Behavioural Brain Research 230 (2012) 259–267

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Research report

Dorsal hippocampal lesions disrupt Pavlovian delay conditioning and conditioned-response timing

Shu K.E. Tam*,[1], Charlotte Bonardi[2]

School of Psychology, University of Nottingham, University Park, Nottingham, NG7 2RD, United Kingdom

ARTICLE INFO

Article history:
Received 24 October 2011
Received in revised form 16 January 2012
Accepted 8 February 2012
Available online 17 February 2012

Keywords:
Hippocampus
Inter-stimulus interval
Delay conditioning
Interval timing

ABSTRACT

The involvement of the rat dorsal hippocampus (dhpc) in Pavlovian conditioning and timing of conditioned responding was examined in an appetitive preparation in which presentation of a relatively long, 40-s auditory conditioned stimulus (CS) was followed immediately by food delivery. Dorsal hippocampal lesions impaired Pavlovian conditioning in this task. They also produced a deficit in interval timing, replicating previous findings with short CSs. The conditioning and timing deficits observed are consistent with the findings from single-unit recording studies in other species, and suggest that the involvement of the dhpc in Pavlovian processes could be more general than is assumed by many of the current theories of hippocampal function.

1. Introduction

The *dorsal* hippocampus (*d*hpc) seems to play only a limited role in Pavlovian processes. Lesions of this area have no effect on aversive delay conditioning, in which the conditioned stimulus (CS) is unimodal and contiguous with delivery of an aversive unconditioned stimulus [US; 1–4]. In contrast, dhpc damage disrupts acquisition of fear contextual conditioning, in which the CS is multidimensional [2,5], and fear trace conditioning, in which the CS and US are separated by an empty interval [1,2,6]. These behavioural dissociations have been taken to suggest that while the dhpc plays no general role in the Pavlovian processes responsible for the formation or retrieval of CS → US associations, it is involved in the formation of contextual or configural representations [7,8], or in the maintenance of stimulus trace across the CS → US interval [9,10]. But findings from *appetitive* conditioning preparations do not support these ideas. Although there is no effect of dhpc lesions on delay conditioning [just as in the aversive case, 11–15], acquisition of appetitive contextual [16] and trace conditioning [17] has also been found to be unaffected by dhpc lesions. These inconsistencies between aversive and appetitive preparations cast doubt on the suggestion that the dhpc plays a fundamental role in the processes underlying performance in contextual or trace conditioning, as this would imply dhpc involvement regardless of the valence of the US employed; although the fact that appetitive and aversive conditioning tasks differ on many dimensions other than US valence should of course be acknowledged as another possible source of this discrepancy.

Findings from electrophysiological studies reveal further inconsistencies with this theoretical analysis. Single-unit recording studies reveal learning-related changes in dhpc pyramidal neuronal activity during Pavlovian delay as well as trace conditioning, in both appetitive and aversive preparations [18–23]; similar changes are also shown, albeit to a lesser extent, in the ventral portion of the structure [vhpc; 23]. These reports suggest that involvement of the dhpc in Pavlovian processes might be more general than is assumed by many of the current theories of hippocampal function [for similar suggestions, see 24–27]. However, if the dhpc *does* play a role in the fundamental conditioning mechanism, one might wonder why no lesion deficit has been found in most reported delay conditioning studies [for exceptions, see 24,28,29], or in any appetitive conditioning task [11–17].

Some light has been thrown on these apparent contradictions in delay conditioning preparations by the study of Beylin et al. [30], in which they investigated the effect of manipulating the CS duration, or inter-stimulus interval (ISI)—the period between CS onset and US delivery, on conditioning in animals with hippocampal damage. They reported a hippocampal lesion deficit in eyeblink delay conditioning when the ISI was relatively long (1.4 s), but not when it was relatively short (0.75 s), suggesting that the length of the ISI might determine whether or not a lesion deficit is observed.

* Corresponding author. Tel.: +44 7854596500.
E-mail addresses: eric.tam@ndcn.ox.ac.uk (S.K.E. Tam), charlotte.bonardi@nottingham.ac.uk (C. Bonardi).
[1] Present address: Nuffield Laboratory of Ophthalmology, University of Oxford, Level 5–6 West Wing, John Radcliffe Hospital, Headley Way, Oxford OX3 9DU, United Kingdom.
[2] Tel.: +44 1158467927; fax: +44 1159515324.

0166-4328
doi:10.1016/j.bbr.2012.02.016

These findings confirm that even Pavlovian delay conditioning can be affected by hippocampal lesions provided that the ISI is long enough, and are thus consistent with the electrophysiological findings [18–23] suggesting a general role of the dhpc in Pavlovian processes. Nevertheless, Beylin et al. [30] damaged the entire hippocampus; it is unclear if a similar deficit could be found after lesions confined to the dhpc. Moreover, there is as yet no evidence that using a longer ISI can also induce a delay conditioning deficit in an *appetitive* preparation after dhpc damage. This is particularly relevant, given that the dhpc-induced deficits listed above have been almost without exception demonstrated in aversively motivated tasks. Accordingly the objective of the present study was to see if a Pavlovian delay conditioning deficit could be observed in an appetitive preparation when the ISI was relatively long. As previous appetitive delay conditioning studies that did not find any lesion deficit employed ISIs of shorter than 20 s in duration [11–17], we employed an ISI duration of 40 s.

We also took the opportunity to examine the involvement of the dhpc in interval timing during Pavlovian conditioning, which we have recently demonstrated with short ISIs [31]. Thus we examined the effect of dhpc lesions on interval timing in the *peak procedure* [32–37]: after delay conditioning, subjects were given a series of non-reinforced *peak* trials, in which the CS was presented for an extended period. This allowed us to determine the time at which the animals anticipated delivery of the US, by examining the time point at which conditioned responding reached a maximum (*i.e. peak time*). Our previous work has shown that, after training with a 15-s CS, although the control subjects appeared to learn that the time of food delivery was 15 s after CS onset, the subjects with dhpc lesions showed maximal conditioned responding at earlier time points [31].

In addition, we examined interval timing performance on non-reinforced *gap* trials, which were identical to the peak trials except that the CS was interrupted for a short period, to establish whether the earlier peak times in the dhpc-lesioned subjects was due to a general disinhibition of appetitive behaviour [38–40]. On the gap trials the control subjects tend to suspend timing during the gap. In contrast, fimbria-fornix lesions, lesions damaging the fibres connecting the hippocampus with other subcortical structures, result in a restart of timing after the gap; this results in *later* peak times than is seen in the control subjects [35–37], a result which is clearly not explicable in terms of a lesion-induced disinhibition of appetitive behaviour. In our recent report [31] dhpc lesions had no effect on timing on the gap trials, suggesting that the later peak times observed after fimbria-fornix lesions [35–37] were not due to dhpc pyramidal neuronal dysfunction. Nevertheless, our failure to see earlier peak times on the gap trials [31] is consistent with the proposal that dhpc damage impairs interval timing, rather than producing a general disinhibitory effect on responding; in the present study we examined if similar results would be found when a longer, 40-s ISI was employed.

2. Method

2.1. Subjects

Twenty-four naïve Lister Hooded male rats (Harlan, UK) were used, and their average weight was 300 g at the start of surgery. They were caged in pairs in a colony with a light–dark cycle of 12 h (light phases started at 07:00). After recovery from surgery, an 85% *ad lib*-weight food deprivation schedule was maintained by feeding each subject a restricted daily ration after each session. The first, magazine training session began one month after surgery, at which point the subjects' average weight was 375 g (range: 325–435 g). Subjects were tested seven days a week for the duration of the entire experiment.

2.2. Surgery

Subjects were anaesthetised with isofluorane. The scalp was incised along the midline and the facial muscles were retracted. Portions of cranial bone above the dhpc were removed with a dental drill. In the dhpc-lesioned group, bilateral dhpc lesions were achieved by injecting ibotenic acid into 14 different sites: anterior-posterior (AP) −2.4 mm, medial-lateral (ML) ±1.0 mm, dorsal-ventral (DV) −3.0 mm; AP −3.0 mm, ML ±1.4 mm, DV −2.1 mm; AP −3.0 mm, ML ±1.4 mm, DV −2.9 mm; AP −3.0 mm, ML ±3.0 mm, DV −2.7 mm; AP −4.0 mm, ML ±2.6 mm, DV −1.8 mm; AP −4.0 mm, ML ±2.6 mm, DV −2.8 mm; and AP −4.0 mm, ML ±3.7 mm, DV −2.7 mm; the AP and ML coordinates were relative to bregma, whereas the DV coordinates were relative to the brain surface. The volume of ibotenic acid injected at sites AP −3.0 mm, ML ±3.0 mm, DV −2.7 mm and AP −4.0 mm, ML ±3.7 mm, DV −2.7 mm was 0.1 μl; the volume injected at all other sites was 0.05 μl. The concentration of the injected ibotenic acid solution was 63 mM, which was made from dissolving 5 mg of ibotenic acid solids (Sigma–Aldrich, UK) into 0.5 ml of 0.1-M phosphate-buffered saline (pH 7.4). Injections were administered by an infusion pump (KD Scientific, Massachusetts) at rates of 0.03 μl min^{-1} using a 2-μl syringe (Hamilton, Switzerland) with a 25-gauge, bevel-tip needle. After each injection the needle was left *in situ* for 1 min before it was withdrawn and moved to the next site. In the sham-lesioned group, the needle was lowered into the same sites but no ibotenic acid was injected. After all sites were visited, the scalp was sutured. All subjects were injected subcutaneously with 1 ml kg^{-1} of Rimadyl (Pfizer, UK) as analgesic and 0.5 ml of warmed saline to prevent dehydration, and they fully recovered within two weeks.

2.3. Apparatus and stimuli

Eight operant chambers (Med Associates, Vermont; length × width × height: 30 cm × 25 cm × 25 cm), each of which was located inside a sound- and light-attenuating chamber (72 cm × 32 cm × 42 cm) equipped with a ventilation fan, were used. The sound level inside the operant chamber with the ventilation fan switched on was 65 dB(A). Each operant chamber had two short aluminium walls and two long transparent plastic walls (the front one served as the door). The ceiling was a piece of transparent plastic. The floor consisted of 19 stainless steel bars spaced 1 cm apart; each had a diameter of 0.5 cm and ran parallel to the short walls; located below the floor was a pan containing a layer of sawdust bedding which was changed weekly. A recessed food magazine was located on one of the short walls, equidistant from the long walls and 3 cm above the floor. The magazine was accessible via a rectangular aperture (width × height: 4 cm × 5 cm); an infrared beam was sent from one side of the magazine and received on the other side; each interruption of the beam was recorded as a discrete response. The operant chambers were not illuminated during an experimental session. The CS was either a white noise or 1-kHz click of 75 dB(A), presented via a speaker located at the upper corner of the short wall, opposite to the wall in which the magazine was located. The US was a 45-mg food pellet (PJAI-0045; Noyes, New Hampshire) delivered into the magazine. Experimental events (delivery of CSs and USs, and head entry responses) were controlled by the Med-PC package (version IV) installed on a PC located in another room, and their occurrence was recorded with a 10-ms resolution.

2.4. Procedure

2.4.1. Acquisition of Pavlovian conditioning: sessions 1–10

The study began with a magazine training session in which USs were delivered according to a variable-time, 240-s schedule; this session was terminated after 40 min (or in the event that 20 USs were delivered in less than 40 min). There followed ten sessions of acquisition; during each session there were 50 delay conditioning trials on which the 40-s auditory CS was followed immediately by delivery of the US (Fig. 1A). Half of the subjects in each group received the click as the CS and the remainder the noise. The inter-trial interval (ITI, the interval between CS termination on one trial and CS onset on the next) comprised a random interval with a mean of 40 s, and a fixed interval of 40 s which served as the pre-CS period; the random portion of the ITI was drawn from an exponential distribution. These sessions lasted 100 min on average.

2.4.2. Interval timing: sessions 11–40

The acquisition phase was followed by the *peak*-trial phase; these peak-trial sessions were identical to the conditioning sessions except that 15 out of 50 of the conditioning trials were replaced by the non-reinforced peak trials, on which the CS lasted for 80 s (Fig. 1B). These trials were used to assess how accurately the subjects had encoded the time of US delivery on the conditioning trials. These sessions lasted 110 min on average.

2.4.3. Interval timing: sessions 41–70

The peak-trial phase was then followed by the *gap*-trial phase; the gap-trial sessions were identical to those in the peak phase, except that each contained 10 peak trials and 10 gap trials, on which the CS was interrupted 10 s after its onset for 5 s; the total CS duration on the gap trials remained 80 s (Fig. 1C). These gap trials were used to assess the extent to which timing of US delivery would be affected by interruption of the CS. These sessions lasted 115 min on average.

In all phases, the rate of conditioned responding (magazine entry) was recorded during each CS presentation, and also during the 40-s pre-CS period that preceded each CS presentation. In the interval timing test phases, the rate of CS responding in each 1-s time bin over the course of a non-reinforced peak or gap trial was recorded;