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Research report

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

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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.

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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;

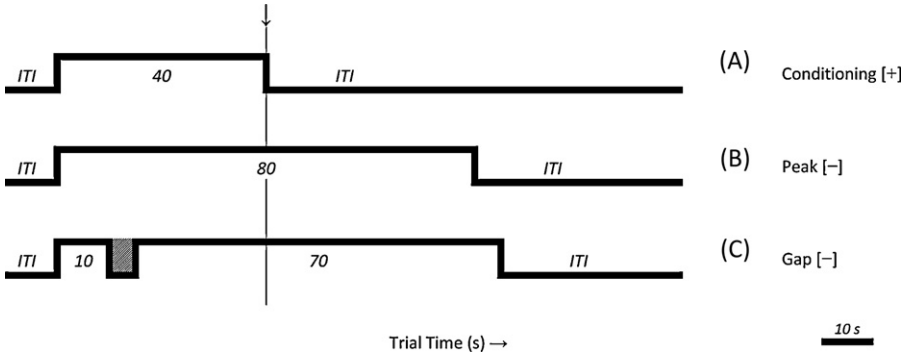

Fig. 1. Pavlovian conditioning trials and non-reinforced test trials. Conditioned stimulus and gap durations are drawn to scale (the bar at the bottom right of the figure represents 10 s). The vertical line indicates the time of US delivery on the conditioning trials (+: trials with US delivery; –: trials with no US delivery). The gap duration (indicated by shading) was 5 s. Conditioned responding was recorded in each 1-s time bin on the non-reinforced peak and gap trials.

the different trial types within a session were randomly selected by the Med-PC programme.

2.5. Histology

Subjects were sacrificed with an overdose of pentobarbitone and perfused intracardially with formal saline. Their brains were stored in formal saline at room temperature for two days, subsequently in 20% sucrose solution at a temperature of 4 °C for two days. The brains were then cut with a cryostat at a temperature of −19 °C; coronal sections were 40 μm in thickness, and every fifth section was collected. The recovered sections were stained with cresyl violet solution and were dried at room temperature. Lesion size was estimated in the following way. For each subject, the AP coordinates of the recovered coronal sections were identified using the Paxinos and Watson atlas [41]. For each identified section, the intact hippocampus in each hemisphere was outlined using ImageJ (version 1.40; National Institutes of Health, Maryland); the hippocampal areas in both hemispheres were estimated (in pixels). The overall hippocampal area (h_i) was estimated for each subject. Subsequently, the mean overall hippocampal area in the sham-lesioned group ($\hbar_{Sham}$) was calculated, and the extent of hippocampal damage of each subject in the dhpc-lesioned group ($damage_i$) was estimated relative to $\hbar_{Sham}$: $damage_i = (1 - h_i/\hbar_{Sham}) \times 100\%$.

2.6. Data treatment

2.6.1. Acquisition of Pavlovian conditioning: sessions 1–10

The magazine entry rates (in responses min^{-1}) during the 40-s CS presentation and during the 40-s pre-CS period that preceded each CS presentation were recorded. The extent of conditioning in each session was expressed in terms of raw CS response rates. In addition, in order to correct for individual variation in responding, and to provide some measure of the degree to which the CS produced an elevation of background responding, a ratio of the form *CS rate*/(*CS + pre-CS rates*) was computed; a ratio of 0.5 indicates no conditioning and a ratio of greater than 0.5 indicates an animal was conditioned to the CS, and responded more during CS presentation that in its absence.

2.6.2. Peak-trial interval timing: sessions 11–70

The data from the non-reinforced peak trials were considered in 20, three-session blocks, so as to capture any transient effect of dhpc lesions [5,32,42,43]. For each subject, magazine entries in 1-s time bins were pooled across three sessions, and each resultant conditioned-response (CR) distribution was smoothed over four 1-s bins. A Gaussian model with three parameters (the peak rate, a; the spread, b; and the peak time, c) was then fitted onto each CR distribution (Fig. 2):

$$\text{response}_i = ae^{-\frac{1}{2}\left(\frac{t_i - c}{b}\right)^2}$$

where i indicates each 1-s time bin. The peak times were used as an indication of timing accuracy, as were timing error scores of the form $|target\ duration - peak\ time|$; the greater the error score, the less accurate was the timing. The spreads were used as an indication of timing precision; the greater the spread, the less precise was the timing. The peak rates were used as an indication of the strength of US expectation [the motivational aspect of interval timing; 44,45]. Finally, to assess how well a three-parameter Gaussian model could be fitted onto each CR distribution, we computed the coefficient of determination of each Gaussian fit (Fig. 2):

$$R^2 = 1 - \frac{SS_{Error}}{SS_{Total}}$$

where SS_{Error} indicates the sum of squares of the differences between the observed and predicted responding in every 1-s bin, and SS_{Total} the sum of squares of the differences between the observed data in every 1-s bin and the observed mean of responding across all bins. A CR distribution with a high R^2 coefficient indicates a high degree of temporal control of conditioned responding (*i.e.* there was a single peak on the peak trials; *e.g.*, Fig. 3B), whereas a distribution with a low R^2 coefficient indicates a low degree of temporal control (*i.e.* there were multiple peaks; *e.g.*, Fig. 3A).

2.6.3. Gap-trial interval timing: sessions 41–70

The data from the non-reinforced gap trials were considered in 10, three-session blocks. A three-parameter Gaussian model was fitted onto each CR distribution, similar to the treatment of the peak-trial data described above. The degree to which timing was disrupted by intervening gaps, the peak shift (Δc), was determined by the calculation of Buhusi and Meck [46–50]:

$$\Delta c = c_{Gap} - c_{Peak} - g$$

where c_{Gap} and c_{Peak} indicate the central tendencies of the CR distributions on the gap and peak trials, respectively, and g the duration of the intervening gap (5 s). If a subject suspended timing during the gap and resumed when the CS started again, c_{Gap} would be longer than c_{Peak} by the gap duration, and the value of shift would be zero (the *stop*-timing strategy). If, however, a subject restarted timing from zero after the gap, c_{Gap} would be longer than c_{Peak} by both the gap duration *and* the CS duration prior to the gap, and the value of shift would be 10 s (the *reset*-timing

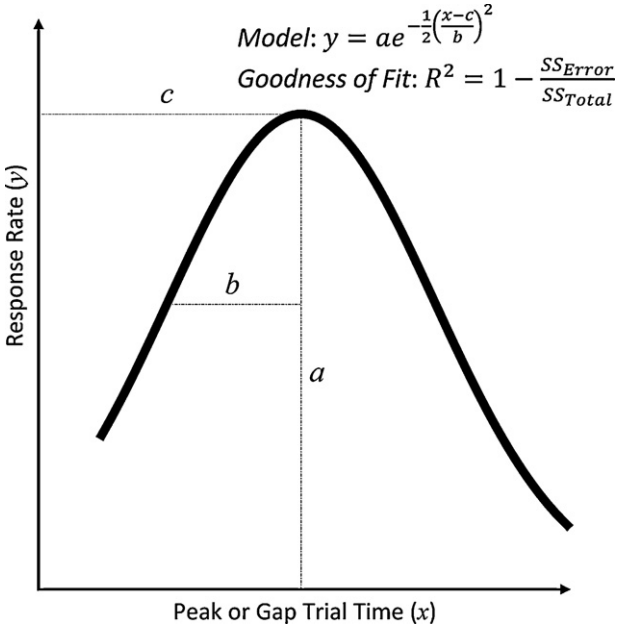

Fig. 2. A Gaussian model with three parameters: the peak rate, a; the spread, b; and the peak time, c. Goodness of each Gaussian fit was determined by the coefficient of determination, R^2. SS_{Error} indicates the sum of squares of the differences between the observed and predicted data in each 1-s time bin, whereas SS_{Total} the sum of squares of the differences between the observed data in each 1-s time bin and the observed mean across all bins. The greater the R^2, the better was the Gaussian fit (and hence the higher was the degree of temporal control of conditioned responding).

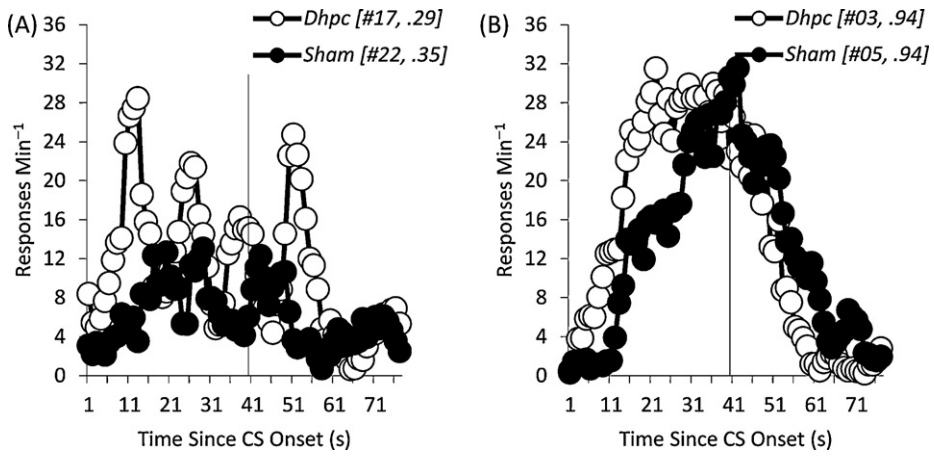

Fig. 3. Conditioned responding under different degrees of temporal control. The examples of CR distributions shown in panel A had relatively low R^2 coefficients (dhpc-lesioned subject #17, $R^2 = 0.29$; sham-lesioned subject #22, $R^2 = 0.35$), whereas those shown in panel B had relatively high R^2 coefficients (dhpc-lesioned subject #03, $R^2 = 0.94$; sham-lesioned subject #05, $R^2 = 0.94$). The vertical lines indicate the time of US delivery on the conditioning trials. Data are from the last block of the interval timing test phase.

strategy). Finally, if a subject continued timing during the gap, c_{Gap} would be equal to c_{Peak}, and the value of shift would be *negative* (the *run*-timing strategy). Note that the value of shift could be greater than 10 s, when the animals adopted the *reset*-timing strategy but did not restart timing at once after the gap [51].

2.7. Statistical analyses

Data were analysed using split-plot analyses of variance (ANOVAs), with Lesion as the between-subjects factor, and Session or Block of Sessions as the within-subjects factor. Whenever the assumption of sphericity was not met for a particular within-subjects main effect or its interactive effect, the Greenhouse-Geisser correction was applied. Significant two-way interactions were examined with simple main effect analyses using the pooled error term for between-subjects comparisons, and individual error terms for within-subjects comparisons [52].

3. Results

3.1. Histology

The 12 subjects that received injections of ibotenic acid sustained bilateral damage to the anterior dorsal portions of the hippocampus, including the dentate gyrus, CA3 and CA1 regions. Hippocampal damage tended to start at AP bregma −1.80 mm [plate #48; from 41] and extend to AP −4.68 mm (plate #72). The mean amount of hippocampal damage was 38% of total hippocampal volume (range: 15–45%); damage to the dorsal subiculum was minimal in all cases. The subjects in the sham-lesioned group did not sustain any damage to the hippocampus. As all subjects in the dhpc-lesioned group had bilateral damage to the dhpc and none in the sham-lesioned group had any hippocampal damage, all subjects were included in the statistical analyses. Photomicrographs from a representative sham-lesioned subject and a dhpc-lesioned subject with an amount of hippocampal damage closest to the group mean are depicted in Fig. 4A and B, respectively.

3.2. Acquisition of Pavlovian conditioning: sessions 1–10

The CS response rates are shown in Fig. 5A. There is some indication that the dhpc-lesioned subjects responded at lower rates than the sham-lesioned subject, but a 2 (Lesion) × 10 (Session) ANOVA revealed no main effect of Lesion or Lesion × Session interaction ($Fs < 1.50$, $ps > 0.20$). There was, however, a main effect of Session [$F(4,88) = 3.00$, $p < 0.005$]. Similarly, a parallel ANOVA conducted on the background rates, shown in Fig. 5C, revealed a main effect of Session only [$F(3,58) = 27.92$, $p < 0.0005$]; the main effect of Lesion and the Lesion × Session interaction were not significant ($Fs < 1.00$, $ps > 0.40$).

As there was no sign of a difference in background responding between the groups, conditioning ratios were also computed, to correct for individual variation, and also to provide a measure of the degree to which the CS elevated background responding. The conditioning ratios are shown in Fig. 5E, and the dhpc-lesioned subjects seemed to have lower ratios, especially in the second half of acquisition. Indeed, there was a main effect of Lesion [$F(1,22) = 11.68$, $p < 0.005$] and a Lesion × Session interaction [$F(4,82) = 2.34$, $p < 0.05$]. Simple main effect analyses revealed dhpc lesion effects in sessions 5–10 ($ps < 0.05$); in addition, there were simple effects of Session in both groups ($ps < 0.005$), but the effect size of Session in the sham-lesioned group was greater than that in the dhpc-lesioned group (partial η^2s = 0.79 *vs.* 0.35, respectively), which could reflect the apparently more rapid increase in ratio scores across sessions in these animals.

Finally, there was no difference in the rates of responding during the magazine training session [means = 7.62 ± 0.73 *vs.* 7.96 ± 0.63 responses min^{-1} in the dhpc- and sham-lesioned groups, respectively; $t(22) = 0.35$, $p = 0.73$].

3.3. Peak-trial interval timing: sessions 11–70

3.3.1. Timing accuracy

The peak times across the interval timing test phases are shown in Fig. 6A, which displays a tendency for the dhpc-lesioned subjects to show earlier peak times than the sham-lesioned subjects. Nevertheless, a 2 (Lesion) × 20 (Block of Three Sessions) ANOVA revealed a main effect of Block only [$F(6,134) = 2.47$, $p < 0.05$], suggesting that both the dhpc- and sham-lesioned subjects overestimated the 40-s target duration on the first few blocks but their peak times became closer to 40 s in subsequent blocks. Neither the main effect of Lesion nor the Lesion × Block interaction was significant ($Fs < 2.50$, $ps > 0.15$). When the peak times were pooled across all blocks, neither the mean overall peak time of the dhpc-lesioned group (36.58 ± 2.36 s) nor that of the sham-lesioned group (40.13 ± 0.71 s) differed from the target duration of 40 s [$t(11)$s < 1.50, $ps > 0.15$].

The timing errors (*i.e.* $|target\ duration - peak\ time|$) across the test phases are shown in Fig. 6B. The dhpc-lesioned subjects seemed to show greater deviation from the target time than did the sham-lesioned subjects. Indeed, this was confirmed by a Lesion × Block ANOVA, which revealed a main effect of Lesion [$F(1,22) = 4.83$, $p < 0.05$]. There was also a main effect of Block [$F(7,155) = 2.75$, $p < 0.05$], suggesting that timing error declined across blocks, but

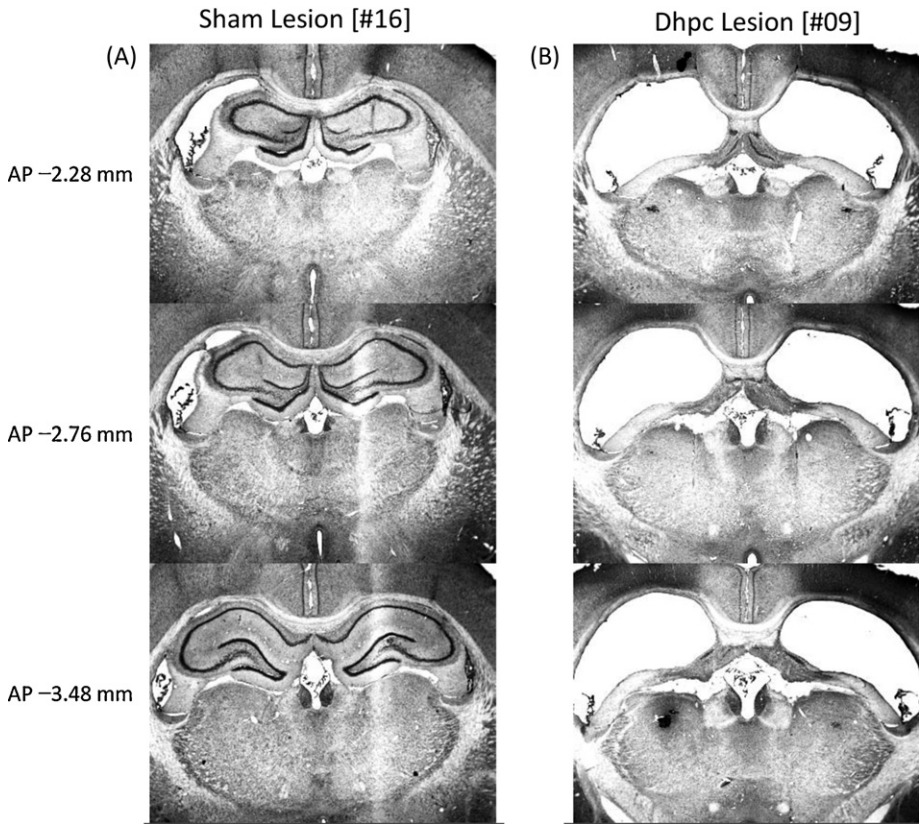

Fig. 4. Photomicrographs of cresyl-violet-stained coronal sections (*top* to *bottom* panels: anterior to posterior) from a representative sham-lesioned subject (#16; panel A) and a dhpc-lesioned subject (#09; panel B) with damage closest to the group mean (38% of total hippocampal volume).

the Lesion × Block interaction was not significant [$F(7,155) = 0.62$, $p = 0.74$].

3.3.2. Timing precision and temporal control of conditioned responding

Although dhpc lesions increased timing error, they did not affect timing precision (*i.e.* the spreads of CR distributions; Fig. 6C). The main effect of Block on the spreads was significant [$F(5,104) = 2.73$, $p < 0.05$], suggesting that interval timing became more precise across the test phases, but the main effect of Lesion and the Lesion × Block interaction were not significant (Fs < 2.00, ps > 0.20). Dorsal hippocampal lesions did not affect temporal control of conditioning responding either, as there was no effect of Lesion on the goodness of Gaussian fit (Fig. 6D). The main effect of Block on the R^2 coefficients was significant [$F(8,178) = 7.29$, $p < 0.0005$], suggesting that the degree of temporal control increased across the test phases, but the main effect of Lesion and the Lesion × Block interaction were not significant (Fs < 1.50, ps > 0.15).

3.3.3. US expectation

The dhpc lesion effects on conditioned responding found in the acquisition phase seemed to persist in the interval timing test phases; this is shown in Fig. 5B, in which the peak rates (*i.e.* maximal CR rates) increased across the test phases. Nevertheless, a Lesion × Block ANOVA revealed a main effect of Block only [$F(5,100) = 7.07$, $p < 0.0005$], suggesting that in both the dhpc- and sham-lesioned groups US expectation around the target time increased across blocks. A parallel ANOVA conducted on the pre-trial background rates (Fig. 5D) found that background responding declined across blocks [$F(4,91) = 4.09$, $p < 0.005$]; the dhpc-lesioned subjects seemed to respond more to the background, but the main effect of Lesion fell just short of significance [$F(1,22) = 3.88$, $p = 0.062$].

To facilitate comparison with the findings from the acquisition phase, conditioning ratios of the form *peak rates*/(*peak* + *background rates*) were computed. In accordance with the findings from the acquisition phase, a Lesion × Block ANOVA conducted on the ratios (Fig. 5F) revealed a main effect of Lesion [$F(1,22) = 6.05$, $p < 0.05$], confirming the persistence of the dhpc lesion effect on Pavlovian conditioning; there was also a main effect of Block [$F(4,97) = 10.54$, $p < 0.0005$]; however, the almost reliable difference in pre-trial responding in these sessions means the significant effect of Lesion on this ratio score should be interpreted with caution.

3.4. Gap-trial interval timing: sessions 41–70

When the 40-s CS was interrupted by a 5-s gap, maximal conditioned responding occurred later in time (*i.e.* $\Delta c \geq 0$), but there did not seem to be any group difference in the effect of gaps on timing accuracy. A 2 (Lesion) × 10 (Block of Three Sessions) ANOVA was conducted on the peak shifts; a shift value of 0 s indicates that a subject adopted the *stop*-timing strategy, whereas a value of 10 s or higher the *reset*-timing strategy (Fig. 7): the main effect of Block fell just short of significance [$F(5,113) = 2.10$, $p = 0.069$]; neither the main effect of Lesion nor the Lesion × Block interaction were significant (Fs < 2.50, ps > 0.15).

4. Discussion

4.1. Pavlovian conditioning

The present study demonstrated involvement of the dhpc in Pavlovian processes. Dorsal hippocampal lesions disrupted delay

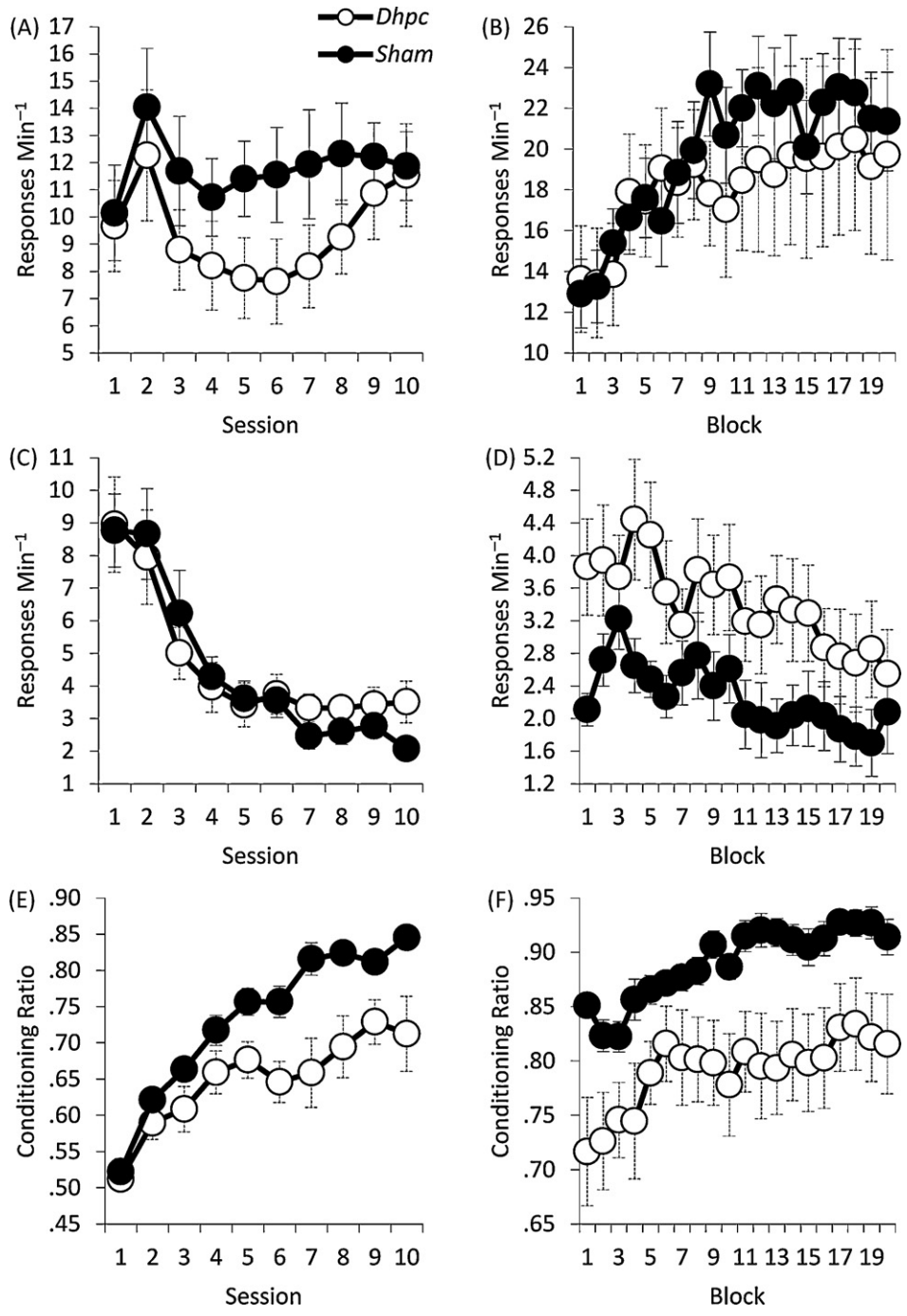

Fig. 5. Pavlovian conditioning: sessions 1–70. The panels on the left show the overall CS response rates (panel A), the overall pre-CS rates (panel C), and the conditioning ratios computed from these rates (panel E), during the acquisition phase of the study (sessions 1–10). The panels on the right show the peak CS response rates (panel B), the overall pre-CS rates (panel D), and the conditioning ratios computed from these rates (panel F), during the interval timing test phases (sessions 11–70). Dorsal hippocampal lesion deficits in conditioning were revealed when the conditioning ratios were considered (panels E and F). In all panels, vertical bars indicate standard errors of means (SEMs).

conditioning, as indicated by the overall CR ratios during the acquisition phase and by the peak CR ratios during the interval timing test phases. Our findings extend those of Beylin et al. [30], not only confirming that delay conditioning can be affected by hippocampal damage provided that the ISI is sufficiently long, but also demonstrating that this occurs in appetitive as well as in aversive preparations. In addition, our findings suggest that damage to the *dorsal* portion of the structure is sufficient to obtain these effects. The conditioning deficits observed confirm the findings from single-unit recording studies, which reveal the involvement of dhpc pyramidal neurons in a variety of Pavlovian conditioning preparations [18–23]. Taken altogether, these findings raise the possibility that the involvement of the dhpc in Pavlovian processes might be more general than is assumed by many of the current theories of hippocampal function [see also 24–27]. Although there is nothing in the present results to rule out the possibility that the hippocampus is also involved in the formation of contextual or configural representations [7,8], or the formation of Pavlovian associations *only if* stimuli are temporally discontiguous [9,10], the results nonetheless invite the speculation that these more complex

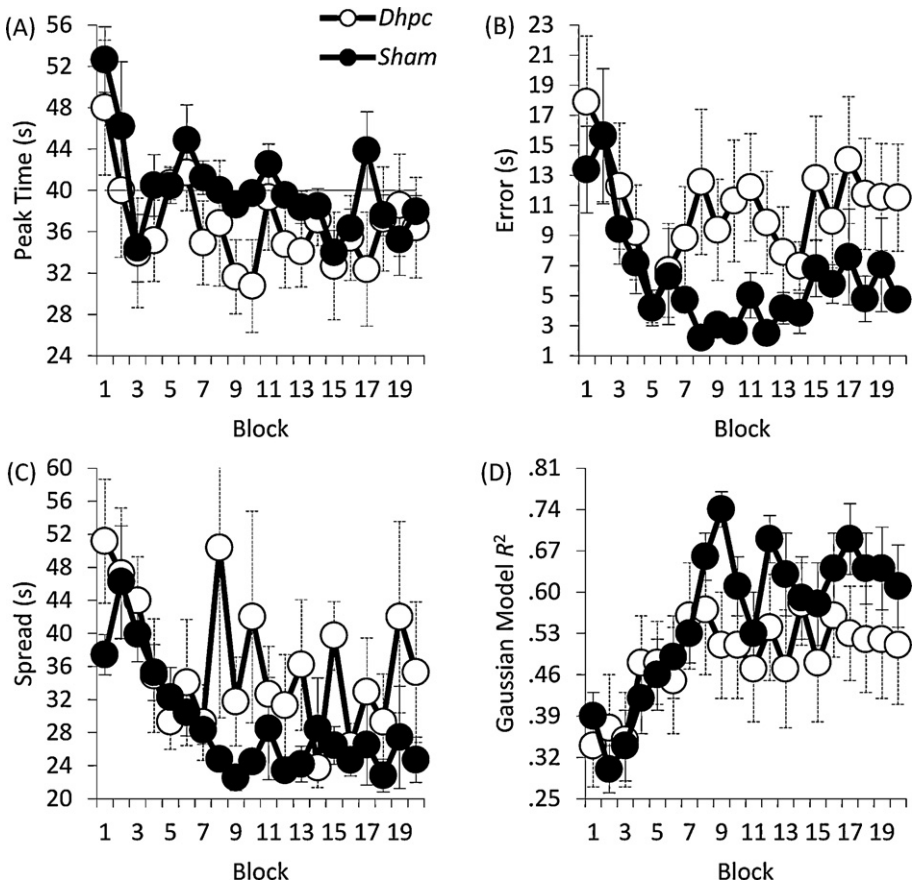

Fig. 6. Peak-trial interval timing: sessions 11–70. Panel A shows the accuracy of CR timing, as measured by the peak times (the horizontal line indicates the time of US delivery on the conditioning trials); panel B shows the timing errors (|*target duration* − *peak times*|); panel C shows the precision of timing, as measured by the spreads; and panel D shows the degree of temporal control, as indicated by the coefficients of determination, R^2. Dorsal hippocampal lesion deficits in timing were revealed when timing errors were considered (panel B). In all panels, vertical bars indicate SEMs.

effects of hippocampal damage could be secondary to a more fundamental effect on associative learning. But it must be acknowledged that such a suggestion could be oversimplistic, as performance in such complex tasks can be multiply determined, and animals with hippocampal damage can sometimes learn to respond like intact animals, presumably by adopting alternative strategies to solve the tasks.

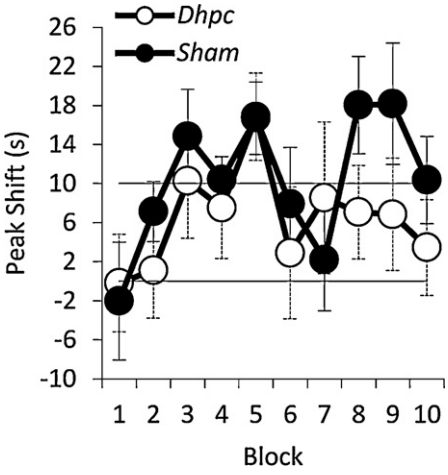

Fig. 7. Gap-trial interval timing: sessions 41–70. Peak shift (Δc) is the difference in peak times on the gap and peak trials, minus the gap duration (5 s). The upper horizontal line indicates the *reset*-timing strategy, and the lower horizontal line the *stop*-timing strategy. Vertical bars indicate SEMs.

4.2. Interval timing

The present study also confirmed our previous findings that dhpc lesions disrupted CR timing accuracy [31]. In the sham-lesioned subjects, the time at which the maximal CR occurred on the non-reinforced peak trials was close to the time at which USs were delivered on the conditioning trials [see also 53,54]; however, in the dhpc-lesioned subjects the time at which the maximal CR occurred was earlier than the target duration of 40 s. Timing precision and overall temporal control of conditioned responding were unaffected. The dhpc lesion effects on CR timing accuracy extend our recent findings, confirming the involvement of the dhpc in timing long as well as short ISI durations. The timing deficits observed confirm the findings from single-unit recording studies, which reveal that some dhpc pyramidal neurons fire maximally or minimally at critical time points during Pavlovian fear conditioning [33] and instrumental conditioning [55], and that different subpopulations of dhpc pyramidal neurons have different, but overlapping, temporally-specific receptive fields [*i.e. time cells*; 56, 57]. Taken altogether, these findings confirm the notion that the hippocampus might be involved in the encoding and retrieval of temporal information within the seconds-to-minutes range [58,59].

The peak procedure is useful in revealing what roles different neural substrates play in interval timing. Dorsal hippocampal lesions disrupted timing accuracy and reduced the strength of US expectation, but had no effect on timing precision. Findings from other lesion studies suggest that neural dysfunction of other kinds might have different effects. For instance, lesions of the ascending serotonergic system (the dorsal and median raphe nuclei) disrupt

timing precision, but have no effect on the strength of reinforcer expectation and timing accuracy [60]. In contrast, lesions of part of the dopaminergic system (the nucleus accumbens core) disrupt the strength of reinforcer expectation, timing accuracy, and timing precision [61]. As the *v*hpc is more innervated by serotonergic and dopaminergic inputs than the dhpc [62], a direction for future studies is to examine whether the effects of vhpc lesions in the peak procedure resemble the effects of dhpc damage, or serotonergic/dopaminergic damage.

4.3. Summary

Dorsal hippocampal lesions disrupted Pavlovian conditioning and CR timing when the ISI was relatively long. The observed lesion effects are consistent with the findings from single-unit recording studies that reveal conditioning- and timing-related firing in the dhpc. Our findings raise the possibility 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.

Disclosure

All procedures were performed in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 and approved by the Home Office under the Project Licence 40/2830.

The authors disclose that they have no actual or potential conflicts of interest, financial or otherwise, related to the present work.

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