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Cameron Meyer-Mueller[1], Pierre-Yves Jacob[1], Jean-Yves Montenay[1], Julien Poitreau[1], Bruno Poucet[1], and Franck A. Chaillan[1]

[1] Laboratory of Cognitive Neuroscience, CNRS – Aix Marseille University, Marseille, France

Correspondence: Franck Chaillan (franck.chaillan@univ-amu.fr) or Bruno Poucet (bruno.poucet@univ-amu.fr), Laboratory of Cognitive Neuroscience, CNRS and Aix Marseille University, Marseille Cedex 13331, France

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Abstract

When facing a choice at a decision point in a maze, rats often display hesitations, pauses and reorientations. Such "vicarious trial and error" (VTE) behavior is thought to reflect decision making about which choice option is best, and thus a deliberation process. Although deliberation relies on a wide neural network, the dorsal hippocampus appears to play a prominent role through both its neural activity and its dynamic interplay with other brain areas. In contrast, the involvement of the ventral hippocampus in deliberation is unexplored. Here, we compared directly the effects of dorsal (dHPC) and ventral intermediate (vHPC) hippocampal inactivations induced by intracerebral muscimol injections on VTE behavior as a model of deliberation. To this aim, we analyzed VTE events as rats were required to switch strategy to a new unlearned reward rule. We used a protocol in which task performance in muscimol-injected animals was minimally altered so as to evidence specific effects on VTE behavior. Our results show subtle alterations in VTE behavior following dHPC, but not vHPC, inactivations, therefore suggesting a specific contribution of the dorsal hippocampus to deliberation through its role in prospective evaluation of future actions.

Introduction

When facing a difficult choice at a decision point in a maze, rats often display "vicarious trial and errors" (VTEs), i.e. a set of behaviors apparently associated with the decision to choose the best option from among several (Redish, 2016 for a review). VTEs can take several forms: animals may simply hesitate for a brief duration, or they may pause for a longer duration and look back and forth, or they may even make go-no-go microchoices, i.e. orient toward arms without entering them (Brown 1992). VTEs are thought to reflect indecision underlying the rat's decision-making and thus a deliberation process. VTEs should occur in early learning or in difficult and changing situations, for example when the rat has to learn a new reward delivery contingency. The rat must already have a schema—a representation of the structure of the environment—to be able to deliberate. Eventually, the rat will learn the task and its behavior will automate. Once automated, VTEs disappear, as the rat is no longer faced with indecision and does not need to deliberate (Redish, 2016).

Deliberation (and thus VTEs, as its overt behavioral correlate) relies on a widespread set of structures including, among others, the medial prefrontal cortex (Hillman and Bilkey, 2012), the ventral striatum (Lansick et al., 2016) and the orbitofrontal cortex (Steiner and Redish, 2012), and in which the hippocampus appears to play a prominent role through its ability to simulate future events. Thus, transient activation of neural ensembles recorded from the dorsal hippocampus of rats during VTE behavior at the choice point of a T-maze was shown to serially represent each of the two possible paths leading to the goal. Since these activations preferentially swept ahead of the animal, they were suggested to represent future possibilities as if, during VTE behavior, the rat was internally deliberating before making its decision (Johnson and Redish, 2007).

While the role of the dorsal hippocampus in deliberation is well documented (Redish, 2016), the involvement of the ventral hippocampus, which hosts cells that project directly to the medial prefrontal cortex (Hoover and Vertes, 2007), is largely unexplored. Here, we compared directly the effects of dorsal (dHPC) and ventral intermediate (vHPC) hippocampal temporary inactivations induced by intracerebral muscimol injections on VTE behavior as a model of deliberation. To this aim, we analyzed VTEs as rats were required to switch strategies to a new unlearned reward rule. One difficulty when assessing VTEs in animals with brain dysfunction is that impaired task performance may obscure subtle alterations in choice behavior (e.g., Hu and Amsel, 1995; Bett et al., 2012). Our protocol was therefore designed to keep task performance in muscimol-injected animals at a control-like level as much as possible so as to evidence specific effects on VTE behavior. Under these circumstances, we found that in spite of nearly normal performance, subtle and specific alterations in VTE behavior were observed following dHPC, but not vHPC, inactivations.

Materials and Methods

Subjects

Eight Long–Evans male rats (R. Janvier, St.-Berthevin, France) weighing 300–350 g were housed one per cage at 20±2°C, under controlled lighting conditions (light on from 07:00 a.m. to 19:00 p.m.). During the experiment, they were mildly food restricted (to 90% free-feeding weight) and were weighed and checked daily. One rat died after surgery reducing group size to 7. All procedures complied with the regulations specified by the European directive (*2010/63/EC)* and French institutional guidelines (authorization n°13-76 to BP). The protocol was approved by the local ethical committee and the French authority under reference number APAFIS#11861-2018020117048590.

Apparatus

The modified T maze apparatus (Figure 1A) was similar to that described by Moussa et al. (2011). It consisted of 4 wooden runways 10 cm wide and painted gray (equipped with 2 cm tall walls on each side), a 100 cm long central stem, a crosspiece 100 cm long forming the two 50 cm long choice arms and two additional runways each connecting the distal end of one choice arm to the base of the central stem. At the cross point between the central stem and the two diagonal runways the walls were raised to 5 cm to prevent animals from making shortcuts. Reward wells were located at the distal end of each choice arm. Food rewards (45 mg sugar pellets) were delivered through two food pellet dispensers (MedAssociates, Vermont, USA) mounted above the wells and activated by remote hand-operated switches. The maze was elevated 40 cm from the ground on a metal frame, located in a room containing several visual cues attached to the walls. The apparatus was illuminated by 4 symmetrical light spots (40 W) fixed to the ceiling. A radio centered above the maze was used to mask uncontrolled sounds. The experimenter was sitting in the adjacent room where the equipment controlling the experiment was located.

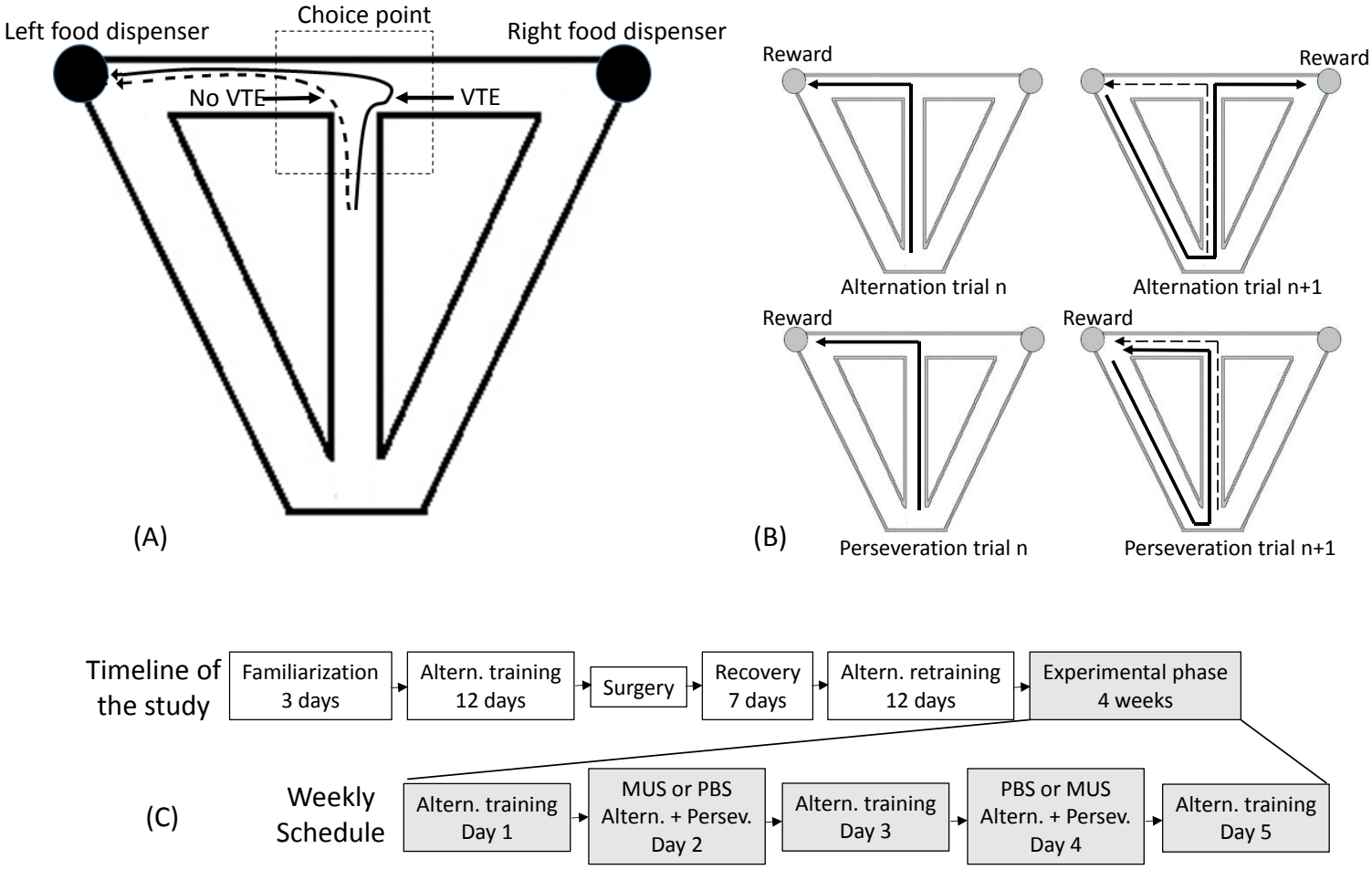

Figure 1. (A) Schematic representation of the continuous T-maze showing an example of a path associated with VTE behavior (black line) compared to a direct path (No VTE, dashed line). The dashed square indicates the choice point zone where VTE behavior was measured. (B) Illustration of the alternation and perseveration reward rules. While rats had to choose the opposite side on two successive trials in the alternation task (top), they had to choose the same side on successive trials in the perseveration task (bottom). Black line is current choice, dashed line is past choice. (C) Timeline of the study and detailed weekly schedule during the experimental phase (see Methods for details).

Pre-surgery behavioral training

Before surgery, rats were first handled daily for one week. They were then familiarized with the maze during 20 min daily sessions for three days, during which they were allowed to freely explore the apparatus and to collect randomly dispersed sugar pellets. Pre-surgery training started the fourth day and required rats to run unidirectional laps, alternating between left and right sides of the continuous T-maze (Figure 1B). Rats were required to run up the central stem and alternatively enter the left or right choice arm in order to obtain a 45 mg sugar pellet. For the first few training sessions, retracing the maze in the incorrect direction was prevented by gently pushing the rat so that it faced the correct movement direction. In the same way, access to the incorrect side of the maze was gently blocked by the experimenter so that the rat used the opposite (correct) arm. A single 45 mg sugar pellet was given each time the animal performed a correct alternation trial. No food was delivered when the rat performed an incorrect trial, either because it perseverated side choice or retraced the maze in the wrong direction. An arm entry was registered when the rat placed four paws into the runaway. Each pre-surgery training session lasted 30 min at most but was stopped if the rat performed 6 successful alternation trials in a row ($p=0.0156$ according to binomial distribution). Pre-surgery training lasted 12 sessions at the end of which all animals had reached the criterion of 6 successive correct choices on one session at least.

Surgery

An analgesic opioid (Buprenorphine, 0.05 mg/kg, SC) was administered at least 30 minutes before any surgery. Then the animals were placed under general anesthesia

(Isoflurane, 1.5%) and the main physiological parameters (temperature, respiratory rate, and heart rate) were monitored.

Four injection cannulas were chronically implanted in each rat, two in the left and right dHPC and two in the left and right vHPC. First the rat was placed in a stereotaxic apparatus (Kopf instruments, Tujunga, CA). After a midline incision of the scalp was made, the skin and muscles were carefully retracted to expose the skull. Holes were drilled above the target regions. Bilateral implantation of guide cannulas was aimed at the following coordinates relative to bregma: dHPC, AP -3mm, L ±2.4mm, and DV -3mm (below the dura); vHPC, AP -5.3mm, L ±5mm, and DV -5mm (Paxinos and Watson, 2005; Saint Blanquat et al., 2013. The guide cannulas were anchored to the skull with four small stainless screws and secured with dental cement. Stainless steel stylets, which extended 0.5mm beyond the tips of the guide cannulas, were placed inside it to prevent occlusion.

At the end of the surgical procedure, a non-steroidal anti-inflammatory drug (Carprofen, 5mg/kg, SC) and an antibiotic (Oxytetracycline, 10 mg/kg, SC) were administered. The rats were placed back in their home cage for at least one week of recovery before post-surgery training.

Post-surgery procedure

After a one-week post-surgery recovery period, rats were retrained in the alternation task as was done before surgery. Habituation to the injection procedure was also progressively introduced by mock treatment during this period: before being placed on the maze, rats were gently restrained while the stylets were removed, cleaned with alcohol and replaced into the guide cannula. Post-surgery retraining lasted 12 sessions after which the

animals were accustomed to the injection procedure and performed consistently 6 successive correct alternation trials per session.

Following post-surgery retraining, the experimental phase started for four weeks, five days a week. The general organization of each week was the following (Figure 1C). Days 1, 3 and 5 simply consisted in retraining the rats in the alternation task such that they maintained performance level (at least 6 successive correct trials per session). On day 2 of each of the four test weeks, animals were intra-cerebrally injected with either phosphate buffer saline (PBS) or muscimol (MUS) in a balanced order across rats and test weeks. Ten min following the injection, the rats were tested successively (i) on performing the well learned alternation task, (ii) on their ability to shift the reward contingency rule by perseverating either right or left choices in a balanced order, (iii) on learning the reverse perseveration rule, and finally (iv) on performing the alternation task. The rule changes between these successive phases were done without any explicit notice once the animal had reached the learning criterion of 6 successive correct choices. On day 4, the rats received the compound MUS or PBS not injected on day 2, and their behavior was assessed in alternation and perseveration reward task rules much as on day 2 except the order of right-left perseveration rules was reversed. This weekly protocol was repeated four times such that MUS was injected twice in dHPC and twice in vHPC on alternate weeks in a pseudo-random order. Similarly, each animal received PBS injections twice in dHPC and twice in vHPC. Thus, eight test sessions were performed for each animal, which included two dHPC MUS sessions, two vHPC MUS sessions, and their respective control PBS sessions.

All test sessions were filmed and simultaneously digitized with a Viewpoint tracking system (Champagne au Mont d'Or, France) for offline analysis of task performance and VTE behavior at the choice point.

Intracerebral drug infusion

Rats were habituated to the infusion procedure during the post-surgery retraining period. On infusion days, rats were gently restrained while the stylets were removed and replaced with sterile infusion needles (30G) that extended 1mm below guide cannulas, and during injection. Ten minutes before the test sessions, rats were given bilateral infusions of either PBS or fluorescent muscimol (Sigma-Aldrich, UK) dissolved at a concentration of 1.65 mM in PBS. Muscimol inhibits local activity within ten minutes following injection, an effect that lasts several hours. Animals received 0.25 µl of either MUS (i.e., 0.41 nM, similar to Rossato et al., 2018) or PBS in both sides of the target structure at a rate of 0.20 µl.min^{-1}. Based on a pilot study and given the injected volume and muscimol concentration, the radius of inactivated brain tissue was 0.5-0.8 mm, which is the width of CA1 in dHPC and vHPC. Needles were connected with PE-20 tubing to a 10 µl Hamilton syringe connected to an infusion pump (Harvard Apparatus). Needles were left in place for 2 min following the infusion to allow diffusion of PBS or MUS. Stylets were replaced after infusion.

Histology

Rats were injected with a lethal dose of sodium pentobarbital (i.p.) and beheaded. The brain was removed and immediately frozen on dry ice. Brains were sectioned (40 µm sections) and stained with cresyl violet. The sections were examined under a light microscope to determine the location of cannula placement. The tips of about half the guide cannulas were located very close dorsally (< 0.5 mm) to the intended target structure while the remaining half was located directly in the target structure (Figure 2). We found no difference in behavior that could be related to this small range in placement. Since injection

needles protruded 1 mm from the cannula tips, infusions affected the intended brain area in all cases.

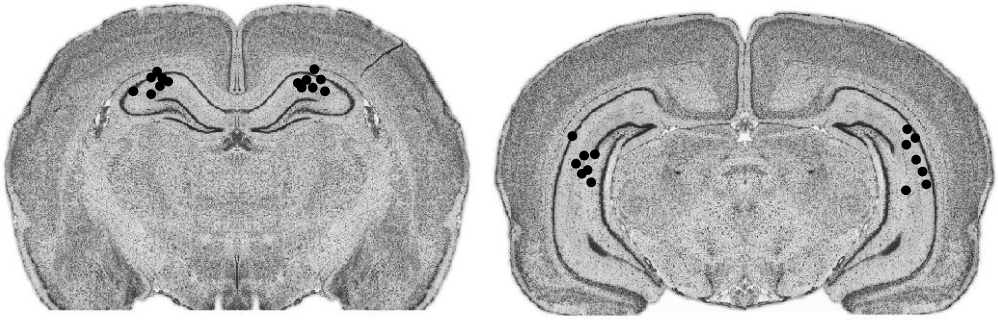

Figure 2. Coronal sections showing the location of dorsal hippocampal (left) and ventral intermediate hippocampal (right) injection sites (i.e., tips of injection needles). From Paxinos and Watson, 2005.

Behavioral analyses and statistics

Behavioral performance was measured using the number of correct and incorrect choices as well as the number of trials to reach 6 successive correct choices in each phase of test sessions.

Vicarious trial and error behavior was measured by digitizing the movements of the animal's nose at the choice point in order to extract the zIdPhi measure, which is the z-scored integrated absolute change in angular velocity of the head in the choice point zone (Papale et al., 2012; Steiner a Redish, 2012). On each choice, the momentary change in motion angle, dPhi, was calculated and integrated over the duration of the choice point pass to yield IdPhi which measures the behavior on a single lap. IdPhi scores were then normalized by z-scoring across laps for each session for each rat (Papale et al., 2012). The z-scored measure, zIdPhi, was compared across injection conditions and test phases. The zIdPhi score is high when the animals show reorientation behaviors (i.e., VTEs) and low when

the animals simply pass through the choice point without a reorientation behavior (Redish, 2016).

The performance and VTE data of each rat were averaged for each injection condition (MUS-D, MUS-V, PBS) and experimental task (i.e., alternation or perseveration). The resulting data were analyzed using within-subject two-way repeated measure ANOVAs with injection condition (MUS-D, MUS-V, PBS) and task phase (reward rule) as the main factors. Specific comparisons were done with paired t-tests when appropriate. A value of $p<0.05$ was considered to indicate a statistically significant difference.

Results

General observations

The total time to complete the different phases of the experiment during test sessions ranged from 17 to 28 minutes (i.e., 113–198 trials) and did not differ across injection conditions ($F_{2,18}$=1.451, p=0.261). Similarly, the mean time required to reach criterion in the alternation phase and in the perseveration phase was similar across injection conditions as shown by the lack of a significant injection x phase interaction ($F_{4,36}$=0.819, p=0.516). Furthermore, no obvious difference in motor behavior was observed between injection conditions. Overall velocity during experimental sessions was similar for all injection conditions (PBS: 23.7 cm/s, dHPC: 23.8 cm/s, vHPC: 24.9 cm/s; $F_{2,18}$=0.17, p=0.845).

Task performance is marginally altered following dorsal hippocampal inactivation

Figure 3A summarizes the main effect of changing the reward rule from spatial alternation to spatial perseveration during test sessions. Inactivations of dHPC or vHPC did

not disrupt task performance in spatial alternation in terms of number of trials to criterion (6 successive correct choices). Expectedly, requiring the rats to shift from the learned alternation rule to the newly introduced perseveration rules (always choosing the same side of the T-maze) increased the number of trials required to reach the learning criterion. This effect was seen to be of the same magnitude in both rats injected with PBS and rats injected with MUS in dHPC and vHPC. Furthermore, shifting back to the alternation rule following the perseveration task was also quickly achieved in both PBS- and MUS-injected animals. These findings were confirmed by the two-way ANOVA which revealed a significant main effect of task phase ($F_{2,36}$=113.048, $p<10^{-6}$) but no effect of injection condition ($F_{2,18}$=0.808, p=0.462) and no injection x phase interaction ($F_{4,36}$=0.412, p=0.799). There was no difference in number of trials to criterion in the two alternation phases that bracketed the perseveration phase for any injection condition (all p>.89; Figure 3A). Furthermore, no effect of repeating test sessions was found. In particular, successive exposures to the change in reward rule did not affect the number of trials to criterion in any injection condition: the ANOVA conducted on the perseveration phase revealed no significant effect of test sessions ($F_{1,18}$=2.026, p=0.172) and injection condition ($F_{2,18}$=1.076, p=0.362), and no interaction between the two factors ($F_{2,18}$=1.968, p=0.169), thus suggesting that shifting from alternation to perseveration was equally difficult across successive test sessions. Finally the proportion of errors to total trials during the perseveration phase was remarkably similar for the three injection conditions (PBS: 32.7±1.2%; MUS-D: 32.5±1.1%; MUS-V: 32.6±1.8%) and significantly greater than during the alternation phase (PBS: 22.1±1.8%; MUS-D: 24.2±5.4%; MUS-V: 27.6±2.9%).

The change in reward contingency required rats to shift to a perseveration rule while they had been extensively trained to alternate. The first sign of a successful shift was therefore the observation of sequences composed of two successive responses to the

correct side of the maze that followed an incorrect choice. We therefore counted the overall number of such sequences (labeled ECC for Error-Correct-Correct) during the perseveration phase and the results showed that it was similar in all conditions (PBS: 10.6±0.8; MUS-D: 9.6±1.0; MUS-V: 9.9±1.2; all $p>0.43$). We then evaluated the distribution of ECC sequences during three periods that were equivalent in numbers of trials and corresponded to the beginning, the middle and the end of the perseveration phase (Figure 3B). As expected, ECC sequences strongly increased across the three periods in all injections conditions (main effect of period, $F_{2,36}=33.298$, $p<10^{-6}$ with no effect of injection condition, $F_{2,18}=0.270$, $p=0.766$ and no period x injection interaction ($F_{4,36}=0.908$, $p=0.47$). However, we noticed that rats displayed less ECC sequences in the MUS-D condition than in the PBS condition during the initial learning period (PBS vs. MUS-D: $t_6=2.97$, $p=0.012$; paired t test), suggesting they were delayed in shifting to the new learning rule. No other significant difference was found in this data (all $p>.10$).

In summary, in all injection conditions rats performed well on the familiar spatial alternation task, shifted gradually to the new spatial perseveration rule (though this shift was slightly slower in the MUS-D condition), and finally shifted quickly back to the familiar spatial alternation strategy.

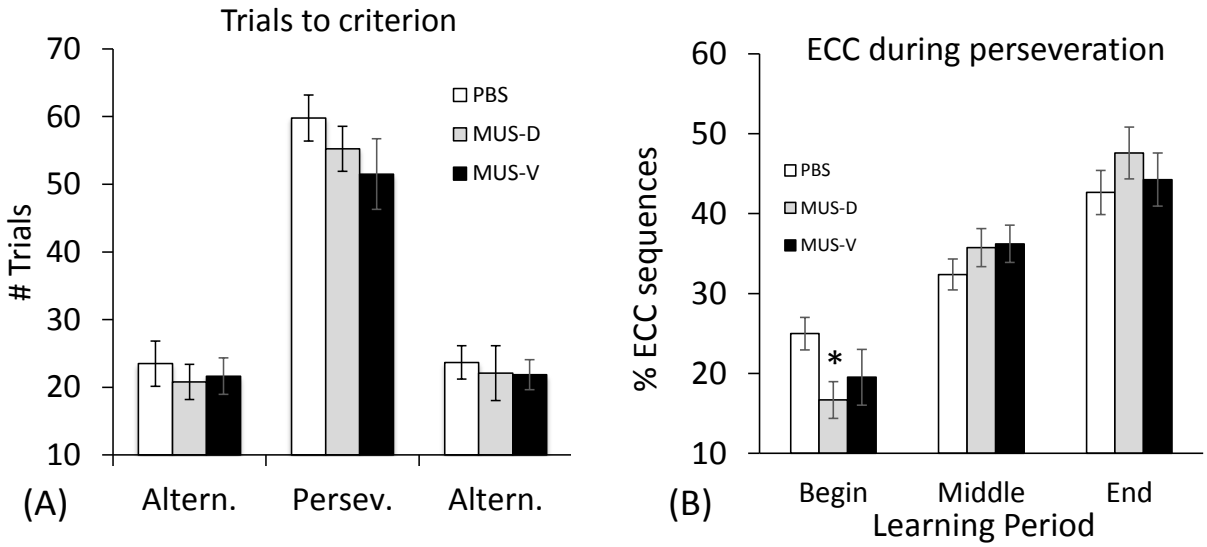

Figure 3. (A) Performance change (mean number trials to criterion ± S.E.M) across the three phases of test sessions. No difference was observed between the three injection conditions in any of the reward rules. (B). Distribution of ECC (Error–Correct–Correct) sequences of choices during three stages of the perseveration phase (means ± S.E.M.). ECC sequences increased during perseveration learning in all conditions, thus reflecting progressive shifting to the new reward rule, but this increase was delayed in MUS-D (* p=0.012 compared to PBS condition).

VTE behavior is altered in a very subtle way by dHPC inactivation

VTE behavior was assessed by calculating the zIdPhi score which measures the change in angular velocity of the head in the choice point zone (see methods). VTE behavior was expected to increase when a new reward rule (i.e. perseveration) was introduced following the familiar alternation rule. Therefore we first focused on the change in VTE behavior by calculating the difference in zIdPhi scores between these two phases (Figure 4A). As expected, VTEs increased following the rule change with no statistically significant effect of injection condition ($F_{2,18}$=1.471, p=0.256). Furthermore, VTEs gradually increased during the perseveration phase (Figure 4B) in all injection conditions. This effect was confirmed by the ANOVA which revealed a significant effect of learning period ($F_{2,36}$=10.029, p=0.0003) with

no effect of injection condition ($F_{2,18}$=2.855, p=0.084) and no significant period x injection interaction ($F_{4,36}$=1.279, p=0.296).

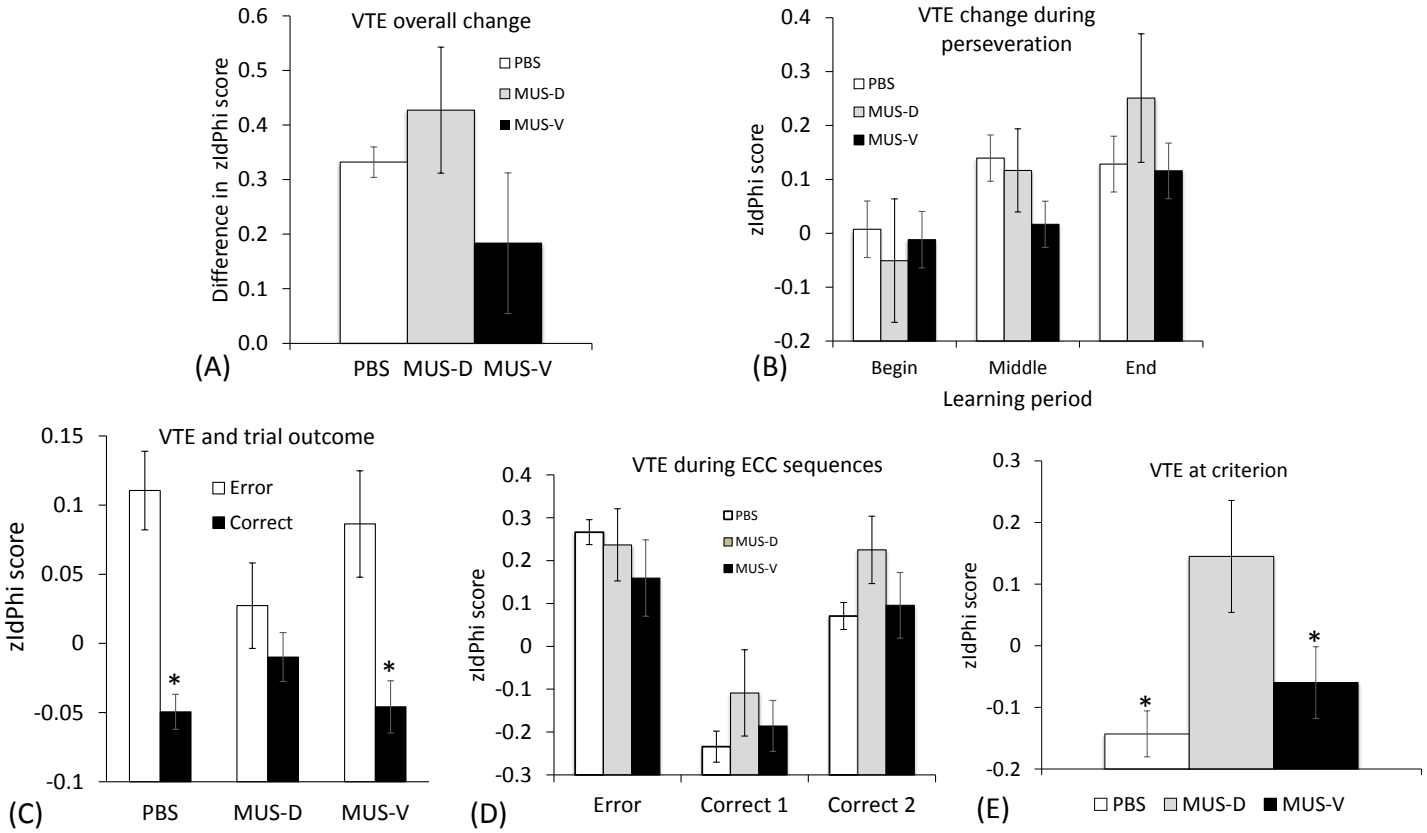

Figure 4. (A) Change in VTE behavior following the introduction of the new reward rule. Each bar shows the mean difference (± S.E.M.) between the zIdPhi scores of the perseveration phase and the preceding alternation phase. Positive values indicate an increase in VTE behavior in all injection conditions. (B) Time-course of VTEs during the perseveration phase (means ± S.E.M.). The zIdPhi scores increased gradually during perseveration learning in all conditions. (C) VTEs and trial outcome (means ± S.E.M.). VTE behavior was significantly greater before an error than before a correct choice in PBS and MUS-V conditions (p=0.003 and p=0.038, respectively), but not in MUS-D condition. (D) VTEs during ECC (Error–Correct–Correct) sequences of choices (means ± S.E.M.). The time-course of VTE behavior was similar in all conditions with a decrease in zIdPhi scores between the error and the first correct choice and a sharp increase between the two successive correct choices. (E) VTE scores at end of the perseveration phase when rats reach the learning criterion (means ± S.E.M.). VTE behavior was exaggerated in MUS-D condition compared to PBS (p=0.015) and MUS-V (p=0.055).

Because the perseveration task required rats to repeatedly choose the same arm after they had been trained to alternate, shifting to the correct rule required avoiding alternation choices leading to errors. It was therefore of interest to see if VTE behavior was different during correct choices and errors. In a first analysis, we simply plotted the zIdPhi scores associated with all correct vs. all incorrect choices (Figure 4C) and found that they were greater before an error than before a correct choice. The two-way ANOVA conducted on this data failed to reveal an effect of injection condition ($F_{2,36}$=0.294, p=0.747) but showed a significant effect of choice ($F_{1,36}$=19.791, p<0.0001) and a significant choice x injection interaction ($F_{2,36}$=3.609, p=0.037). While the choice effect was statistically significant in PBS (t_6=4.015, p=0.003) and MUS-V (t_6=2.139, p=0.038) conditions, it was absent in the MUS-D condition (t_6=0.779, p=0.233).

In a second analysis, we looked at the change in VTEs during ECC (Error-Correct-Correct) sequences which are associated with successful shifting to the perseveration rule (see above). We found that the time-course of VTE behavior was similar in all conditions, with greater zIdPhi scores before an error than before the following correct choice and a sharp increase between the two successive correct choices following an error (Figure 4D). This observation was confirmed by the ANOVA which revealed a significant effect of choice ($F_{2,36}$=32.530, p<10^{-8}) with no effect of injection condition ($F_{2,18}$=1.205, p=0.323) and no significant choice x injection interaction ($F_{4,36}$=0.729, p=0.571). When combined with the lack of an overall difference in zIdPhi scores between errors and correct choices, this data suggests that control-like VTE behavior in MUS-D injected rats occurred only upon successful rule shifting. This conclusion is further supported by the finding that zIdPhi scores at the very end of the perseveration phase, when rats have reached the learning criterion, were greater in MUS-D than in PBS and MUS-V conditions (Figure 4E). The ANOVA revealed a significant

effect of injection condition ($F_{2,18}$=5.063, p=0.018) and specific paired comparisons showed that the difference was significant between MUS-D and PBS (t_6=2.890, p=0.014), and almost significant between MUS-D and MUS-V (t_6=1.872, p=0.055), but not significant between PBS and MUS-V (t_6=1.313, p=0.119).

Discussion

Because impaired performance following brain dysfunction may be a confounding factor masking changes in VTE behavior, our protocol was designed to study VTEs in the absence of altered performance. In this protocol, rats were extensively pre-trained on a continuous spatial alternation task which, once automatized, is insensitive to hippocampal dysfunction when there is no delay between successive trials (Ainge et al., 2007). More generally, well-trained spatial behavior involves hippocampal activity to a minimal extent, as shown by reduced expression of the immediate early gene Arc (Gardner et al., 2016). During test sessions, following brief retraining in the alternation task, the rats were subjected, without notice and during the same session, to a change in the reward delivery contingencies, in which they now had to select the same arm repeatedly. This perseveration strategy is known to be insensitive by hippocampal inactivation (White & McDonald, 2001). Nevertheless, the unexpected change in reward rule required rats firstly to recognize that the former alternation rule was no longer correct, and secondly to discover the new reward rule so as to modify their behavior accordingly and optimize their gains. We reasoned that switching to the perseveration rule would therefore involve a deliberation process, thus allowing us to study changes in VTE behavior *per se* since no strong alteration in task performance was expected following disruption of hippocampal activity. Using a within-subject design in which rats received small amounts of muscimol or PBS in dHPC or vHPC on

alternate days, we found that in spite of nearly normal performance, subtle and specific alterations in VTE behavior were observed following dHPC, but not vHPC, inactivations.

As expected, muscimol injections in the dorsal (MUS-D) or ventral intermediate hippocampus (MUS-V) did not impair performance of the familiar alternation task, nor did they alter learning of the perseveration rule, whether this was measured by trials to criterion or by percent of errors. One noticeable effect, however, was that rats continued to alternate longer in the MUS-D than in the PBS condition, which resulted in the production of less numerous ECC (Error–Correct–Correct) sequences at the beginning of perseveration learning. This slight delay in shifting to the perseveration rule was not observed in the MUS-V condition.

VTE behavior was also altered in a very subtle, though interesting way in the perseveration task following dorsal hippocampal muscimol injections. As expected (Schmidt et al., 2013), VTEs increased after the rule change and continued to increase as learning proceeded. Although this increase was of the same magnitude for all injection conditions, VTE behavior was found to be much greater before an error than before a correct choice only in the PBS and MUS-V conditions, but not in the MUS-D condition. Because the perseveration task required rats to repeatedly choose the same arm, the same magnitude of VTE behavior before a correct choice and before an error reflected a failure of MUS-D rats to notice that the reward rule had changed and to anticipate that their choice was wrong. It was only when they successfully shifted to the perseveration rule, as shown by the production of ECC sequences, that they would display the same pattern as in PBS and MUS-V conditions, with both strong VTE behavior before an error and before successive correct choices. The finding that VTE behavior was associated with understanding the new reward rule in MUS-D rats was further supported by the observation that zIdPhi scores were much

greater in the MUS-D condition than in the PBS condition at the very end of the perseveration phase, when rats had reached the learning criterion. Together this data indicate impaired prospective evaluation of choice outcome during early learning stage following dorsal hippocampal inactivation.

Overall, our results are consistent with several previous reports (Bett et al., 2012; Papale et al., 2012). In the Bett et al. study, extensive lesions of the hippocampus produced a significant impairment in the performance of a spatial reversal task and a significant delay in the production of VTEs. While control animals displayed more VTEs before finding the new reward location (i.e., presumably during errors) than after identifying the correct reward location, such a difference was not observed in lesioned animals, much like in the present work MUS-D rats display VTE mostly when they switch to the appropriate perseveration rule. Nevertheless, in the Bett et al. study the same animals were also tested in a simple two-choice visual discrimination task in a completely different setup, in which no deficit was found in hippocampal rats in either learning performance or VTE behavior. A possible explanation for this discrepancy may be that the rats, contrary to our procedure, did not have to abandon a previously successful strategy to switch to a new one. However, it is also interesting to note that when the visual discrimination was made slightly more difficult by the addition of a third choice option with no change in reward contingency, the dynamics of VTE behavior in control and hippocampal rats closely resembled those observed in our study. Thus following the shift from two-choice to three-choice visual discrimination, the rise in VTE behavior was initially greater in control rats than in hippocampal rats whereas VTE behavior remained at a higher level in hippocampal rats than in control rats later in learning, much as for MUS-D rats in the present study.

None of the effect reported above for MUS-D rats was observed in the MUS-V condition. Although MUS-V rats often had some scores that were intermediary between PBS and MUS-D rats, their overall pattern closely resembled the PBS pattern in all respects. The absence of any effect in the MUS-V condition was somewhat unexpected, given the central position of the ventral intermediate hippocampus and the interdependence between dorsal and ventral hippocampus in information processing (Lee et al., 2019) as well as its connections with the medial prefrontal cortex, a structure important for behavioral flexibility (Ragozzino et al., 1999; Hoover and Vertes, 2007). Although it is possible that more extensive inactivations could yield significant alterations of VTE behavior, they would also likely result in performance deficits, thus making it difficult to disentangle specific effects on VTEs. The same limitation applies to dHPC inactivation: even though some effects were observed in MUS-D rats, they were rather modest. There are several possible reasons for this observation. First, the volume of hippocampal tissue affected by diffusion of muscimol was small (and perhaps too small for vHPC which is slightly larger than dHPC), though it was in the range observed in previous studies (Rossato et al., 2018). Again, larger diffusion volumes would probably produce greater effects at the expense, however, of lower specificity of alterations in VTE behavior if execution of the task is too strongly disrupted. Second, it could be argued that small effects were observed because neither well-trained spatial alternation nor response learning (i.e. perseveration) put much burden on the hippocampus, therefore making the shift from the alternation task to the perseveration task too easy to involve the hippocampus. However, our focus was on the process of deliberation underlying decision making when behavioral flexibility is required. If the hippocampus is involved in such process (Redish, 2016), then what matters is not the difficulty of each task separately but rather the hippocampus role when a flexible switch in strategy (and therefore

a deliberation about which choice option is best) is required. With regard to this point, our results point to a deficit in MUS-D rats which, though it is modest, reveals a potentially interesting contribution of the dorsal hippocampus to decision making.

Recent explanations of the deliberation process have proposed that it is associated with the tendency for dHPC place cells to display firing sequences, proceeding far ahead of the rat when it displays VTEs at the choice point and alternating serially between goal-related options, as if the rat was deliberating about possible outcomes (Redish, 2016 for review). With this in consideration, dHPC inactivation would therefore result in impaired ability to prospectively assess possible outcomes during VTEs by altering the production of such sequences of firing. Nevertheless, that VTE behavior was observed once dHPC rats have resolved the task, a stage at which sweep ahead are rarely observed (Redish, 2016), would also indicate a relative disconnection between the two processes. Turning to the ventral hippocampus, our results show little contribution of vHPC to VTE behavior. Assuming there is a causal link between VTEs and dHPC firing sequences, the implication is that one should not observe vHPC firing sweeps similar to those seen in dHPC. This issue is out of the scope of the present study and will require dedicated work to be clarified.

In conclusion, we found that selective dHPC inactivation did not abolish VTE behavior but did alter, at an early learning stage, the production of VTE events in relation to choice outcome. Although these findings support the view that dHPC contributes to deliberation through its role in prospective evaluation of future actions, they also suggest that normal VTE behavior can occur once the rat understands the new reward rule.

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References

Ainge, J. A., van der Meer, M. A. A., Langston, R. F. and Wood, E. R. (2017). Exploring the role of context-dependent hippocampal activity in spatial alternation behavior. Hippocampus, 17, 988–1002.

Bett, D., Allison, E., Murdoch, L. H., Kaefer, K., Wood, E. R. and Dudchenko, P. A. (2012). The neural substrates of deliberative decision making: contrasting effects of hippocampus lesions on performance and vicarious trial-and-error behavior in a spatial memory task and a visual discrimination task. Frontiers in Behavioral Neuroscience, 6, 70.

Brown, M. F. (1992). Does a cognitive map guide choices in the radial arm maze? Journal of Experimental Psychology: Animal Behavior Processes, 18, 56–66.

Gardner, R. S., Suarez, D. F., Robinson-Burton, N. K., Rudnicky, C. J., Gulati, A., Ascoli, G. A. and Dumas, T. C. (2016). Differential Arc expression in the hippocampus and striatum during the transition from attentive to automatic navigation on a plus maze. Neurobiology of Learning and Memory, 131, 36–45.

Hillman, K. L. and Bilkey, D. K. (2012). Neural encoding of competitive effort in the anterior cingulate cortex. Nature Neuroscience, 15, 1290–1297.

Hoover, W. B. and Vertes, R. P. (2007). Anatomical analysis of afferent projections to the medial, prefrontal cortex in the rat. Brain Structure and Function, 212, 149–179.

Hu, D. and Amsel, A. (1995). A simple test of the vicarious trial-and-error hypothesis of hippocampal function. Proceedings of the National Academy of Sciences USA, 92, 5506–5509.

Johnson, A., and Redish A. D. (2007). Neural ensembles in CA3 transiently encode paths forward of the animal at a decision point. Journal of Neuroscience, 27, 12176-12189.

Lansink, C. S., Meijer, G. T., Lankelma, J. V., Vinck, M. A., Jackson, J. C. and Pennartz, C. M. A. (2016). Reward expectancy strengthens CA1 theta and beta band synchronization and hippocampal–ventral striatal coupling. Journal of Neuroscience, 36, 10598–10610.

Lee, S. L., Lew, D., Wickenheisser, V. and Markus, E. J. (2019). Interdependence between dorsal and ventral hippocampus during spatial navigation. Brain and Behavior, 9, e01410.

Moussa, R., Poucet, B., Amalric, M. and Sargolini, F. (2011). Contributions of dorsal striatal subregions to spatial alternation behavior. Learning and Memory, 18, 444–451.

Papale, A., Stott, J. J., Powell, N. J., Regier, P. S. and Redish, A. D. (2012). Interactions between deliberation and delay-discounting in rats. Cognitive, Affective, and Behavioral Neuroscience, 12, 513–526.

Paxinos, G. and Watson, C. (2005) The Rat Brain in Stereotaxic Coordinates. Compact 6th Edition, Academic Press, New York, 400p.

Ragozzino, M. E., Detrick, S. and Kesner, R. P. (1999). Involvement of the prelimbic–infralimbic areas of the rodent prefrontal cortex in behavioral flexibility for place and response learning. Journal of Neuroscience, 19, 4585–4594.

Redish, A.D. (2016). Vicarious trial and error. Nature Reviews Neuroscience, 17, 147-159.

Rossato, J. I., Moreno, A., Genzel, L., Yamasaki, M., Takeuchi, T., Canals, S. and Morris, R.G.M. (2018). Silent learning. Current Biology, 28, 3508+

Saint-Blanquat, P., Hok, V., Save, E., Poucet, B. and Chaillan, F. (2013). Differential role of the dorsal hippocampus, ventro-intermediate hippocampus and medial prefrontal cortex in updating the value of a spatial goal. Hippocampus, 23, 342–351.

Schmidt, B. J., Papale, A. E., Redish, A. D. and Markus, E. J. (2013). Conflict between place and response navigation strategies: effects on vicarious trial and error (VTE) behaviors. Learning and Memory, 20, 130–138.

Steiner, A. P. and Redish, A. D. (2012). The road not taken: neural correlates of decision making in orbitofrontal cortex. Frontiers in Neuroscience, 6, 131.

White, N. M. and McDonald, R.J. (2002). Multiple parallel memory systems in the brain of the rat. Neurobiology of Learning and Memory, 77, 125–184.