations adequately. Figure 2a shows the mean rate of magazine entry responding during the course of acquisition. Inspection of this figure shows that rats acquired a discriminated Pavlovian conditioned approach response to the reinforced auditory stimulus (A1) and visual stimulus (V1) but not the non-reinforced stimulus (A2). This was confirmed by a within subjects ANOVA with within factors of training session (1-10) and CS (A1, A2, V1) and between subjects factor of drug (MK-801, saline). Rats successfully acquired discriminated approach over training sessions (Session: $F_{(9, 153)} = 20.695$, $p < 0.001$; CS: $F_{(2, 34)} = 62.147$, $p < 0.001$; Session x CS: $F_{(18, 306)} = 7.378$, $p < 0.001$). Importantly, there was no difference in acquisition between saline and MK-801 rats (session x CS x drug: $F_{(18, 306)} < 1$; CS x drug: $F_{(2, 34)} = 3.55$, $p < 0.05$; drug: $F_{(1, 17)} = 1.723$, $p = 0.207$).

Compound training

Figure 2b shows the mean rate of magazine entry responding to the auditory, visual and compound stimuli during the four days of compound training. Inspection of this figure shows that saline and MK-801 treated rats showed equal levels of approach to A1V1 and V1, and less approach to A2 this was confirmed by a mixed ANOVA with within factors of CS (A1V1, A2, V1) and session (1-4) and between subjects factor of drug (MK-801, saline) which revealed a significant main effect of CS ($F_{(2, 34)} = 159.403$, $p < 0.001$) and drug ($F_{(1, 17)} = 5.352$, $p < 0.05$) and no significant main effect of session ($F_{(3, 51)} < 1$). A significant session x drug interaction was observed ($F_{(3, 51)} = 3.948$, $p < 0.05$), no

significant interactions between the drug x CS ($F_{(2, 34)} = 2.02, p=0.148$), session x CS ($F_{(6, 102)} < 1$) and session x CS x drug ($F_{(6, 102)} = 1.163, p=0.332$) were observed.

Inspection of the simple main effects of the session x drug interaction revealed a significant effect of drug in session 1 of compound training ($F_{(1, 17)} = 23.966, p<0.001$), but not in sessions 2-4 (F 's < 2.009). No significant effects of MK-801 ($F_{(1, 17)} = 2.781, p=0.077$) or saline ($F_{(1, 17)} = 1.960, p=0.163$) were observed across sessions. Thus, this interaction was driven by the increased responding by saline injected rats in session 1 in comparison to the MK-801 injected rats.

Test performance

Figure 2c shows the mean rate of magazine entry responding to the auditory stimuli during the extinction test trials in rats administered saline or MK-801 prior to compound training sessions. Inspection of this figure shows whereas the saline group maintained discrimination between A1, V1 and A2, the MK-801 group did not – showing higher levels of approach during A2 presentations compared to saline-treated rats. This observation was confirmed by a mixed ANOVA with within factors of CS (A1, V1, A2) and between subjects factor of drug (MK-801, saline) which revealed a significant main effect of CS ($F_{(1, 17)} = 28.287, p<0.001$) and no significant main effect of drug ($F_{(1, 17)} = 3.629, p=0.074$). Importantly, a significant interaction between the drug x CS was observed ($F_{(1, 17)} = 4.34, p<0.05$) which revealed a significant effect of CS type in rats administered pre-reactivation saline ($F_{(2, 16)} = 22.638, p<0.001$) and in rats administered MK-801 ($F_{(2, 16)} = 5.199, p < 0.05$).

Due to the presence of significant simple effects of CS in both groups, post-hoc analyses ($p<0.05$, Bonferroni-corrected) were performed which indicated that rats administered MK-801 prior to compound training showed no difference in responding to A1 and A2, whereas rats administered saline did discriminate between A1 and A2. In addition, rats continued to discriminate between V1 and A2 in both the MK-801 and saline groups.

Planned comparison between performance during A1 and V1 presentations indicated a significant main effect of CS ($F_{(1, 17)} = 7.33, p < 0.05$) but no significant main effect of drug ($F_{(1, 17)} < 1$). There was no significant interaction between drug x CS ($F_{(1, 17)} < 1$).

In addition, no differences were observed in PreCS responding between drug conditions (data not shown). This was confirmed by a mixed ANOVA with within factors of CS (A1, V1, A2) and between subjects factor of drug (MK-801, saline). MK-801 had no effect on PreCS responding (drug x CS: $F_{(2, 34)} < 1$, drug: $F_{(1, 17)} < 1$), and PreCS responding did not differ across CS ($F_{(2, 34)} = 1.938, p=0.16$). Thus the impaired discrimination observed during A2 presentations was not due to rate differences during PreCS responding.

Planned comparison between performance during A1 and V1 presentations indicated no effect of MK-801 (drug x CS: $F_{(1, 17)} < 1$; drug: $F_{(1, 17)} < 1$), and a significant discrimination between the cues ($F_{(1, 17)} = 7.33$, $p < 0.05$). Therefore, rats suppressed responding to A1 in comparison to V1, indicating over-expectation.

Analysis of the raw magazine approach responding during CS presentations further indicated a disruptive effect of MK-801 (CS x drug: $F_{(2, 34)} = 6.082$, $p < 0.001$) (data not shown). However, there was no effect of MK-801 on levels of responding overall (drug: $F_{(1, 17)} = 1.213$, $p = 0.286$). Analysis of simple effects revealed that the failure to discriminate at test between A1 and A2 in the MK-801-treated group is due to high levels of A2 approach in the MK-801 group ($F_{(1, 17)} = 4.63$, $p < 0.05$).

----- FIGURE 2 HERE -----

Four days compound training – Non-reactivation controls

Acquisition of Pavlovian discriminations

Sixteen experimentally naive rats were used in this study. Figure 3a shows the mean rate of magazine entry responding during the course of acquisition. Inspection of this figure shows that rats acquired a discriminated Pavlovian conditioned approach response to the reinforced auditory stimulus (A1) and visual stimulus (V1) but not the non-reinforced stimulus (A2). This was confirmed by a within subjects ANOVA with within factors of training session (1-10) and CS (A1, A2, V1) and between subjects factor of drug (MK-801, saline). Rats successfully acquired discriminated approach over training sessions (Session: $F_{(9, 126)} = 30.037$, $p < 0.001$; CS: $F_{(2, 28)} = 73.678$, $p < 0.001$; Session x CS: $F_{(18, 252)} = 6.352$, $p < 0.001$). Importantly, there was no difference in acquisition between saline and MK-801 rats (session x CS x drug: $F_{(18, 306)} < 1$; CS x drug: $F_{(2, 28)} < 1$; drug: $F_{(1, 14)} < 1$).

Compound training

Figure 3b shows the mean rate of magazine entry responding to the auditory, visual and compound stimuli during four days of compound training. Inspection of this figure shows that saline and MK-801 treated rats showed equal levels of approach to A1V1 and V1, and less approach to A2. This was confirmed by a mixed ANOVA with within factors of CS (A1V1, A2, V1) and between subjects factor of drug (MK-801, saline) revealed a significant main effect of CS ($F_{(2, 28)} = 6.183$, $p < 0.001$) and no significant main effect of session ($F_{(3, 42)} = 1.443$, $p = 0.244$) or drug ($F_{(1, 14)} < 1$). A significant interaction between session x CS was observed ($F_{(6, 84)} = 2.328$, $p < 0.05$). No significant interactions

were observed between drug x CS ($F_{(3, 42)} < 1$), session x drug ($F_{(3, 42)} = 1.565$, $p = 0.212$) and session x CS x drug ($F_{(6, 84)} = 1.321$, $p = 0.257$) were observed.

Simple effects analysis of the session x CS interaction revealed significant effects of A1V1 ($F_{(3, 12)} = 5.338$, $p < 0.05$), but not V1 ($F_{(3, 12)} = 2.366$, $p = 0.122$) or A2 ($F < 1$). Significant differences between responding to the cues were observed in session 1-4 (F 's > 9.907).

Test performance

Figure 3c shows the mean rate of magazine entry responding to the auditory stimuli during the extinction test trials in rats administered saline or MK-801 prior to compound training sessions. Inspection of this figure shows that rats administered saline or MK-801 maintained discriminated performance, characterised by a Pavlovian conditioned approach response to A1 and V1, and less approach to A2. This was confirmed statistically by a mixed ANOVA with within factors of CS (A1, V1, A2) and between subjects factor of drug (MK-801, saline) revealed a significant main effect of CS ($F_{(2, 28)} = 19.591$, $p < 0.001$) and no significant main effect of drug ($F_{(1, 14)} < 1$).

Planned comparison between performance during A1 and V1 presentations indicated no effect of MK-801 (drug x CS: ($F_{(1, 14)} < 1$; drug: $F_{(1, 14)} < 1$), and a significant discrimination between the cues ($F_{(1, 14)} = 4.99$, $p < 0.05$). Therefore, rats suppressed responding to A1 in comparison to V1, indicating over-expectation.

In addition, no differences were observed in PreCS responding between drug conditions (data not shown). This was confirmed by a mixed ANOVA with within factors of CS (A1, V1, A2) and between subjects factor of drug (MK-801, saline). MK-801 had no effect on PreCS responding (drug x CS: $F_{(2, 28)} < 1$, drug: $F_{(1, 14)} < 1$), and PreCS responding did not differ across CS ($F_{(2, 28)} < 1$). Thus, MK-801 administration had no effect on response rates during PreCS.

----- FIGURE 3 HERE -----

Discussion

Here we have demonstrated that memory reconsolidation is engaged in a pavlovian discriminative approach setting by compound training that likely induces pavlovian over-expectation. Systemic injection of MK-801 on four days shortly prior to pavlovian compound training resulted in impaired discrimination between auditory cues previously predictive and non-predictive of reward. This disruptive effect of MK-801 was critically dependent upon close temporal proximity with the

compound training sessions, as no impairment was observed when the MK-801 injections were delayed by 6 hours. Moreover, the observation of amnesia required repeated MK-801 injection-compound training treatments, as there was no effect with a single day of treatment. These results suggest that compound training triggers NMDA receptor-dependent pavlovian memory reconsolidation.

Over-expectation is a behavioural phenomenon providing evidence of an error correction mechanism that can influence responding to conditioned stimuli (Rescorla, 2007). In a typical over-expectation experiment (e.g., Lattal and Nakajima 1998; Rescorla 1970), two conditioned stimuli (A and B) are separately followed by an unconditioned stimulus (US), and then their AB compound is followed by the same US. The additional compound conditioning results in decreased responding to A and B. The associative strength of the AB compound is greater than the actual outcome and the discrepancy induced by reinforcement of the compound is negative, resulting in reduced responding to the elements separately (Rescorla 2007).

In the present study, following four days of compound training, rats suppressed responding to A1 in comparison to V1. Therefore, compound training reduced responding to A1, indicating that over-expectation was evoked during training in these conditions, but not following one day of training. While we did not have a further reinforced auditory stimulus that did not undergo compound training to test against, the similarity to the study of Takahashi et al. (2009) and analysis of these data suggests that compound training did evoke over-expectation. Importantly, there was continued discrimination between reinforced (A1) and non-reinforced (A2) auditory cues at test in rats administered saline. This is because A1 was still a better predictor of food than A2.

In contrast to saline-injected controls, rats administered MK-801 in the four-day compound training condition did not discriminate between A1 and A2. The lack of discriminated performance demonstrates that the appetitive pavlovian memory was impaired in the MK-801 group. Interestingly, the impaired discrimination appeared to be driven primarily by an increase in responding to A2, and hence performance was characterised by indiscriminate approach to the magazine in the presence of both the CS+ and CS-. This pattern of results has previously been observed in reconsolidation studies using MK-801 in appetitive pavlovian settings.

MK-801 acts as an antagonist at the NMDA subtype of glutamate receptor (NMDAR), and has been studied extensively in memory reconsolidation studies (Lee et al. 2006a; Przybylski and Sara 1997) including in appetitive settings (Kelley et al. 2007; Lee and Everitt 2008; Sadler et al. 2007). Of particular relevance to the current study are those of pavlovian sign-tracking and instrumental transfer with both sucrose (Lee and Everitt 2008) and alcohol (Milton et al. 2012) rewards. In these studies,

the MK-801-treated rats also showed no discrimination between the CS+ and CS-. Moreover, the impairment was characterised as much by an increase in responding to the CS- as by any decrease in responding to the CS+. Indeed, there was no evidence that responding in MK-801 treated rats decreased to the CS+ at all in the alcohol sign-tracking and pavlovian instrumental transfer settings (Milton et al. 2012). As observed in the current study and previous experiments (Lee & Everitt, 2008; Milton et al., 2012), the amnesic rats responded vigorously and in an indiscriminate manner to the CS's and the resulting drug by CS interaction is driven by the increased responding to A2, the previously unreinforced cue. Previous appetitive studies have posited that manipulations to the predictive value of the context during testing by including levers or decreasing CS presentation durations may drive the suppression of responding (Milton et al., 2008, 2012). However, the current experiment and others where increased CS- responding underpinned reactivation dependent amnesia (Milton et al., 2012, Lee & Everitt, 2008), the response context changed little between the training and the testing phases; potentially allowing the general incentive properties of the context to invigorate responding in an undirected manner in amnesic rats. Thus, the disruption of specific A1–food and A2–no food associations lead to a remaining auditory cue–food association which becomes activated within a context also associated with food, thus indiscriminate responding to A1 and A2 was observed. Whether this effect would be observed in a novel test context or when compound training was performed in a separate context is yet to be tested.

We have also demonstrated that the amnesic effect was dependent on MK-801 application within the “reconsolidation window”, the period in which an aversive (Monfils et al. 2009; Nader et al. 2000; Schiller et al. 2010) or appetitive (Alberini 2005; Mark and Watts 1971; Milekic and Alberini 2002; Xue et al. 2012) memory is restabilised following reactivation. Application of MK-801 6 hours post compound training did not disrupt reconsolidation of memory, and at test, these rats continued to bias responding to the auditory cue associated with reward. Although this method of delayed MK-801 application was not a true non-reactivation control, a true non-reactivation group would have had no over-expectation and hence no reduction in responding to the CS+ (at least in the saline group). This would, therefore, have artificially magnified the apparent reactivation-dependence of the effect.

The discrepancy between the actual outcome and prior expectation during compound training theoretically generates a negative prediction error that would neuronally signal this incongruity to allow the modulation of behaviour. A neural correlate of this negative prediction error has been localised to the responding of dopamine neurons within the ventral tegmental area (VTA (Takahashi et al. 2009)). It has previously been electrophysiologically demonstrated that a similar goal-tracking task was capable of generating a negative prediction error within the VTA, whereas the OFC was important in the encoding of expectancies (Takahashi et al. 2009). Given that memory reconsolidation

may mediate memory updating, the presence of a prediction error signal may be of critical importance in triggering memory destabilisation (Lee 2009). The current data are consistent with such an account, as a behavioural manipulation that elicits a prediction error signal should be conducive to inducing memory destabilisation.

In this study, we used one or four days of over-expectation compound training. Following one day of over-expectation training, rats administered either saline or MK-801 continued to discriminate between the auditory stimuli. This observation indicated that one day of over-expectation training did not induce memory reactivation and reconsolidation mechanisms. However, four days of over-expectation training was demonstrated to be sufficient to induce memory reconsolidation. This was illustrated by impaired discrimination between the auditory cues at test in rats administered MK-801 30 minutes prior to each compound training session. This necessity for repeated treatment and reactivation sessions may account for the previous failure to observe reconsolidation impairments in a similar, albeit less complex, goal-tracking setting. While Blaiss and Janak (2007) varied the extent of training and stimulus re-exposure, they did not include a condition with repeated treatment sessions. The requirement for multiple reactivation-treatment sessions to disrupt memory reconsolidation in an appetitive memory has been previously demonstrated, especially in the appetitive reconsolidation literature (Fricks-Gleason and Marshall 2008; Sadler et al. 2007). The present study, in conjunction with others (Fricks-Gleason and Marshall 2008; Sadler et al. 2007) supports the interpretation that repeated administrations of amnesic agents may interfere with reconsolidation in a cumulative manner.

While we argue that the observed effects of MK-801 in the present study are most likely due to impairment in memory reconsolidation, there are other interpretations than must be considered. First, given the dependence of new learning upon NMDA receptor mechanisms (Lee and Kim 1998; Santini et al. 2001), it is possible that MK-801 simply impairs the learning and/or consolidation of the compound training, rather than reconsolidation of the original training. There are two reasons that make such an interpretation unlikely. First, there was no evidence that MK-801 impaired learning during the 4 days of compound training, as there were no differences between the MK-801 and saline administered groups. Second, even if MK-801 did impair new learning during compound training, this does not easily explain why there was impairment in discrimination between A1 and A2, which should have been maintained by the persisting memory for the original training.

One further alternative explanation of our results is based on the possibility that there was generalisation decrement between the compound training and test. The test session is characterised by a return to presentation of A1 individually. Therefore, impaired performance at test may result from a failure to generalise learning from the A1V1 compound to simple A1 presentations. Within such an

interpretation, it is such a decrement that accounts for the reduced responding to A1 compared to V1, the latter having been reinforced individually during compound training. This might have been controlled for by comparing to a further reinforced stimulus that is then compounded with a novel unreinforced stimulus. However, it has previously been demonstrated that A1V1 compound presentations, exactly as used in the present study, do induce a VTA dopaminergic negative prediction error signal (Takahashi et al., 2009). Therefore, even if there were generalisation decrement, this would be occurring in parallel with the prediction error signal. Hence, the effect of MK-801 to impair discriminated responding at test might be due to a magnification of the generalisation decrement. This may be achieved through state-dependent effects of MK-801 (Ceretta et al. 2008), such that the compound learning under MK-801 is less likely to generalise to individual stimulus presentations in the absence of MK-801 at test.

Returning to the reconsolidation-based interpretation, the effectiveness of four days of over-expectation training compared to one day of over-expectation training is not optimal in terms of a memory reconsolidation study (in which only a single memory reactivation session is usually necessary). However, there are a number of studies that have demonstrated the efficacy of repeated reconsolidation treatment in appetitive settings in the absence of any amnesic effect with a single treatment session e.g. (Fricks-Gleason and Marshall 2008; Sadler et al. 2007). It can be proposed that the summative nature of responding to the compound may have driven continued responding and repeated negative prediction error signals that trigger memory reconsolidation. This observation may indicate that a negative prediction error driven by over-expectation is weaker than a prediction error generated by omission of a reward entirely and thus multiple reconsolidation treatments are required to overcome a reactivation boundary. Whether the prediction error signal becomes greater with repeated training sessions, or is summative in character is as yet unknown. Whether the magnitude of the amnesic effect bears some correlation to the magnitude of the prediction error signal and if the magnitude of a prediction error signal generated via extinction or over-expectation differs quantitatively has not been demonstrated electrophysiologically. Indeed, whether over-expectation (discrepancy in magnitude of reward) and extinction (non-reinforcement) are fundamentally similar is also questioned. Both over-expectation and extinction generate dopaminergic prediction error signals in the VTA (Pan et al. 2008; Takahashi et al. 2009) and according to Rescorla the observation of renewal following over-expectation and extinction indicates that these phenomena are supported by similar mechanisms (Rescorla 2006; 2007). However, Witnauer & Miller (2009) suggest that over-expectation and extinction are not driven by a common mechanism based on differential sensitivities to the effects of overtraining, demonstrating that extinction is enhanced by increased nonreinforced trials whereas over-expectation is unaffected.

The variable magnitude of a prediction error signal in extinction has been demonstrated via immunohistochemical analysis of phospho-ERK signalling (Huh et al. 2009). This study demonstrated that the rate of error detection measured by hippocampal ERK signalling, and fear extinction was dependent on shock expectancy and the aversive valence of the context, demonstrated by comparing groups trained with single, continuous, or partial reinforcement (Huh et al. 2009). During reevaluation of an aversive CS-US association, the presence of a CS but absence of the otherwise expected US generates a negative predictive error (actual outcome < expected outcome) and loss of fear through extinction learning. If the decrements in responding from over-expectation and non-reinforcement (CS-noUS learning) involve the same mechanism, this suggests that over-expectation could provide an alternative procedure for the study of extinction, one that avoids any disruptive effects of omitting the US (Garfield and McNally 2009).

Conclusions

This study demonstrated that over-expectation training in a complex goal-tracking procedure was sufficient to induce memory reconsolidation in rats following four days, but not one day, of compound conditioning. Application of the NMDAR antagonist MK-801 prior to, but not 6 hours post, over-expectation training resulted in amnesia in rats, whereby discrimination to previously reinforced and non-reinforced auditory cues was abolished. Thus, this indicates that memory updating can be induced by a discrepancy in an expected outcome, not just an omission, and links the initiation of reconsolidation to prediction error signals. However, it remains to be determined whether prediction error signals are necessary to induce memory reconsolidation.

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References

- Alberini CM (2005) Mechanisms of memory stabilization: are consolidation and reconsolidation similar or distinct processes? *Trends in neurosciences* 28: 51-6.
- Ceretta AP, Camera K, Mello CF, Rubin MA (2008) Arcaine and MK-801 make recall state-dependent in rats. *Psychopharmacology (Berl)* 201: 405-11.
- Debiec J, Doyere V, Nader K, LeDoux JE (2006) Directly reactivated, but not indirectly reactivated, memories undergo reconsolidation in the amygdala. *Proceedings of the National Academy of Sciences of the United States of America* 103: 3428-3433.
- Duvarci S, Nader K (2004) Characterization of fear memory reconsolidation. *Journal of Neuroscience* 24: 9269-9275.
- Flavell CR, Barber DJ, Lee JL (2011) Behavioural memory reconsolidation of food and fear memories. *Nature communications* 2: 504.
- Fricks-Gleason AN, Marshall JF (2008) Post-retrieval beta-adrenergic receptor blockade: effects on extinction and reconsolidation of cocaine-cue memories. *Learn Mem* 15: 643-8.
- Garfield JB, McNally GP (2009) The effects of FG7142 on overexpectation of Pavlovian fear conditioning. *Behavioral neuroscience* 123: 75-85.
- Huh KH, Guzman YF, Tronson NC, Guedea AL, Gao C, Radulovic J (2009) Hippocampal Erk mechanisms linking prediction error to fear extinction: roles of shock expectancy and contextual aversive valence. *Learn Mem* 16: 273-8.
- Kelley BJ, Yeager KR, Pepper TH, Bornstein RA, Beversdorf DQ (2007) The effect of propranolol on cognitive flexibility and memory in acute cocaine withdrawal. *Neurocase* 13: 320-327.
- Lattal K, Nakajima S (1998) Overexpectation in appetitive Pavlovian and instrumental conditioning. *Learn Behav* 26: 351-360.
- Lee H, Kim JJ (1998) Amygdalar NMDA receptors are critical for new fear learning in previously fear-conditioned rats. *J Neurosci* 18: 8444-54.
- Lee JL (2008) Memory reconsolidation mediates the strengthening of memories by additional learning. *Nature neuroscience* 11: 1264-6.
- Lee JL (2009) Reconsolidation: maintaining memory relevance. *Trends in neurosciences* 32: 413-20.
- Lee JL, Everitt BJ (2008) Appetitive memory reconsolidation depends upon NMDA receptor-mediated neurotransmission. *Neurobiology of learning and memory* 90: 147-54.
- Lee JL, Milton AL, Everitt BJ (2006a) Cue-induced cocaine seeking and relapse are reduced by disruption of drug memory reconsolidation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 26: 5881-7.
- Lee JL, Milton AL, Everitt BJ (2006b) Reconsolidation and extinction of conditioned fear: inhibition and potentiation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 26: 10051-6.
- Mark RF, Watts ME (1971) Drug inhibition of memory formation in chickens. I. Long-term memory. *Proc R Soc Lond B Biol Sci* 178: 439-54.
- Milekic MH, Alberini CM (2002) Temporally graded requirement for protein synthesis following memory reactivation. *Neuron* 36: 521-5.
- Milekic MH, Brown SD, Castellini C, Alberini CM (2006) Persistent disruption of an established morphine conditioned place preference. *Journal of Neuroscience* 26: 3010-3020.
- Milton AL, Lee JL, Everitt BJ (2008) Reconsolidation of appetitive memories for both natural and drug reinforcement is dependent on {beta}-adrenergic receptors. *Learn Mem* 15: 88-92.
- Milton AL, Schramm MJ, Wawrzynski JR, Gore F, Oikonomou-Mpegeti F, Wang NQ, Samuel D, Economidou D, Everitt BJ (2012) Antagonism at NMDA receptors, but not beta-adrenergic receptors, disrupts the reconsolidation of pavlovian conditioned approach and instrumental transfer for ethanol-associated conditioned stimuli. *Psychopharmacology (Berl)* 219: 751-61.
- Milton AL, Schramm MJW, Everitt BJ (2011) Molecular Targets for the Disruption of Alcohol and Cocaine Memory. *Behav Pharmacol* 22: E6-E6.

- Monfils MH, Cowansage KK, Klann E, LeDoux JE (2009) Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories. *Science* 324: 951-5.
- Nader K, Hardt O (2009) A single standard for memory: the case for reconsolidation. *Nature Reviews Neuroscience* 10: 224-234.
- Nader K, Schafe GE, LeDoux JE (2000) The labile nature of consolidation theory. *Nature reviews Neuroscience* 1: 216-9.
- Pan WX, Schmidt R, Wickens JR, Hyland BI (2008) Tripartite mechanism of extinction suggested by dopamine neuron activity and temporal difference model. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 28: 9619-31.
- Przybylski J, Sara SJ (1997) Reconsolidation of memory after its reactivation. *Behav Brain Res* 84: 241-246.
- Rescorla RA (1970) Reduction in Effectiveness of Reinforcement after Prior Excitatory Conditioning. *Learn Motiv* 1: 372-381.
- Rescorla RA (2006) Spontaneous recovery from overexpectation. *Learn Behav* 34: 13-20.
- Rescorla RA (2007) Spontaneous recovery after reversal and partial reinforcement. *Learn Behav* 35: 191-200.
- Sadler R, Herzig V, Schimdt WJ (2007) Repeated treatment with the NMDA antagonist MK-801 disrupts reconsolidation of memory for amphetamine-conditioned place preference. *Behav Pharmacol* 18: 699-703.
- Santini E, Muller RU, Quirk GJ (2001) Consolidation of extinction learning involves transfer from NMDA-independent to NMDA-dependent memory. *J Neurosci* 21: 9009-17.
- Schiller D, Monfils MH, Raio CM, Johnson DC, Ledoux JE, Phelps EA (2010) Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature* 463: 49-53.
- Takahashi YK, Roesch MR, Stalnaker TA, Haney RZ, Calu DJ, Taylor AR, Burke KA, Schoenbaum G (2009) The orbitofrontal cortex and ventral tegmental area are necessary for learning from unexpected outcomes. *Neuron* 62: 269-80.
- Witnauer JE, Miller RR (2009) Contrasting the overexpectation and extinction effects. *Behav Process* 81: 322-327.
- Xue Y-X, Luo Y-X, Wu P, Shi H-S, Xue L-F, Chen C, Zhu W-L, Ding Z-B, Bao Y-p, Shi J, Epstein DH, Shaham Y, Lu L (2012) A Memory Retrieval-Extinction Procedure to Prevent Drug Craving and Relapse. *Science* 336: 241-245.

Table Legend

Table 1. Experimental design and reward contingencies. A1 and A2 are auditory stimuli (clicker or tone) and V1 is a visual stimulus (illumination of stimulus lights).

Table 1

Behavioural training	Compound conditioning	Test
A1 → 3 pellets	A1V1 → 1 pellet	A1 → ∅
A2 → ∅	A2 → ∅	A2 → ∅
V1 → 3 pellets	V1 → 3 pellets	V1 → ∅

Figure Legends

Figure 1. a) Acquisition of individual auditory (A1, A2) and visual (V1) discriminations across the 10 day training protocol. b) Compound conditioning to A1V1 and continued discrimination to individual V1 and A2 presentations during the 1 day training protocol. c) Probe test responding to auditory stimuli A1 and A2 in extinction. Dashed line indicates a ratio of 0.5 – no discriminative performance. Error bars represent ± 1 S.E.M.

Figure 2. a) Acquisition of individual auditory (A1, A2) and visual (V1) discriminations across the 10 day training protocol. b) Compound conditioning to A1V1 and continued discrimination to individual V1 and A2 presentations during the 4 day training protocol. c) Probe test responding to auditory stimuli A1 and A2 in extinction. Dashed line indicates a ratio of 0.5 – no discriminative performance. Error bars represent ± 1 S.E.M.

Figure 3. a) Acquisition of individual auditory (A1, A2) and visual (V1) discriminations across the 10 day training protocol. b) Compound conditioning to A1V1 and continued discrimination to individual V1 and A2 presentations during the 4 day training protocol. c) Probe test responding to auditory stimuli A1 and A2 in extinction. Dashed line indicates a ratio of 0.5 – no discriminative performance. Error bars represent ± 1 S.E.M.

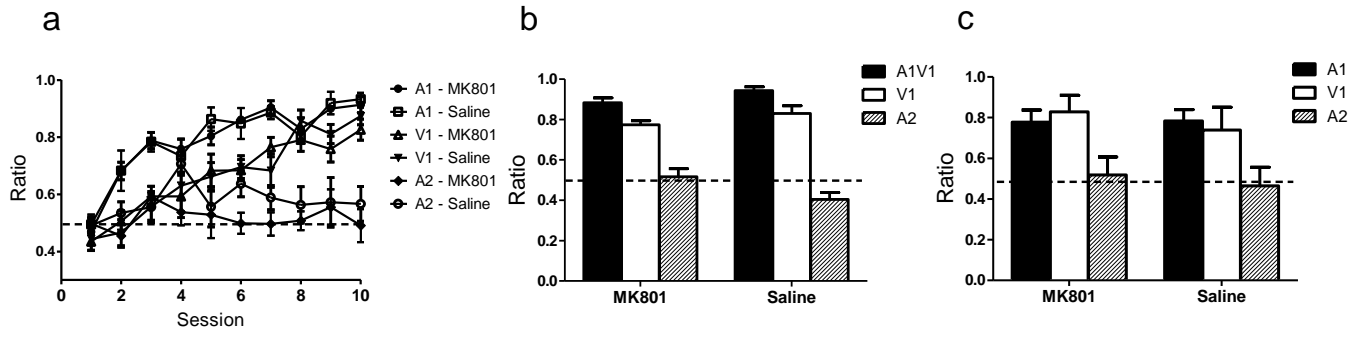


Figure 1

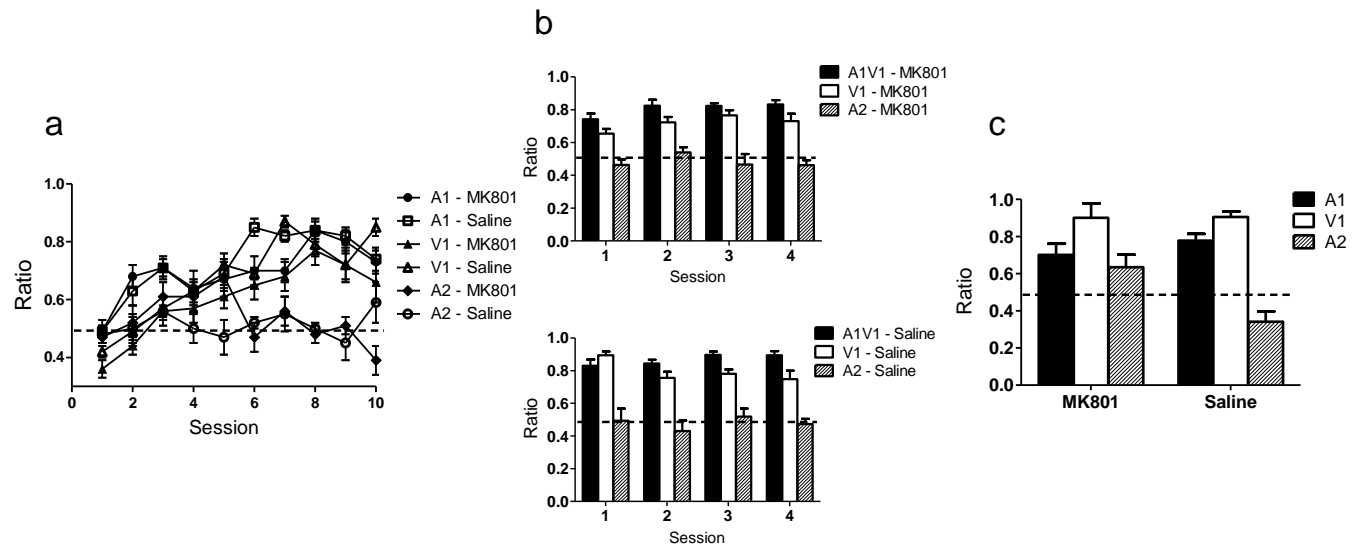


Figure 2

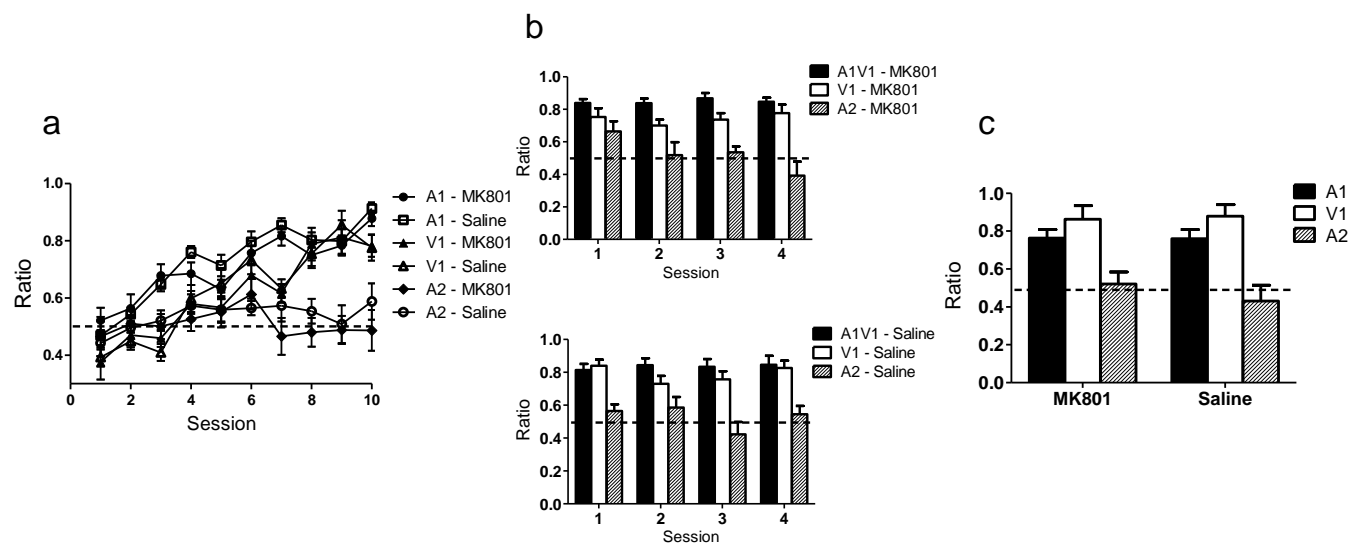


Figure 3