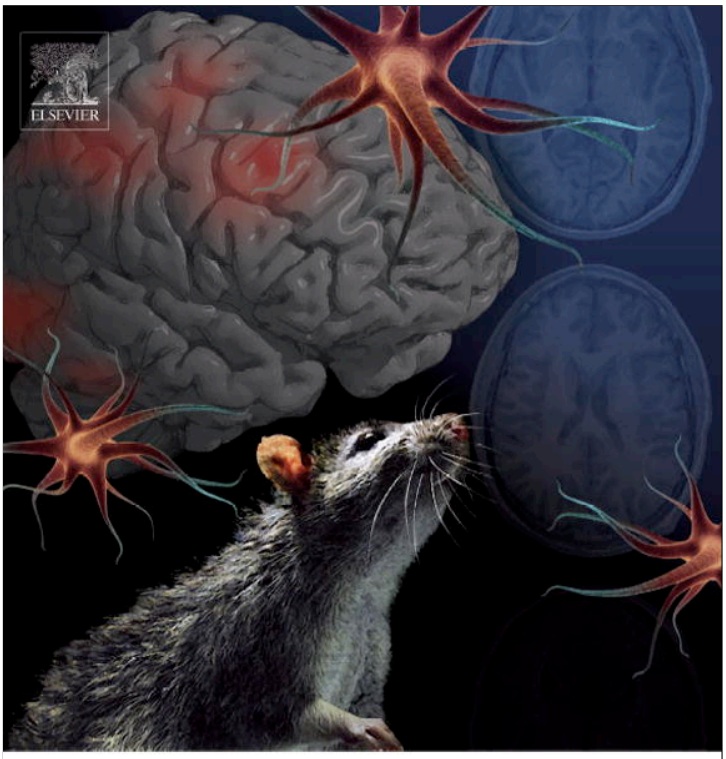

Behavioural
Brain
Research

Volume 211 Issue 2 25 August 2010

Behavioural Brain Research 211 (2010) 240–247

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Some appetitive procedures for examining associative learning in the mouse: Implications for psychopathology

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ARTICLE INFO

Article history:
Received 27 January 2010
Received in revised form 19 March 2010
Accepted 23 March 2010
Available online 31 March 2010

Keywords:
Mice
Classical conditioning
Appetitive
Latent inhibition
Blocking
Overshadowing
Conditioned inhibition

ABSTRACT

There are few demonstrations of basic associative learning phenomena using appetitive procedures in mice. This article describes procedures for obtaining four associative learning phenomena in mice, using an appetitive conditioning procedure in which the reinforcer was delivery of a sucrose pellet, and the conditioned response head entry into the food tray. Experiment 1 demonstrated latent inhibition in a within-subjects procedure. Experiment 2 demonstrates both overshadowing and blocking, and Experiment 3 Pavlovian conditioned inhibition, which was evaluated by both summation and retardation tests. These procedures all have potential relevance to current translational research questions. The specific advantages of using appetitive tasks are discussed.

Recent years have seen an extraordinary increase in the amount of research conducted with genetically modified animals, usually mice, which reflects their usefulness as a tool in understanding the mechanisms underlying behaviour. However, in purely behavioural studies of learning and memory the mammalian species of choice is typically the rat. Thus, despite the fact that there exists a coherent battery of tests for use in rat subjects, not all have yet been successfully adapted for use in mice. Consequently the experimental paradigms available for use in mice, and hence in transgenic studies, is limited, and the range of behavioural and cognitive processes that can be examined necessarily curtailed. This has implications for our understanding of brain disorders, and for our ability to test the efficacy of treatments. The studies presented here were designed to address this issue. We focussed on four different learning phenomena that, we argue, have theoretical relevance to current translational work related to psychopathology. One, latent inhibition, is already widely used in mice, but to date only in aversively motivated tasks; failure to show normal latent inhibition has been implicated in conditions such as schizophrenia and ADHD [e.g., [16,18]]. The others, overshadowing, blocking and conditioned inhibition, are to our knowledge undocumented in the mouse literature, despite their major theoretical significance. Overshadowing and blocking illustrate the basic principle that learning depends on prediction error rather than simple contiguity, and abnormal activity in dopamine neurons that appear to encode prediction error [36] has been linked to psychosis [e.g., [26]; see also [28]]. In contrast, conditioned inhibition is arguably related to the phenomenon of impulsivity [15], and abnormalities in impulsivity have been associated with conditions such as ADHD and schizophrenia. We present some simple procedures for obtaining these effects in mice, and suggest that these tasks might have both theoretical and practical relevance to current translational research.

The majority of tasks used with mice are aversively motivated - while research using rats also routinely uses appetitive procedures. Although the rapid aversive conditioning procedures can be well suited to certain manipulations, such as examining the acute effects of drugs, appetitive preparations can have certain advantages over their aversive counterparts. First, they are to be favoured from a welfare perspective. Both standing and proposed welfare legislation at European level will result in increased regulation of aversive procedures, making it likely that appetitive paradigms will become increasingly favoured. Moreover, they conform with UK Home Office recommendations by refining existing procedures. Second, using both appetitive and aversive tasks can help rule out potential artefacts; for example, if a mutation is known to affect anxiety, then this might complicate interpretation of effects obtained in aversively motivated tasks. Finally, as deficits may be obtained in one preparation but not in another [see [3]], methods for increasing the generality of an observed effect are of obvious importance.

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doi:10.1016/j.bbr.2010.03.047

The tasks described below all employ an appetitive Pavlovian conditioning procedure which is routinely used in rat subjects. The conditioned stimulus (CS) signals the delivery of a sucrose pellet to a food hopper, whose entrance is equipped with photobeams. Breaking the photobeams during the CS, but before the sucrose unconditioned stimulus (US) is delivered, is the conditioned response (CR).

1. Experiment 1

Latent inhibition (LI) refers to the observation that preexposure to a stimulus reduces the speed with which it subsequently conditions [17]. LI is often viewed as a loss of attention to the preexposed cue [cf. [20,30,41]], and failure to show normal LI has been implicated in conditions such as schizophrenia [e.g., [18]] and ADHD [e.g., [16]]. Consequently evaluation of LI in translational models is increasingly common [e.g., [1,2,4,8,14,31,32,40]], and so sound methodologies for obtaining the effect are of theoretical and practical importance.

The preparations used to study LI in mice typically use aversive outcomes; such tasks have the advantage of being quick, which can be important for certain experimental protocols. However, there are attendant problems with such an approach. For example, in many of these preparations the conditioned response is suppression of behaviour (for example freezing [e.g. [31,32]], lick suppression [e.g., [1,40]] and suppression of drinking in the conditioned taste aversion procedure [e.g., [2,8]]) and so is similar to the *unconditioned* response (UCR) to the stimulus when it is novel. This introduces the possibility that the preexposed stimulus elicits less responding at test because it has also undergone habituation during preexposure, and this attenuates its ability to elicit unconditioned suppression of behaviour. This possibility introduces the need for further control experiments, or adaptation of the task employed [but see e.g., [4,14]; cf., [7]]—and so in this respect appetitive aversions of the LI task are useful, as any unconditioned suppression produced by the nonpreexposed stimulus will interfere with, rather than mimic, the conditioned response, ruling out this potential artefact.

Another potential issue is the fact that in many aversive tasks animals are tested after, rather than during, the conditioning phase. LI is a transitory effect, as conditioning in preexposed and nonpreexposed groups eventually reaches the same asymptote; thus it is possible that differences in LI could go undetected, because they have dissipated by the time the animals are tested. In appetitive tasks conditioning occurs slowly and can be measured directly throughout the conditioning stage, making it a potentially more sensitive test.

A further issue relates to the fact that latent inhibition is *context specific* [5]—if there is a context change between preexposure and conditioning then LI is attenuated. But the outcome itself may be regarded as part of the context—so that in a typical aversive preparation the start of conditioning is accompanied by a marked change of context, from the motivationally neutral environment in which preexposure occurred to an aversive one. This could result in underestimation of the latent inhibition effect obtained. As it seems likely that in the appetitive counterpart the unconditioned stimulus is less salient, the context change would be less marked, and LI relatively preserved.

To our knowledge there is no published demonstration of latent inhibition in mice using a food-motivated task, and so this was the purpose of the first experiment. A within-subjects design was employed, based on procedures successfully used in rat subjects. In the initial, preexposure phase animals received repeated presentations of an auditory stimulus. In the subsequent, conditioning phase this preexposed stimulus and a second, novel stimulus were paired with the delivery of a sucrose pellet. The physical identity of the two stimuli was counterbalanced. It was anticipated that conditioning to the preexposed stimulus would proceed more slowly than to the novel stimulus.

1.1. Method

1.1.1. Subjects

The subjects were 12 experimentally naïve C57BL/6 mice, 6 male and 6 female, with mean *ad libitum* weight 19.85 g (range 17.0–23.2 g). They were housed in groups of three of the same sex, and had water freely available. The holding room was on a 12-h light–dark cycle (lights on 7 am to 7 pm). The animals' food intake was restricted before the start of the experiment, and they were maintained at approximately 85% of their *ad libitum* weight, by being fed a restricted daily ration of food at the end of each experimental session. This 85% target weight was increased at 3-weekly intervals, according to growth curves for the appropriate sex. To avoid singly housing the animals all the time, part of the restricted food ration (typically 1 g per mouse per day) was given in the home cages, and part in feeding cages. Immediately after their experimental session, mice that needed more than the home cage ration to achieve their target weight were placed in a feeding cage with an extra ration and returned to the home cage when this was consumed. When all mice from a particular cage had been returned they were given their home cage ration.

1.1.2. Apparatus

All the experiments reported below were conducted in 6 identical fully automated conditioning chambers housed within sound-attenuating cases containing ventilation fans (Med Associates). Each of the conditioning chambers consisted of a box (15.9 cm × 14.0 cm × 12.7 cm) with stainless steel sides, a transparent polycarbonate ceiling and back wall, and front-loading door. The floor consisted of 24 stainless steel bars, with 7.9 mm spacing between them, above a stainless steel waste pan. Mounted in the centre of the right wall was a foodcup with an opening measuring 2.5 cm × 2.5 cm × 1.9 cm. This was located 1 cm above the grid floor and was connected to a pellet dispenser through which 12-mg sucrose pellets (Formula P) could be delivered. Head entry to the foodcup was detected and recorded by the breaking of an infra-red photobeam across the opening. Mounted at the top of the back left wall was the house light, a .12-W bulb, operated at 28 V, mounted in a partially open hood that directed the light upwards. Loudspeakers for the presentation of the auditory stimuli were set in the right wall of the chamber, to the right and left of the food magazine; that to the left was connected to a home-made audio-generator that could deliver a 2 Hz, 75 dB-clicker and a 75 dB-white noise. At the rear of the chamber was an aperture through which water could be delivered, but no water was provided during these experiments. Med-PC for Windows [38] controlled experimental events, and recorded the time at which events occurred with 10-ms resolution.

Data treatment. In all the experiments that follow responding (entries into the foodcup) was measured during CS presentations, and also during the preCS periods immediately preceding each stimulus presentation. The measure of conditioning for each type of trial was then calculated as an elevation score, by subtracting the rate of responding (in responses per minute—rpm) during the preCS period from the rate of responding during the CS, pooled over all trials of that type in the session. The data were analysed using factorial ANOVA; significant interactions were examined with simple main effects analysis, using the pooled error term. Mean rates of preCS responding were obtained by pooling the rates of preCS responding over the various types of trial in each session. Finally, all the analyses reported below were performed both with and without gender as a factor. In most cases this factor did not have any impact on any of the effects of interest, and so the analyses reported below are those without gender as a factor. The two instances in which significant differences between males and females were observed are noted in the text—although as the number of animals per cell is between 4 and 6 when gender is included, these reports should be treated with caution.

1.2. Procedure

Pilot work has shown that the animals find and consume the food pellets readily in this apparatus, and so no magazine training was given in any of the experiments that follow.

Preexposure phase. All animals received preexposure to one of the experimental stimuli; three males and three females were preexposed to the clicker, and the remaining animals were preexposed to the noise. All preexposures were of 20-s duration, and each was separated by an intertrial interval of 60 s, plus a further interval of variable duration with a mean of 30 s. (In this and all the following experiments the variable portion was added to the intertrial interval so that the animals could not easily predict the time of occurrence of the following trial, and respond on the basis of this rather than of stimulus identity.) In addition each stimulus

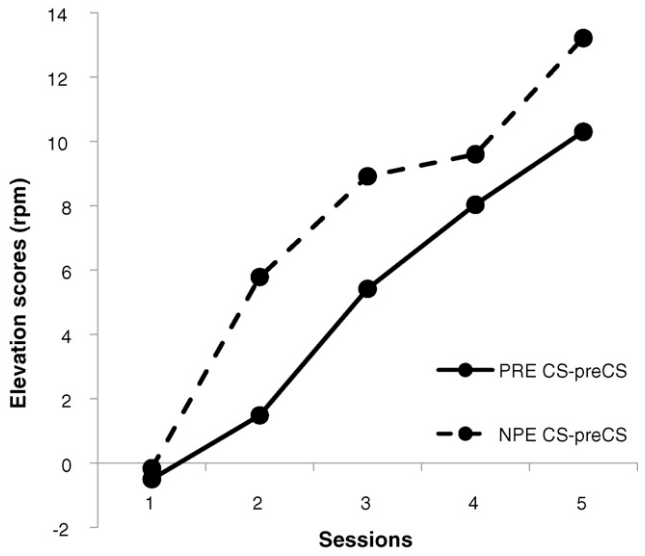

Fig. 1. Elevation scores for the preexposed (PRE) and nonpreexposed (NPE) conditioned stimuli during the 5 test sessions of Experiment 1.

presentation was preceded by a 20-s preCS period during which responding was also recorded; this yielded a total interstimulus interval (ISI) of 110 s. There were 7 sessions in this stage, and 40 trials per session. Throughout this experiment and all that follow, animals only received one session per day.

Conditioning phase. Each of the five sessions of the conditioning phase comprised 15 presentations of the preexposed stimulus and 15 of the nonpreexposed stimulus (the noise for those preexposed to the clicker, and the clicker for those preexposed to the noise), presented in a random order; offset of each stimulus was accompanied by the delivery of a sucrose pellet. In other respects these sessions were identical to those of the preexposure phase of the experiment, except for the fact that the mean duration of the variable portion of the intertrial interval was increased from 30 to 60 s, yielding a total ISI of 140 s.

1.3. Results

Preeexposure. In the initial sessions of preexposure there was a tendency for the mice to suppress their background responding during CS presentations, but this appeared to dissipate over the course of preexposure, possibly reflecting habituation to the preexposed stimulus: the mean elevation scores in sessions 1–7 were −.375, −.338, −.019, .119, .169, .081 and .15 rpm respectively; however this trend was not statistically significant, $F(6,66) = 1.22$, $p = .307$.

Conditioning. The results of the conditioning phase may be seen in Fig. 1. Responding to both cues increased over the course of this phase, but critically responding was lower to the preexposed stimulus. This impression was supported by the results of an analysis of variance with stimulus (preexposed or not) and sessions as factors, which revealed main effects of stimulus, $F(1,11) = 38.31$, $p = .0001$, sessions, $F(4,44) = 7.37$, $p = .0001$ and an interaction between these two factors, $F(4,44) = 2.68$, $p = .044$. Simple main effects analysis revealed that responding to the two stimuli differed on sessions 2, 3 and 5, $F(1,11) = 22.25$, 14.74 and 10.24 respectively, all $ps < .01$; there was also a significant effect of sessions for both stimuli, $F(4,44) = 3.36$, $p = .018$ and 4.23, $p = .006$ for preexposed and nonpreexposed stimuli respectively. PreCS rates of responding were 6.15, 10.38, 5.87, 4.78 and 4.47 rpm for sessions 1–5 respectively; ANOVA revealed that this apparent downward trend was significant, $F(4,44) = 9.96$, $p < .001$.

Table 1
Design of Experiment 2. + denotes delivery of a sucrose pellet, and − no outcome.

Group	Stage 1	Stage 2	Test
B	Light+Noise−	Click/light+Noise−	Click
O		Click/light+Noise−	Click
C		Click+ Noise−	Click

1.4. Discussion

The results of this experiment provide clear evidence of latent inhibition in an appetitive conditioning procedure. Animals responded at a significantly lower rate to the stimulus that had been preexposed than to the novel stimulus. This experiment demonstrates a relatively simple technique for obtaining LI in mice using an appetitive procedure, and the within-subject design also economises on the number of animals required, thus providing a potentially useful alternative means of assessing LI in transgenic animals.

2. Experiment 2

The second experiment examined two further key learning phenomena, overshadowing and blocking. In an overshadowing task a stimulus is conditioned in compound with another stimulus, and this results in less learning than if the stimulus had been conditioned in isolation [21]. In a blocking task two stimuli are again conditioned in compound; however in one group the added CS has previously been paired with the US, whereas in the other, control group it has not. Less learning occurs to the target CS if the CS that accompanies it during conditioning has received prior training—conditioning to it is *blocked*. Both phenomena rely on the principle that learning depends not just on CS/US contiguity, but on the discrepancy between the magnitude of the US that is *predicted* and that which actually *occurs* [34; although some view these phenomena as retrieval rather than learning deficits—see e.g. [9]]. To our knowledge there is only one published demonstration of overshadowing in mice [11], and this used an unautomated odour conditioning procedure; we believe there to be no explicit demonstrations of blocking in this species [although see e.g., [24,4]]. The purpose of this experiment was to demonstrate overshadowing and blocking with an automated appetitive conditioning procedure.

Both effects were demonstrated in the same experiment. There were three groups, and the experiment was conducted over three phases. In the first phase animals in Group B (blocking) received training in which a light (the blocking stimulus) was paired with food, while Groups O (overshadowing) and C (control) were placed in the chambers for an equivalent amount of time, but received no stimuli or food pellets. In the second phase Groups B and O received trials in which a compound of the light and a clicker were reinforced. Group C received the same training as the other two groups, except that the light was omitted. Finally animals were tested with the click (see Table 1; animals also received nonreinforced presentations of a noise in these conditioning sessions, as pilot work had shown that this increased the effectiveness of the conditioning trials).

Overshadowing was assessed by comparing responding to the click in Groups O and C. Group C was conditioned to the clicker alone whereas Group O was conditioned to the click in compound with the light. If the light overshadowed responding to the clicker, then responding should be lower to the click in Group O than in Group C at test. Blocking was assessed by comparing Groups B and O. Both these groups were conditioned to a compound of the click and the light; but Group B had received prior conditioning to the light whereas Group O had not. Blocking would thus be evident as less responding to the click in Group B than in Group O at test.

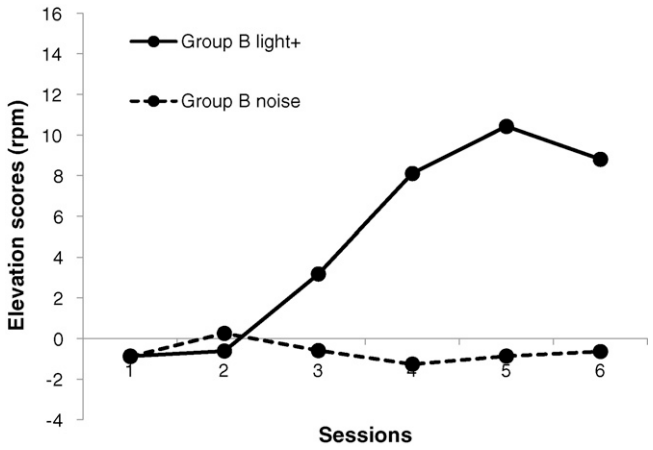

Fig. 2. Elevation scores for Group B during the reinforced light and nonreinforced noise during the six stage1 training sessions of Experiment 2.

2.1. Method

2.1.1. Subjects

The subjects were 24 experimentally naïve C57BL/6 mice, 12 male and 12 female, with mean *ad libitum* weight 21.05 g (range 16.1–27.8 g). They were housed and maintained exactly as in the previous experiment.

2.1.2. Apparatus

The apparatus was the same as that of the previous experiment.

2.2. Procedure

Stage 1. For Group B the six sessions of this stage comprised 15 presentations of the light followed by a sucrose pellet, and 15 of the noise with no consequence. The trials were presented in a random order. All stimulus presentations and preCS periods were of 20-s duration, and the intertrial interval was 60 s plus a variable interval with a mean duration of 60 s, yielding a total mean ISI of 140 s. Animals in Groups O and C remained in the chambers for the same amount of time as Group B, but received no stimulus presentations or sucrose deliveries.

Stage 2. Sessions in this stage comprised reinforced presentations of a simultaneous compound of the click and the light (Groups B and O) or a presentation of the click alone (Group C) and nonreinforced presentations of the noise. There were 12 of each trial type. In all other respects these sessions were identical to those of stage 1.

Test 1. The first test comprised 8 presentations of each of the two trial types from stage 2, plus 8 nonreinforced presentations of the click alone. There were two sessions in this stage. In all other respects these sessions were identical to those of stage 2.

Test 2. The second test was administered to Groups O and C only, and comprised two sessions each consisting of 5 trials each of the two trial types from stage 2, and 20 nonreinforced clicker presentations. In all other respects these sessions were identical to those of stage 2.

2.3. Results

Stage 1. It is clear from Fig. 2 that in Group B a discrimination rapidly developed between the reinforced light and nonreinforced noise. Analysis of variance performed on the difference scores revealed significant main effect of session, $F(5,35) = 7.77$, $p = .0001$, and trial type, $F(1,7) = 17.34$, $p = .004$, and a significant interaction between these two factors, $F(5,35) = 8.13$, $p < .0001$. Simple main effects revealed that responding differed between light and noise on sessions 4, 5 and 6, $F(1,7) = 8.37$, 12.16 and 8.51 and $p = .023$ .01 and .022 respectively; in addition there was an effect of sessions on responding to the light, $F(5,35) = 17.29$, $p < .001$, but not to the noise, $F < 1$. Responding in the preCS periods was 3.94, 4.15, 4.21, 3.24, 2.40,d 2.49 rpm for sessions 1–6 respectively; ANOVA showed that preCS responding declined significantly over the course of this stage, $F(5,35) = 3.49$, $p = .011$.

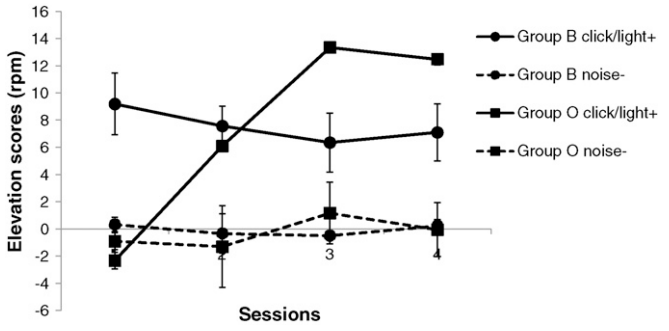

Fig. 3. Group mean elevation scores for Groups O and B, during reinforced and nonreinforced trials in the four stage 2 training sessions of Experiment 2. The bars show standard errors.

Compound conditioning. As the experiment involved two independent comparisons, between Groups C and O, and O and B, these groups' data were analysed separately.

Group O versus Group B: During the compound stage responding to the nonreinforced noise remained low in both groups; in Group B responding to the click/light compound began high, as the light had already been conditioned to asymptote for these animals, and remained steady throughout this phase (Fig. 3). In contrast, responding to the compound started low in Group O, for whom both components of the compound were novel, but increased steadily, finishing higher than Group B. These impressions were supported by the results of an ANOVA with group, trial type and sessions as factors, which revealed a significant three-way interaction, $F(3,42) = 8.47$, $p < .001$. In order to explore this interaction further, responding on reinforced and nonreinforced trials was analysed separately. An analysis of responding on reinforced trials, with group and sessions as factors, revealed a significant interaction between these two factors, $F(3,42) = 18.61$, $p < .0001$; simple main effects revealed that the two groups differed on sessions 1 and 3, $F(1,56) = 15.19$ and 5.60, $p < .001$ and $p = .022$ respectively; moreover there was an effect of sessions in Group O but not in Group B, $F(3,42) = 27.56$, $p < .0001$, and $F < 1$ respectively.

A corresponding analysis of responding on *noise*- trials revealed no effects or interactions, largest $F(3,42) = 1.78$. Finally, the rate of preCS responding was 1.89, 1.67, 1.16 and 1.52 rpm for Group B, and 4.67, 5.92, 2.78 and 2.48 rpm for Group O, for sessions 1–4 respectively; an analysis of variance, with group and sessions as factors, revealed a significant interaction between these factors, $F(3,14) = 5.54$, $p = .003$; simple main effects revealed that the preCS scores differed on sessions 1, 2 and 3, $F(1,56) = 12.20$, 28.28 and 4.17, $p < .001$, $p < .0001$ and $p = .046$ respectively; there was a main effect of sessions in Group O only, $F(3,42) = 14.14$, $p < .0001$.

Group O versus Group C: Responding in these two groups appeared quite similar over the course of this phase; responding to the noise remained low, while responding to the compound/click alone increased steadily in both groups, and preCS responding decreased (Fig. 4). An ANOVA with group, trial type and sessions as factors revealed a main effect of trial type, $F(1,14) = 30.36$, $p = .0001$ and of sessions, $F(3,42) = 30.48$, $p < .0001$ and an interaction between these two factors, ($F(3,42) = 15.45$, $p < .0001$; no effect or interaction involving group was significant, largest $F(3,42) = 1.26$. Simple main effects analysis revealed that the discrimination between reinforced and nonreinforced trials was significant on sessions 2, 3 and 4, $F(1,56) = 6.29$, 29.48 and 51.62, $p = .015$, $p < .0001$

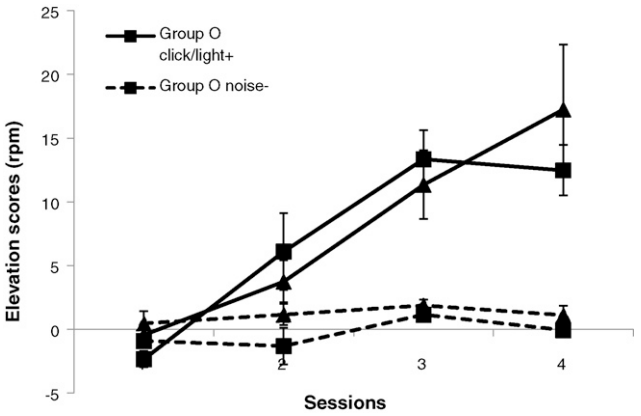

Fig. 4. Group mean elevation scores for Groups O and C during reinforced and nonreinforced trials in the four stage 2 training sessions of Experiment 2. The bars show standard errors.

and $p < .0001$ respectively, and that there was an effect of sessions on reinforced trials only, $F(3,84) = 42.76$, $p < .0001$ and $F < 1$ for reinforced and nonreinforced trials respectively. The group mean rates of preCS responding for these four sessions were 4.67, 5.91, 2.78 and 2.48 rpm for Group O, and 5.08, 7.11, 3.67, and 2.55 rpm for Group C, for sessions 1–4 respectively; analysis of variance revealed only a significant main effect of sessions, $F(3,42) = 13.60$, $p < .0001$.

2.3.1. Test

Group O versus Group B: The results of the test may be seen in Fig. 5. As predicted, responding in Group B was substantially below that in Group O in the first test session; this result was supported by the results of an analysis of variance with group and sessions as factors, which revealed a significant interaction between these two factors, $F(1,14) = 11.09$, $p = .005$; simple main effects revealed that responding in the two groups differed on session 1, $F(1,28) = 10.68$, $p = .003$, but not on session 2, $F < 1$. Rates of preCS responding for sessions 1 and 2 were 1.53 and 0.91 rpm for Group B, and 1.55 and 1.06 rpm for Group O; analysis of variance revealed only a main effect of sessions, $F(1,14) = 6.36$, $p = .024$; critically the main effect of group and interaction were not significant, $Fs < 1$, suggesting that there was no group difference in preCS rates that could compromise interpretation of responding during the click. Although it is unclear why blocking was only observed in the first test block, it seems likely that is was due in part to rapid extinction in Group O.

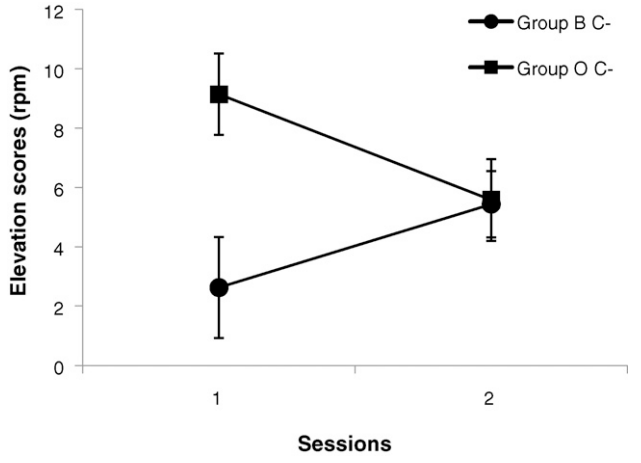

Fig. 5. Group mean elevation scores for Groups O and B during test trials of the first two test sessions of Experiment 2. The bars show standard errors.

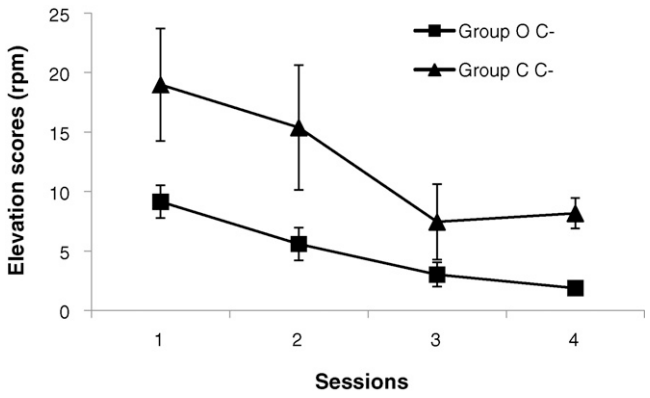

Fig. 6. Group mean elevation scores for Groups O and C during test trials of the four test sessions of Experiment 2. The bars show standard errors.

Group O versus Group C: The results of the four test sessions may be seen on Fig. 6, and here it is clear that, as predicted, Group O was responding at a lower rate than Group C. An ANOVA with group and sessions as factors revealed a main effect of group, $F(1,14) = 4.91$, $p = .044$; there was also a main effect of sessions, $F(3,42) = 11.70$, $p < .0001$, but the interaction between these factors was not significant, $F(3,42) = 1.09$. Rates of preCS responding were 1.55, 1.06, 1.30 and 0.94 rpm for Group O, and 2.73, 3.56, 3.55 and 2.13 rpm for Group C, for sessions 1–4 respectively; analysis of variance revealed main effects of group and session, $F(1,14) = 29.48$, $p = .0001$ and $F(3,42) = 2.95$, $p = .043$ and no interaction, $F(3,42) = 1.34$. Although preCS rates were higher in the control group, the difference was very small in comparison to that seen during CS responding; thus the overshadowing effect is unlikely to reflect only a greater tendency to respond in Group C.

2.4. Discussion

This experiment provides clear demonstrations of both overshadowing and blocking in an appetitive conditioning procedure with C57BL/6 mice. In this experiment, our analyses with gender as a factor revealed a small difference between male and female mice in Groups O and C, showing that males responded more on reinforced trials; also the group difference in preCS responding at test appeared greater in the female animals; however the key test results were statistically identical in males and females.

The design of our blocking experiment is very simple, and thus alternative explanations could be considered. For example, Group B experienced reinforcement in the context during the initial stage of training whereas Group O did not. Although it is not clear how this could be responsible for the pattern of results that was observed, it is a potential confound. A better approach might be to have given all groups training with a CS in the first stage—but while for Group B this CS would also be present in the second stage of training, for Groups O and C it would not.

3. Experiment 3

The third experiment examined the phenomenon of conditioned inhibition [29]. In a conditioned inhibition (CI) task a stimulus signals the absence of an otherwise expected outcome. As a result of such training the inhibitor suppresses conditioned responding to other signals for the US, and is slower to become a signal for the US than a preexposed stimulus. Conditioned inhibition is potentially related to the variety of conditions said to be characterised by a problem with impulsivity [cf. [25,6]], such as ADHD and personality disorders. For example, some tasks used to measure impulsivity in animals (such as the DRL procedure [e.g.

Table 2
Design of Experiment 3. + denotes delivery of a sucrose pellet, and − no outcome.

Group	Stage 1	Summation test	Retardation test
E	Noise+Click+Clicklight−	Noise−Noiselight−	Light+
C	Noise+ Click+ Light−	Noise−Noiselight−	Light+

[39], also [10]] which refers to a situation in which responding to stimuli associated with reinforcement prevents the occurrence of that reinforcement) may be regarded as variant of conditioned inhibition tasks [cf. [19,37]].

Two groups of animals received training during which a click signalled food delivery. The experimental group (Group E) also received trials on which a click/light compound was paired with no outcome, so that the light would signal the absence of food and become a conditioned inhibitor (Table 2). The control group (C) received nonreinforced trials with the light alone, so that although its exposure was equated to that in the experimental group, it should acquire no inhibitory properties. Conditioned inhibition was then assessed using a *summation* test, in which the ability of the light to suppress responding to another signal for food was evaluated; to this end all animals were also given pairings of a white noise, with food, and the effect of the light on responding to the noise was then assessed. CI would be evident as greater suppression of responding to the noise in the experimental animals. In addition, in order to rule out the possibility that this effect was due to attentional factors (specifically that the light was without effect in the control group because these animals had learned to ignore it during the inhibitory training phase) animals were also given a *retardation* test of CI, in which the light itself was paired with food. Here CI would be evident as slower learning in the experimental animals—an effect which clearly could not result from poor attention to the light in control subjects [cf. [33]]. In addition, all animals were given brief preexposure to the light, both alone and in compound with the other auditory cues, before the start of training proper. This was because our pilot work has demonstrated that the unconditioned suppression of responding elicited by the unfamiliar click/light compound at test can mask the expression of conditioned inhibition in the summation test—possibly because animals in Group E have experienced auditory visual compounds throughout training, whereas those in Group C have not.

3.1. Method

3.1.1. Subjects

The subjects were 18 experimentally naïve C57BL/6 mice, 9 males and 9 females, with mean *ad libitum* weight 19.57 g (range 15.2–23.4 g). There were 8 subjects in Group E (3 males and 5 females) and 10 in Group C (6 males and 4 females). They were housed and maintained exactly as in the previous experiment.

3.1.2. Apparatus

The apparatus was identical to that of the previous experiment.

3.2. Procedure

Preexposure. This comprised two sessions, in each of which animals received 10 preexposures each of the light, click/light and noise/light compounds. In this stage and the rest of the experiment the intertrial interval was 60 s plus a variable interval of mean duration 60 s, and the preCS and CS periods were 20 s, yielding a total mean SI of 140 s. The different trial types were presented in a semi-random order.

Excitor training. Then subjects received four sessions in which the click and the noise were paired with food; there were 15 of each type of trial per session.

Inhibition training. All subjects continued to receive reinforced trials with the clicker and the noise. However animals in Group E also received nonreinforced presentations of the click/light compound, while those in Group C simply received nonreinforced trials with the light. In the first 8 sessions of this stage subjects received 6 trials each with the click and the noise, and 18 with the light or click/light; in the remaining 7 sessions all received 8 trials with the click and only four with the noise, while continuing to receive 18 with the light or click/light.

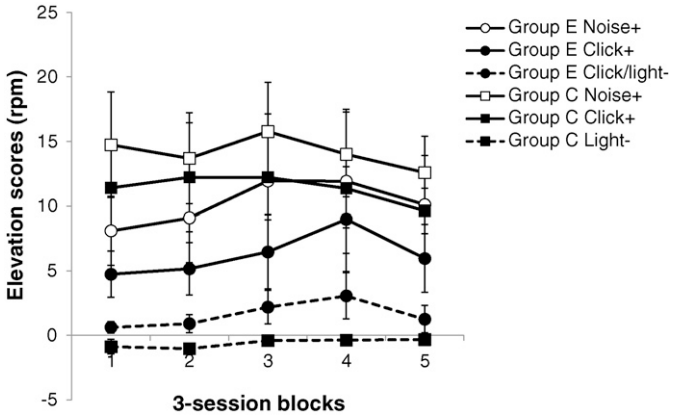

Fig. 7. Group mean elevation scores for the three trial types of the five 3-session blocks of inhibition training in Experiment 3. The bars show standard errors.

Summation test. The summation test session comprised 15 trials with the noise and 15 with the noise/light compound. No reinforcers were delivered in this session.

Retardation test. The five retardation sessions each comprised 30 reinforced trials with the light.

3.3. Results

Excitor training. During this stage animals learned to respond to the click and the noise; in the last session of this stage the mean difference score to the noise and the click was, for Group E, 8.00 and 5.18 rpm respectively, and 13.52 and 8.88 rpm for Group C; ANOVA with group and trial type as factors revealed that responding to noise and click differed significantly, $F(1,16) = 16.58$, $p = .001$, but that responding in Groups E and C did not, $F < 1$; there was no interaction between these two factors, $F < .1$.

Inhibition training. The data from this stage may be seen in Fig. 7; they were pooled into five 3-session blocks. It is clear that a good discrimination developed between the click and the click/light compound in both groups. Analysis of variance with Group (E or C), trial type and block as factors revealed only a significant main effect of trial type, $F(1,16) = 20.43$, $p = .0003$; the interaction between trial type and group approached, but did not quite attain statistical significance, $F(1,16) = 3.95$, $p = .064$, probably reflecting the fact that the discrimination appeared slightly larger in Group C. Nothing else was significant, largest $F(4,64) = 1.68$. There was no group difference in responding to the noise over these sessions, $F < 1$. The preCS response rates were 1.46, 1.53, 0.98, 1.17 and 0.59 rpm for Group E, and 2.67, 2.05, 1.49, 1.18, and 0.80 rpm for Group C, for blocks 1–5 respectively; this slight group difference in preCS responding was almost significant, $F(1,16) = 4.20$, $p < .057$, and preCS responding decreased over blocks, $F(4,64) = 11.51$, $p < .0001$.

Summation test: It is common in tests of this type to employ suppression ratios, of form $A/A + B$, where A is the rate of responding to the noise/light compound, and B the rate of responding to the noise alone; this is in order to eliminate variability due to differences in responding to the noise. The resulting ratios may be seen in Fig. 8. Analysis of variance revealed that the apparent difference, greater suppression in Group E, was highly significant, $F(1,16) = 15.62$, $p = .001$. The rates of responding to the noise were 7.54 rpm in Group E and 2.91 rpm in Group C; this differ-

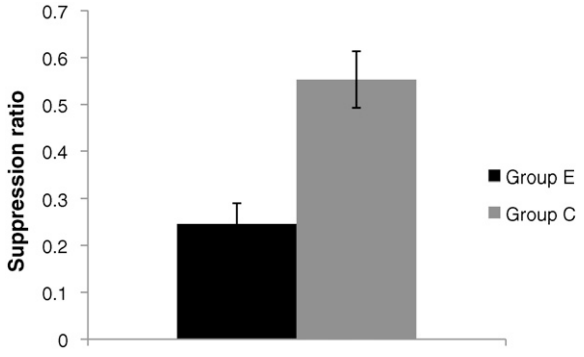

Fig. 8. Group mean suppression ratios from the summation test sessions of Experiment 3. The bars show standard errors.

ence, although numerically large was not significant, $F(1,16) = 2.05$, $p = .17$, and appeared to be due to one animal in Group E who was responding at an exceptionally high rate, and more than two standard deviations from the mean; the corresponding rates during the noise/light compound were 2.14 and 2.64 rpm respectively. To rule out the possibility that the differences we observed were in some way due to this high responder, we also tried excluding this subject; this yielded a mean rate of responding to the noise of 4.29 rpm for Group E, which did not differ from the corresponding score in Group C, $F < 1$. The suppression ratio in Group E after excluding this subject was 0.255, and this differed significantly from the mean ratio in Group C, $F(1,15) = 12.94$, $p = .003$. The rates of preCS responding were 0.23 rpm in Group E and 0.25 rpm in Group C, and these rates did not differ, $F < 1$.

Retardation test: Data from the retardation test are seen in Fig. 9, and again it seems clear that although both groups increased their levels of responding to the light, Group E responded at a lower rate than Group C. Analysis of variance confirmed this description, revealing a main effect of group, $F(1,16) = 4.64$, $p = .047$, and of sessions, $F(4,64) = 2.80$, $p = .033$; there was no interaction between these two factors, $F < 1$. The mean rate of preCS responding was 0.68, 1.16, 0.70, 0.86, 0.68 and 1.24 rpm for Group E, and 1.31, 1.57, 1.01, 1.27, 1.85 and 2.38 rpm for group C, for sessions 1–5 respectively; ANOVA revealed that preCS responding did not differ between the two groups, $F(1,16) = 3.80$, $p = .069$. Although levels of responding were low by the end of testing, we do not feel this compromises the reliability of the result, as the deficit in retardation tests of this type can be quite transient, and disappear with extended training.

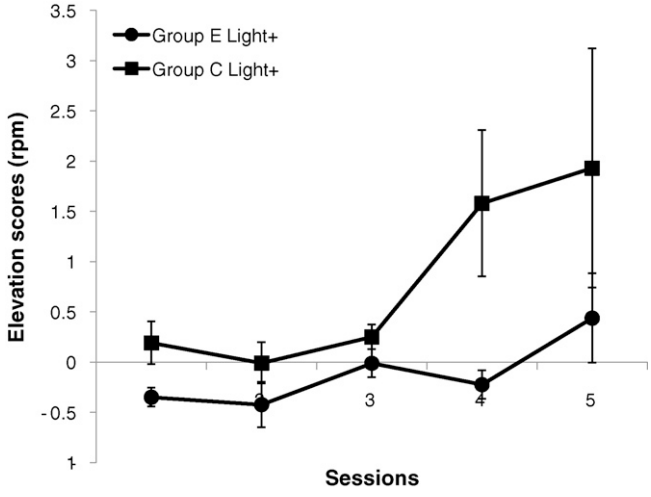

Fig. 9. Group mean elevation scores to the light in the five retardation test sessions of Experiment 3. The bars show standard errors.

3.4. Discussion

The final experiment provided evidence for conditioned inhibition. In Group E the light passed both summation and retardation tests, suppressing responding to the white noise more effectively than the control stimulus, but itself becoming a signal for sucrose less effectively than the control stimulus. Because the light passed both tests we may rule out alternative explanations of the results, such as differences in attention to the light in the two groups; although lack of suppression in Group C in the summation test could be due to poor attention to the light in this group, more rapid acquisition in the retardation test cannot.

We have argued that the light should acquire no inhibitory properties in Group C, as these animals simply experienced nonreinforced trials with the light, and reinforced trials with the noise and the click, during the initial training stage. Nonetheless, sometimes this *differential conditioning procedure* can produce inhibitory conditioning to the nonreinforced CS, an effect that is usually attributed to the fact that the nonreinforced CS signals the absence of the US signalled by the context [cf. [19], p. 34]. The extent to which inhibition develops in these circumstances thus depends on the degree to which the context signals the US—and in a procedure with widely spaced trials, it is unlikely that the context is going to be a stronger signal for the outcome than an explicit conditioned stimulus. Thus, although some inhibition might have accrued to the light in Group C, it is likely to have been weaker than that accruing in Group E, simply because the light was presented with a more potent signal for the outcome in the latter group. In this sense our control condition provided a conservative estimate of conditioned inhibition in the experimental animals.

4. General discussion

We have demonstrated four key learning phenomena in mice, using an appetitive conditioning procedure. Experiment 1 demonstrated latent inhibition. Although LI has been reported in a variety of aversive conditioning tasks, we believe this to be the first demonstration in an appetitive conditioning procedure. Operationally LI refers to the ease with which a stimulus may be *learned* about, and this is reflected in theoretical interpretations of the effect. For example, [30] Pearce and Hall used the concept of controlled and automatic processing [35] to discriminate between the attention required to condition to a stimulus (which they termed *associability* and which is reduced during latent inhibition) and the attention required to *respond* to a stimulus that has been conditioned (which is not). They argued that associability depends on prior experience of what a stimulus predicts, distinct from its intrinsic salience which is a function of its physical characteristics. But LI has also been explained as a failure not of learning, but of performance [see [9]]. Nonetheless, whichever view is taken, the appetitive procedure presented here provides a useful alternative means of examining this theoretically important phenomenon.

Experiment 2 demonstrated overshadowing and blocking effects—both of which represent failures of learning because the US is unsurprising [34]. This explanation derived from the Rescorla–Wagner model relies on the lack of prediction error reducing *US* processing. But others [e.g. [30]] argue that a predicted US does not retard learning directly, as Rescorla–Wagner asserts, but *indirectly*, by reducing the associability of the CSs that precede it—and that this is responsible for both overshadowing and blocking effects. Like LI, these effects are mediated by changes in the *associability* of the CS; and because of this type of analysis, overshadowing and blocking have been regarded as selective attention/learning tasks, of interest for the same reasons as latent inhibition [e.g. [13,27]]. There is now a considerable amount of

independent evidence for both CS and US processing accounts of overshadowing and blocking [see, e.g., [12,22]], and so these effects could in principle be used to investigate abnormalities in both CS and US processing [22]. However, a conservative view would be that both are probably multiply determined, and so if a definitive interpretation is necessary, more analytical experiments will probably be required.

Finally, Experiment 3 provided evidence of conditioned inhibition, assessed conservatively by both summation and retardation tests. Inhibition is a broad term that can be taken to refer to a whole range of situations in which responding is suppressed, commonly indexed by tasks such as go/no go and pre-pulse inhibition. The conditioned inhibition task presented here provides a different index of inhibitory processing ability.

In summary, the work here provides a framework that should be of use to those with interests in attentional abnormalities, prediction error, and inhibitory processing. Moreover, adopting these well established tests allows direct experimental contact with the huge body of evidence accumulated through research into learning theory. Thus there is the potential to substantially increase the range and subtlety of experimental questions that may be asked in studies with genetically modified animals. This in turn will have important implications for the questions that may be asked about the mechanisms underlying many types of psychopathology, and the ways in which they may be treated.

Acknowledgements

Thanks to Helen Cassaday, Jasper Robinson, and Marie Pardon for helpful comments on the manuscript.

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