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RESEARCH ARTICLE

Dorsal hippocampal involvement in conditioned-response timing and maintenance of temporal information in the absence of the CS

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Abstract Involvement of the dorsal hippocampus (DHPC) in conditioned-response timing and maintaining temporal information across time gaps was examined in an appetitive Pavlovian conditioning task, in which rats with sham and DHPC lesions were first conditioned to a 15-s visual cue. After acquisition, the subjects received a series of non-reinforced test trials, on which the visual cue was extended (45 s) and gaps of different duration, 0.5, 2.5, and 7.5 s, interrupted the early portion of the cue. Dorsal hippocampal-lesioned subjects underestimated the target duration of 15 s and showed broader response distributions than the control subjects on the no-gap trials in the first few blocks of test, but the accuracy and precision of their timing reached the level of that of the control subjects by the last block. On the gap trials, the DHPC-lesioned subjects showed greater rightward shifts in response distributions than the control subjects. We discussed these lesion effects in terms of temporal versus non-temporal processing (response inhibition, generalisation decrement, and inhibitory conditioning).

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Introduction

Interval timing refers to the ability to time the occurrence of biologically significant events (with respect to some temporal landmarks) within the seconds-to-minutes range (Balci et al. 2009; Coull et al. 2011). Findings from single-unit recording studies suggest that the hippocampus, more specifically, the *dorsal* pole of the structure (*D*HPC), mediates interval timing. In the differential reinforcement of low rates task in which instrumental responses are rewarded only if they are at least *t* seconds apart from each other, pyramidal neurons of the rat DHPC show high firing rates after each response is emitted, but the firing rates decline gradually across time and reach a minimum at the criterion time (Young and McNaughton 2000). In the Pavlovian peak procedure, animals are first conditioned to a stimulus of *t* seconds, the termination of which is followed by delivery of an unconditioned stimulus (US); on non-reinforced test trials, pyramidal neurons of the rabbit DHPC show low firing rates at the beginning of the trial, but the firing rates increase across time and reach a maximum *t* seconds after trial onset (McEchron et al. 2003). More recently, in a recognition memory task in which an empty interval (a gap) intervenes between the sample and test phases, it has been revealed that rat DHPC pyramidal neurons have temporally specific receptive fields during the gap: Different DHPC neurons are preferentially activated at different points in time during the gap (MacDonald et al. 2011). It is suggested that these temporally selective signals are important for the maintenance of information experienced during the sample phase, giving rise to appropriate recognition

behaviour at test (MacDonald et al. 2011); similar ideas have been put forward by other investigators (e.g. Rawlins 1985; Rodriguez and Levy 2001; Woodruff-Pak and Disterhoft 2008; Ludvig et al. 2009).

In accordance with the presence of temporal signals in the DHPC (Young and McNaughton 2000; McEchron et al. 2003), we have recently demonstrated that ibotenic acid lesions of the DHPC disrupted interval timing in the appetitive Pavlovian peak procedure: DHPC-lesioned and control rats were first conditioned to a stimulus of 15 s; they were then given non-reinforced test trials on which the duration of the conditioned stimulus (CS) was extended (45 s), and the conditioned-response rate at each moment of the CS was recorded. On these test trials, the control subjects showed little responding in the early and late portions of the CS, but showed the highest response rates at time points at which the US was delivered on the conditioning trials; such a Gaussian-shaped response distribution suggests that these subjects timed the CS → US interval in an accurate and precise manner. The DHPC-lesioned subjects also had Gaussian-shaped response distributions, but they showed the highest response rates at significantly earlier time points, that is, they *underestimated* the CS → US interval (Tam and Bonardi 2012).

In that study, we also used the peak procedure to examine whether DHPC lesions disrupted the maintenance of (temporal) information in the presence of intervening gaps, as suggested by recent electrophysiological findings (MacDonald et al. 2011): The DHPC- and sham-lesioned subjects were given a second type of test trial on which the CS was extended as before, but a 5-s gap interrupted the early portion of the test trial. If the DHPC is important for the maintenance of temporal information across gaps, the DHPC-lesioned subjects would tend to restart timing from 0 s after gaps, as the CS duration experienced prior to the gaps would not be retained. In contrast, it was predicted that the sham-lesioned subjects would maintain in memory the CS duration prior to the gap and so be more likely to resume timing after the gap from the time point at which the CS was interrupted (Church 1984; Meck et al. 1984); thus, the DHPC-lesioned subjects' response distributions would be shifted rightward (i.e. later in time) to a greater extent than those of the sham-lesioned subjects. However, we found that the extent of rightward shift did not differ between the groups (Tam and Bonardi 2012).

The failure to reveal any lesion effect on the gap trials, however, might be related to the fact that only one gap duration was used. For example, it is possible that the 5-s gap duration was too long; in our study sham-lesioned subjects also appeared to restart timing from 0 s after gaps, that is, their response distributions also shifted significantly rightward (Tam and Bonardi 2012, Fig. 6), which would have tended to mask any potential DHPC lesion effect. Accordingly, to explore the possibility that absolute gap duration might influence the magnitude of any effect observed, the present study examined the effect of DHPC lesions on timing of a 15-s CS in the presence of gaps of three different durations, 0.5, 2.5, and 7.5 s. If the use of shorter gap durations is critical, then we would anticipate that, on the test trials with shorter gaps, the DHPC-lesioned subjects would restart timing from 0 s after gaps, but the sham-lesioned subjects would not, resulting in significant rightward shifts in the DHPC-lesioned subjects' response distributions. In contrast, no group difference would be expected on the longest, 7.5-s gap trials, as both the DHPC- and sham-lesioned groups would reset their timing after such a relatively long gap (Buhusi and Meck 2009a, b).

Methods

Animals

Twenty-four naïve Lister Hooded male rats (Harlan, Bicester, UK) were used, and their average weight was 300 g at the start of surgery. Half of them were assigned to the DHPC-lesioned group, and the remaining half to the sham-lesioned group. Subjects of the same group were caged in pairs in a colony with a light–dark cycle of 12 h (light phases started at 0700). After recovery from surgery, an 85 *%-ad-lib*-weight food deprivation schedule was maintained by feeding each pair a restricted ration after each session. The first session of the study began 3 weeks after surgery; the subjects' average weight was 387 g (range: 350–435 g) at that time. Subjects were tested 7 days a week during the acquisition, peak, and gap phases.

Surgical procedure

At the beginning of surgery, subjects were anaesthetised with isoflurane. The scalp was then incised along the midline and the facial muscles retracted. Portions of cranial bone above the DHPC were removed with a dental drill. In the DHPC-lesioned group, bilateral lesions were achieved by injecting ibotenic acid into the following 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, Dorset, UK) into 0.5 ml of 0.1 M phosphate-buffered saline (pH 7.4). Injections were administered by an infusion pump (KD Scientific, Holliston, Massachusetts) at rates of 0.03 μl min^{-1} using a 2-μl syringe (Hamilton, Bonaduz, 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. Subjects were injected subcutaneously with 1 ml kg^{-1} of Rimadyl (Pfizer, Surrey, UK) as analgesic and 0.5 ml of warmed saline to prevent dehydration; all of them fully recovered within 2 weeks.

Apparatus and stimuli

Eight operant chambers (Med Associates, St. Albans, Vermont; length × width × height: 30 cm × 25 cm × 25 cm), each of which was located inside a sound- and light-attenuating chamber (70 cm × 30 cm × 40 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 long wall 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 that was changed regularly. 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 CS was presentation of a 2.8-W houselight, the bottom half of which was shielded and located 11 cm above the magazine. When the CS was not present, the chambers were not illuminated. The US was delivery of a 45-mg food pellet (Noyes, Lancaster, New Hampshire) into the magazine. Experimental events (presentation of CSs and USs, and magazine entries) were timed and recorded by the Med-PC programme (version IV; Med Associates, St. Albans, Vermont), and their occurrence was recorded with a 10-ms resolution.

Behavioural procedure

Sessions 1–6: acquisition phase

The study began with a 40-min magazine training session in which USs were delivered according to a variable-time, 240-s schedule. There followed six sessions of acquisition; each session contained 64 delay conditioning trials on which the 15-s houselight CS was followed immediately by US delivery. The inter-trial interval comprised a random interval with a mean of 60 s, drawn from an exponential distribution, plus a fixed interval of 30 s.

Sessions 7–22 (Test Blocks 1–4): peak phase

The acquisition sessions were followed by sixteen *peak-trial* sessions, which were identical to the acquisition sessions except that half of the conditioning trials (32 trials) were replaced by the peak trials, on which the CS lasted for 45 s and was terminated without US delivery. These non-reinforced peak trials were used to assess the accuracy and precision of conditioned-response timing (Kirkpatrick and Church 2000; Balsam et al. 2002; McEchron et al. 2003; Tam and Bonardi 2012). The conditioning and peak trials were presented in a randomised order, with the constraint that each session began with a conditioning trial.

Sessions 23–38 (Test Blocks 5–8): gap phase

The peak-trial sessions were followed by sixteen *gap-trial* sessions, which were identical to the peak-trial sessions except that there were eight of each of the following types of test trial presented in an intermixed order: (a) peak (no-gap) trials; (b) 0.5-s gap trials; (c) 2.5-s gap trials; and (d) 7.5-s gap trials. On each of the three types of gap trial, the CS was presented for 7.5 s, off for the required duration, and presented again for 37.5 s. These gap trials of different duration were used to assess the extent to which interval timing would be affected by the presence of intervening gaps (Buhusi and Meck 2000, 2002, 2006a, b, 2009a, b).

Histological procedure

After the gap phase, subjects were killed with an overdose of pentobarbitone and perfused intracardially with formal saline. Their brains were stored in formal saline at room temperature for 2 days, subsequently in 20 % sucrose solution at a temperature of 4 °C for 2 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. For each subject, the AP coordinates of the recovered

coronal sections were identified using the Paxinos and Watson (2005) atlas. For each identified section, the intact hippocampus in each hemisphere was outlined using ImageJ (version 1.40; National Institutes of Health, Bethesda, Maryland); the hippocampal areas in both hemispheres were estimated (in pixels), and the total hippocampal area was calculated for each subject. Subsequently, the mean total hippocampal area of the sham-lesioned group was calculated, and the extent of hippocampal damage of each subject in the DHPC-lesioned group was expressed as a percentage of the mean of the sham-lesioned group.

Data treatment

Sessions 1–6: acquisition phase

During the acquisition phase, magazine entries were recorded during each CS presentation, and during the 15-s pre-CS period that preceded each CS presentation. The magazine entry rates, in response min^{-1}, during the 15-s CS presentation were used as an indication of the strength of Pavlovian conditioning. The magazine entry rates during the 15-s period that preceded each CS presentation were used as a measure of the strength of conditioning to the background cues.

Sessions 7–38: peak and gap phases

During the peak- and gap-trial phases, magazine entries in each 1-s time bin over the course of a non-reinforced peak or gap trial were recorded in order to examine timing accuracy and precision. The data from the peak trials in sessions 7–38 were considered in eight, four-session blocks. For each subject, magazine entries in 1-s time bins were pooled across four sessions, and each resultant response distribution was smoothed over four 1-s bins. A Gaussian model,

$$response_i = a \times \exp(-0.5 \times (t_i - c)^2/b^2),$$

was then fitted onto each response distribution. The central tendency of the fitted distribution, c, was used as an indication of timing accuracy; the closer it was to the target duration of 15 s, the less was the error, and hence the more accurate the timing. We anticipated that the DHPC-lesioned subjects would show an earlier mean c than the sham-lesioned subjects (Tam and Bonardi 2012). The width, or dispersion, of the fitted distribution, b, was used as a measure of timing precision; smaller values of b indicated more precise timing. The maximum height of the distribution, a, was an index of the strength of US expectation around the time of US delivery. Finally, the coefficient of determination of the regression model, R^2, was a measure of the goodness of fit; the higher the value, the better the fit and hence the greater the temporal control of conditioned responding. The data from the gap trials in sessions 23–38 were analysed in a similar way. The degree to which timing was affected by gaps was determined by relative shifts in central tendency, $c_{Gap}/(c_{Peak} + c_{Gap})$, where c_{Gap} and c_{Peak} indicate the central tendencies of the gap and no-gap distributions, respectively. If a subject continued timing during the gap, c_{Gap} would be equal to c_{Peak}, and the value of shift would be 0.5; but if the subject suspended timing during the gap, there would be a rightward shift in the peak of responding on gap trials such that $c_{Gap} > c_{Peak}$; the greater this rightward shift, the higher the value of c_{Gap} relative to c_{Peak}, and the higher would be the ratio score.

Results

Histology

Seven out of the twelve subjects that received ibotenic acid injections sustained bilateral damage to the anterior dorsal portions of the CA3 and CA1 subregions. Damage to the dentate gyrus, however, was minimal in most cases. Hippocampal damage tended to start at AP bregma −1.80 mm (Plate 48; from Paxinos and Watson 2005) and extend to AP −4.68 mm (Plate 72). The mean hippocampal damage was approximately 20 % of total hippocampal volume among these seven subjects (range: 15–25 %); no dorsal subicular damage was detected in these cases. The remaining five subjects in the DHPC-lesioned group were excluded from the behavioural analyses, as their hippocampal damage was mostly unilateral. One subject in the sham-lesioned group was also excluded, as some of its coronal sections were lost during the staining process, and hence, its overall hippocampal volume could not be determined; no hippocampal or subicular damage was detected in the remaining eleven sham-lesioned subjects. Example photomicrographs from a representative sham-lesioned subject and a representative DHPC-lesioned subject are shown in Fig. 1a, b respectively.

Sessions 1–6: acquisition of Pavlovian conditioning

Dorsal hippocampal lesions did not disrupt Pavlovian conditioning; nor did they have any effect on the speed with which responding to the background context declined across sessions. The magazine entry rates during the CS increased across the six sessions of acquisition in both groups [$F(5,80) = 10.01$, $p < 0.005$; Fig. 2]; the main effect of Lesion and the Lesion × Session interaction were not significant [$F(1,16) = 0.01$, $p = 0.91$ and $F(5,80) = 0.41$, $p = 0.84$, respectively]. The corresponding response rates during the pre-CS periods declined across sessions [$F(5,80) = 20.14$, $p < 0.0005$; Fig. 2], but again the main effect of Lesion and the Lesion × Session interaction were not significant ($ps > 0.10$).

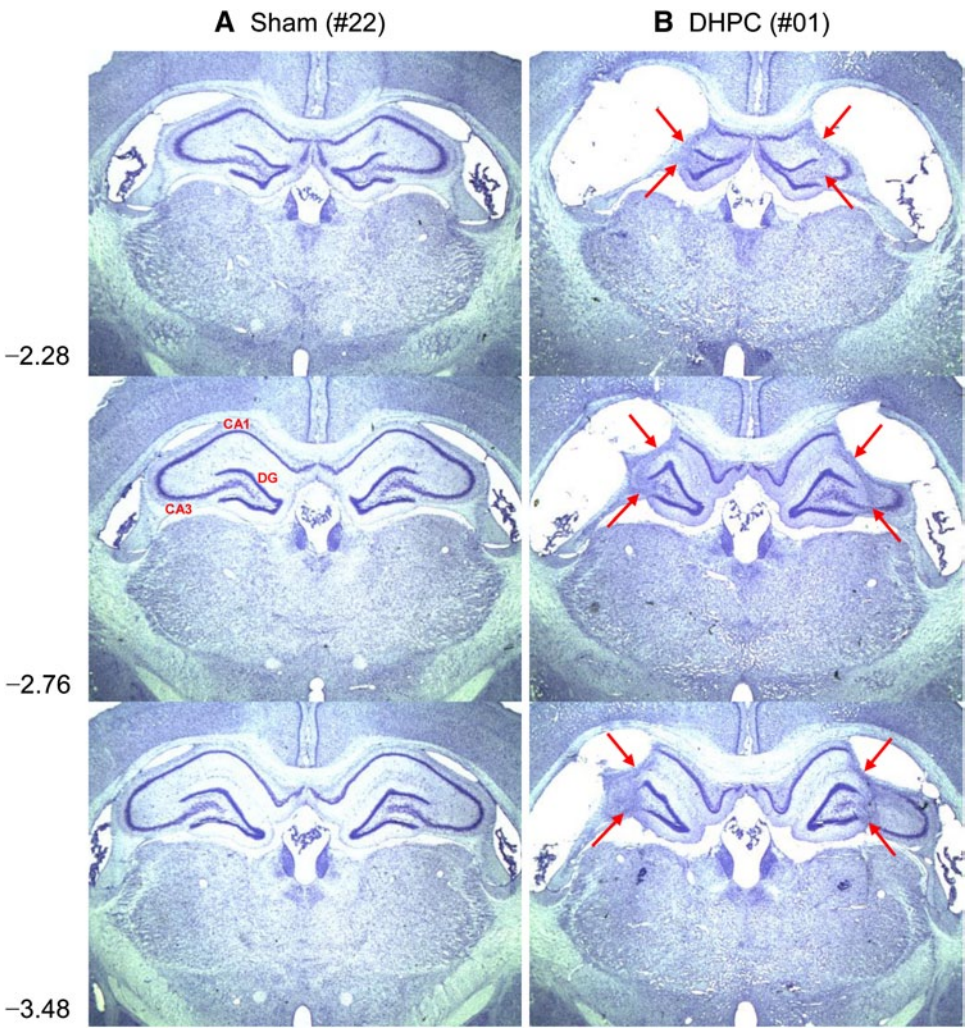

Fig. 1 Example photomicrographs of coronal sections from a representative sham-lesioned subject (**a**) and a representative DHPC-lesioned subject (**b**). The *top*, *middle*, and *bottom rows* show, respectively, sections about 2.28, 2.76, and 3.48 mm posterior to bregma, which correspond to Plates 52, 56, and 62 in the Paxinos and Watson (2005) atlas. Dentate gyrus (DG), CA3, and CA1 subregions are marked in **a**. Loss of CA3 and CA1 cells is marked with *arrows* in **b**

Sessions 7–38 (Test Blocks 1–8): conditioned-response timing on peak trials

Overview

Figure 3 shows the group mean response distributions on the conditioning trials of the acquisition phase (Fig. 3a, b) and on the non-reinforced peak trials of the peak (Fig. 3c, d) and gap (Fig. 3e, f) phases.

For the acquisition phase, data from the first and last sessions are shown in Fig. 3a, b, respectively. It is clear that as training progressed, the subjects learned that the termination of the 15-s CS was followed by US delivery, and came to show substantially more conditioned responding in the late portion of the CS than in the early portion of the cue, so that the response gradients became steeper as training progressed. Data from the first and last blocks of the peak phase are shown, respectively, in Fig. 3c, d. The response distributions were Gaussian shaped, and their peaks were close to the time at which the US had been delivered on the conditioning trials, suggesting temporal control of conditioned responding had developed. Moreover, although both groups seemed to underestimate the target duration, this effect seemed to be more substantial in the DHPC-lesioned group; in addition, the response distributions seemed to be broader in this group, suggesting less precise timing. The DHPC-lesioned subjects continued to time less accurately and precisely in the first block of the gap phase (Fig. 3e), although these effects seemed to have disappeared by the last block of the gap phase (Fig. 3f). In addition, comparing Fig. 3c–f suggests that as training progressed, both groups showed peaks progressively closer to the reinforced 15-s time point and their response distributions became less dispersed, suggesting an overall increase in timing accuracy and precision.

Timing accuracy

The findings from the statistical analyses are consistent with the above description of the data. The parameters

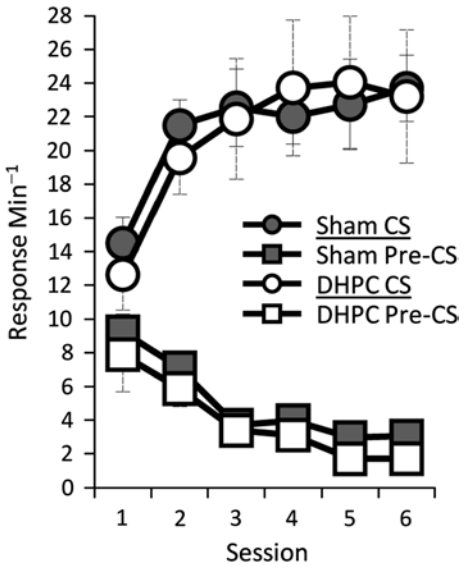

Fig. 2 Overall responding in the acquisition phase. Responding was recorded during the 15-s CS periods and the 15-s background periods prior to CS presentation. *Vertical bars* indicate the standard errors of the means

derived from fitting Gaussian models to these response distributions, calculated for each session block, are presented in Fig. 4. Figure 4a shows the central tendencies for each block of the peak and gap phases, and it is clear that there was a consistent tendency for the DHPC-lesioned group to show maximal responding at earlier time points than the sham-lesioned group. This impression was supported by the results of a 2 (Lesion: Sham or DHPC) × 2 (Phase: Peak or Gap) × 4 (Block of Four Sessions) ANOVA, which revealed a main effect of Lesion [$F(1,16) = 6.46$, $p < 0.05$]. There was also a main effect of Phase [$F(1,16) = 12.54$, $p < 0.005$], supporting the observation that all subjects tended to underestimate the target duration of 15 s initially, but they timed more accurately as training progressed. When the central tendencies were pooled across both phases and all blocks, the mean central tendency of the DHPC-lesioned subjects, 13.11 ± 0.57 s, was significantly different from 15 s [$t(6) = 3.35$, $p < 0.025$ (2-tailed)], but that of the sham-lesioned subjects, 14.91 ± 0.44 s, was not [$t(10) = 0.21$, $p = 0.84$], further suggesting that

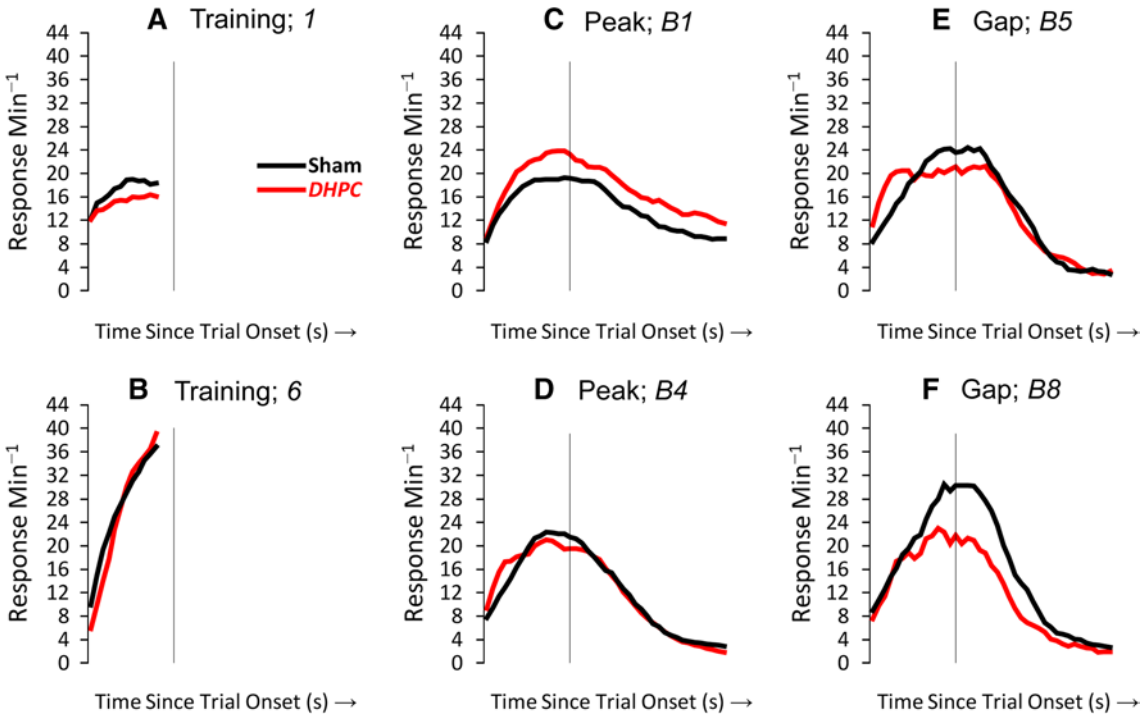

Fig. 3 Conditioned-response distributions on the 15-s conditioning trials and 45-s non-reinforced peak trials at the beginning (*top panels*) and end (*bottom panels*) of each phase. **a**, **b** show data from conditioning trials in the first and final sessions of the acquisition phase (training); **c** and **d** show data from the peak trials in the first and final 4-session blocks of the peak phase (Blocks 1 and 4: sessions 7–10 and 19–22 respectively); **e** and **f** show data from the peak trials in the first and final 4-session blocks of the gap phase (Blocks 5 and 8: sessions 23–26 and 35–38 respectively). *Vertical lines* indicate the time point of US delivery on the conditioning trials. The response traces of the DHPC-lesioned group are highlighted in *red*. Note that the response traces on the conditioning trials in **a** and **b** end earlier than the target duration of 15 s due to smoothing (colour figure online)

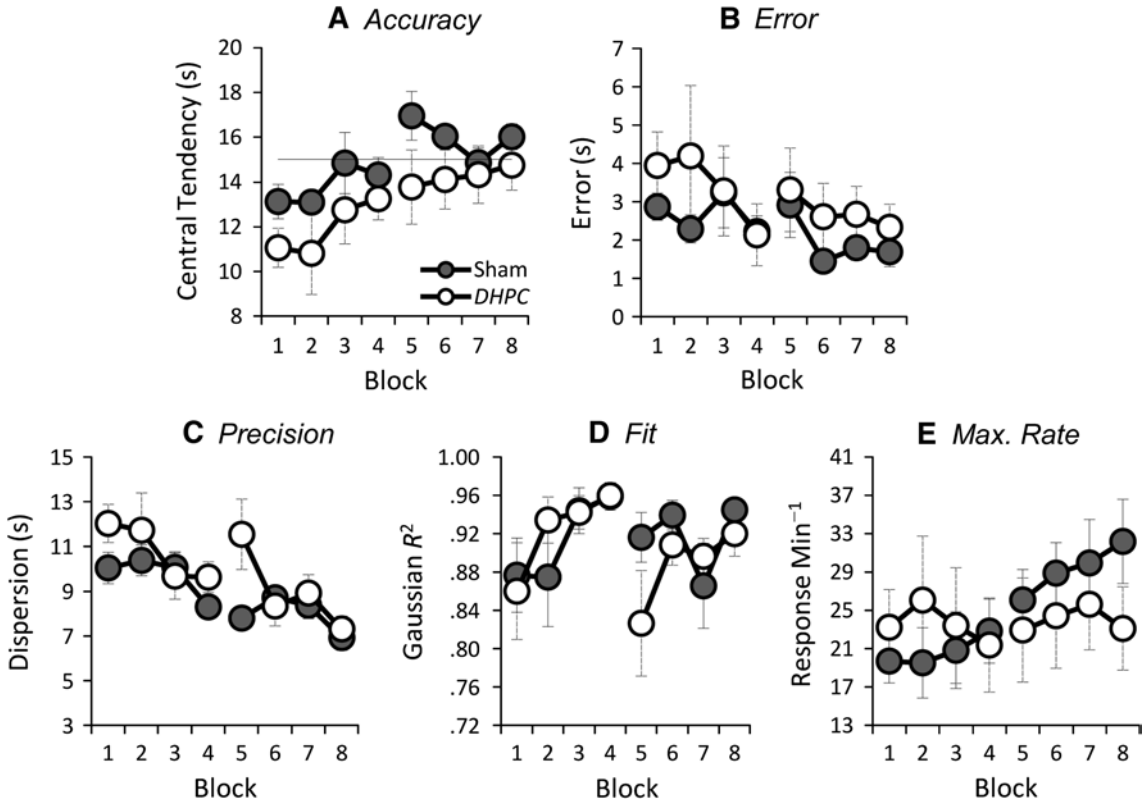

Fig. 4 Conditioned-response timing measures on the non-reinforced peak trials in the peak (Blocks 1–4) and gap phases (Blocks 5–8). **a** shows the central tendencies of the conditioned-response distributions, and **b** shows the timing errors, |15 s − *central tendency*|; these two measures reflect the accuracy of timing. **c** shows the dispersion of the response distributions, which indicates the precision of timing, and **d** shows the goodness of fit (R^2) of the Gaussian models, which indicates the overall degree of temporal control. **e** shows the maximal conditioned-response rates, which indicate the strength of US expectation around the target time. Dorsal hippocampal lesion effects were found on timing accuracy (**a**) and precision (**c**), although these effects seemed to be transient. *Vertical bars* indicate the standard errors of the means; the *horizontal line* in **a** indicates the time point of US delivery on the conditioning trials

the DHPC-lesioned subjects underestimated the target duration more substantially than the sham-lesioned subjects. Figure 4b shows the timing errors, |15 s−*central tendency*|, which suggest that the DHPC-lesioned subjects also appeared to have higher errors than the sham-lesioned subjects. However, this was not significant: a parallel Lesion × Phase × Block ANOVA conducted on these data found only a main effect of Phase [$F(1,16) = 4.43$, $p = 0.05$]; no other effect was significant (all ps > 0.08).

Timing precision and degree of temporal control

The width, or dispersion, of the response distributions—a measure of timing precision—is shown in Fig. 4c. Dorsal hippocampal-lesioned subjects appeared to have broader distributions, indicating less precise timing—a suggestion that was supported by the results of a Lesion × Phase × Block ANOVA, which revealed a significant effect of Lesion [$F(1,16) = 7.03$, $p < 0.05$]. The main effects of Phase and Block were also significant [$F(1,16) = 14.09$, $p < 0.005$ and $F(3,48) = 6.87$, $p < 0.005$, respectively], confirming that timing became more precise as training progressed. In addition, the Lesion × Block interaction approached significance [$F(3,48) = 2.70$, $p = 0.060$], possibly reflecting the fact that the lesion effect on timing precision seemed more substantial in the first block of each phase.

The R^2 coefficients—a measure of the temporal control of responding—are shown in Fig. 4d; these did not appear to differ between groups; a Lesion × Phase × Block ANOVA conducted on these data found a main effect of Block [$F(3,48) = 4.96$, $p < 0.005$], suggesting that the degree of temporal control increased across blocks within each phase; the Phase × Block interaction was also significant [$F(3,48) = 2.74$, $p = 0.05$], possibly due to the transient decline in R^2 in the penultimate block of the gap phase in the sham-lesioned subjects. Nothing else was significant (all ps > 0.09).

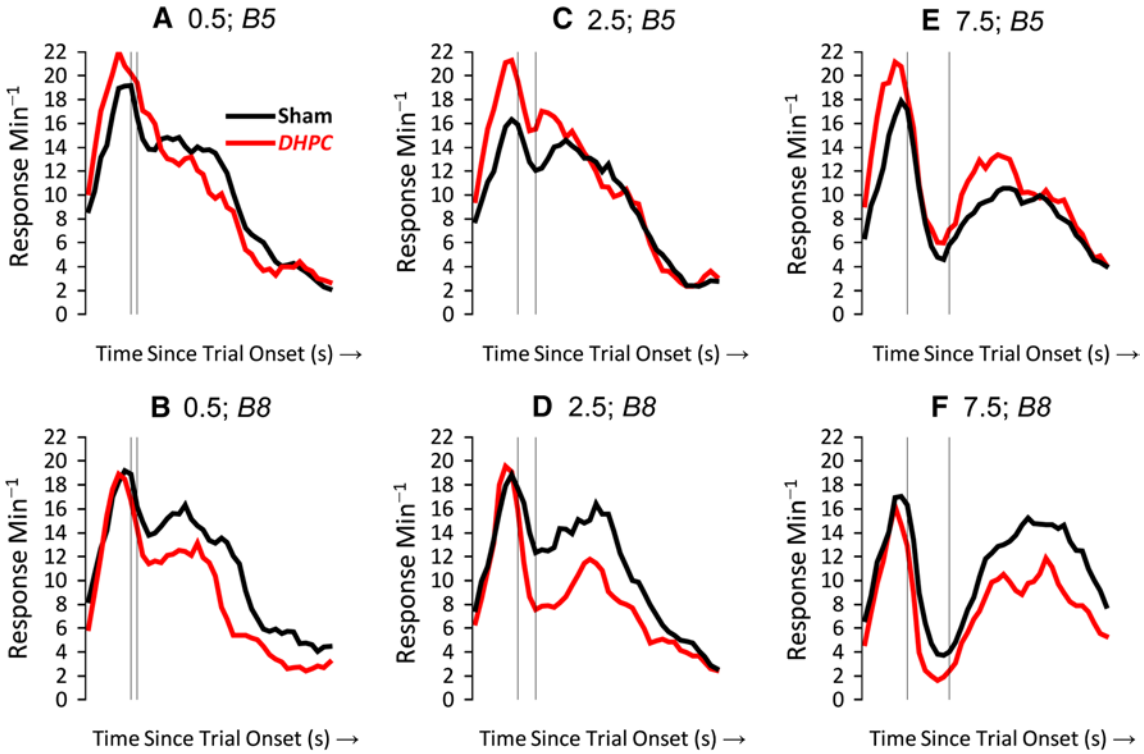

Fig. 5 Conditioned-response distributions on the non-reinforced gap trials. **a**, **c**, and **e** show, respectively, the response distributions on the 0.5-, 2.5-, and 7.5-s gap trials in the first block of four sessions in the gap phase (Block 5: sessions 23–26), whereas **b**, **d**, and **f** show, respectively, the response distributions on the 0.5-, 2.5-, and 7.5-s gap trials in the final block of four sessions (Block 8: sessions 35–38). *Vertical lines* indicate the onset and termination of the gap periods. The response traces of the DHPC-lesioned group are highlighted in *red* (colour figure online)

Strength of US expectation

The maximal rates of conditioned responding, which are taken to reflect the strength of US expectation around the time of reinforcement, are shown in Fig. 4e. The figure suggests that these rates increased across blocks in the sham-lesioned subjects, but not in the DHPC-lesioned subjects. Consistent with this observation, a Lesion × Phase × Block ANOVA found a main effect of Phase [$F(1,16) = 10.00$, $p < 0.01$] and a Lesion × Phase interaction [$F(1,16) = 7.89$, $p < 0.05$]. Simple effect analyses revealed that there was a linear increase in maximal rates across blocks in the sham-lesioned subjects [$F(1,10) = 30.79$, $p < 0.001$], but not in the DHPC-lesioned subjects [$F(1,6) = 0.001$, $p = 0.98$]; there was no simple effect of Lesion in either phase (both $ps > 0.10$).

Sessions 23–38 (Test Blocks 5–8): conditioned-response timing on gap trials

Overview

Group mean response distributions on the gap trials are shown in Fig. 5; distributions from the 0.5-, 2.5- and 7.5-s gap trials are shown in the left, centre, and right panels, respectively; data from the first block of the gap phase are shown at the top, and those from the last block at the bottom.

In the first block of the gap phase, on trials with 0.5- and 2.5-s gaps, the response distributions were only slightly bimodal (Fig. 5a, c) and did not seem to be qualitatively different from the distributions observed on the peak trials; this suggests that the subjects might have continued timing, or only transiently suspended timing, during these shorter gaps. However, when 7.5-s gaps were employed, the response distributions were clearly bimodal (Fig. 5e), although the magnitude of the second response peak did not reach the level of that prior to the gaps. In addition, the second peak of responding on the 7.5-s gap trials occurred later in time than the peaks on the 0.5- and 2.5-s gap trials, and the target duration of 15 s, suggesting that subjects showed the greatest tendency to reset their timing after the longest gaps.

In the final block of the gap phase, the response distributions on *all* types of gap trial were bimodal (Fig. 5b, d, and f), and the longer the gap duration, the later the second peak of responding occurred; furthermore, the second peak of responding on the 7.5-s gap trials occurred later in

time in the final block than in the first block (Fig. 5e vs. f). Overall, these observations suggest that subjects timed differently on gap trials of different duration and that they timed differently in the first versus final blocks of the gap phase. Finally, and consistent with our hypothesis, there was a suggestion that the second peak of responding in the DHPC-lesioned subjects occurred later in time than that of the sham-lesioned subjects, this being especially evident on the 0.5- and 7.5-s gap trials in the final block.

Timing accuracy

To quantify the extent to which gaps of different duration affected timing accuracy (compared to the no-gap trials), relative shifts in central tendency, $c_{Gap}/(c_{Peak} + c_{Gap})$, were computed. The resulting data from the 0.5-, 2.5-, and 7.5-s gap trials are shown in Fig. 6a–c. There seemed to be a consistent tendency for the DHPC-lesioned group to show higher ratios than the sham-lesioned group, and this was true regardless of gap duration. In addition, the ratio scores appeared to increase with gap duration, consistent with the idea that the longer the gap duration, the greater the rightward shift in response distribution. These impressions were supported by the results of a 2 (Lesion) × 3 (Gap Duration) × 4 (Block) ANOVA, which revealed a main effect of Lesion [$F(1,16) = 4.60$, $p < 0.05$], confirming that the DHPC-lesioned subjects showed greater rightward shifts in central tendency than the sham-lesioned subjects. There was also a main effect of Gap Duration [$F(2,32) = 98.58$, $p < 0.0005$], and the linear increase in shifts across gap durations was significant [$F(1,16) = 164.33$, $p < 0.0005$], confirming the suggestion that the longer the gap duration, the greater the rightward shift in central tendency. No other effect was significant (all ps > 0.09).

Strength of US expectation before versus after gaps

There is some suggestion from Fig. 5 that, by the end of the gap phase, the drop in conditioned responding across the gap might be more rapid in the DHPC-lesioned subjects than in the sham-lesioned subjects. This raises the possibility that DHPC lesions might also affect the rate of decay of US representation across time. However, further analyses suggested that this effect was not significant. Conditioned-response rates during the 3-s bins before and after gaps (pooled across blocks) were extracted; these data are shown in Fig. 7a–c. A 2 (Lesion) × 3 (Gap Duration) × 2 (Period: Pre- vs. Post-gap) ANOVA conducted on these data revealed main effects of Gap Duration and Period [$F(2,32) = 38.71$, $p < 0.005$ and $F(1,16) = 24.85$, $p < 0.005$, respectively], as well as an interaction between the two factors [$F(2,32) = 19.88$, $p < 0.005$], suggesting that the drop in conditioned responding was greater when the gap was extended. There was no main effect of Lesion [$F(1,16) = 0.049$, $p = 0.83$], and there were no interactions involving Lesion (all ps > 0.50).

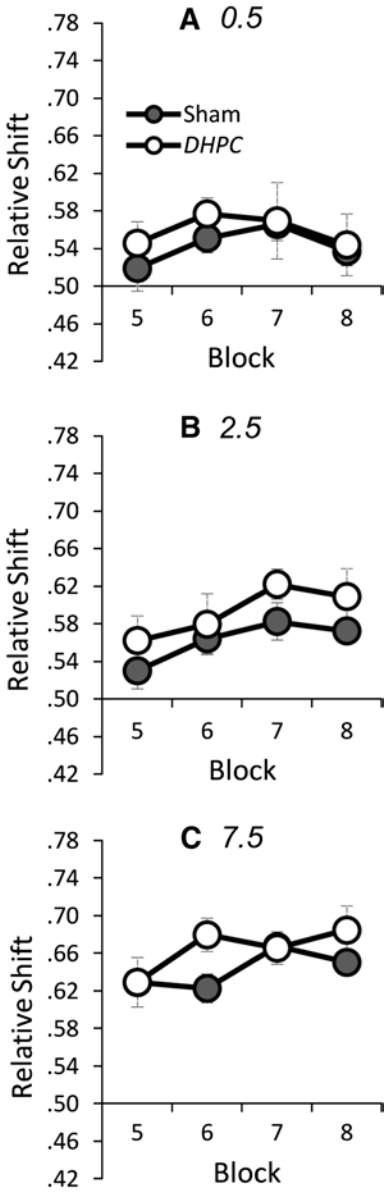

Fig. 6 Relative shifts in central tendency on the non-reinforced gap trials. **a**, **b**, and **c** show, respectively, the relative shifts in central tendency on the 0.5-, 2.5-, and 7.5-s gap trials (relative to the peak trials) in each of the four blocks of four sessions in the gap phase. *Vertical bars* indicate the standard errors of the means

Discussion

Acquisition of conditioned-response timing

In accordance with the presence of temporal signals in DHPC pyramidal neurons during Pavlovian fear

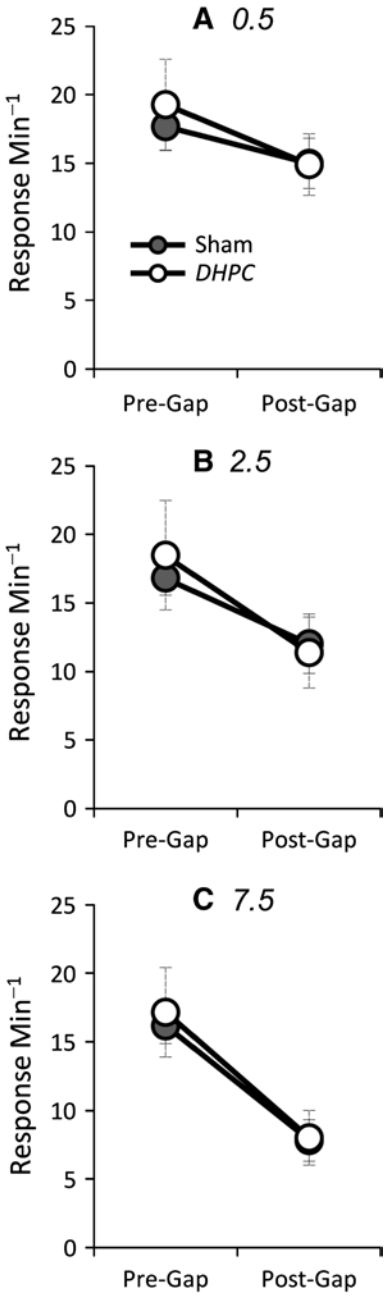

Fig. 7 Conditioned responding before versus after gaps. **a**, **b**, and **c** show the conditioned-response rates during the 3-s bins before and after 0.5-, 2.5-, and 7.5-s gaps (data were pooled across all blocks). *Vertical bars* indicate the standard errors of the means

conditioning (McEchron et al. 2003) and the behavioural findings from our previous study (Tam and Bonardi 2012), DHPC lesions disrupted appetitive conditioned-response timing accuracy. The lesioned subjects showed maximal conditioned responding at earlier time points than the control subjects. However, the lesion effect on timing accuracy did not seem to be permanent, as by the end of the study the lesioned subjects timed as accurately as the control subjects (Fig. 4a). This suggests that neural substrates other than the DHPC, such as striatal dopaminergic neurons (e.g. Malapani et al. 1998; Matell et al. 2003; Meck 2006), could also be involved in temporal learning, but that the rate of acquisition of temporal information of extra-hippocampal systems is slower than that of the hippocampal system. In fact, it has often been demonstrated that animals with partial or complete hippocampal lesions are able to acquire spatial and contextual information, but at slower rates (Rudy et al. 2002; Wiltgen et al. 2006; Bast et al. 2009).

There is at least one discrepancy between the current and previous findings. In our previous study (Tam and Bonardi 2012) DHPC lesions did not affect timing precision, whereas in the current study the lesioned subjects timed less precisely than the control subjects. But similar to the lesion effect on timing accuracy, the lesion effect on timing precision was transient. It remains to be determined whether this discrepancy is related to differences in training protocol (e.g. proportions of reinforced vs. non-reinforced trials) or the extent of lesion (20 % vs. 35 % of total hippocampal volume in the current and previous studies).

Alternative interpretation: failure to inhibit premature responses

An alternative interpretation of the lesion effect on timing accuracy is that DHPC lesions might have transiently induced impulsivity or a response inhibition deficit (Davidson and Jarrard 2004; Cheung and Cardinal 2005; McHugh et al. 2008) rather than a temporal learning or memory deficit, leading to a leftward shift in central tendency in the first few blocks of the test phase. Indeed, the fact that the response distributions of the lesioned subjects were more dispersed than those of the control subjects is consistent with such a proposal. It is difficult to provide conclusive evidence against this possibility; however, a number of arguments may be made against it. For example, such a hypothesis would predict that the lesioned subjects would show leftward shifts in central tendency even after the gaps; thus, the fact that DHPC lesions induced leftward shifts in central tendency on the peak trials but greater *rightward* shifts on the gap trials is at face value not consistent with the impulsivity or response inhibition hypothesis. In addition, inspection of the response distributions shown in Fig. 3c suggests that the magnitude of conditioned responding in the first few time bins of the peak trials was almost identical in the lesioned and control groups in the *first* block of test (sessions 7–10), during which the size of the timing deficit was the greatest; if the lesioned subjects failed to inhibit premature responses, one might expect them to show more responding in the first few time bins. Furthermore, DHPC lesions did not affect the decline of responding in the pre-CS periods that occurred

over training, which could be taken as evidence against the suggestion that the lesioned subjects suffered from a general deficit in response inhibition. Finally, it remains to be determined whether the lesion effect on timing precision is reliable, as no such an effect was found in our previous study (Tam and Bonardi 2012).

Maintaining temporal information in the absence of the CS

The novel finding is that, in accordance with the electrophysiological findings (MacDonald et al. 2011), DHPC lesions affected the maintenance of temporal information across intervening gaps. On the gap trials, the lesioned subjects showed greater rightward shifts in response distribution than the control subjects, suggesting that the lesioned subjects tended to restart timing from 0 s after gaps of different duration (i.e. they adopted the reset-timing strategy), compared to the control subjects who were more likely to adopt the stop-timing strategy (Church 1984; Meck et al. 1984). We observed a similar, albeit non-significant, pattern of results in our previous study (Tam and Bonardi 2012); it is not clear why the effect attained significance in the present experiment, although there were several differences in experimental procedure, perhaps most notably the use of a variety of different gap durations. However, there was no evidence that the enhanced rightward shift seen in the lesioned group was influenced by gap duration.

The DHPC lesion effect on the shifts in response distribution can be interpreted in terms of the hypothesis that, in the absence of the CS, temporal information about the CS decays, or subjectively shortens, over time (Church 1984; Meck et al. 1984; Buhusi and Meck 2000, 2002, 2006a, b, 2009a, b), and that DHPC pyramidal neuronal loss accelerates the rate of decay or subjective shortening of temporal information.[1] Such an interpretation is consistent with the more general suggestion that the hippocampus is involved in maintaining stimulus representations across time (e.g. Rawlins 1985; Rodriguez and Levy 2001; Woodruff-Pak and Disterhoft 2008; Ludvig et al. 2009). However, it must be acknowledged that this hypothesis has to be incomplete, as it has been reported that subjects with complete hippocampal lesions are still able to form associations between CSs and appetitive USs separated by relatively long gaps (Kyd et al. 2007; Lin and Honey 2011). Perhaps the DHPC is responsible for maintaining specifically temporal aspects of the stimulus trace that are not required for successful trace conditioning, but in the absence of further experimental work, this must remain speculative.

Alternative interpretation 1: the role of generalisation decrement

Conditioned-response timing after CS interruption might be determined not by the rate of decay of temporal information, but rather by the degree of generalisation between the intervening gaps and inter-trial intervals (ITIs), which elicit little conditioned responding as they predict the occurrence of no US for a mean duration of 90 s. According to this hypothesis, the longer the duration of a gap, the more it resembles the ITI, and hence the *less* likely that the subjects will treat the CS presentation after the gap as a continuation of the previous cue (Sherburne et al. 1998; Zentall and Kaiser 2005); this provides an explanation for the linear increase in shifts across 0.5-, 2.5-, and 7.5-s gaps. From this perspective, the exaggerated shift in the lesioned subjects across gaps of different duration (Fig. 6a–c) might have been due to *enhanced* generalisation from the ITIs to the gaps, or a failure to discriminate between the variable-duration ITIs and gaps (means = 90 s vs. 3.5 s, respectively). A failure to discriminate between 90-s vs. 3.5-s intervals, however, seems unlikely, given that lesioned subjects are able to discriminate between 15-s versus 30-s intervals (Tam and Bonardi 2012), which is more difficult than a 90-s versus 3.5-s discrimination. In addition, partial hippocampal lesions do not affect temporal discrimination in the temporal bisection task (Bueno and Bueno-Júnior 2011).

Alternative interpretation 2: the role of conditioned inhibition

During the gap phase, the subjects received a larger number of reinforced and non-reinforced trials (512 conditioning vs. 384 gap trials), and this is equivalent to a feature-negative discrimination task involving two types of trial, CS $\rightarrow$ US and CS + x $\rightarrow$ no US trials, where x (the gap) predicts no US. Thus, the gap stimuli might have gradually acquired negative associative strength over the course of the gap phase (Rescorla 1980); after sufficient training, the gap stimuli might have led to a cessation of conditioned responding and timing. From this perspective, the exaggerated effect of shifts in the lesioned subjects (Fig. 6a–c) might have been due to *more* rapid inhibitory conditioning. Such an effect, however, seems unlikely, given that hippocampal-lesioned animals are often thought to be impaired in feature-negative discrimination (McNaughton and Wickens 2003; Davidson and Jarrard 2004). Another

[1] Another view, suggested by the reviewer, is that DHPC lesions increase the probability of resetting after gaps. For example, this could be due to a deficit in attention: the lesioned subjects might be more likely to distribute their attentional resources to the background context as soon as the CS was terminated, and thus when the CS reappeared, they had a higher probability of restarting response timing from 0 s; when the data were averaged across individual trials as in the present study, it would result in an overall rightward shift in response distribution.

problem is that there is no way to demonstrate explicitly the hypothesised negative associative strength of the gap stimuli by the standard tests of conditioned inhibition (summation and retardation tests; Rescorla 1980), as the gap stimuli are, by nature, the absence of the CS rather than the presence of a different cue.

Summary and conclusions

The present study examined the role of the DHPC in conditioned-response timing and maintaining temporal information in the absence of the CS. Dorsal hippocampal lesions transiently disrupted timing accuracy and precision, and they led to a more rapid decay of temporal information across gaps. Alternative interpretations unrelated to temporal processing, including response inhibition, generalisation decrement, and conditioned inhibition, were considered, but the evidence for these possibilities is limited. Thus, our present findings are consistent with the suggestion that DHPC pyramidal neurons are involved in acquisition of conditioned-response timing and maintenance of temporal information across time gaps.

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