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

Charlotte Bonardi[a,*], Craig Bartle[a], Kathryn Bowles[a], Felicity de Pulford[a], Dómhnall J. Jennings[b]

[a] School of Psychology, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom
[b] Institute of Neuroscience, Newcastle University, Henry Wellcome Building, Framlington Place, Newcastle NE2 4HH, United Kingdom

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.

* Corresponding author. Tel.: +44 115 8467927; fax: +44 115 9515324.
E-mail address: cmb@psychology.nottingham.ac.uk (C. Bonardi).

0166-4328/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
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 audiogenerator 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