Temporary inhibition of dorsal or ventral hippocampus by muscimol: distinct effects on measures of innate anxiety on the elevated plus maze, but similar disruption of contextual fear conditioning

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Abstract

Studies in rats, involving hippocampal lesions and hippocampal drug infusions, have implicated the hippocampus in the modulation of anxiety-related behaviors and conditioned fear. The ventral hippocampus is considered to be more important for anxiety- and fear-related behaviors than the dorsal hippocampus. In the present study, we compared the role of dorsal and ventral hippocampus in innate anxiety and classical fear conditioning in Wistar rats, examining the effects of temporary pharmacological inhibition by the GABA-A agonist muscimol (0.5 ug/0.5 ul/side) in the elevated plus maze and on fear conditioning to a tone and the conditioning context. In the elevated plus maze, dorsal and ventral hippocampal muscimol caused distinct behavioral changes. The effects of ventral hippocampal muscimol were consistent with suppression of locomotion, possibly accompanied by anxiolytic effects, whereas the pattern of changes caused by dorsal hippocampal muscimol was consistent with anxiogenic effects. In contrast, dorsal and ventral hippocampal muscimol caused similar effects in the fear conditioning experiments, disrupting contextual, but not tone, fear conditioning.

1. INTRODUCTION

Studies examining the effects of lesion or pharmacological manipulations of the hippocampus in rats have provided compelling evidence that the hippocampus is important for unconditioned anxiety/fear responses, as well as the formation and expression of conditioned fear responses to elemental (e.g., auditory) and contextual stimuli[1] Moreover, the weight of evidence from studies using separate ventral or dorsal hippocampal manipulations suggests that the ventral hippocampus plays a rather general role in unconditioned anxiety and conditioned fear, whereas dorsal hippocampal contributions are more restricted to specific mnemonic aspects of fear conditioning, such as context learning; this is consistent with the ventral hippocampus featuring stronger direct connectivity to amygdala and hypothalamus, key components of the brain's anxiety and fear circuit, whereas the dorsal hippocampus is more closely linked to parts of the entorhinal cortex that are implicated in visuo-spatial information encoding (Moser & Moser, 1998; Anagnostaras et al., 2001; Bast et al., 2001b; Kjelstrup et al., 2002; Bast et al., 2003; Bannerman et al., 2004; Maren & Holt, 2004; Pentkowski, et al., 2006; Bast, 2007; Engin & Treit, 2007; Fanselow & Dong, 2010; Bast, 2011).

The present paper reports three experiments, in which we compared further the contributions of dorsal and ventral hippocampus to unconditioned anxiety and conditioned fear. We examined the effects of bilateral functional inhibition of neurons within dorsal or ventral hippocampus by local microinfusion of the GABA-A agonist muscimol (0.5 µg / 0.5 µl / side) on measures of unconditioned anxiety on the elevated plus maze (Experiment 1) and on the formation of conditioned fear (measured as freezing) to a tone or the conditioning context (Experiments 2 and 3). The elevated plus maze experiment (Experiment 1) addressed the hypothesis that ventral hippocampal muscimol would cause more pronounced anxiolytic effects than dorsal muscimol. This hypothesis is consistent with the idea that the ventral hippocampus plays a more important role in

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[1] Anxiety and fear both refer to responses to aversive stimuli and situations. Anxiety is more commonly used to refer to unconditioned, rather tonic, responses to more diffuse stimuli or situations associated with behavioral conflict and uncertainty, whereas fear commonly refers to rather phasic responses to stimuli associated with explicit danger (compare Gray and McNaughton, 2000, and Bannerman et al., 2004, and references therein).

unconditioned anxiety than the dorsal hippocampus, which is based on wide range of evidence Bannerman et al., 2004; Pentkowski, et al., 2006; Engin & Treit, 2007). More specifically, ventral cytotoxic lesions have been found to cause more pronounced anxiolytic effects than dorsal lesions on a variety of measures of innate anxiety, including elevated plus maze measures (Kjelstrup et al., 2002; Bannerman et al., 2002, 2004), and ventral infusion of the local anaesthetic lidocaine (a sodium channel blocker inactivating neurons and fibers of passage) significantly increased the proportion of open-arm entries on the elevated plus maze test, whereas dorsal lidocaine had no significant effect (Bertoglio et al., 2006). Moreover, ventral, but not dorsal, hippocampal muscimol reduced unconditioned fear, as assessed by the shock-probe burying test (McEown and Treit, 2010). However, even though one study reported that dorsal hippocampal muscimol reduced measures of unconditioned anxiety on the elevated plus maze (Rezayat et al., 2005), the effects of dorsal and ventral hippocampal muscimol infusions on the elevated plus maze remain to be compared directly. Furthermore, in the present study, the effects of dorsal and ventral hippocampal muscimol infusions are examined alongside the effects of these manipulations on fear conditioning (Experiments 2 and 3), allowing a direct comparison. In the fear conditioning experiments (Experiment 2 and 3), we aimed to corroborate our previous finding that ventral hippocampal muscimol (1 µg / 0.5 µl / side) disrupts contextual, but not tone, fear conditioning (Bast et al., 2001a) and to extend this finding by demonstrating similar effects of dorsal hippocampal muscimol. Such an outcome would be consistent with the idea that contextual fear conditioning requires dorsal hippocampal mechanisms mediating the formation of context representations, and ventral hippocampal mechanisms relating the context representations to fear processing via subcortical structures, including the amygdala (Maren and Fanselow, 1995; Anagnostaras et al., 2001; Bast et al., 2001a; Bast et al., 2003; Bannerman et al., 2004; Fanselow & Dong, 2010). While the ventral hippocampus has also been implicated in tone fear conditioning (Bast et al., 2001b; Bannerman et al., 2004), ventral hippocampal muscimol did not significantly reduce tone fear conditioning in our previous study (even though there was a numerical reduction), and we argued that partial inhibition of neuronal

activity in the ventral hippocampus via GABA-A receptor stimulation may not sufficiently interfere with ventral hippocampal processing to affect tone fear conditioning (in contrast, more general ventral hippocampal inactivation by the sodium channel blocker tetrodotoxin markedly impaired tone fear conditioning) (Bast et al., 2001a). Following our initial 2001 study (Bast et al., 2001a), a number of studies examined the effects of hippocampal muscimol infusions on fear conditioning, with somewhat discrepant outcomes. Maren & Holt (2004) reported that ventral hippocampal muscimol (0.25 µg / 0.25 µl / side) disrupted tone, but not contextual (background), fear conditioning, whereas dorsal infusions had no effect. Consistent with two main findings by Maren & Holt (2004), additional studies reported that dorsal hippocampal muscimol (0.5 µg / 0.5 µl / side; Matus-Amat et al., 2004) and muscimol infusion into the ventral hippocampus (subiculum; 0.5 µg / 1 µl / side; Biedenkapp and Rudy, 2008) did not cause anterograde contextual fear conditioning deficits. Such absence of anterograde contextual fear conditioning deficits following hippocampal muscimol (and also lesions) was explained by the competition hypothesis (Maren et al., 1997; Biedenkapp and Rudy, 2008; Fanselow, 2010). This hypothesis suggests that, while hippocampal mechanisms are normally important for contextual fear conditioning, they compete with an extra-hippocampal system that can also support contextual fear conditioning, albeit less efficiently; the hippocampus normally suppresses the alternative system, but this suppression is released during hippocampal inactivation or inhibition, so that the extra-hippocampal system can support contextual fear conditioning. Most recently, however, Esclassan et al. (2009) reported that ventral hippocampal muscimol (0.25 µg /0.25 µl / side) disrupted both tone and contextual fear conditioning, whereas dorsal muscimol selectively reduced contextual fear conditioning, and Wang et al. (2012) also reported that dorsal hippocampal muscimol (0.5 µg / 0.5 µl / side) impaired contextual fear conditioning (Wang et al., 2012). Considering the different findings made in different laboratories, we found it important to re-examine the anterograde effects of ventral hippocampal muscimol infusions (Bast et al., 2001a) and to compare directly the effects of ventral and dorsal hippocampal muscimol on fear conditioning in our laboratory.

2. MATERIALS AND METHODS

2.1. Subjects

A total of 40 adult male Wistar rats (Zur:WIST[HanIbm], Research Unit Schwerzenbach, Schwerzenbach, Switzerland), weighing about 250 g and aged about 2 to 3 months at the time of surgery, were used in this study. They 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 animals were allowed free access to food and water. Eighteen rats received bilateral implantation of guide cannulae aiming at the dorsal hippocampus and twenty-two rats received bilateral implantation of guide cannulae aiming at the ventral hippocampus. After surgery, all rats were caged singly. Starting one day before surgery and throughout the study, all rats were handled daily. Behavioural testing was carried out in the dark phase of the cycle, between 9 and 18 h. Principles of laboratory animal care (NIH publication no. 86-23, revised 1985) and Swiss regulations for animal experimentation were followed.

2.2. Apparatus & Procedures

2.2.1. Surgery

Rats were anesthetized (i.p.) with Nembutal (sodium pentobarbital, Abbott Labs, North Chicago, IL; 50mg/kg body weight), together with a mixture of midazolam hydrochloride (Dormicum®, Hoffman–LaRoche, Switzerland; 2 mg/kg body weight) and medetomidin hydrochloride (Dormitor, Orion Corporation, Espoo, Finland; 0.15 mg/kg body weight) given intramuscularly. Then their head was placed in a Kopf stereotaxic frame. After application of a local anesthetic (lidocaine), the scalp was incised to expose the skull. Bregma and lambda were aligned in the same horizontal plane. A small hole (1.5 mm in diameter) was drilled on each side of the skull to reveal the dura covering the cortex overlying the hippocampus. Stainless steel guide

cannulae (26 gauge, 9 mm or 7 mm for ventral or dorsal hippocampus, respectively) in a custom-made Perspex holder were implanted bilaterally into the brain aiming above 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 et al., 2001a; Zhang et al., 2002a, 2002b; Bast et al., 2003). 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 during which the experimenter gave the rat daily health checks and gentle handling, and replaced missing stylets. The behavioral procedures started five days after surgery.

2.2.2. Hippocampal drug infusions

Muscimol [$C_4H_6N_2O_2$(1/2 H_2O); Tocris, Bristol, UK] was dissolved in 0.9% saline to obtain solutions with a concentration of 0.5 µg/0.5 µl for bilateral intracerebral infusion on the day of infusion. This dose, which is half the dose used in our previous fear conditioning experiments involving ventral hippocampal infusion (Bast et al., 2001a), was chosen based on watermaze studies where infusion into dorsal or ventral hippocampus caused significant impairments (Zhang et al., unpublished data) and because it caused only a moderate reduction of locomotor activity following ventral hippocampal infusion, whereas 1 µg per side caused very pronounced locomotor suppression (Bast et al., 2001a). Infusions of 0.5 µl of 0.9 % saline were used as control.

For the intracerebral infusion, 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 hippocampus. Thus, the final dorso-ventral coordinate for the ventral and dorsal hippocampus was 6.5 mm and 4.0 mm below the dura, respectively, as in previous studies (Bast et al., 2001a; Zhang et al., 2002a, b; Bast et al., 2003). The infusion cannulae were connected

to 10-µl Hamilton microsyringes by flexible polyetheretherketone (PEEK) tubing. The syringes were mounted on a Kds micro-infusion pump. All rats were infused bilaterally with an infusion volume of 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 absorption of the infusion bolus before being replaced by the stylets. Using a small infusion volume (0.5 µl) and fine infusion cannulae (34 g) as in the present study, the infused drug is estimated to spread 1 mm or less in any direction (Myers, 1966). While one study suggested that the spread of muscimol may be quite wide-spread following infusion into the nucleus basalis magnocellularis or the reticular nucleus of the thalamus (Edeline et al., 2002), recent studies using infusion of fluorescent muscimol into prefrontal cortex (Allen et al., 2008) or into dorsal and ventral hippocampus (Jacobs et al., 2013) with doses and infusion volumes similar to the present study suggested that spread of muscimol is largely restricted to 0.5-1 mm. In addition, the densely packed fiber bundles surrounding the hippocampus also seem to prevent diffusion out of the hippocampus (Morris et al., 1998). Behavioral testing began 5 min after replacement of the stylets. Our previous experiments indicate that, by this time, hippocampal muscimol infusion exerts significant behavioral (locomotor) effects which last for at least 60 min (Bast et al., 2001a; Bast & Feldon, 2003).

2.2.3. Apparatus for behavioral testing

2.2.3.1. Elevated plus maze.

The maze was constructed of black-painted wood with four elevated arms raised by a single central support to a height of 62 cm above the floor. It was arranged as a cross with two open arms (45 cm × 10 cm) facing each other, and two other arms enclosed by high walls (45 cm x 10 cm x 40 cm). The four arms extended from a common central platform (10 x 10 cm). Ridges of 0.5 cm bordering the open arms were added to provide an additional grip. The illumination above the central platform was around 20 lx. Behavior on the maze was recorded by a video camera mounted on the ceiling above the center of the maze and relayed to a monitor and a Video tracking Motion

Analysis and Behaviour Recognition System (EthoVision®, Noldus, Wageningen, The Netherlands). The maze was divided into five areas, one for each arm and one for the center (central platform). Equipment programming and data recording were controlled by a PC computer.

2.2.3.2. Fear conditioning.

Behavioral tests of conditioned freezing behavior were conducted in four shock chambers (Habitest; Coulborn Instruments, Allentown, PA) and four no-shock plexiglas cylinders (diameter, 29cm; height, 28cm) enclosed in ventilated sound-attenuating boxes. Shock chambers were used for conditioning and context-test sessions, while the no-shock cylinders were used for the tone test sessions. Shock chambers were fitted with a parallel grid shock floor (16 parallel bars; E10-10RF; Coulborn Instruments), through which scrambled shocks could be delivered. These chambers had two side walls of aluminum and a rear and front wall of clear Perspex. A brown empty waste tray was situated below the grid floor. The four no-shock cylinders were fitted with a lattice grid and a brown waste tray was situated below the lattice grid. Waste trays, grids and chambers were cleaned with wet paper towels between rats and sessions. All testing in the no-shock cylinders was conducted with a house light on (1.12 W, light level in the no-shock cylinders, 1.5 lx); however, the shock chambers were not illuminated. The four shock boxes and the four no-shock cylinders were placed in two different rooms. Delivery of electric foot shock were controlled by a PC computer with dedicated software (S. Frank, Psychology Department, University of Tel Aviv, Israel) connected to a Coulborn Universal Environment Interface (E91-12) with Coulborn Universal Environment Port (L91-12). Shocks were delivered with a Coulbourn Precision Animal Shocker (E13-12) which generated bipolar rectangular 10-ms current pulses with a frequency of 10 Hz. Background noise was provided by a ventilation fan affixed to the light- and sound-attenuating chambers during all sessions. A monochrome minivideo camera with a wide angle (100°) 2.5-mm lens (VPC-465B; CES AG, Zurich, Switzerland) was attached to the center of the ceiling of each operant chamber. Four infrared (875 nm) light-emitting diodes (HSDL-4220; Hewlett Packard) positioned in the ceiling of each operant chamber provided light sufficient for camera function.

Throughout all sessions, images from each of the four shock or no-shock cylinders, respectively, were provided by these cameras, integrated into a four-quarter single image (100000 pixels) by a multiplexer (DX216CE, Sony), and recorded by a video-recorder (SVT1000; Sony). The video images were transferred to a PC computer equipped with an analysis program (Image; http://rsb.info.nih.gov/nih-image) and a macroprogram (P Schmid, Behavioral Neurobiology Laboratory, Swiss Federal Institute of Technology Zurich) to carry out automated analysis of freezing by comparing adjacent 1-s frames of videotape. Total immobility except respiratory movements is measured as freezing by our automated system. The validation and principle of the automated analysis of freezing behavior has been described in previous publications (Richmond et al., 1999).

2.3. Experimental design and procedures for behavioral testing

2.3.1. Experiment 1: *Effects of muscimol infusion into the dorsal or ventral hippocampus on the elevated plus maze.*

A total of 21 rats were tested individually on the plus-maze. The infusion groups were as follows: Saline (n=9, five into ventral and four into dorsal hippocampus), dorsal hippocampal muscimol (n=6) and ventral hippocampal muscimol (n=6). The rats were individually brought to the experimental room at least 20 min before the experiment started. Five minutes after infusion, rats were placed on the central platform facing one of the open arms and allowed to explore the maze for 15 min, during which the rats' behavior was videotaped. The rat was then removed from the maze and returned to its home cage. The maze was carefully cleaned before the next rat was tested. Based on the videotape recordings, several parameters were measured. Time spent and distance moved on the open and closed arms was measured using Ethovision, and entries into open and closed arms were scored by the experimenter when the rat entered its four paws into the arm. Relative increases of open arm as compared to closed arm parameters are typically considered to reflect anxiolytic effects, whereas relative decreases in closed arm as compared to open arm

parameters are thought to reflect anxiogenic effects (Pellow, Chopin, File, Briley, 1985; Rodgers & Dalvi, 1997; for more recent studies combining the elevated plus maze with hippocampal manipulations: Kjelstrup et al., 2002; Bannerman et al., 2002; Rezayat et al., 2005; Bertoglio et al., 2006).

2.3.2. Fear conditioning experiments

Two experiments were conducted to test the effects of muscimol infusion into the dorsal or ventral hippocampus on classical fear conditioning. Rats were tested in batches of four. The different testing boxes and the order of testing were counterbalanced among the experimental groups as far as possible.

2.3.2.1. Experiment 2: *Effects of muscimol infusion into the dorsal or ventral hippocampus on foreground contextual fear conditioning.*

One week after the elevated plus maze experiment, all 21 rats from the elevated plus maze experiment were used for a fear conditioning experiment involving contextual conditioning without tone presentation. They were assigned to either the saline or muscimol group, so as to match these groups with respect to the rats' infusion history during the elevated plus maze experiment (i.e., half, or about half, of the rats in each group of the fear conditioning experiment had received muscimol during the elevated plus maze experiment, the other half had received saline.). The infusion groups were as follows: Saline (n=9, five ventral and four dorsal hippocampal infusions), dorsal hippocampal muscimol (n=6) and ventral hippocampal muscimol (n=6). In this experiment, conditioning was conducted without an auditory CS to achieve a strong association between the electrical foot shock and the contextual CS, i.e. the conditioning chamber where the rats received the foot shock. Conditioning started 5 min after completion of the infusions. For conditioning, rats were put in the shock boxes for a total of 21 min and 6 s and were exposed to six 1-s foot shocks (0.5 mA) separated by 3-min blocks between an initial and a final 3-min block. The proportion of time spent freezing was calculated for the seven 3-min blocks preceding and following the 1-s foot

shock. One day after conditioning, in the context test session, rats were tested for freezing to the contextual CS (shock boxes). For that purpose, rats were placed in the shock box for 8 min and the proportion of time spent freezing was calculated in 1-min time block.

2.3.2.2. Experiment 3: *Effects of muscimol infusion into the dorsal or ventral hippocampus on tone and background contextual fear conditioning.*

A total of 19 naïve rats were used for a fear-conditioning experiment involving simultaneous tone and contextual conditioning. The infusion groups were as follows: Saline (n=6, four ventral and two dorsal hippocampal infusions), dorsal hippocampal muscimol (n=6) and ventral hippocampal muscimol (n=7). For conditioning (Day 1), rats were put in the shock boxes (context A) for a total of 24 min and 6s and were exposed to six pairings of a 30s auditory CS [85 dB(A), 2.9 kHz] and a 1s foot shock (0.5 mA), with the 1-s footshock being contiguous with the last 1 s of the auditory CS and pairings separated by 3min blocks between an initial and a final 3min block. Use of fixed, predictable, intervals between the unconditioned stimuli (e.g., foot shocks) has been suggested to minimize overshadowing of the conditioning context by the explicit CS (LoLordo et al., 2001); indeed, the present background contextual fear conditioning resulted in similar context freezing as the foreground contextual conditioning procedure in the previous experiment (compare Figs 3B and 4C). The proportion of time spent freezing was calculated for the seven 3min blocks preceding and following the 30s CS and for the duration of each 30s CS. One day after conditioning, in the context test session (Day 2), rats were tested for freezing to the contextual CS. For that purpose, rats were placed in the shock box (context A) for 8 min, without presentation of the auditory CS or application of the foot shock. Two days after conditioning, in the tone test session (Day 3), rats were tested for freezing to the auditory CS. For that purpose, they were put in the no-shock cylinder (context B) for a total of 11 min. After initial 3 min, the auditory CS was presented for the remaining 8 min without the presentation of shock. During all the test sessions, the proportion of time spent freezing was calculated for each 1-min block.

2.4. Data analysis

Statistical analyses were performed with the StatView software system (Abacus Concepts, Inc., Berkeley, CA, 1992). Data were first subjected to ANOVA, using groups as between-subjects factor and time as within-subjects factor. Post hoc comparisons were conducted using Fisher's protected least significant difference test. Significant differences were accepted at $P < 0.05$. All values are presented as means $\pm$ S.E.M.. Since the groups receiving ventral and dorsal hippocampal saline infusion did not differ in any experiment (all Fs < 1), data from these groups were collapsed into one control group (Saline) for the analysis presented in the Results section.

ANOVA is based on the assumptions of normality and homoscedasticity (equal variance); as has been pointed out by statisticians, most real data only meet these assumptions to some degree (Glass et al., 1972; Judd et al., 1995). Data transformations may help to improve compliance with these assumptions (Judd et al., 1995; Osborne, 2002). However, freezing data are commonly analyzed using ANOVA without any prior transformation of data, even though some authors have applied data transformation (e.g., Esclassan et al., 2009). Similarly, many elevated plus maze studies use ANOVA without prior data transformation (e.g., Bertoglio and Carobrez, 2002; Rezayat et al., 2005; Pohorecky, 2008), even though some studies also use nonparametric tests (e.g., Bannerman et al., 2004) or data transformation prior to ANOVA (e.g., Bertoglio et al., 2006). Eyeballing the distributions of our data and F ratio tests for equal variance suggest that our data do, overall, not grossly violate the assumptions underlying ANOVA; the exceptions are distance moved in closed arms, which significantly violated the assumption of equal variance (mainly due to one outlier in the dorsal hippocampal muscimol group, which did not at all enter the closed arm during the first 5-min block of testing) and arm entries, which were noticeably skewed. Importantly, statisticians have highlighted that it is less important whether ANOVA assumptions are exactly met, but more important to consider what the consequences of such violations might be (Glass et al., 1972). It is widely agreed that ANOVA is robust with respect to violations of the assumptions of normality and homoscedasticity, affecting type I and II errors only minimally (Glass et al., 1972;

Judd et al., 1995, Ann Rev Psychol). Furthermore, even though this is not widely realized, nonparametric tests rely on other strong assumptions, which may be difficult to verify or meet (Judd et al., 1995); common nonparametric tests are also unsuitable for multifactorial analysis (such as combined analysis of treatment and time effects, which is relevant in the present study). Moreover, transformations are also associated with problems and may confound data interpretation (Games, 1984; Osborne, 2002). For these reasons, we chose ANOVA without data transformation as a suitable approach to provide a quantitative, albeit approximate, measure of the statistical reliability of our findings.

2.5. Histology

After completion of the behavioral experiments, the 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% saline, followed by 120 ml of 4% formalin (4°C) to fix the brain tissue. Brains were extracted from the skull, post-fixed in 4% formalin solution, and subsequently cut into 40-µm coronal sections on a freezing microtome. To verify the injection sites, every fifth section through the dorsal or ventral hippocampus was mounted on gelatine-treated slides and stained with cresyl violet. After staining, the sections were dehydrated through alcohol series, cleared with xylene, and coverslipped with Eukitt (Kindler, Freiburg, Germany). Subsequently, the sections were examined with a light microscope to verify the location of the tips of the infusion cannulae and marked on plates taken from the rat brain atlas of Paxinos and Watson (1998).

3. RESULTS

3.1. Histology

In all 40 cannulated rats, the tips of the infusion cannulae were located in the targeted areas within the border of the dorsal or ventral hippocampus (**Fig. 1**). Visible tissue damage was restricted to the area immediately surrounding the guide and infusion cannulae.

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Fig 1 about here

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3.2. Experiment 1: *Distinct effects of muscimol infusion into the dorsal or ventral hippocampus on the elevated plus maze*

During the experiment, one ventral hippocampal muscimol rat fell off the maze and was, therefore, not included in the behavioral analysis, leaving final group sizes of: n= 6 for dorsal hippocampal muscimol, n= 5 for ventral hippocampal muscimol and n= 9 for saline infusions.

Both ventral and dorsal hippocampal muscimol infusions reduced distance travelled within closed arms, and neither of the two manipulations affected entries into closed arms; interestingly, dorsal hippocampal muscimol increased time spent in the closed arms (mainly during the third 5-min block of the test session), pointing toward an anxiogenic effect, whereas ventral hippocampal muscimol did not significantly affect this measure (**Fig. 2, left panels**). Dorsal hippocampal muscimol infusion also decreased distance moved within open arms and entries into open arms, supporting an anxiogenic effect, whereas ventral hippocampal muscimol did not affect these measures; neither dorsal nor ventral hippocampal muscimol infusion affected open arm time (**Fig. 2, right panels**). A 3 x 3 ANOVA (group x 5-min block) of the distance moved in closed arms only yielded a significant main effect of group ($F_{2, 17} = 7.32$, $P < 0.006$), without an interaction ($F_{4, 34} < 1$) or main effect ($F_{2, 34} = 2.09$, $P = 0.14$) involving 5-min block. Post hoc comparisons revealed that saline rats moved significantly more in the closed arms than rats infused with muscimol into the ventral hippocampus ($P < 0.03$) or the dorsal hippocampus ($P < 0.003$), with no difference between the two muscimol groups ($P = 0.41$). A 3 x 3 ANOVA of the entries into closed arms did not yield a significant effect of group ($F_{2, 17} = 1.17$, $P = 0.33$) or 5-min block ($F_{2, 34} = 1.77$, $P = 0.10$), nor an interaction between the two ($F_{4, 34} < 1$). However, a 3X3 ANOVA of time spent in closed arms

revealed a significant interaction of group x 5-min block ($F_{4, 34} = 5.1$, $P < 0.03$). This reflected that during the third 5-min block, dorsal hippocampal muscimol increased time in the closed arms as compared to the two other groups, whereas groups did not differ during the first two 5-min blocks (**Fig. 2, bottom panel, left, inset**). In support of this interpretation, separate ANOVAs for each 5-min block revealed a significant group effect during the third 5-min block ($F_{2, 17} = 4.6$, $P < 0.03$, but not during the first two 5-min blocks ($F_{2, 17} < 1.7$, $P > 0.2$). Post hoc comparisons showed a significantly higher time in the doral hippocampal muscimol group (48.8±7.0 s), as compared with

the ventral hippocampal muscimol group (26.6 ±4.9 s, $P < 0.02$) and the saline group (32.3 s ±3.6, $P < 0.03$). The ventral hippocampal muscimol and the saline group did not differ ($P = 0.45$). Furthermore, a 3 x 3 ANOVA of the distance moved in open arms yielded a strong trend toward a main effect of group ($F_{2, 17} = 3.02$, $P = 0.075$), without an interaction ($F_{4, 34} < 1$) or main effect ($F_{2, 34} < 1$) involving 5-min block. Post hoc comparisons showed that rats infused with muscimol into the dorsal hippocampus moved significantly less in the open arms than the saline group ($P < 0.03$) and also tended to move less than rats infused with muscimol into the ventral hippocampus ($P = 0.084$); the latter two groups did not differ (P = 0.8). Similarly, a 3 x 3 ANOVA (group x 5-min block) of the entries into open arms revealed a significant effect of group ($F_{2, 17} = 4.35$, $P < 0.03$), without an interaction ($F_{4, 34} = 1.35$, P = 0.43) or main effect ($F_{2, 34} < 1$) involving 5-min block. Post hoc comparisons showed that rats infused with muscimol into the dorsal hippocampus entered open arms significantly less than the saline group ($P < 0.02$) or rats infused with muscimol into the ventral hippocampus ($P < 0.03$); the latter two groups did not differ from each other (P = 0.98). ANOVA did not reveal a main effect or interaction involving group for open-arm time (F < 1.4, P > 0.28).

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The ratio of open arm entries to total entries (open arm + closed arm) may reflect anxiolytic or anxiogenic effects, respectively, relatively unconfounded by locomotor effects, because locomotor effects would affect open and closed arm entries similarly and, thus, have only little influence on the ratio. However, an analysis of ratios, as compared to a separate analysis of open arm and closed arm measures, also reduces overall sensitivity to treatment effects, because the ratio measure has a considerably larger variance than the separate measures; the latter reflects that the variances of both separate measures contribute to the variance of the ratio measure. In the present study, dorsal hippocampal muscimol numerically decreased the proportion of open arm entries ($24.7 \pm 11.2\%$), whereas ventral hippocampal muscimol increased the proportion ($41.2 \pm 7.1\%$), as compared to saline ($33.1 \pm 3.9\%$), supporting opposite effects of dorsal and ventral hippocampal muscimol. However, in contrast to the separate analysis of open and closed arm entries (see above), a 3X3 ANOVA of the ratio measure failed to reveal a significant effect of group, and there was also no interaction group X 5-min block (Fs < 1.1, Ps > 0.35). This is likely to reflect at least partially the relatively high relative variance of the ratio measure.

3.3. Experiment 2: *Muscimol infusion into the dorsal or ventral hippocampus reduces foreground contextual fear conditioning*.

In the first fear conditioning experiment, one dorsal hippocampal muscimol rat fell ill and had to be culled; therefore this rat was not included in the behavioral analysis, leaving final group sizes of n= 5 for dorsal hippocampal muscimol, n= 6 for ventral hippocampal muscimol and n=9 for saline.

During the conditioning session, all groups spent a similar percentage of time freezing during the seven 3-min blocks preceding and following the 1-s foot shocks (**Fig.3A**). A 3 x 7 (group x time-blocks) ANOVA of the percentage of time spent immobile yielded only a significant effect of the seven 3-min blocks ($F_{6, 102} = 22.831$, $P < 0.0001$), but neither an effect of groups ($F_{2, 17} = 0.32$, $P > 0.73$) nor an interaction of groups and time-blocks ($F_{12, 102} = 1.527$, $P > 0.12$). The significant effect

of time-blocks reflected that in all groups freezing developed throughout the six applications of the 1-s foot shock.

During the context test, both dorsal and ventral hippocampal muscimol rats showed virtually no conditioned fear to the conditioning context, whereas the Saline rats exhibited conditioned freezing during the first 2-3 min (**Fig. 3B**). A 3 x 8 (groups x time-blocks) ANOVA of the percentage of time spent immobile revealed a significant interaction of groups and 1-min blocks ($F_{14, 119} = 3.05$, $P < 0.001$). This reflected that in the Saline group freezing gradually increased to a maximum throughout the first three 1-min blocks of the context test session, followed by a subsequent gradual decline in freezing (reflecting extinction), whereas the dorsal and ventral hippocampal muscimol rats exhibited virtually no freezing throughout the total 8 min. Separate ANOVAs for each 1-min block revealed a significant group effect during the third 1-min block ($F_{2, 17} = 4.99$, $P < 0.02$; all other 1-min block $F < 2.43$, $P > 0.11$). Post hoc comparisons showed a significantly higher percentage of time spent freezing in the Saline group (32.41 ± 10.03) as compared with the ventral hippocampal muscimol group (4.72 ± 2.49, $P < 0.03$) and with the dorsal hippocampal muscimol group (0.67 ± 0.41, $P < 0.02$). The two muscimol groups did not differ ($P = 0.75$).

———————————————

Fig 3 about here

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3.4. Experiment 3: *Effects of muscimol infusion into the dorsal or ventral hippocampus on background contextual fear conditioning and on tone fear conditioning*

During the conditioning session, all groups spent a similar percentage of time freezing during the six 30-s CS presentations ($F_{2, 16} = 1.71$, $P > 0.21$) or during the seven 3-min blocks preceding and following the CS presentations ($F_{2, 16} = 1.13$, $P > 0.34$; **Fig. 4A, B**). ANOVA of the percentage of time spent immobile yielded only a significant effect of the six CS presentations ($F_{5, 80} = 10.33$, $P < 0.0001$) and the seven 3-min blocks ($F_{6, 96} = 16.69$, $P < 0.0001$). There was no interaction of

groups and six CS presentations ($F_{10, 80} = 1.51$, $P > 0.15$) or the seven 3-min blocks ($F_{12, 96} = 1.63$, $P = 0.095$). This reflected that in all groups freezing increased similarly due to foot-shock applications.

During the context text, both dorsal and ventral hippocampal muscimol groups appeared to exhibit less conditioned fear to the context (conditioning box) than the Saline rats (**Fig. 4C**), even though statistical analysis only partly supported this impression. A 3 x 8 (groups x time blocks) ANOVA of the percentage of time spent freezing during the total 8 min of the context session yielded a strong trend for a main effect of group ($F_{2, 16} = 2.83$, $P = 0.08$) and a significant main effect of time blocks ($F_{7, 112} = 2.59$, $P < 0.02$), without an interaction group x time ($F_{14, 112} = 0.52$, $P = 0.91$). Post hoc tests on the basis of the statistical trend for a group effect revealed that freezing in ventral hippocampal muscimol rats (4.73 ± 3.34) was significantly reduced as compared to the Saline group (30.49 ± 12.35; $P < 0.05$) (inset in **Fig. 4C**). Even though the DH-MUS group froze only about half as much (17.01 ± 5.84) as the Saline group, this difference failed to reach significance ($P = 0.24$). The two muscimol groups did not differ from each other ($P > 0.27$).

During the tone test, all groups exhibited similar conditioned fear to the tone CS (**Fig. 4D**). During the 3 min preceding the tone CS presentation, freezing levels were very low in all groups with no difference between groups ($F_{2, 16} = 0.64$, $P > 0.53$). However, during the 8 min of tone CS presentation, all groups exhibited marked freezing, i.e., conditioned fear. A 3 x 8 (Group x time-blocks) ANOVA of the percentage of time spent immobile during these 8 min yielded only a significant main effect of time-blocks ($F_{7, 112} = 9.64$, $P < 0.0001$), but neither a significant effect of group ($F_{2, 16} < 1$) nor an interaction of groups x 1-min blocks ($F_{14, 112} < 1$). The significant effect of time-blocks reflected an immediate increase of conditioned fear to a maximum due to the tone CS presentation at min 4–6 and a subsequent extinction of conditioned fear in all groups.

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Fig 4 about here

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4. DISCUSSION

In the elevated plus maze experiments, ventral and dorsal hippocampal muscimol infusions had distinct effects. Ventral hippocampal muscimol infusions decreased movement only on the closed arms, consistent with decreased locomotion found in a previous study (Bast et al., 2001a), but left open arm measures unaffected; the latter may reflect anxiolytic effects (which would increase movement in open arms) countering the locomotor suppressing effects of ventral hippocampal muscimol. In contrast, dorsal hippocampal muscimol decreased movement on both open and closed arms and selectively decreased entries into open arms and increased time spent in closed arms; given that dorsal hippocampal muscimol infusion did not decrease, but rather increased, locomotor activity in previous open-field experiments (Bast & Feldon, 2003), our elevated plus maze data indicate that dorsal hippocampal muscimol may have anxiogenic effects. In the fear conditioning experiments, both ventral and dorsal hippocampal muscimol impaired contextual fear conditioning (even thouth the effects of dorsal hippocampal muscimol were less reliable), whereas tone fear conditioning was unaffected.

4.1. Distinct effects of ventral and dorsal hippocampal muscimol on the elevated plus maze

Previous studies examining the effects of partial hippocampal lesions and temporary pharmacological inactivation on a range of tests of unconditioned anxiety suggested that both ventral and dorsal hippocampal inactivation may be anxiolytic, with the effects of ventral hippocampal manipulations tending to be more pronounced (Kjelstrup et al., 2002; Bannerman et al., 2002, 2004; Rezayat et al., 2005; Bertoglio et al., 2006; Engin & Treit, 2007; McEown & Treit, 2010). However, the effects of hippocampal manipulations on tests of anxiety, such as the elevated plus maze, show considerable variability across studies, depending on a variety of factors (see, for example, discussion in Bannerman et al., 2002). Two factors that may be particularly relevant concerning the present results are locomotor effects of the hippocampal manipulations and baseline anxiety levels. As to ventral hippocampal muscimol infusions, we found previously that this

manipulation decreases open-field locomotor activity (Bast et al., 2001a), consistent with the view that ventral hippocampal activity positively modulates locomotor activity possibly through its positive modulation of ascending dopamine systems (Bast and Feldon, 2003). A non-specific suppression of behavioral activity, as suggested by these open field findings, may account for the reduction in movement on closed arms. With respect to movement on or into open arms, the locomotor suppressing effects of ventral hippocampal muscimol infusion and its anxiolytic effects (which would increase movement in open arms) may cancel each other out, so that ventral hippocampal muscimol infusions do not change open arm parameters compared to saline infusions. In contrast, dorsal hippocampal muscimol does not suppress open field locomotor activity, but rather increases it, potentially reflecting deficits in habituation to the open field environment, which may rely on dorsal hippocampal spatial/contextual processing (Bast & Feldon, 2003; also compare Anagnostaras et al., 2001). Therefore, the reduction in locomotion on both open and closed arms of the elevated plus maze by dorsal hippocampal muscimol is unlikely to reflect a non-specific suppression of behavioral activity, but is more consistent with anxiogenic effects. The finding that dorsal hippocampal muscimol also reduced open arm, but not closed arm, entries, and increased closed arm time (albeit only during the last 5 min of the 15-min test session), but not time in open arms, also supports anxiogenic effects. Our findings contrast with a previous report that dorsal hippocampal muscimol increased open arm entries and time, alongside a numerical increase in locomotor counts (Rezayat et al., 2005). It is possible that the different findings reflect an interaction of baseline anxiety levels and the effects of dorsal hippocampal muscimol on locomotor activity. Thus, in our study higher baseline anxiety levels, due to procedural differences, may have prevented the expression of locomotor hyperactivity that may result from dorsal hippocampal muscimol infusion (Bast & Feldon, 2003), so that anxiogenic effects were detected; for example, we single-housed rats after surgery and tested them in their dark phase, whereas Rezayat et al. (2005) group housed rats and tested them in their light phase, both of which may have contributed to increased anxiety on the elevated plus maze as compared to the study by Rezayat et al. (2005)

(Hogg, 1996; Bertoglio and Carobrez, 2002; Pohorecky, 2008). In contrast, if baseline anxiety levels are lower, dorsal hippocampal muscimol may induce locomotor hyperactivity and this may contribute to the increase of entries to and time on open arms.

Overall, while our findings are consistent with the idea that activity in the ventral hippocampus contributes to anxiety, the present effects of dorsal hippocampal muscimol infusion raise the possibility that dorsal hippocampal mechanisms may, under some circumstances, contribute to reduced anxiety. The latter may be related to the mnemonic functions of the dorsal hippocampus in the encoding and storage of episodic-like memory (Morris, 2006; Bast, 2007): for example it is possible that dorsal hippocampus-dependent episodic-like memories of safe experiences within the laboratory normally act to moderate anxiety-related behavior, such as on the elevated plus maze.

4.2. Ventral and dorsal hippocampal muscimol impair contextual, but not tone, fear conditioning

The weight of evidence from studies using partial hippocampal cytotoxic lesions or temporary inactivation suggests that both ventral and dorsal hippocampus play a role in forming conditioned fear memories, with the effects of dorsal hippocampal lesions or inactivation largely limited to contextual fear memory (Anagnostaras et al., 2001; Bast et al., 2001a, 2001b; Bannerman et al., 2004; Maren & Holt, 2004; Pentkowski, et al., 2006; Esclassan et al., 2009).

However, as outlined in the Introduction, there is substantial variability between effects reported by different studies, including studies using temporary functional inhibition by muscimol (also see discussion by Maren & Holt, 2004, and Esclassan et al., 2009). Importantly, in the present study, we replicated our previous findings that ventral hippocampal muscimol impairs contextual fear conditioning in both foreground and background procedures, while not significantly affecting tone fear conditioning (Bast et al., 2001a), and we showed similarly selective effects on contextual fear conditioning for dorsal hippocampal muscimol infusions. In the present study, the effects of dorsal hippocampal muscimol on contextual fear conditioning was not totally reliable, with this

effect failing to reach significance in Experiment 3; this is consistent with the variable results reported in the literature, with some studies reporting significant disruption of contextual fear by dorsal hippocampal muscimol (Esclassan et al., 2009; Wang et al., 2012), while others failed to find a significant effect (Maren & Holt, 2004; Matus-Amat et al., 2004). Interestingly, the selective impairment of contextual, but not tone, fear conditioning by hippocampal muscimol resembles the selective anterograde deficits in contextual fear conditioning following infusion of NMDA-receptor antagonists into the ventral (Zhang et al., 2001) or dorsal hippocampus (Bast et al., 2003; Schenberg and Oliveira, 2008). Given that stimulation of hippocampal GABA receptors inhibits NMDA receptor-dependent long-term potentiation (LTP) (Collingridge, 2003), it is possible that the selective deficits in contextual fear conditioning caused by muscimol stimulation of hippocampal GABA-A receptors reflect interference with LTP-like plasticity mechanisms.

Which firm conclusions can we draw from these findings and which factors determine the effects of hippocampal muscimol infusions on fear conditioning? First, out of three studies (present study; Maren & Holt, 2004; Esclassan et al., 2009), not one study reported effects of dorsal hippocampal muscimol (0.5 µg / 0.5 µl / side in the present study, 0.25 µg / 0.25 µl / side in the other two studies) on tone fear conditioning, supporting that the dorsal hippocampus is not required for elemental fear conditioning. Second, the strength of fear conditioning may play a role in determining the effects of dorsal and ventral hippocampal muscimol infusions. Thus, both dorsal and ventral hippocampal muscimol infusions disrupted contextual fear conditioning when the resulting conditioned context fear in the control group was relatively moderate (20-50% freezing) (present study, Experiments 2 and 3, 0.5 µg / 0.5 µl / side; Esclassan et al., 2009, 0.25 µg / 0.25 µl / side), whereas neither dorsal nor ventral hippocampal muscimol infusions affected contextual fear conditioning when conditioning resulted in stronger conditioned freezing (50-70%) (Maren & Holt, 2004, 0.25 µg / 0.25 µl / side; Matus-Amat et al., 2004, 0.5 µg / 0.5 µl / side; Biedenkapp and Rudy, 2008, 0.5 µg / 1 µl / side) (with the exception of Wang et al. (2012), who reported anterograde context fear deficits following dorsal hippocampal muscimol (0.5 µg / 0.5 µl / side) with context

freezing levels of nearly 60%). That dorsal and ventral hippocampal muscimol infusions cause anterograde deficits in contextual fear conditioning, depending on the strength of the conditioing is consistent with the following view: i) contextual fear conditioning normally requires dorsal and ventral hippocampus (with dorsal hippocampus mediating the formation of contextual representations and ventral hippocampus relating these representations to fear processing via subcortical sites, such as the amygdala; Maren and Fanselow, 1995; Anagnostaras et al., 2001; Bast et al., 2001a; Bast et al., 2003; Bannerman et al., 2004; Fanselow & Dong, 2010); ii) an alternative extra-hippocampal system can support contextual fear conditioning, but is less efficient than the hippocampus and, thus, is not able to sustain conditioning under more demanding circumstances, such as those that would result in weak contextual fear (Fanselow, 2010). The strength of conditioning may also partly determine whether or not ventral hippocampal muscimol infusions disrupt tone fear conditioning. Thus, in our experiments (present study, Experiment 2; Bast et al., 2001a), tone fear conditioning resulted in higher levels of freezing than contextual fear conditioning and was not affected by ventral hippocampal muscimol infusions, whereas in the experiment by Maren & Holt (2004) tone fear conditioning was weaker than contextual fear conditioning and was disrupted by ventral hippocampal muscimol infusion (whereas contextual fear conditioning was not). However, Esclassan et al. (2009) reported very high levels of tone fear conditioning in their control condition (nearly 80% freezing) and yet found a marked disruption by ventral hippocampal muscimol. Thus, while the strength of conditioning may essentially determine whether dorsal and ventral hippocampal muscimol infusions disrupt contextual fear conditioning across studies, the effects of ventral hippocampal muscimol infusions on tone fear conditioning are less consistently linked to conditioning strength across studies.

5. CONCLUSIONS

On the elevated plus maze, the effects of ventral hippocampal muscimol infusion were consistent with a reduction in locomotor activity, possibly accompanied by anxiolytic effects. In

contrast, the effects of dorsal hippocampal inhibition by muscimol were more consistent with anxiogenic effects. Our fear conditioning experiments, corroborate that both ventral and dorsal hippocampus are required for contextual fear conditioning; a comparison with previous studies using dorsal and ventral hippocampal muscimol infusions suggests that this requirement may be limited to conditions normally resulting in moderate context fear. In addition, our fear conditioning data corroborate previous findings that dorsal hippocampal muscimol infusions do not affect tone fear conditioning. Finally, in our hands, ventral hippocampal muscimol infusions do not significantly affect tone fear conditioning (also see Bast et al., 2001a), contrasting with studies by other groups (and with our own finding of disrupted tone fear conditioning following ventral hippocampal inactivation by tetrodotoxin; Bast et al., 2001a). The reasons for the variable effects of ventral hippocampal inactivation on tone fear conditioning are not clear.

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FIGURE LEGENDS

Fig. 1 Infusion sites in the dorsal **(A)** and ventral **(B)** hippocampus. Photomicrograph of a coronal brain section with the tracks of the guide cannulas visible in both hemispheres (top) and approximate location of the tips of the infusion cannulae depicted on plates of coronal sections through the rat brain (Paxinos and Watson, 1998) (bottom). Values on the right represent distance in mm from bregma. The photomicrographs have been taken at an anterior-posterior level corresponding to approximately -3.6 mm (**A**) and -5.6 mm (**B**) from bregma in the atlas by Paxinos and Watson (1998).

Fig. 2 Experiment 1: Effects of muscimol infusion into the dorsal or ventral hippocampus on the elevated plus maze. Rats were bilaterally infused with saline into dorsal or ventral hippocampus (0.5 µl/side; Saline, n=9) or with muscimol (0.5 µg/0.5 µl/side) into dorsal hippocampus (DH-MUS, n=6) or into ventral hippocampus (VH-MUS, n=5) 5-min prior to the test. *Top*, Distance (cm) moved in the closed and open arms; *middle*, Entries into the closed or open arms; *bottom,* Time (s) spent in the closed or open arms; because of a significant interaction group x 5-min block of testing for time spent in closed arms (see Results, 3.2.), the inset shows values separately for each 5-min block (B1 to B3). Values show the average per 5-min block as mean±SEM; *$P < 0.05$ *vs* saline; [§]*$P < 0.02$ *vs* ventral hippocampal muscimol.
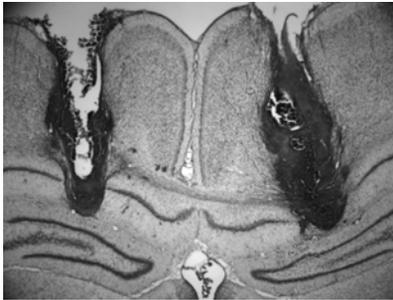
Fig. 3 Experiment 2: Effects of muscimol infusion into the dorsal or ventral hippocampus on foreground contextual fear conditioning. Rats were bilaterally infused with saline into dorsal or ventral hippocampus (0.5 µl/side; Saline, n=9) or with muscimol (0.5 µg/0.5 µl/side) into the dorsal (DH-MUS, n=5) or ventral hippocampus (VH-MUS, n=6) 5-min before conditioning. **(A)** Proportion of time spent freezing during the seven 3-min blocks preceding and following the six 1-s foot shocks in the conditioning session. **(B)** Proportion of time spent freezing during the eight 1-min

blocks of the context test session; *P* < 0.05 *vs* VH-MUS and DH-MUS. All values show means±SEM.
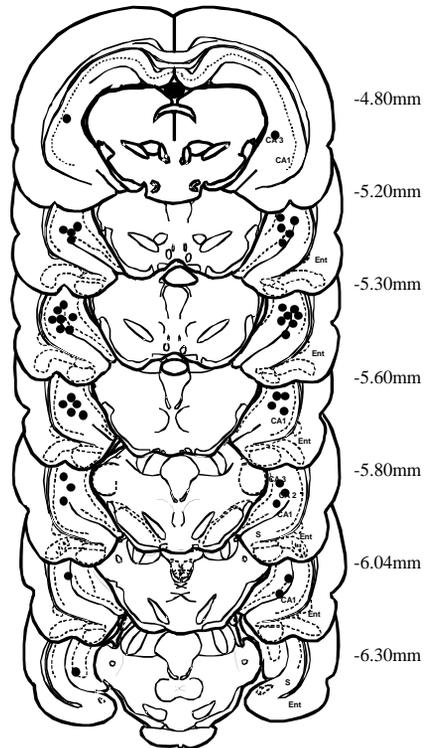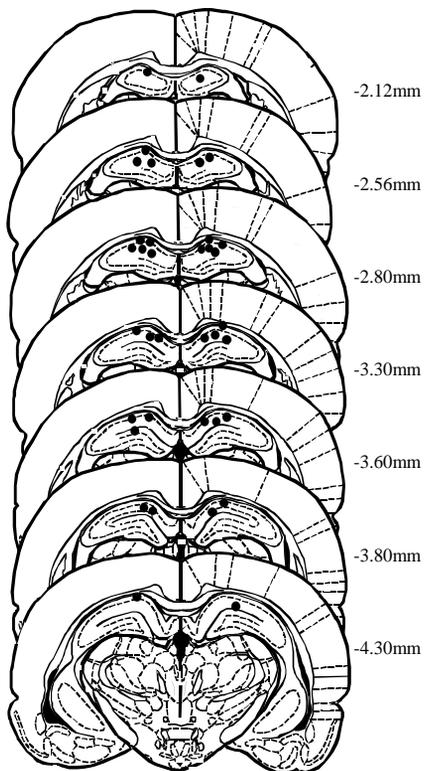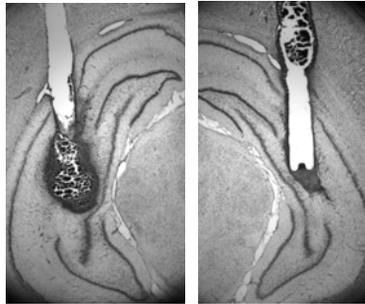
Fig. 4 Experiment 3: Effects of muscimol infusion into the dorsal or ventral hippocampus on background contextual fear conditioning and on tone fear conditioning. Rats were bilaterally infused with saline into dorsal or ventral hippocampus (0.5 µl/side; Saline, n=6) or with muscimol (0.5 µg/0.5 µl/side) into the dorsal (DH-MUS, n=6) or ventral hippocampus (VH-MUS, n=7) 5-min before conditioning. **(A)** Freezing during the six 30-s blocks of conditioned stimulus (*CS*) presentation in the conditioning session. **(B)** Freezing during the seven 3-min blocks preceding and following the *CS* presentations during the conditioning session. **(C)** Freezing during the eight 1-min blocks of the context test. The inset shows freezing per 1-min block, averaged across the 8-min test session to reveal the main effect of group. The asterisk indicates a significant difference from the Saline group. **(D)** Freezing during the three 1-min blocks preceding the CS presentation and the subsequent eight 1-min blocks of tone-CS presentation of the tone test. All values show means±SEM.

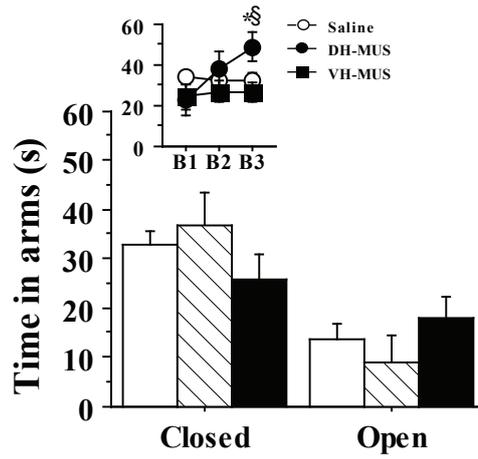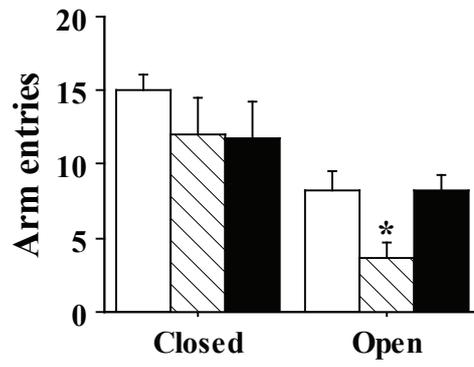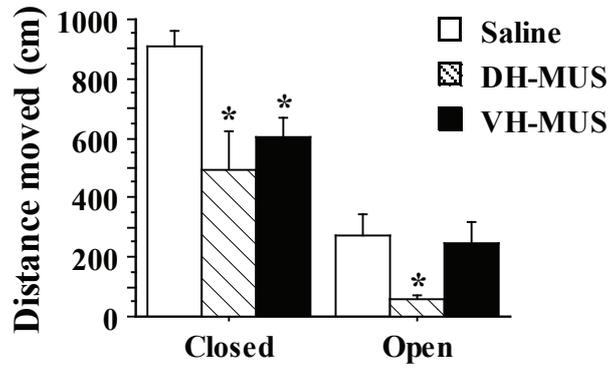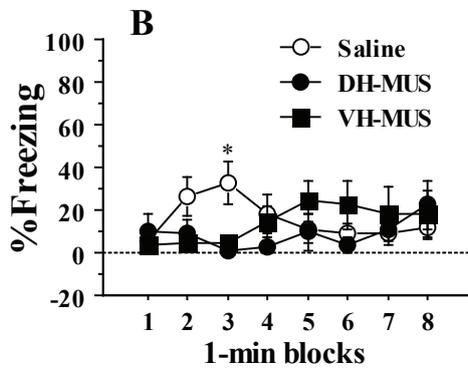# Fig. 1

A Dorsal hippocampus

B Ventral hippocampus

-2.12mm

-2.56mm

-2.80mm

-3.30mm

-3.60mm

-3.80mm

-4.30mm

-4.80mm

-5.20mm

-5.30mm

-5.60mm

-5.80mm

-6.04mm

-6.30mm

Fig. 4