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Potentiation rather than distraction in a trace fear conditioning procedure

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ABSTRACT

Trace conditioning procedures are defined by the introduction of a trace interval between conditioned stimulus (CS, e.g. noise or light) offset and unconditioned stimulus (US, e.g. footshock). The introduction of an additional stimulus as a distractor has been suggested to increase the attentional demands of the task and to extend the usefulness of the behavioural model. In Experiment 1, the CS was noise and the distractor was provided by an intermittent light. In Experiment 2, the CS was light and the distractor was provided by an intermittent noise. In both experiments, the introduction of a 10s trace interval weakened associative learning compared with that seen in a 0s delay conditioned group. However, there was no consistent evidence of distraction. On the contrary, in Experiment 1, associative learning was stronger (in both trace and delay conditioned groups) for rats conditioned also in the presence of the intermittent light. In Experiment 2, there was no such effect when the roles of the stimuli were reversed. The results of Experiment 2 did however confirm the particular salience of the noise stimulus. The finding of increased associative learning dependent on salience is consistent with arousal-mediated effects on associative learning.

1. Introduction

Trace conditioning procedures are defined by the introduction of a trace interval between conditioned stimulus (CS, e.g. noise) offset and unconditioned stimulus (US, e.g. food or footshock) onset (Kamin, 1965). The characteristic result – of reduced conditioning in consequence of temporal discontiguity – can be demonstrated in a variety of Pavlovian conditioning procedures (both appetitive and aversive) but aversive procedures have been much more widely adopted, both because acquisition is rapid and the neural circuitry necessary to basic fear conditioning is well documented.

The ability to bridge time delays to show associative learning in a trace conditioning procedure allows animals to associate what goes with what, when potentially causally-related events are separated in time. Thus, as a measure of working memory, trace conditioning holds promise as a behavioural assay for age-related memory decline: it is impaired in aged rabbits (Graves and Solomon, 1985), rats (McEchron et al., 2004; Moyer and Brown, 2006) and mice (Galvez et al., 2011; Kishimoto et al., 2001), as well as in a mouse model of senescence (Lopez-Ramos et al., 2012).

In younger adult animals, trace conditioning has been shown to require an intact hippocampus to process the temporal gap between the CS and US (McEchron et al., 1998; Weiss et al., 1999; McEchron et al., 2000; Beylin et al., 2001; Quinn et al., 2002; Rogers and Kesner, 2006) and – as is the case for tasks which measure declarative memory – seems to depend upon awareness (Clark and Squire, 1998). Consistent with known projections from hippocampus, medial prefrontal cortex (mPFC) has also been shown to be part of the trace conditioning network. Comparing across a variety of trace conditioning preparations, the emerging pattern seems to be a role for the prelimbic (PL) sub-region when memory processes are directly engaged, for example when retention is tested (Runyan et al., 2004; Oswald et al., 2008, 2010), when neuronal activity is examined during a relatively long trace interval (Gilmartin and McEchron, 2005) or when longer CS durations compound the memory load (McLaughlin et al., 2002). In contrast, there is evidence to suggest that the anterior cingulate (AC) sub-region is important for earlier acquisition-related processes (Kronforst-Collins and Disterhoft, 1998; Weible et al., 2003, 2000; Kalmbach et al., 2009; Hattori et al., 2014). This distinction may relate to the role of AC in attentional processes and – consistent with this interpretation – excitoxic lesions of the AC sub-region of mPFC were reported to reduce trace conditioning in a mouse fear conditioning

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procedure which was sensitive to the effects of an experimental distractor stimulus (Han et al., 2003).

In eye-blink trace conditioning procedures, human participants' ability to report on the CS-US relationship is similarly impaired by concurrent distraction, and this finding has also been confirmed using a trace fear conditioning procedure, in this case with a finger shock US Carter et al. (2003). This latter study was designed to be analogous to the Han et al. rodent study, though the nature of the experimental distraction was different. Carter et al. (2003) used a concurrent *n*-back task, which required participants to track previously presented digits in a list of numbers, by way of a distractor intended to compete for working memory capacity. As was the case in the Han et al. (2003) mouse conditioning study, distractor stimuli were similarly found to interfere with trace fear conditioning, delay conditioning being much more resilient to the effects of distraction (Carter et al., 2003).

Thus, it has been argued that the use of a distractor is an important procedural modification in order to model the putative attentional role of AC in a task with demonstrated sensitivity to attentional parameters and high translational relevance to our understanding of normal human ageing. Moreover, it follows that increased attentional load may be a contributing factor in the event trace conditioning deficits are demonstrated in rodent models, at least to the extent that these depend on attentional processes mediated by the AC (Pezze et al., 2016).

In a series of trace conditioning experiments using rat fear conditioning procedures, we have routinely used an extended background stimulus (Norman and Cassaday, 2003; Horsley and Cassaday, 2007; Grimond-Billa et al., 2008; Nelson et al., 2011; Pezze et al., 2016). This was provided by a continuously flashing light presented for the full duration of the conditioning session and has been intended to provide an experimental context rather than a distractor stimulus. The distractor stimulus used by Han et al. (2003) was also provided by a flashing light, different only in its temporal properties. Therefore, since distraction is of both theoretical and practical importance – to both the interpretation and demonstration of trace conditioning impairments – we adapted our existing fear conditioning procedure in an attempt to establish a reliable distractor suitable for use in rats.

It must be noted that under some experimental circumstances the introduction of extraneous stimuli is already known to result in potentiation rather than distraction (Durlach and Rescorla, 1980; Pearce et al., 1981; Rescorla, 1982; Hall and Honey, 1993). It was not our objective to add to this body of knowledge. Rather the present study sought to explore the feasibility of adapting a published distractor procedure, in order (in the longer term) to further examine the role of AC in working memory. This behavioural work was done in a rat rather than a mouse model and using a different variant of trace fear conditioning (suppression of licking rather than freezing), as per a number of earlier studies conducted to examine the neuropharmacological substrates of trace conditioning (Norman and Cassaday, 2003; Horsley and Cassaday, 2007; Grimond-Billa et al., 2008; Nelson et al., 2011; Pezze et al., 2016).

2. Methods

2.1. Animals

In each of two experiments, 48 experimentally naïve adult male Wistar rats (Charles River, UK) were caged in groups of 4 in individually ventilated cages (IVCs), on a 12:12 h light/dark cycle with food and water *ad libitum*. Cages were cleaned out twice per week and cardboard tubes and nesting materials were provided as environmental enrichment. The rats were handled for approximately 5 min per day for 1 week and then at mean weight 199 g (range 168–224 g) in Experiment 1 and 218 g (range 193–246 g) in Experiment 2 were placed on water deprivation immediately prior the conditioning procedures. One rat (in Experiment 1) was humanely killed for an unrelated reason, on the advice of the Named Veterinary Surgeon. All procedures were carried out in accordance with the United Kingdom (UK) Animals Scientific Procedures Act 1986, Project License number PPL 40/3716, which ensures full compliance with the EU Directive 2010/63/EU for animal experiments.

2.2. Behavioural conditioning apparatus

Four identical fully automated conditioning boxes, housed within sound-attenuating cases containing ventilation fans (Cambridge Cognition, Cambridge, UK), were used. The inner conditioning box walls consisted of plain steel (25 cm × 25 cm × 22 cm high) with a Plexiglas door (27 cm × 21 cm high), at the front. The floor was a shock grid with steel bars 1 cm apart and 1 cm above the lip of a 7 cm deep sawdust tray. A waterspout was mounted on one wall. The spout was 5 cm above the floor and connected to a lickometer supplied by a pump. Licks were registered by a break in the photobeam within the spout, which also triggered water delivery of 0.05 ml per lick. The waterspout was illuminated when water was available. A loudspeaker for the presentation of auditory stimuli was set in the roof. In Experiment 1, a 5s mixed frequency continuous noise set at 80 dB served as the CS and the distractor was an intermittent light provided by the three wall-mounted dome-shaped stimulus lights and the house light set to flash intermittently (130 ms on/off, at 8 lx, for 3 s duration with an interstimulus interval randomly chosen from 5, 10, 15 or 20 s). In Experiment 2, a 5 s flashing light served as the CS (in this case provided by the three wall mounted stimulus lights and the house light flashing (500 ms on/off, at 8 lx) and the distractor was an intermittent noise (130 ms on/off for 3 s, set at 80 dB with an interstimulus interval sequence randomly chosen from 5, 10, 15 or 20 s). Footshock of 1 s duration and 1 mA intensity provided the UCS. This was delivered through the grid floor by a constant current shock generator (pulsed voltage: output square wave 10 ms on, 80 ms off, 370 V peak under no load conditions, MISAC Systems, Newbury, UK). Stimulus control and data collection was by an Acorn Archimedes RISC computer programmed in Basic with additional interfacing using an Arachnid extension (Cambridge Cognition).

2.3. Behavioural conditioning procedure

Water deprivation was introduced 1 day prior to shaping and all rats received 1 h of *ad libitum* access to water in their home cage at the same time each day, in addition to access to water in the conditioning apparatus on all the experimental days except conditioning. The stages of the trace conditioning procedure were as follows:

2.3.1. Pre-conditioning to establish baseline lick response

To initiate licking, rats were placed in the conditioning boxes with one of their cage mates and were shaped for 1 day until all drank from the waterspout. No data were recorded. Thereafter, animals were individually assigned to a conditioning box for the duration of the experiment (counterbalanced by experimental group). There then followed 5 days of pre-training, in which rats drank in their conditioning boxes for 15 min each day (timed from first lick). The drinking spout was illuminated throughout, but no other stimuli were presented in this phase. Latency to first lick was recorded to assess any pre-existing differences in readiness to drink (prior to conditioning).