Wang, Jia and Bast, Tobias and Wang, Yu-Cong and Zhang, Wei-Ning Hippocampus and two way active avoidance conditioning: contrasting effects of cytotoxic lesion and temporary inactivation. Hippocampus . ISSN 1050-9631

Hippocampus

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Journal:	*Hippocampus*
Manuscript ID:	Draft
Wiley - Manuscript type:	Research Article
Keywords:	active avoidance learning, excitotoxic hippocampal lesion, temporary deactivation, tetrodotoxin (TTX), muscimol

Hippocampus and two way active avoidance conditioning: contrasting effects of cytotoxic lesion and temporary inactivation

Running title: Hippocampus and two way active avoidance conditioning

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Text pages: 37; figures: 5; tables: 1

Grant sponsor: Swiss Federal Institute of Technology (ETH) Zurich to Joram Feldon; additional support from JiangSu University, JiangSu province, China (BL2014068, 2012M521019, Nr. 13JDG001, 11JDG112, and NSFC Nr. 31201346) to WZ and JW.

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ABSTRACT

Hippocampal lesions tend to facilitate two way active avoidance (2WAA) conditioning, where rats learn to cross to the opposite side of a conditioning chamber to avoid a tone-signaled foot shock. This classical finding has been suggested to reflect that hippocampus-dependent place/context memory inhibits 2WAA (a crossing response to the opposite side is inhibited by the memory that this is the place where a shock was received on the previous trial). However, more recent research suggests other aspects of hippocampal function that may support 2WAA learning. More specifically, the ventral hippocampus has been shown to contribute to behavioral responses to aversive stimuli and to positively modulate the meso-accumbens dopamine system, whose activation has been implicated in 2WAA learning. Permanent hippocampal lesions may not reveal these contributions because, following complete and permanent loss of hippocampal output, other brain regions may mediate these processes or because deficits could be masked by lesion-induced extra-hippocampal changes, including an upregulation of accumbal dopamine transmission. Here, we re-examined the hippocampal role in 2WAA learning in Wistar rats, using permanent NMDA-induced neurotoxic lesions and temporary functional inhibition by muscimol or tetrodotoxin (TTX) infusion. Complete hippocampal lesions tended to facilitate 2WAA learning, whereas ventral or dorsal hippocampal lesions had no effect. In contrast, ventral or dorsal hippocampal muscimol or TTX infusions impaired 2WAA learning. Ventral infusions caused an immediate impairment, whereas after dorsal infusions rats showed intact 2WAA learning for 40-50 min, before a marked deficit emerged. These data show that functional inhibition of ventral hippocampus disrupts 2WAA learning, while the delayed impairment following dorsal infusions may reflect the time required for drug diffusion to ventral hippocampus. Overall, using temporary functional inhibition, our study shows that the

ventral hippocampus contributes to 2WAA learning. Permanent lesions may not reveal these contributions due to functional compensation and extra-hippocampal lesion effects.

INTRODUCTION

A classical finding from hippocampal lesion studies is that damage to the hippocampal system facilitates two way active avoidance (2WAA) conditioning in rats (Gray and McNaughton, 1983; Guillazo-Blanch et al., 2002; O'Keefe and Nadel, 1978; Olton and Isaacson, 1968; Pouzet et al., 1999; Weiner et al., 1998; Tonkiss et al., 1990). In 2WAA conditioning, rats are trained to avoid a foot shock by crossing to the opposite side of a conditioning chamber in response to a conditioned stimulus (CS) predicting the foot shock. Facilitation of 2WAA conditioning by hippocampal damage has been suggested to reflect the disruption of place or contextual memory, a well-established consequence of hippocampal lesions (Anagnostaras et al., 2001; Bannerman et al., 2004; Bast, 2007; Bast et al., 2009; Morris et al., 1980; Morris et al., 1990; Nadel and Hardt, 2004; O'Keefe and Nadel, 1978; Rudy, 2009). More specifically, it was proposed that 2WAA requires the rat to overcome fear of a place or context to return to an area of the conditioning chamber where it has just received a foot shock. Hippocampal damage, disrupting place or context memory, might reduce such fear and thereby facilitate 2WAA (Guillazo-Blanch et al., 2002; O'Keefe and Nadel, 1978; Olton and Isaacson, 1968). Indeed, hippocampal lesions especially disrupt the rapid, one-trial, place and contextual learning required to remember the place or context of events, such as a shock, experienced on a specific trial (Bast et al., 2009; Morris et al., 1990; Wiltgen et al., 2006).

However, while hippocampus-dependent one-trial place or context memory may inhibit 2WAA, other aspects of hippocampal function might be expected to support such behavior. First, the hippocampus, especially the ventral part, supports behavioral responses and fear conditioning to aversive stimuli in a variety of paradigms (Bannerman et al., 2004; Bast et al., 2001a, Bast et al., 2001b, Bast, 2007; Bast, 2011; Fanselow and Dong, 2010; Kjelstrup et al., 2002; Pentkowski et al., 2006). Second, activity of the hippocampus,

especially the ventral part, positively modulates midbrain dopamine projections to the forebrain, including nucleus accumbens (Bast, 2007; Bast, 2011; Grace et al., 2007; Taepavarapruk et al., 2008), and stimulation of midbrain dopamine projections and accumbens dopamine transmission have been implicated in the facilitation of 2WAA conditioning (Darvas et al., 2011; Ilango et al., 2012; Shumake et al., 2010; Wadenberg and Hicks, 1999; Boschen et al. 2011; Smith et al., 2007; Dombrowski et al., 2013;).

Hippocampal lesions may fail to reveal such hippocampal contributions to 2WAA conditioning due to functional compensation and secondary changes in other brain regions. First, other brain structures implicated in aversively motivated responses (Maren and Quirk, 2004) or in the modulation of the meso-accumbens dopamine system (Sesack and Grace, 2010) may compensate for the permanent loss of hippocampal contributions. Second, there is evidence that hippocampal lesions result in secondary changes in the nucleus accumbens that facilitate local dopamine transmission, including dopamine receptor hypersensitivity (Mittleman et al., 1993) and enhanced dopamine transmission (Lipska et al., 1992; Wilkinson et al., 1993).

Compared to permanent hippocampal lesions, temporary functional inactivation of the hippocampus may afford less opportunity for compensatory adaptations and cause less secondary changes in efferent sites (Lomber, 1999). Therefore, temporary inactivation may reveal some aspects of hippocampal function that have eluded lesion studies. In support of this possibility, we have successfully used functional inactivation to reveal a previously undiscovered hippocampal role in certain sensorimotor processes (consistent with functional links to prefrontal and subcortical sites involved in these processes). More specifically, temporary hippocampal inactivation by the GABA-A receptor agonist muscimol or the sodium channel blocker tetrodotoxin (TTX) reduces both locomotor activity and prepulse inhibition, whereas hippocampal lesions do either not affect or, in the case of locomotor

activity, even tend to increase these measures (Bast and Feldon, 2003; Bast et al., 2001b; Zhang et al., 2002).

In the present study, we examined the contribution of the hippocampus to 2WAA conditioning in rats, using temporary functional inactivation by bilateral infusions of the GABA-A receptor agonist muscimol or the sodium channel blocker TTX into the dorsal or ventral hippocampus. For comparison, we also examined the effect of NMDA-induced neurotoxic lesions to the dorsal, ventral or complete hippocampus. We predicted that temporary hippocampal inactivation, especially if targeting the ventral part, would impair 2WAA conditioning, whereas hippocampal lesions would, if at all, facilitate conditioning.

MATERIALS AND METHODS

Subjects

The subjects were 108 male Wistar rats (Zur:WIST[HanIbm], Research Unit Schwerzenbach, Schwerzenbach, Switzerland), weighing about 250 g and aged 2 to 2.5 months at the time of surgery. Forty-seven rats were used for the lesion experiment and 61 rats for the infusion experiments. Rats were housed in groups of four per cage under a reversed light-dark cycle (lights on: 19:00-07:00) in a temperature (21 ± 1°C) and humidity (55 ± 5%) controlled room. All rats were allowed free access to food and water. After surgery, they were caged individually. Starting one day before surgery and then throughout the studies, all rats were handled daily. Behavioral testing was carried out in the dark phase of the cycle. All experiments were conducted in accordance with the principles of laboratory animal care (NIH publication no. 86-23, revised 1985) and Swiss regulations for animal experimentation.

Stereotaxic surgery

Rats were anesthetized with Nembutal (sodium pentobarbital, 50 mg/ml, Abbott Labs, North Chicago, IL) at a dose of 1 ml/kg (i.p.) and their head was placed in a Kopf stereotaxic frame. After application of a local anesthetic (lidocaine), an incision was made on the scalp and the skull surface exposed. Bregma and lambda were aligned in the same horizontal plane.

Hippocampal neurotoxic lesion

Forty-seven rats were allocated to four groups: 10 rats received bilateral lesions of the dorsal hippocampus, 10 received bilateral lesions of the ventral hippocampus, 10 received bilateral lesions of the complete hippocampus, 8 rats receiving sham surgery and 9 unoperated rats served as controls. For each of the lesion groups, the smallest possible craniotomy was made above the injection sites on each side of the brain. The procedure used to make the lesions was the same as described in Zhang et al (2004). Rats received multiple injections of N-methyl-D-aspartate (NMDA, in volumes between 0.025 and 0.10 µl per injection) dissolved in 0.1M phosphate-buffered saline (PBS, pH 7.4) at a concentration of 10 mg/ml. Rats in the complete hippocampal lesion group received injections at 36 sites, rats in the dorsal hippocampal lesion group at 22 sites and rats in the ventral hippocampal lesion group at 14 sites (Table 1). The injection cannula was left in place at each injection site for 60 s before being retracted. Rats in the sham surgery group were placed in the frame, had the skull exposed and were given microinjections of PBS, as a vehicle control (four rats received PBS injections at the 22 sites used in the dorsal hippocampal lesion group and the other four at the 14 sites used in the ventral hippocampal lesion group). The scalps were then stitched. After surgery, all rats were allowed at least 2 weeks to recover before the beginning of the 2WAA experiment.

Table 1

Implantation of guide cannulae for intrahippocampal infusions

Sixty-one rats were used for the hippocampal infusion experiments. Rats were placed in the stereotaxic frame and a small hole (1.5 mm in diameter) was drilled on each side of the skull to reveal the cortex overlying the hippocampus. Stainless steel guide cannulae (26 gauge, 9 mm or 7 mm for ventral or dorsal hippocampus, respectively) in a Perspex holder (custom made) were implanted bilaterally into the brain aiming at the ventral (-5.2 mm posterior and ±5.0 mm lateral to bregma, and -5.0 mm ventral to dura) or dorsal (-3.0 mm posterior and ±1.5 mm lateral to bregma, and -2.5 mm ventral to dura) hippocampus, using the same coordinates as in previous studies (Bast and Feldon, 2003; Bast et al, 2001b; Zhang et al, 2002; Zhang et al., 2014). The guide cannulae were fixed to the skull with three anchoring skull screws and dental cement. Stainless steel stylets (34 gauge) extending 0.5 mm beyond the tips of the guide cannulae were placed inside the guide cannulae to prevent occlusion. After surgery, rats were allowed to recover for five days before the beginning of the 2WAA experiments. During this time, the experimenter conducted daily health checks, gently habituated the rats to the handling required for the infusions, and replaced any missing stylet.

Intracerebral infusions

The rats were manually restrained, the stylets removed carefully, and infusion cannulae (34 gauge, stainless steel) were inserted into the brain through the previously implanted guide cannulae. The tips of the infusion cannulae protruded 1.5 mm beyond the tip of the guide cannulae into the ventral or dorsal hippocampus, resulting in final dorso-ventral coordinates of 6.5 and 4.0 mm below dura in the ventral and dorsal hippocampus, respectively, as in our

previous studies (Bast and Feldon, 2003; Bast et al, 2001b; Zhang et al., 2002; Zhang et al., 2014). The infusion cannulae were connected to 10-µl Hamilton microsyringes by flexible PEEK tubing. The syringes were mounted on a Kds microinfusion pump. All rats were infused bilaterally and the infusion volume was 0.5 µl/side, delivered at the rate of 0.5 µl/min. Afterwards, the infusion cannulae were kept in place for an additional 60 s to allow for tissue absorption of the infusion bolus before being replaced by the stylets. As in our previous studies (Bast et al., 2001b, Zhang et al., 2002), muscimol (1 µg/0.5 µl/side) was infused immediately and TTX (10 ng/0.5 ml/side) 20 min before behavioral testing. Accordingly, half of the rats infused with vehicle, i.e. 0.9% saline (0.5 µl/side), received infusion immediately before the behavioral sessions, the other half 20 min before the behavioral sessions.

Drugs

Muscimol [$C_4H_6N_2O_2$(1/2 H_2O); Tocris, Bristol, UK] was dissolved in 0.9% saline at a concentration of 2 µg/µl on the day of infusion. TTX ($C_{11}H_{17}N_3O_8$; Tocris, Bristol, UK) was stored at –40°C in aliquots containing 40 ng/µl in 0.9% saline. On the day of infusion, these aliquots were thawed and diluted with 0.9% saline to obtain a solution with a concentration of 20 ng/µl for intra-hippocampal infusion.

Two-way active avoidance paradigm

Apparatus

The apparatus consisted of four identical 2-way shuttle boxes (Coulbourn Instruments, model E10-16TC), each set in a ventilated, sound- and light-attenuating shell (model E10-20). The internal dimensions of each chamber were 35 x 17 x 21.5 cm as measured from the raised grid floor. The box was divided by an aluminium hurdle (17 cm long, 4 cm high) into two identical compartments. The hurdle was low enough to allow the subject to shuttle freely

between the two compartments and thin enough to ensure that the rats could not stand on it to avoid foot shocks. The modular shock floor (model E10-16RF) consisted of 24 stainless steel rods 0.48 cm in diameter and spaced 1.5 cm apart, center to center. The grid floor was hinged in the middle of the box and thus displacement of the subject from one compartment to the other (i.e., a shuttle) could be detected by the corresponding pivoting of the gird floor unit. Scrambled shocks could be delivered from a constant direct current shock generator (CI, model E13-14) and scanner (model E13-13) set at 0.5 mA. The chamber was illuminated during the whole experimental session by two small light bulbs (1.8W, houselights), mounted 19 cm above the grid floor in the middle of the side walls. The CS was an 85-dB tone produced by a 2.9 kHz tone module (model E12-02) placed behind the shuttle box on the floor of the isolation cubicle. Background noise was provided by a ventilation fan affixed to each isolation cubicle. Data acquisition and stimulus parameters were controlled by a Compaq PC computer using a DOS-based software program developed in our laboratory.

Procedures

Two-way active avoidance procedures were based on previous studies (Pouzet et al., 1999; Weiner et al., 1998). Testing was carried out over 4 days, with habituation to the test apparatus on day 1, 2WAA acquisition on day 2 and a session to test retention of the learned avoidance response on day 4. Individual rats completed all stages of the experiment in the same shuttle box.

Habituation to the apparatus: Rats were placed in the shuttle box with the house lights on for 60 min and then returned to their home cage. The number of spontaneous crosses between the two sides of the shuttle box was recorded during the habituation session, providing a measure of basal activity. Rats in the intracerebral infusion study were

subsequently matched for this measure of activity prior to their assignment to one of the three drug infusion groups.

Acquisition of two way active avoidance: Acquisition training was carried out one day after the habituation session. In the infusion experiments, the infusions were conducted before acquisition training. Each animal was placed into the experimental chamber and received 100 avoidance trials, presented on a variable inter-trial interval (ITI), ranging from 10 to 90 s (average 50 s). Each avoidance trial began with the onset of a 10 s tone CS. If the animal did not shuttle to the opposite compartment during the 10 s tone (avoidance response), a foot shock (unconditioned stimulus, US) of 0.5 mA was delivered, the tone remaining on with the shock. The maximal duration of the shock was 2 s. A shuttle response during this period (escape response) terminated the shock as well as the CS. If the animal did not cross during the entire 12 s tone-shock trial, the response was recorded as an escape failure. Shuttle response latency was calculated as a combined avoidance / escape latency throughout the 100-trial test session, such that a value of 0-12 seconds was assigned to each animal regardless of whether an animal avoided (0-10 s), escaped (10-12 s) or did not escape the shock (maximal 12 s).

Test of two way active avoidance retention: Two days after the initial acquisition training, all of the rats were subjected to a retention test of 2WAA. The procedure was the same as used in acquisition training. Avoidance responses and the latencies were recorded as in the acquisition training. The aim of this test was to assess the retention of the 2WAA response learnt two days earlier, as well as the possible long-term effects of the infusion.

Measures of two way active avoidance and other behavioral measures: As measures of 2WAA, the number of avoidance responses and response latencies were recorded in 10 trial blocks. As a control measure for potential non-specific motor effects, crossings during the ITI

were also recorded in 10 trial blocks. In addition, the overall number of escape failures across the 100 trial sessions was recorded.

Experimental design

Rats were tested in batches of 4. The different testing boxes and the order of testing were counterbalanced among the experimental groups as far as possible.

Lesion experiment (Experiment 1)

There were four groups: bilateral dorsal hippocampal lesion group (n=10), bilateral ventral hippocampal lesion group (n=10), bilateral complete hippocampal lesion group (n=10) and control group, consisting of sham (n=8) operated and unoperated (n=9) rats (overall n=17). Before combining sham operated and unoperated rats into one control group, separate analysis confirmed that these two groups did not differ in any of the behavioral measures examined (all $F < 0.68$).

Infusion experiments (Experiments 2 and 3)

Rats with bilateral implantation of guide cannulae targeting ventral (n = 17) or dorsal hippocampus (n = 44) were used to test the effects of ventral (Experiment 2) or dorsal hippocampal infusions (Experiment 3). Infusions were only made before the acquisition session. Based on matched activity measures during the habituation session, the cannulated rats were allocated to one of three infusion groups to receive bilateral infusion of 0.5 µl saline/side, 10 ng TTX/0.5µl/side, or 1 µg muscimol/0.5 µl/side into either the ventral or the dorsal hippocampus. In the experiment involving ventral hippocampal infusions, group sizes were: saline, n=6; TTX, n=4; muscimol, n=7. In the experiments involving dorsal hippocampal infusions, group sizes were: saline, n=14; TTX, n=15; muscimol, n=15.

Histology

After the completion of behavioral testing, all hippocampal lesioned, cannulated and five randomly selected sham-operated rats were deeply anesthetized with an overdose of 2.5 ml/kg Nembutal (sodium pentobarbital, 50 mg/ml, i.p.) and transcardially perfused with 0.9% NaCl solution, followed by 120 ml of 4% formol saline (4°C) to fix the brain tissue. The brains were extracted from the skull, post-fixed in 4% formalin solution, and subsequently cut into 40-µm coronal sections on a freezing microtome. For the examination of the hippocampal lesions or the injection sites, every fourth section through the hippocampus was mounted onto gelatine coated slides and stained with cresyl violet. After staining, the sections were dehydrated through an alcohol series, cleared with xylene, and coverslipped with Eukitt (Kindler, Freiburg, Germany). Subsequently, the sections were examined with a light microscope to verify lesions and cannula placements. Lesions were quantified as outlined below and infusion sites were mapped onto plates taken from the atlas of Paxinos and Watson (1998).

Quantification of lesion size

Hippocampal lesion size was measured using a method adapted from Bast et al. (2009). Briefly, for each rat from the lesion and sham groups, the relative volume of intact/spared hippocampal tissue was measured. The intact hippocampus (including CA1, CA3, and dentate gyrus) in each coronal section was outlined using the light microscope connected via a digital camera to a computer running ImageJ software (version 1.7, National Institutes of Health, Maryland). The total hippocampal area was measured in pixels for each brain and the mean hippocampal area in pixels was calculated for each group. The proportion of spared tissue in individual brains from the lesion group was calculated by dividing the spared hippocampal

area by the mean hippocampal area in the sham group, and the extent of hippocampal damage of each subject for each group was calculated as 100% minus percentage of spared tissue. From these values, the mean % of hippocampal damage was calculated for each lesion group.

Data analysis

Statistical analyses were performed with StatView software (Abacus Concepts, Inc., Berkeley, CA, 1992). Groups were used as between-subjects factor and blocks of 10 trials as repeated measures. *Post hoc* comparisons were conducted using Fisher's protected least significant difference test. Significant differences were accepted at $P < 0.05$. Values are presented as means. In the text, variability is indicated by the standard error of the mean (S.E.M.). In the figures, for the sake of clarity, the standard error (S.E.) derived from the appropriate mean square of the ANOVA indicates variability.

RESULTS

Histology

Experiment 1: Neurotoxic hippocampal lesions

Photomicrographs of coronal sections taken from representative rats with bilateral dorsal, ventral and complete hippocampal excitotoxic lesions together with schematic reconstructions of the minimal (solid areas) and maximal (solid and shaded areas) damage are depicted in Fig. 1A. Sham lesioned rats showed no discernable damage to the hippocampus or to extra-hippocampal areas, apart from occasional small traces of the needle tracks.

Rats with lesions targeting the dorsal hippocampus showed substantial cell loss and extensive gliosis in the dorsal half to two-thirds of the hippocampus (including the dentate gyrus, CA1 and CA3), while the ventral third was intact. In the most anterior part of the

dorsal hippocampus, minor sparing was seen in the medial dentate gyrus and CA1 subfield. The mean amount of damage ± SEM was 58.5 ± 2.4% of total hippocampal volume (range: 49.8 - 67.2%). In addition to the intended hippocampal damage, there was some damage to the dorsal subiculum and to the cortex overlying the hippocampus. Rats with lesions targeting the ventral hippocampus typically showed extensive cell loss and gliosis in the ventral half to two thirds of the hippocampus, while the dorsal third remained intact. In some cases, minor damage was seen in the ventral subiculum and the ventral pre- and parasubiculum; however, this damage never extended into the entorhinal cortex. In three of the ventral lesioned rats, only very limited damage could be discerned in the ventral hippocampus (less than 10% of total hippocampal volume), and these three rats were therefore excluded from further analysis. The mean amount of hippocampal damage in the rest of the ventral hippocampal lesion group was 55.8 ± 4.1% of total hippocampal volume (range: 38.0 - 72.6%).

The complete hippocampal lesion group was characterized by substantial cell loss and intense gliosis throughout the entire longitudinal extent of the hippocampus. In some cases, minor sparing of the most caudo-medial part of the dorsal hippocampus (dentate gyrus and CA1) was observed, while in other cases sparing of the dentate gyrus granule cells at the most ventral tip of the hippocampus was observed. No signs of damage to the amygdala, or dorsal thalamus were noted. In some cases, some damage to the ventral and dorsal subiculum and the ventral and dorsal pre- and parasubiculum was observed, yet this damage did not extend into the entorhinal cortex. In general, the damage present in the complete hippocampal lesion group was comparable to the extent and location of the damage seen in the dorsal and ventral hippocampal lesion groups separately. One complete hippocampal lesioned rat showed very limited damage (less than 10% of total hippocampal volume), and consequently this rat was excluded from further analysis. The mean amount of hippocampal damage in the rest of the complete hippocampal lesion group was 100 ± 3.6% of the total hippocampal volume (range:

84.4 – 116.7%). The final number of rats used in the behavioural analysis was 17 Cont (9 unoperated, 8 sham-lesioned), 10 DH, 7 VH and 9 CH.

Experiments 2 and 3: Hippocampal infusion sites

In all 61 cannulated rats, the centers of the infusion sites, i.e. the tips of the infusion cannulae, were located within or around the border of the ventral (n = 17, Experiment 2) or dorsal (n = 44, Experiment 3) hippocampus as intended (Fig. 1B). Tissue damage was found in the hippocampus and the cortex overlying the hippocampus. This damage was restricted to the area immediately surrounding the guide and infusion cannulae.

Fig 1 insert about here

Experiment 1: Dorsal or ventral hippocampal lesions do not affect two-way active avoidance conditioning, whereas complete hippocampal lesions tend to improve performance

Dorsal or ventral hippocampal lesions did not alter conditioned 2WAA acquisition, while complete hippocampal lesions tended to enhance acquisition; this was supported by the analysis of avoidance responses (Fig. 2A, left panel) and of the latencies to avoid or escape the foot shook following CS onset (Fig. 2B, left panel). The analysis of percent avoidance response during acquisition test using a 4 x 10 (group x blocks of 10 trials) ANOVA only yielded a significant main effect of blocks ($F_{9, 351} = 46.4$, $P < 0.0001$), indicating an overall increase in avoidance response as a function of training. Neither the main effect of group ($F_{3, 39} = 1.36$, $P > 0.26$) nor the group x blocks interaction ($F_{27, 351} = 1.12$, $P > 0.31$) was significant. However, consistent with previous evidence for improved acquisition of 2WAA

behavior following substantial damage to the hippocampus or fimbria fornix (see Introduction), rats with complete hippocampal lesions tended to show more avoidance responses as compared to the other groups during the first half (50 trials) of the acquisition session. Indeed, a separate 4 x 5 (group x blocks of 10 trials) ANOVA of the percent avoidance responses during the first 50 trials of the acquisition test yielded a strong trend towards a main effect of group ($F_{3, 39} = 2.57$, $P = 0.06$). Post hoc comparisons revealed that the average percentage of avoidance responses across the first 50 trials was increased in the complete hippocampal lesion group (58.0 ± 8.6%) as compared to the control (33.5 ± 5.6%, $P < 0.02$) and dorsal hippocampal lesion group (33.6 ± 6.8%, $P < 0.03$). There was no significant difference between the complete and the ventral lesion (44.8 ± 8.6%) groups ($P > 0.26$) and between the control, dorsal and ventral groups (all P's > 0.28). Analysis of the response latencies yielded similar results. A 4 x 10 (group x blocks of 10-trials) ANOVA of response latencies during acquisition training revealed only a significant main effect of blocks ($F_{9, 351} = 42.2$, $P < 0.0001$), indicating an overall decrease of response latencies as a function of blocks, but neither the main effect of group ($F_{3, 39} = 2.02$, $P > 0.12$) nor the group x blocks interaction ($F_{27, 351} = 1.06$, $P > 0.38$) attained significance. However, rats with complete hippocampal lesions exhibited shorter response latencies than the three other groups during the first 50 trials of acquisition training. A separate 4 x 5 (group x blocks of 10-trials) ANOVA of the crossing response latency during the first 50 trials revealed a significant main effect of group ($F_{3, 39} = 3.31$, $P < 0.03$), alongside a highly significant main effect of blocks ($F_{4, 156} = 61.4$, $P < 0.0001$) with no interaction group X block ($F_{12, 156} = 1.23$, $P > 0.26$). Post hoc comparisons revealed that the average response latencies of the complete hippocampal lesion group (6.2 ± 0.8 s) across the first 50 trials were significantly shorter than those of the control (8.5 ± 0.4 s, $P < 0.01$) and dorsal hippocampal lesion group (8.6 ± 0.6 s, P

< 0.01). There were no significant differences between complete and ventral lesion (7.6 ± 0.7 s) group (P> 0.15) and between control, dorsal and ventral group (all Ps > 0.32).

Two days after acquisition training, all groups showed similar 2WAA behavior during the retention test in terms of avoidance responses (Fig. 2A, right panel). However, the control group tended to show slightly higher response latencies, especially as compared to the complete hippocampal lesion group, during the beginning of the retention session (Fig. 2B, right panel). A 4 x 10 (group x blocks of 10 trials) ANOVA of avoidance responses yielded only a significant main effect of blocks ($F_{9, 351} = 20.3$, $P < 0.0001$), reflecting an improvement in 2WAA responding during the first 20-30 trials. Neither the main effect of group ($F_{3, 39} = 1.03$, $P > 0.39$) nor the group x blocks interaction ($F_{27, 351} = 1.05$, $P > 0.39$) were significant. A 4 x 10 (group x blocks of 10 trials) ANOVA of response latencies during retention test yielded a significant main effect of blocks ($F_{9, 351} = 11.8$, $P < 0.0001$), no main effect of group ($F_{3, 39} = 0.58$, $P > 0.6$), but a strong trend towards an interaction of group x blocks ($F_{27, 351} = 1.46$, $P = 0.069$). This trend reflected lower latencies in the lesion groups, especially in the complete hippocampal lesion group, as compared to the control group during the first 20 trials, before asymptotic values were reached by all groups. The latency data indicate that the complete hippocampal lesion group carried over some of the facilitated 2WAA performance from the acquisition to the retention test session.

Fig 2 insert about here

Experiment 2: Tetrodotoxin or muscimol infusion into the ventral hippocampus disrupt the acquisition of two-way active avoidance behavior

Tetrodotoxin and muscimol infusion into the ventral hippocampus markedly disrupted acquisition of 2WAA behavior, with avoidance responses remaining at a very low level (Fig. 3A, left panel) and response latencies remaining high (Fig. 3B, left panel) throughout the whole acquisition session. A 3 x 10 (group x blocks of 10 trials) ANOVA of the percent avoidance response during acquisition test yielded highly significant main effects of group ($F_{2, 14} = 14.23$, $P < 0.0005$) and of blocks ($F_{9, 126} = 10.65$, $P < 0.0001$) and a highly significant interaction of group x blocks ($F_{18, 126} = 6.03$, $P < 0.0001$). The significant interaction group x blocks of 10 trials reflected that the number of avoidance responses in the saline group increased as training progressed, whereas the TTX and muscimol rats showed very low levels of avoidance responses throughout the acquisition session. Analysis of response latencies produced similar results. An overall 3 x 10 (groups x blocks of 10-trials) ANOVA of response latencies during acquisition training showed a significant main effect of group ($F_{2, 14} = 9.70$, $P < 0.003$) and of blocks ($F_{9, 126} = 9.14$, $P < 0.0001$), as well as a significant group x blocks interaction ($F_{18, 126} = 6.68$, $P < 0.0001$). The significant interaction of group x blocks of 10-trials reflected that response latencies in the saline group decreased as a function of acquisition training, whereas latencies remained high in the TTX and muscimol groups.

Two days after acquisition training, the retention test was carried out without infusion (Fig. 3A and B, right panels). Rats that had received TTX or muscimol into the ventral hippocampus before acquisition training still showed evidence for slightly impaired 2WAA behavior, probably reflecting that, in contrast to the saline group, they benefited only little from the preceding acquisition training. However, all three groups showed a similar increase in avoidance response as the session progressed, suggesting that the impairment in 2WAA acquisition induced by TTX or muscimol infusion was temporary and reversible. A 3 x 10 (group x blocks of 10 trials) ANOVA of percent avoidance response during retention test

revealed a strong trend towards a main effect of group ($F_{2, 14} = 3.5$, $P = 0.059$) and a main effect of blocks ($F_{9, 126} = 23.28$, $P < 0.0001$), but no interaction group x block ($F_{18, 126} = 1.53$, $P = 0.92$). Pairwise comparisons between groups revealed that the overall percentage of avoidance responses during the retention test was higher in the saline group (88.83 ± 0.40%) than in the muscimol (65.71 ± 4.9%, $P < 0.03$) and TTX (69.00 ± 15.27%, $P = 0.08$) groups, which did not differ from each other ($P = 0.75$). Analysis of response latencies using a 3 x 10 (groups x blocks of 10 trials) ANOVA only yielded a main effect of blocks ($F_{9, 126} = 14.75$, $P < 0.0001$), without a main effect of groups ($F_{2, 14} = 2.15$, $P > 0.15$) or an interaction of groups x blocks ($F_{18, 126} = 1.21$, $P > 0.26$), even though numerically latencies were higher in the muscimol and TTX groups compared to the saline group.

Fig 3 insert about here

Experiment 3: Tetrodotoxin or muscimol infusion into the dorsal hippocampus cause a delayed disruption in the acquisition of two-way active avoidance behavior

TTX and muscimol infusions into the dorsal hippocampus disrupted 2WAA acquisition, as indicated by reduced avoidance responses (Fig. 4A, left panel) and increased response latencies (Fig. 4B, right panel). Interestingly, the deficit only emerged during the last 40-50 trials of acquisition training. A 3 x 10 (group x blocks of 10 trials) ANOVA of the percent avoidance response during acquisition training revealed a strong trend towards a main effect of group ($F_{2, 41} = 2.95$, $P = 0.06$), a main effect of blocks ($F_{9, 369} = 30.68$, $P < 0.0001$), as well as a highly significant group x blocks interaction ($F_{18, 369} = 4.12$, $P < 0.0001$). The significant interaction of group x block of 10-trials reflected that the reduction of avoidance responses in the TTX and muscimol groups as compared to the saline group emerged during

the second half of acquisition training. Similarly, a 3 x 10 (groups x blocks of 10-trials) ANOVA of response latencies during acquisition training yielded a significant main effect of group ($F_{2, 41} = 4.0$, $P < 0.03$), a highly significant main effect of blocks ($F_{9, 369} = 28.2$, $P < 0.0001$) and a significant groups x blocks interaction ($F_{18, 369} = 5.73$, $P < 0.0001$). The significant interaction of groups x blocks of 10-trials reflected that the increase of response latencies in the TTX and muscimol groups in comparison to the saline group emerged during the second half of acquisition training. Two days after acquisition training, when tested in the absence of infusion, the TTX and muscimol groups still showed impaired 2WAA behavior as compared to the saline group during the first half of the retention session, but had acquired similar performance levels by the beginning of the second half (Fig. 4A and B, right panels). A 3 x 10 (group x blocks of 10 trials) ANOVA of percent avoidance responses yielded a significant main effect of blocks ($F_{9, 369} = 38.6$, $P < 0.0001$) and a significant groups x blocks interaction ($F_{18, 369} = 3.10$, $P < 0.001$), but no main effect of group ($F_{2, 41} = 1.47$, $P > 0.24$). The interaction of groups x blocks reflected that the saline group reached asymptotic levels of active avoidance responses within the first block of 10 trials, whereas the TTX and muscimol groups only showed such high levels of avoidance responses during the second half of the session. Analysis of response latencies yielded similar results. A 3 x 10 (groups x blocks of 10 trials) ANOVA of response latencies during the retention test yielded a significant main effect of blocks ($F_{9, 369} = 27.5$, $P < 0.0001$) and a significant groups x blocks interaction ($F_{18, 369} = 3.12$, $P < 0.0001$), but no main effect of groups ($F_{2, 41} = 1.72$, $P > 0.19$). The significant groups x blocks interaction reflected that the saline rats reached asymptotically low levels of response latencies within the first block of 10 trials, whereas the TTX and muscimol rats showed similarly low latencies only during the second half of the retention test.

Fig 4 insert about here

Experiments 1-3: Changes in ITI crossings or escape failures cannot account for the effects of hippocampal lesions or inactivation on measures of two way active avoidance learning

Changes in ITI crossings are often used to assess changes in motor activity that may account for improved or impaired 2WAA learning (Boschen et al., 2011; Darvas et al., 2011; Guillazo-Blanch et al., 2002; Shumake et al., 2010; Vinader-Caerols et al., 1996). The pattern of ITI crossings during acquisition and retention session of Experiments 1 to 3 (Fig. 5 left) does not support that group differences in motor activity, as reflected by ITI crossings, can account for the group differences in 2WAA learning (Figs 2-4). In Experiment 1, ITI crossings during acquisition did not clearly differ between lesion groups (main effect of group: $F_{3,39} = 1.74$, $P = 0.17$; interaction group x block of 10 trials: $F_{27,351} < 1$); during retention, there was an interaction between group and block of 10 trials ($F_{27,351} = 1{,}69$, $P = 0.019$), mainly reflecting that complete and ventral hippocampal lesion groups showed less ITI crossings, as compared to the other groups, during blocks 3 to 5. While these differences are not easy to explain and might reflect a chance finding, any differences in ITI crossings during retention can clearly not account for the differences in 2WAA learning observed during acquisition. In Experiment 2, there was a strong trend toward an interaction of ventral hippocampal infusion group with block of 10 trials during acquisition ($F_{18,126} = 1.62$, $P = 0.06$), reflecting that the saline group tended to show the highest number of ITI crossings during block 1, 3, 9 and 10, whereas, during block 2, the saline group showed the lowest number of crossings and, during the remaining blocks, TTX infused rats tended to show the highest number of crossings, with the muscimol group tending to show the lowest levels.

Again these differences were likely due to chance and it is difficult to see how they could account for the reduced 2WAA learning in the muscimol and TTX groups. During retention, numbers of ITI crossings were lower in the group receiving muscimol into the ventral hippocampus, as compared to the saline and TTX groups ($F_{2,14} = 4.11$, $P = 0.04$; interaction group X block of 10 trial: $F < 1$), even though only the difference between muscimol and saline was significant ($P = 0.012$, two other $Ps > 0.17$). This difference is unlikely to reflect a direct motor effect of the muscimol infusion, given that infusions were applied before the acquisition session, and cannot account for the reduced 2WAA learning in both TTX and muscimol groups. Finally, in Experiment 3, involving dorsal hippocampal infusions, the muscimol group showed more ITI crossing than the saline and TTX groups during blocks 2 to 9 of acquisition (interaction group X blocks of 10 trials: $F_{18,369} = 2.05$, $P = 0.007$), whereas the saline group showed more ITI crossing than the other two groups during blocks 1 to 3 of retention (interaction group X blocks of 10 trials: $F_{18,369} = 2.23$, $P = 0.003$). Again, it is difficult to see how these differences could account for the impaired 2WAA learning in both the muscimol and TTX group. Moreover, analysis of escape failures did not reveal any significant group differences during acquisition or retention testing (all $Fs < 2.59$, $Ps > 0.11$) (Fig. 5 right). Overall, these data do not support that group differences in 2WAA learning were due to changes in motor activity (as reflected by ITI crossings) or by a failure to respond to the foot shock (as reflected by escape failure).

Fig 5 insert about here

DISCUSSION

The main new finding of the present study is that temporary inhibition of hippocampal activity, using muscimol or TTX infusions into the dorsal or ventral hippocampus, disrupted 2WAA learning. Muscimol or TTX infusions into the ventral hippocampus caused an immediate deficit in 2WAA performance, whereas following dorsal infusions rats performed similar to the control group for 40-50 min before a performance deficit emerged during the second half of the 2WAA acquisition session. In contrast to the impairments observed following temporary functional inhibition of the hippocampus by muscimol or TTX, NMDA-induced neurotoxic lesions to the complete hippocampus tended to facilitate, while neuronal lesions restricted to the dorsal or ventral hippocampus did not affect 2WAA learning.

Effects of permanent hippocampal lesions

Many previous studies have reported enhanced 2WAA learning following non-fiber sparing hippocampal lesions or fornix transections (Gray and McNaughton, 1983; Guillazo-Blanch et al., 2002; O'Keefe and Nadel, 1978; Olton and Isaacson, 1968; Pouzet et al., 1999; Tonkiss and Galler, 1990; Weiner et al., 1998). In the present study, we found a similar, albeit weaker, effect following neurotoxic lesions to the complete hippocampus. This new finding supports that permanent damage to hippocampal neurons, rather than to fibers of passage, leads to the facilitation of 2WAA learning. One interpretation of improved 2WAA learning following hippocampal lesions is that some aspects of hippocampal processing hinder 2WAA learning. As outlined in the Introduction, hippocampus-dependent one-trial place or context fear conditioning, inhibiting escape responses to a part of the chamber where the rat received a foot-shock on the previous trial, might hinder 2WAA learning. In addition, upregulation of nucleus accumbens dopamine transmission as a consequence of permanent hippocampal lesions (Lipska et al., 1992; Mittleman et al., 1993; Wilkinson et al., 1993) may

contribute to the facilitation of 2WAA learning, given that meso-accumbens dopamine transmission plays an important facilitating role in 2WAA learning (Boschen et al., 2011; Darvas et al., 2011; Dombroski et al., 2013; Shumake et al., 2010; Wadenberg and Hicks, 1999). The upregulation of dopamine transmission following hippocampal lesion may also cause locomotor hyperactivity, which is often observed in open-field testing of rats with hippocampal lesions (Bast and Feldon, 2003; Gray and McNaughton, 1983). However, in the present study, hippocampal lesions did not cause any clear effects on ITI crossings, a measure of motor activity during 2WAA testing. This argues against non-specific motor effects as an explanation for the improved 2WAA learning following hippocampal lesions, consistent with previous studies (Gray and McNaughton, 1983; Olton and Isaacson, 1968). Therefore, the tendency of cytotoxic lesions of the complete hippocampus to facilitate 2WAA conditioning may reflect the disruption of rapid place or context conditioning, the upregulation of accumbens dopamine transmission or a combination of these two mechanisms.

Effects of hippocampal muscimol and TTX infusion: ventral, but not dorsal, hippocampus contributes to two-way active avoidance learning

In contrast to permanent hippocampal lesions, temporary functional inhibition of the hippocampus, using muscimol or TTX infusions into the ventral or dorsal hippocampus, markedly impaired 2WAA learning. The GABA-A agonist muscimol selectively inhibits the functions of neurons, whereas the sodium-channel blocker TTX also affects fibers of passage. In the present study, muscimol and TTX caused similar behavioral effects, suggesting that these effects mainly reflect the functional inhibition of hippocampal neurons, not inactivation of fibers of passage. Two-way active avoidance learning was markedly impaired throughout the complete acquisition session following ventral infusions, whereas following dorsal infusions an impairment only emerged during the second half of the acquisition session (i.e.,

from 40-50 min after infusion). This suggests that functional inactivation of more ventral portions of the hippocampus, but not of the dorsal hippocampus, impairs 2WAA learning. The delayed impairment following dorsal drug infusions may reflect the time required for drug spread from the dorsal infusion site to more ventral parts of the hippocampus. Recent experiments combining muscimol infusion (0.5 ug/1 ul) with neuronal recordings in the dorsal hippocampus suggest that the extent of muscimol-induced functional inhibition of hippocampal neurons can spread by 0.5 mm in the horizontal direction within the first 6 min after infusion (Barry et al., 2012). Muscimol concentrations will fall below an effective concentration at further distance from the infusion site and it is difficult to accurately estimate the functional spread of the muscimol infusion (1 ug/0.5 ul) in our study. Nevertheless, it is plausible that during the first half (i.e., 40-50 min) of the acquisition session functional inhibition by muscimol might have spread from the dorsal infusion site to at least intermediate regions of the hippocampus, which are about 1-2 mm away from the dorsal infusion site. Drug spread outside of the hippocampus is unlikely given that the dense fiber bundles surrounding the hippocampal surface may largely prevent extra-hippocampal drug spread (Morris et al., 1989). In any event, our finding that muscimol and TTX infusions into the ventral hippocampus impaired 2WAA learning from the onset of the acquisition session (when neural effects of the drugs would have been restricted to the vicinity of the infusion site), whereas following dorsal infusions an impairment did not emerge before 40-50 min into the session, suggest that activity of more ventral regions of the hippocampus, but not the dorsal hippocampus, is required for 2WAA learning. Interestingly, in line with a preferential involvement of the ventral hippocampus in 2WAA learning suggested by our findings, a previous study showed that electrical kindling of the ventral, but not dorsal, hippocampus impaired 2WAA learning (Becker et al., 1997).

Contributions of the ventral hippocampus to two-way active avoidance learning

Why does functional inhibition of ventral to intermediate regions of the hippocampus impair 2WAA learning? Even though ventral hippocampal muscimol and TTX infusions reduce open field locomotor activity (Bast et al., 2001b), our analysis of ITI crossings indicated that non-specific changes in motor activity could not account for the impairment of 2WAA learning following these manipulations. Instead, functional inhibition of the ventral hippocampus may disrupt specific neural processes underpinning 2WAA learning. First, ventral hippocampal activity has been implicated in elemental classical fear conditioning, i.e. the formation of associations between an elemental CS, such as a tone, and an aversive US, such as a footshock (Bannerman et al., 2004; Fanselow and Dong, 2010). In fact, in our previous studies, we found that ventral hippocampal TTX infusion impaired the formation of elemental fear conditioning, even though ventral hippocampal muscimol (similar to dorsal hippocampal muscimol) only impaired contextual fear conditioning (i.e., formation of an association between a context and a footshock (Bast et al., 2001b; Zhang et al., 2014)). The two-process view of 2WAA suggests that the acquisition of fear to the CS is necessary for learning 2WAA, because the fear to the CS is necessary to motivate the avoidance response (Choi et al., 2010). According to this view, ventral hippocampal processing may contribute to 2WAA learning by supporting classical fear conditioning. In line with this suggestion, the lateral and basal nuclei of the amygdala, which are necessary for classical fear conditioning, are also required for 2WAA conditioning (Choi et al., 2010). These nuclei also feature strong anatomical links to the ventral hippocampus (Pitkanen et al., 2000). Second, the ventral to intermediate hippocampus exerts a positive control over the dopamine projections from the ventral tegmental area to the forebrain, including to the nucleus accumbens (Bast, 2007; Bast, 2011; Grace et al., 2007; Taepavarapruk et al., 2008). It has also been directly demonstrated that TTX infusion into the ventral subiculum prevents activation of nucleus accumbens

dopamine transmission by novelty (Legault and Wise, 2001). There is strong evidence that activation of the meso-accumbens dopamine system supports 2WAA learning (Smith et al., 2007; Boschen et al., 2011; Darvas et al., 2011; Dombroski et al., 2013; Ilango et al., 2012; Wadenberg and Hicks, 1999). Therefore, ventral hippocampal activity may also support 2WAA learning by contributing to the activation of meso-accumbal dopamine transmission.

Why may permanent lesions fail to reveal the contributions of the ventral hippocampus to two way active avoidance learning?

One important consideration is that the ventral hippocampus may not make unique contributions to 2WAA learning. That is to say, the ventral hippocampal contributions to elemental classical fear conditioning and to the activation of the meso-accumbens dopamine system overlap with the contributions of other brain regions. For example, the lateral and basal nuclei of the amygdala are also important (and probably more important than the ventral hippocampus) for the association of an elemental CS and an aversive US (Fanselow and LeDoux, 1999.; Maren and Quirk, 2004) and several regions, including the basal and lateral amygdala and the prefrontal cortex, can activate the meso-accumbens dopamine pathway (Sesack and Grace, 2010). Therefore, the loss of hippocampal contributions may be compensated for by the contributions of other brain regions. Such compensation may particularly be possible following a complete and permanent loss of hippocampal activity, as resulting from permanent lesions. In contrast, residual, but severely disrupted, hippocampal activity, as can be expected following TTX or muscimol infusions, may less allow for compensation by other brain regions, because of residual, albeit faulty, hippocampal output (also compare Lomber, 1999). In addition, as already discussed above, permanent hippocampal lesions have been demonstrated to lead to an upregulation of accumbal dopamine transmission (Lipska et al., 1992; Mittleman et al., 1993; Wilkinson et al., 1993), a

secondary lesion effect that may even overcompensate for the loss of positive modulation of dopamine transmission by the ventral to intermediate hippocampus.

Conclusions

Using temporary functional inhibition by muscimol or TTX, the present study reveals that the ventral hippocampus contributes to 2WAA learning. These contributions may reflect the participation of the ventral hippocampus (1) in classical elemental fear conditioning and (2) in the activation of the meso-accumbens dopamine system, both of which processes that have been strongly implicated in 2WAA learning. The contributions of the ventral hippocampus to these two processes are not unique, but overlap with those by other brain regions. Two main reasons may explain why hippocampal lesions do not reveal these contributions. First, if hippocampal activity is permanently and completely lost, other brain regions may compensate for the loss of hippocampal function in 2WAA learning, whereas the temporarily limited and incomplete reduction of hippocampal function resulting from TTX and muscimol infusion may not equally allow for such compensation. Second, permanent hippocampal lesions cause an upregulation of meso-accumbens dopamine transmission, which may facilitate 2WAA learning.

Acknowledgements The authors thank Joram Feldon for his support of this work, Liz Weber and Helen H.J. Pothuizen for their help with histology, and Peter Schmid for the set-up and maintenance of the computerized systems for behavioral analysis.

Figure legends

Fig. 1 *Hippocampal lesions (Experiment 1) and infusions sites (Experiment 2 and 3).* (**A**) Experiment 1: Photomicrographs of coronal sections showing a dorsal hippocampal (DH), ventral hippocampal (VH) and complete hippocampal (CH)excitotoxic lesion (top) and schematic reconstruction of the smallest (solid black areas) and the largest (solid grey areas) extents of damage to the hippocampal region and the overlying cortex (bottom). (**B**) Infusion sites in the ventral (Experiment 2, n=17, left) and dorsal (Experiment 3, n=44, right) hippocampus: photomicrographs of a coronal brain section with the tracks of the guide cannula and the infusion sites visible in both hemispheres (top) and a schematic reconstruction of infusion sites on coronal sections. Coronal sections are adapted from the atlas of Paxinos and Watson (1998) and numbers indicate the distance from bregma.

Fig. 2 *Experiment 1: Effects of ventral, dorsal and complete hippocampal excitotoxic lesion on two way active avoidance performance.* Percent avoidance responses (**A**) and avoidance/escape latencies (**B**) during acquisition and retention (two days apart). Ventral hippocampal (VH), dorsal hippocampal (DH) or complete hippocampal (CH) lesions had been performed before acquisition. The control (Cont) group included sham-lesioned and unoperated rats. Avoidance response and latency data are expressed as the averages of 10 trial blocks. Values are means, error bars represent 1 standard error (S.E.) derived from ANOVA.

Fig. 3 *Experiment 2: Effects of tetrodotoxin or muscimol infusion into the ventral hippocampus on two way active avoidance performance.* Percent avoidance responses (**A**) and avoidance/escape latencies (**B**) during acquisition and retention (two days apart). Rats were bilaterally infused with saline (0.5µl per side), muscimol (MUS, 1 µg/0.5µl per side), or

tetrodotoxin (TTX, 10 ng/0.5µl per side) into the ventral hippocampus 20 min (TTX group, half of the saline group) or immediately (muscimol group, half of the saline group) before acquisition. All groups were tested again in the absence of infusion (retention) two days after the infusion day. Avoidance response and latency data are expressed as the averages of 10 trial blocks. Values are means, error bars represent 1 standard error (S.E.) derived from ANOVA.

Fig. 4 *Experiment 3: Effects of tetrodotoxin or muscimol infusion into the dorsal hippocampus on two way active avoidance performance.* Percent avoidance responses (**A**) and avoidance/escape latencies (**B**) during acquisition and retention (two days apart). Rats were bilaterally infused with saline (0.5µl per side), muscimol (MUS, 1 µg/0.5µl per side), or tetrodotoxin (TTX, 10 ng/0.5µl per side) into the dorsal hippocampus 20 min (TTX group, half of the saline group) or immediately (muscimol group, half of the saline group) before acquisition. All groups were tested again in the absence of infusion (retention) two days after the infusion day. Avoidance response and latency data are expressed as the averages of 10 trial blocks. Values are means, error bars represent 1 standard error (S.E.) derived from ANOVA.

Fig. 5 *Experiment 1 to 3: ITI crossings and escape failures.* ITI crossings (left) and escape failures (right) during acquisition and retention in Experiment 1 (hippocampal lesions before acquisition: Cont, control group; VH, ventral hippocampal lesion; DH, dorsal hippocampal lesion; CH, complete hippocampal lesions), Experiment 2 (ventral hippocampal muscimol, MUS, or TTX infusion before acquisition) and Experiment 3 (dorsal hippocampal muscimol, MUS, or TTX infusion before acquisition). ITI crossing are presented as the averages of 10 trial blocks, with values showing means and error bars representing 1 standard error (S.E.)

derived from ANOVA. Escape failures are presented as total number throughout the complete 100 trial acquisition or retention sessions, with values showing mean ± SEM.

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Table 1.

Complete Hippocampal Lesions			
AP	**ML**	**DV**	**vol(ul)**
-2.2	±1.2	-3.3	0.075
-2.7	±1.9	-3.3	0.075
-3.0	±1.4	-3.3	0.050
-3.0	±1.4	-2.6	0.050
-3.0	±3.0	-3.1	0.100
-3.5	±3.5	-3.1	0.075
-4.3	±2.8	-3.3	0.050
-4.3	±2.8	-2.3	0.050
-4.3	±4.2	-5.0	0.025
-4.3	±4.2	-4.0	0.050
-4.3	±4.2	-3.0	0.050
-4.8	±4.8	-6.0	0.075
-4.8	±4.8	-5.0	0.050
-5.1	±4.2	-7.5	0.100
-5.1	±4.2	-5.5	0.075
-5.1	±4.2	-4.5	0.050
-5.4	±5.0	-6.5	0.100
-5.4	±5.0	-5.5	0.075

Dorsal Hippocampal Lesions			
AP	**ML**	**DV**	**vol(ul)**
-2.4	±1.0	-3.3	0.075
-2.8	±1.8	-3.3	0.075
-3.2	±1.4	-3.3	0.050
-3.2	±1.4	-2.6	0.050
-3.2	±3.0	-3.1	0.100
-3.6	±3.5	-3.1	0.075
-4.4	±2.8	-3.3	0.050
-4.4	±2.8	-2.3	0.050
-4.4	±4.0	-3.3	0.050
-4.4	±4.0	-2.3	0.050
-5.4	±4.1	-3.5	0.250

Ventral Hippocampal Lesions			
AP	**ML**	**DV**	**vol(ul)**
-4.4	±4.0	-4.0	0.025
-4.9	±4.8	-6.2	0.075
-4.9	±4.8	-5.2	0.050
-5.2	±4.2	-7.5	0.100
-5.2	±4.2	-4.8	0.075
-5.5	±5.0	-6.0	0.100
-5.5	±5.0	-4.9	0.075

Table 1. Stereotaxic coordinates and injection volumes used for NMDA injections (10 mg/ ml) to induce neurotoxic lesions to the complete, dorsal, or ventral hippocampus . Anterior-posterior (AP), medio-lateral (ML) and dorsoventral (DV) coordinates are in mm. AP and ML were measured with respect to bregma, and DV relative to dura.

Fig 1

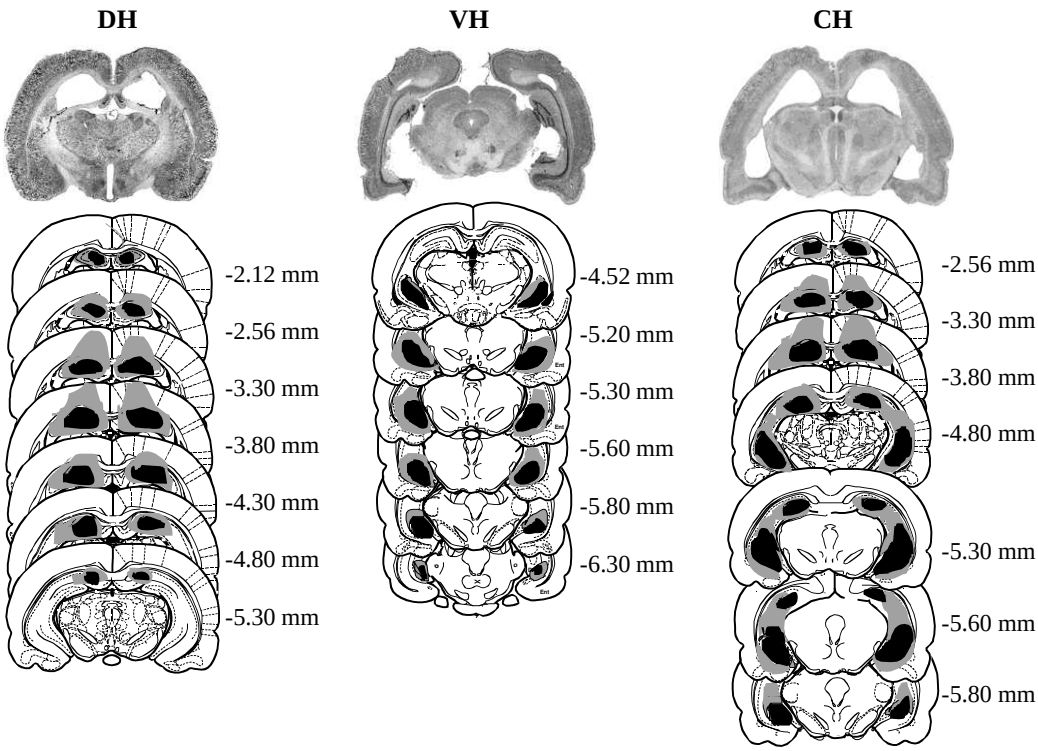

A
DH
VH
CH
-2.12 mm
-2.56 mm
-3.30 mm
-3.80 mm
-4.30 mm
-4.80 mm
-5.30 mm
-4.52 mm
-5.20 mm
-5.30 mm
-5.60 mm
-5.80 mm
-6.30 mm
-2.56 mm
-3.30 mm
-3.80 mm
-4.80 mm
-5.30 mm
-5.60 mm
-5.80 mm
B
Ventral hippocampus
Dorsal hippocampus
-4.80mm
-5.20mm
-5.30mm
-5.60mm
-5.80mm
-6.04mm
-6.30mm
-2.12mm
-2.56mm
-2.80mm
-3.30mm
-3.60mm
-3.80mm
-4.30mm

Fig 2

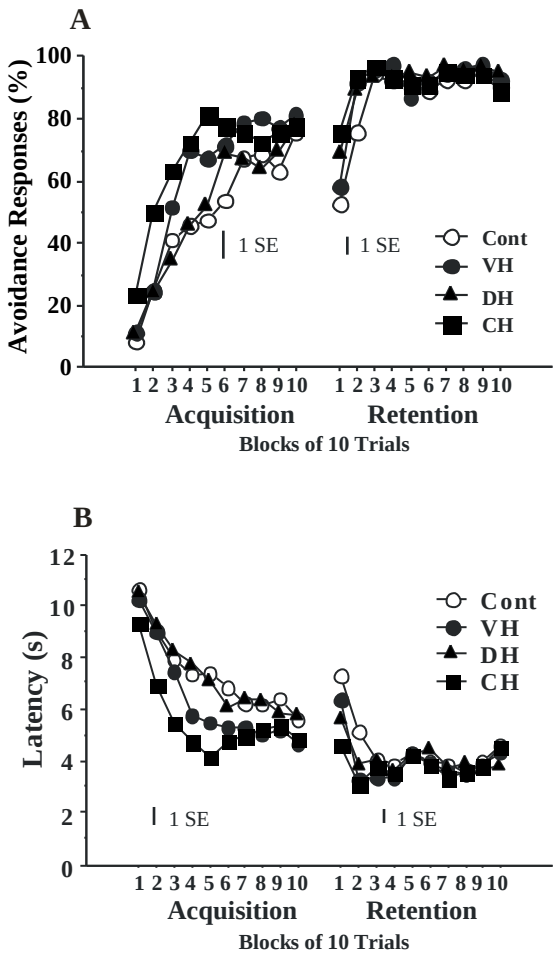

A
Avoidance Responses (%)
100
80
60
40
20
0
1 SE
1 SE
Cont
VH
DH
CH
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10
Acquisition
Retention
Blocks of 10 Trials
B
Latency (s)
12
10
8
6
4
2
0
1 SE
1 SE
Cont
VH
DH
CH
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10
Acquisition
Retention
Blocks of 10 Trials

Fig 3

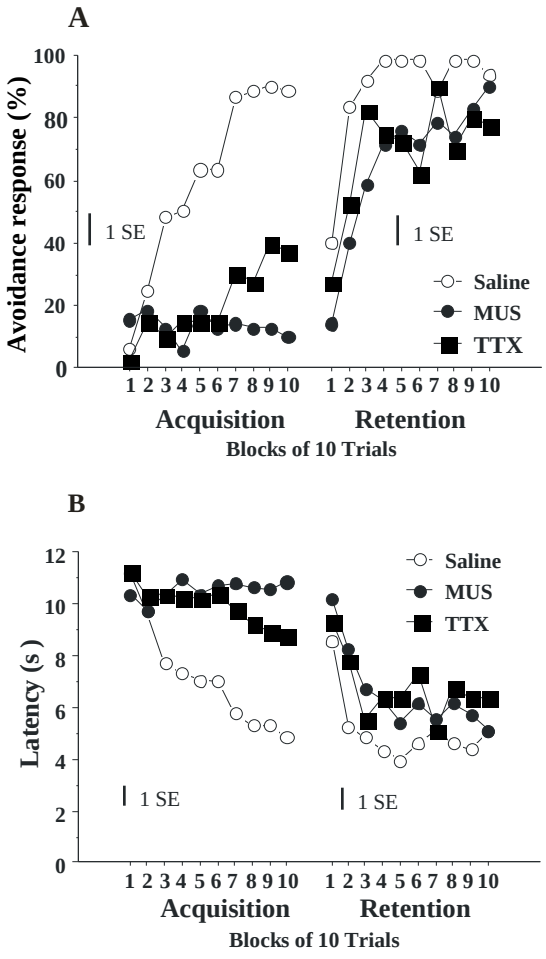
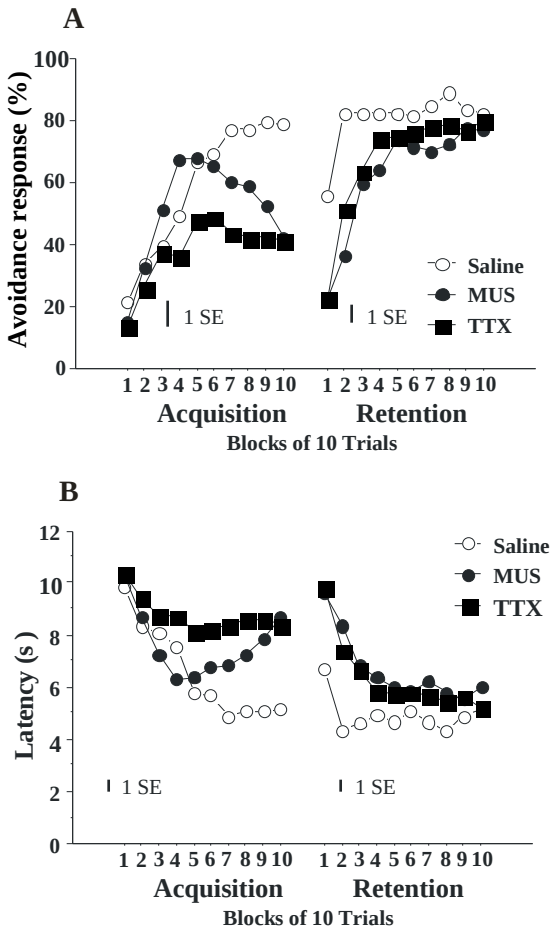
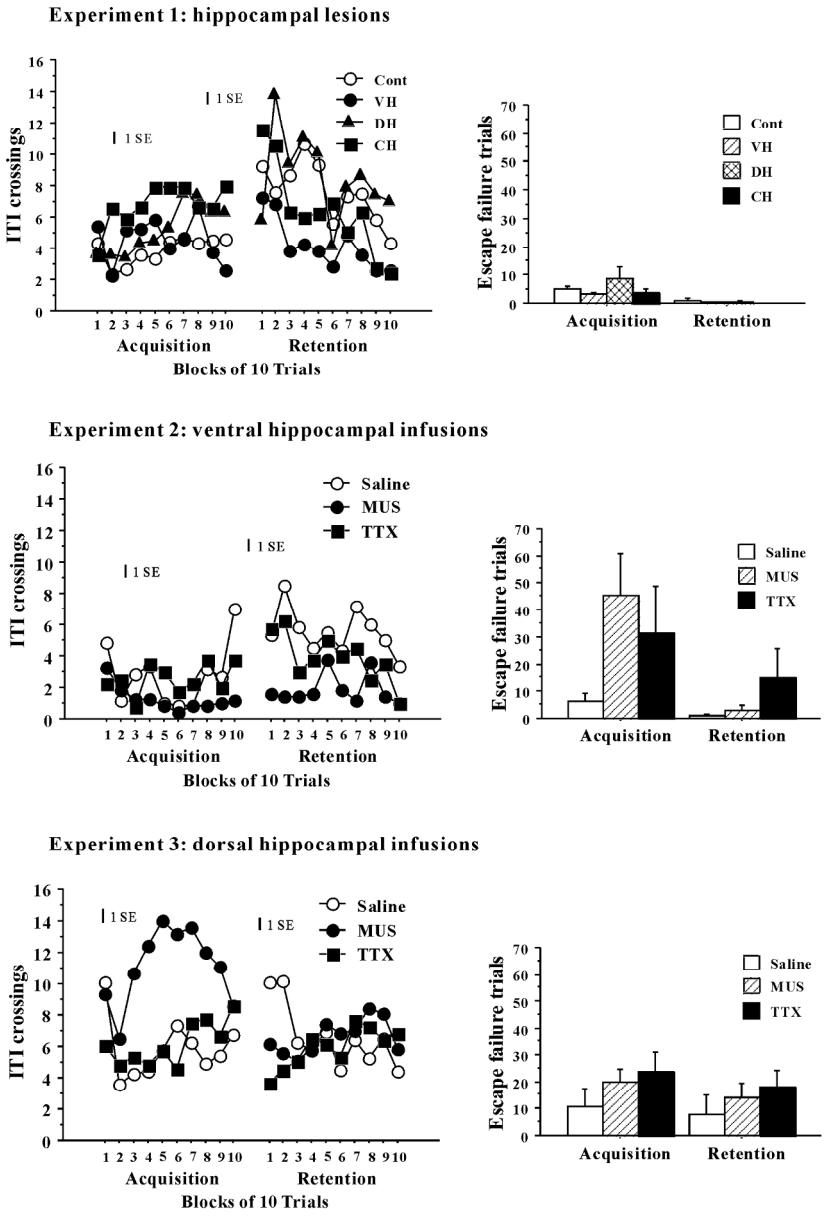

A
Avoidance response (%)
100
80
60
40
20
0
1 SE
1 SE
Saline
MUS
TTX
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10
Acquisition
Retention
Blocks of 10 Trials
B
Latency (s)
12
10
8
6
4
2
0
Saline
MUS
TTX
1 SE
1 SE
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10
Acquisition
Retention
Blocks of 10 Trials

Fig 4


A
Avoidance response (%)
100
80
60
40
20
0
1 SE
1 SE
Saline
MUS
TTX
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10
Acquisition
Retention
Blocks of 10 Trials
B
Latency (s)
12
10
8
6
4
2
0
1 SE
1 SE
Saline
MUS
TTX
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10
Acquisition
Retention
Blocks of 10 Trials


Experiment 1: hippocampal lesions
ITI crossings
1 SE
1 SE
Cont
VH
DH
CH
Acquisition
Retention
Blocks of 10 Trials
Escape failure trials
Cont
VH
DH
CH
Acquisition
Retention
Experiment 2: ventral hippocampal infusions
ITI crossings
1 SE
1 SE
Saline
MUS
TTX
Acquisition
Retention
Blocks of 10 Trials
Escape failure trials
Saline
MUS
TTX
Acquisition
Retention
Experiment 3: dorsal hippocampal infusions
ITI crossings
1 SE
1 SE
Saline
MUS
TTX
Acquisition
Retention
Blocks of 10 Trials
Escape failure trials
Saline
MUS
TTX
Acquisition
Retention