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Dorsal Hippocampus and Classical Fear Conditioning to Tone and Context in Rats: Effects of Local NMDA-Receptor Blockade and Stimulation

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ABSTRACT: Consistent with the importance of the hippocampus in learning more complex stimulus relations, but not in simple associative learning, the dorsal hippocampus has commonly been implicated in classical fear conditioning to context, but not to discrete stimuli, such as a tone. In particular, a specific and central role in contextual fear conditioning has been attributed to mechanisms mediated by dorsal hippocampal N-methyl-D-aspartate (NMDA)-type glutamate receptors. The present study characterized the effects of blockade or tonic stimulation of dorsal hippocampal NMDA receptors by bilateral local infusion of the noncompetitive NMDA receptor antagonist MK-801 (dizocilpine maleate; 6.25μg/side) or of NMDA (0.7μg/side), respectively, on classical fear conditioning to tone and context in Wistar rats. Freezing was used to measure conditioned fear. Regardless of whether conditioning was conducted with tone-shock pairings or unsignaled footshocks (background or foreground contextual conditioning), both NMDA and MK-801 infusion before conditioning resulted in reduced freezing during subsequent exposure to the conditioning context. Freezing during subsequent tone presentation in a new context, normally resulting from conditioning with tone-shock pairings, was not impaired by MK-801 but was strongly reduced by NMDA infusion before conditioning; this freezing was also reduced by NMDA infusion before tone presentation (in an experiment involving NMDA infusions before conditioning and subsequent tone presentation to assess the role of state-dependent learning). It was assessed whether unspecific infusion effects (altered sensorimotor functions, state dependency) or infusion-induced dorsal hippocampal damage contributed to the observed reductions in conditioned freezing. Our data suggest that formation of fear conditioning to context, but not tone, requires NMDA receptor-mediated mechanisms in the dorsal hippocampus. As indicated by the effects of NMDA, some dorsal hippocampal processes may also contribute to fear conditioning to tone. The role of the dorsal hippocampus and local NMDA receptor-mediated processes in fear conditioning to tone and context is discussed in comparison with ventral hippocampal processes. © **2003 Wiley-Liss, Inc.**

KEY WORDS: freezing; glutamate; intracerebral microinfusion; learning and memory; MK-801

INTRODUCTION

In classical fear conditioning in rats, a conditioned stimulus (CS) is paired with an inescapable aversive unconditioned stimulus (US), such as an electrical footshock, so that it elicits conditioned fear responses, such as freezing. A discrete stimulus, such as a tone, as well as the environmental context, can serve as CS. With a discrete CS, fear conditioning is simple associative learning. With a contextual CS, it may also involve relational learning to form a unified representation of environmental stimuli and their mutual relations. This relational learning has been linked to processes underlying the formation of spatial and human declarative (in particular, episodic) memory and, in contrast to simple associative learning, has commonly been associated with the hippocampus (Nadel and Willner, 1980; Eichenbaum, 1996; Fanselow, 2000; Anagnostaras et al., 2001; Kandel, 2001; Morris, 2001).

Recent studies yielded strong evidence that the ventral hippocampus is not only involved in contextual fear conditioning, but also in simple fear conditioning to tone (Maren, 1999; Richmond et al., 1999; Bast et al., 2001b,d; Zhang et al., 2001). It is still commonly held, however, that the dorsal hippocampus, which indeed may differ functionally from the ventral hippocampus (Moser and Moser, 1998; Zhang et al., 2002), contributes to contextual fear conditioning by supporting a unified context representation, but is not required for fear conditioning to tone (Fanselow, 2000; Anagnostaras et al., 2001; Gale et al., 2001; Rudy and O'Reilly, 2001; Wallenstein and Vago, 2001; but see Maren et al., 1997). In particular, fear conditioning to context, similar to spatial and episodic-like learning (Morris et al., 1989; Steele and Morris, 1999; Lee and Kesner, 2002; but see Cain, 1997), has been suggested to require processes mediated by N-methyl-D-aspartate (NMDA)-type glutamate receptors in the dorsal hippocampus, and prevalent concepts of fear conditioning to context imply a central contribution of NMDA receptor-mediated synaptic plasticity in the dorsal hippocampus to context representa-

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tion (Fanselow et al., 1994; Young et al., 1994; Anagnostaras et al., 2001; Stiedl et al., 2000; Gale et al., 2001). However, although we recently provided respective data for the ventral hippocampus (Zhang et al., 2001), it remains to be demonstrated directly that blockade of NMDA receptors in the rat dorsal hippocampus impairs only fear conditioning to context, but not to tone.

The four experiments (experiments 1–4) described in the present article further examined the role of the dorsal hippocampus and local NMDA receptor-mediated processes in classical fear conditioning to tone and context in Wistar rats. Freezing was used as a measure of conditioned fear. NMDA receptor-mediated signaling in the dorsal hippocampus was manipulated by local microinfusion of the noncompetitive NMDA receptor antagonist MK-801 (dizocilpine), which blocks the pore of the receptor channel, or the prototypic and selective NMDA receptor agonist NMDA (Collingridge and Lester, 1989). MK-801 impairs any type of NMDA receptor-mediated signaling. NMDA tonically stimulates NMDA receptor-mediated transmission and thereby disrupts processes depending on the temporal and synaptic order of NMDA receptor activation, such as NMDA receptor-mediated synaptic plasticity (Martin et al., 2000). Moreover, by inducing strong excitation of the local neuronal network, NMDA in the dorsal hippocampus may also interfere with coordinated dorsal hippocampal neurotransmission not primarily mediated by NMDA receptors. Prevalent concepts of the role of the dorsal hippocampus and local NMDA receptor-mediated signaling in classical fear conditioning (see above) would predict that both MK-801 and NMDA in the dorsal hippocampus impair fear conditioning to context, but not to tone. Part of the presented results has previously been published in a preliminary form (Bast et al., 2001a).

MATERIALS AND METHODS

Subjects

One hundred-forty male adult Wistar rats (Zur:Wist[HanIbm]; Research Unit Schwerzenbach, Schwerzenbach, Switzerland), weighing ~250–300 g, and ~10 weeks old at surgery, were included in the four experiments of the present study (experiments 1, 2, and 4: 40 rats each; experiment 3: 20 rats). Animals were housed under a reversed light-dark cycle (lights on: 19:00–07:00) in a temperature (21 ± 1°C)- and humidity (55 ± 5%)-controlled room and were allowed free access to food and water. All rats received bilateral implantation of guide cannulae aimed at the dorsal hippocampus. Before surgery, rats were housed in groups of four per cage; after surgery, they were individually caged. Beginning 3 days before surgery and thereafter until the beginning of the behavioral experiments, all rats were handled daily. All experimental procedures were carried out during the dark phase of the cycle. The Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1985) and Swiss regulations for animal experimentation were followed.

Implantation of Guide Cannulae for Intracerebral Microinfusions

Rats were anesthetized with 1 ml of Nembutal (sodium pentobarbital, 50 mg/ml, Abbott Laboratories, North Chicago, IL) per kg body weight, with heads placed in a stereotactic frame (Kopf Instruments, Tujunga, CA). 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 pair of guide cannulae (9-mm, 26-gauge, stainless steel) in a Perspex holder were implanted through small holes (1.5-mm diameter) drilled on each side of the skull. The tips of the guide cannulae were aimed at the following coordinates above the dorsal hippocampus (in mm): 3.0 posterior and ±1.5 lateral to bregma, and 2.5 ventral to dura. These coordinates have been used in previous studies examining the behavioral effects of dorsal hippocampal infusions (Zhang et al., 2000, 2002). The guide cannulae were fixed with dental cement for which three small stainless screws, previously screwed into the skull, served as anchors. Stainless steel stylets (34-gauge), which extended 0.5 mm beyond the tips of the guide cannulae, were placed inside the guide cannulae to prevent occlusion. After surgery, the health of the rats was checked daily and lost stylets were replaced. The behavioral experiments commenced 7 days after surgery.

Intracerebral Microinfusions and Drugs

For microinfusions into the dorsal hippocampus, rats were manually restrained, and the stylets were removed from the guide cannulae. Infusion cannulae (34-gauge, stainless steel), connected via flexible polyetheretherketone (PEEK) tubing to 10-μl Hamilton microsyringes mounted on a microinfusion pump (KD scientific or WPI sp200i), were then inserted into the guide cannulae. The tips of the infusion cannulae protruded 1.5 mm from the guide cannulae into the dorsal hippocampus, thus aiming at a final dorsoventral coordinate of 4 mm below dura. The rats were bilaterally infused with NMDA (0.7 μg; $C_5H_9NO_4$; Sigma, Switzerland) or MK-801 (6.25 μg; dizocilpine maleate; $C_{16}H_{15}NC_4H_4O_4$; Merck, Sharp & Dohme, UK) in 0.5 μl vehicle (0.9% saline) or with 0.5 μl vehicle (VEH) only per side. The infusion rate was 0.5 μl/min. To allow for absorption of the infusion bolus by the brain tissue, infusion cannulae were left in the brain for 60 s after infusion before being replaced by the stylets. Immediately (experiments 1–3) or 4 min (experiment 4) afterward, the rats were subjected to the experimental sessions. Drug solutions for infusions were freshly prepared on the day of infusion. Solution of MK-801 in isotonic 0.9% saline at the required concentration was facilitated by slight sonification. Doses and time points for the drug infusions were chosen on the basis of our previous experiments (Zhang et al., 2000, 2002). Dorsal hippocampal NMDA infusion at the same dose used in the present study resulted in a slight decrease of startle reactivity, which disappeared within 24 h after infusion, while not affecting prepulse inhibition of the startle reflex or locomotor activity in the open field (Zhang et al., 2002). Importantly, the NMDA dose used in the present study does not induce convulsions and, in a study developing a rat model of hippocampal seizures, much higher doses of NMDA (5–30 μg/side)

have been infused into the dorsal hippocampus to induce convulsions (Hallak et al., 1993). Dorsal hippocampal MK-801 infusion at the dose used in the present study increased startle reactivity, as well as locomotor activity in the open field (Zhang et al., 2000). Both effects disappeared within 24 h after infusion.

Histological Examination of Cannula Placement and Infusion-Induced Neuronal Damage

After completion of the behavioral experiments, all brains were examined histologically, to verify correct cannula placement. In view of the neurotoxic potential of NMDA (Hajos et al., 1986) and MK-801 (Olney et al., 1989), some brains were subjected to a closer histological analysis in order to examine whether NMDA and MK-801 caused any additional neuronal damage as compared with VEH infusions. For that purpose, we used immunohistochemical staining of the neuronal marker protein NeuN in order to selectively and unequivocally visualize neuronal cells (Wolf et al., 1996; Jongen-Rêlo et al., 2002).

For verification of cannulae placements, 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 at room temperature to rinse out the blood, followed by ~100 ml of 4% formalin (4°C) to fix the brain tissue. During perfusion, the aorta was clamped. After extraction from the skull, the brains were postfixed in 4% formalin solution and were subsequently cut into 40-μm coronal sections on a freezing microtome. Every fifth section through the dorsal hippocampus was mounted on a gelatin-treated slide and was stained with cresyl violet. After staining, the sections were dehydrated and coverslipped. Subsequently, they were examined with a light microscope to verify that the tips of the infusion cannulae were placed in the dorsal hippocampus, and their approximate locations were noted onto corresponding plates taken from the rat brain atlas of Paxinos and Watson (1998).

For comparison of neuronal damage induced by one single infusion of VEH (n = 2), NMDA (n = 2), or MK-801 (n = 4), rats were transcardially perfused 3–4 weeks after the infusion. Perfusion was conducted as above. However, the 4% formalin solution was prepared freshly in 0.1 M phosphate-buffered solution (PBS; pH 7.2). Further, it was ensured that the flow rate (14 ml/min) as well as the volume of saline (28 ml) and formalin (140 ml) were the same for each rat, to permit comparison of histological results. After extraction from the skull, the brains were postfixed in 4% formalin for at least 1 week and were then transferred into a cryoprotectant solution of 30% sucrose in PBS. Serial 40-μm coronal sections were prepared with a freezing microtome and were collected in PBS. One series was mounted on gelatin-treated slides, stained with cresyl violet, dehydrated, and coverslipped. An adjacent series was subjected to immunohistochemical staining of the neuronal marker protein NeuN according to the immuno-ABC technique, using a primary monoclonal mouse antibody against NeuN (mNeuN, Chemicon, Switzerland) and a biotinylated secondary antibody (biotinylated horse and mouse IgG; Vector, Switzerland), before being mounted on slides and coverslipped (for further details, see Jongen-Rêlo et al., 2002). For assessment of neuronal damage in the hippocampus, the brain sections were examined with a light microscope. A digital camera controlled by Neurolucida software was used to prepare photomicrographs of immunostained tissue for documentation.

Apparatus for Behavioral Testing

Eight operant test boxes (i.e., four shock boxes and four no-shock boxes) (Habitest; Coulborn Instruments, Allentown, PA) were used. Shock boxes were used for conditioning and context-test sessions, while the no-shock boxes were used to assess fear to the tone CS in an environment distinct from that during conditioning (see Basic Experimental Design, below). Shock boxes were fitted with a parallel grid shock floor (16 parallel bars; E10-10RF; Coulborn Instruments), through which scrambled shocks could be delivered, and placed in light- and sound-attenuating chambers measuring 55 cm × 40 cm × 55 cm. These chambers had two side walls of aluminum and a rear and front wall of clear Perspex. A white waste tray was situated below the grid floor. The four no-shock boxes were fitted with a lattice grid (E10-18NS; Coulborn Instruments); each was placed in a light- and sound-attenuating chamber measuring 72 cm × 45 cm × 45 cm and had three black walls and a front wall of clear Perspex. A brown waste tray was situated below the lattice grid. The four shock and the four no-shock boxes were placed in two different rooms. Presentation of the tone CS and delivery of electric footshock were controlled by a PC with dedicated software (S. Frank, Psychology Department, University of Tel Aviv, Tel Aviv, Israel) connected to a Coulborn Universal Environment Interface (E91-12) with Coulborn Universal Environment Port (L91-12). The tone CS [85 dB(A)] was produced by a 2.9-kHz tone module (E12-02) fixed on one wall of the operant chamber. Shocks were delivered with a Coulborn 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 the test boxes were provided by these cameras, integrated into a four-quarter single image (100,000 pixels) by a multiplexer (DX216CE, Sony), and recorded by a video-recorder (SVT1000; Sony).

Automated Measurement of Activity and Freezing

The video images were transferred to a computer (7600/120 Power Macintosh) equipped with an analysis program (NIH-Image; http://rsb.info.nih.gov/nih-image/download.html) and a macroprogram (P. Schmid, Behavioral Neurobiology Laboratory, Swiss Federal Institute of Technology, Zurich). The percentage of changed pixels between two adjacent 1-s quarter images recorded from a box was used as a measure of activity (for further details, see Richmond et al., 1998). Freezing is commonly identified as cessa-

TABLE 1.

*Summary of Procedures in the Four Experiments (Experiments 1–4) of the Present Study**

Experiment	Infusion[a]	Conditioning (in shock box)	Context test (in shock box)	Tone test (in no-shock box)
1	Immediately before conditioning VEH(16), MK-801(8), NMDA(16)	5 unsignaled shocks each preceded and followed by 5-min blocks	1 day after conditioning	No tone test
2	As in Exp. 1	10 tone-shock pairings each preceded and followed by 2-min blocks	1 day after conditioning	2 days after conditioning
3	Immediately before conditioning: VEH, MK-801 (10 each)	5 unsignaled shocks, then 5 tone-shock pairings, each shock and pairing preceded and followed by 2.5-min blocks	1 day after conditioning + additional extinction 2 days after conditioning	3 days after conditioning
4	4 min before conditioning and tone test 1: VEH-VEH, VEH-NMDA, NMDA-VEH, NMDA-NMDA (10 each)	8 tone-shock pairings each preceded and followed by 1-min blocks	6, 10, and 13 days after conditioning	7, 11, and 14 days after conditioning

*Tone: 30 s, 2.9 kHz, 85 dB(A). Shock: 1-s, 0.5-mA footshock. During pairings, last tone second was congruent with the shock. Infusion parameters and concentrations: 6.25 μg/side MK-801, 0.7 μg/side NMDA, 0.5 μl/side saline as vehicle (VEH) infused within 1 min. Conditioned fear during conditioning and testing was automatically measured in the form of freezing.
[a]Numbers of animals per group are given in parentheses.

tion of any movement except for respiratory movements (e.g., Fanselow et al., 1994; Young et al., 1994). If the percentage of changed pixels between two adjacent 1-s images was $<0.05\%$, this corresponded well to such movement cessation, and the behavior of the rat was scored as "freezing" for the respective second. Validation and principle of the automated analysis of freezing behavior have been described in detail in previous publications (Richmond et al., 1998; Pryce et al., 1999).

Basic Experimental Design, Behavioral Measures, and Data Analysis

Altogether, four fear-conditioning experiments (experiments 1–4) were conducted in the present study. Rats were always subjected to the behavioral sessions in squads of four, with the experimental boxes and testing order being counterbalanced between the different groups as far as possible. The groups differed only with respect to the infusions they received into the dorsal hippocampus and were otherwise treated identically. Infusions of VEH, MK-801, or NMDA (see Intracerebral Microinfusions and Drugs, above) were applied only before conditioning in experiments 1–3, and before conditioning, as well as before the first tone test, in experiment 4. The different conditioning and test sessions were at least 24 h apart. Conditioning sessions consisted of presentations of different combinations of unsignaled 1-s footshocks (0.5-mA current-pulse amplitude) or tone-shock pairings (30-s tone coterminating with a 1-s footshock) in the shock boxes and had different duration depending on the experiment. Context-test sessions were conducted to assess long-term conditioned fear (i.e., conditioned fear persisting beyond the conditioning session) to the context in which the footshocks were experienced. For that purpose, the rats were put into the shock boxes and were left undisturbed for 8 min. Tone-test sessions were conducted in experiments 2–4, in which rats received tone-shock pairings during conditioning, so that long-term conditioned fear to the tone could be assessed. Tone tests were conducted in the no-shock boxes, i.e., an environment distinct from the conditioning context, and consisted of a continuous 8-min tone presentation, preceded by 2 (experiments 1–3) or 3 (experiment 4) min without stimulation. The infusion groups as well as the conditioning and testing procedures applied in experiments 1–4 are described in detail below and summarized in Table 1.

During all sessions, freezing was assessed by the automated analysis system as a measure of conditioned fear. Freezing during conditioning of experiments 2–4, in which rats were conditioned with tone-shock pairings, was analyzed separately for the time blocks surrounding the tone-shock pairings and the 30-s tone presentations. This type of separate analysis may yield some hints as to whether a treatment differently affects the development of freezing

to the context and to the discrete CS during conditioning, even though freezing during the tone presentations is certainly "contaminated" by freezing to the conditioning context, as is freezing between the tone presentations by freezing to the tone. During conditioning sessions, some unconditioned behaviors were also assessed. The video images were watched in order to assess whether all infusion groups reacted to the shock by similar vigorous twitching or jumping, marked components of the unconditioned immediate shock response (Anagnostaras et al., 1999b). Activity before the first tone or shock was assessed using the percentage of changed pixels between adjacent 1-s video images that was given by the automated system. Finally, in experiments 2 and 4, the unconditioned activity response to the first tone presentation was analyzed by comparing the activity scores during the 30 s immediately before the tone with those during the 30-s tone presentation. In these experiments, the tone was the first salient stimulus presented during conditioning. Under these conditions, a tone may induce a pattern of behavioral arousal, including increased activity (Inglis and Fibiger, 1995).

Statistical analysis was conducted with the Statview software system (SAS Institute, NC). From the freezing scores obtained for each second ("freezing" or "not freezing"), the percentage of time spent freezing in a given time block was calculated. By averaging the percentage of pixels changed between adjacent 1-s video images in a given time block, a relative activity measure for this time block was calculated. Data were subjected to analysis of variance (ANOVA), using the different infusions as between-subjects factor and the different time blocks of testing as repeated-measures factor. When there were more than two infusion groups, Fisher's protected least significant difference post hoc comparisons were used for further analysis of the main effects of infusion. The level of significance was set at $P < 0.05$.

Experiment 1: foreground contextual fear conditioning after dorsal hippocampal NMDA or MK-801 infusion

Foreground contextual fear conditioning, i.e., fear conditioning to a context in which unsignaled shocks were presented, has been found to be impaired by dorsal hippocampal lesions in rats (Kim et al., 1993; Young et al., 1994), even though another study did not confirm this effect (Phillips and LeDoux, 1994), and by dorsal hippocampal infusion of the competitive NMDA-receptor antagonist D,L-2-amino-5-phosphonovalerate (APV) in both rats (Young et al., 1994) and mice (Stiedl et al., 2000). Experiment 1 was carried out to confirm the requirement of dorsal hippocampal NMDA receptors, as well as to examine the effect of tonic stimulation of these receptors, in foreground contextual fear conditioning. There were three infusion groups to receive VEH (n = 16), NMDA (n = 16), or MK-801 (n = 8) in the dorsal hippocampus before conditioning. The rats were experimentally naive, except for eight rats each of the VEH and NMDA groups, which received three infusions, one or two of them with NMDA (0.1, 0.25, or 0.7 μg/side), in prepulse-inhibition and open-field experiments finished 1 week before. Conditioning sessions lasted a total of 30 min and 5 s and consisted of presentation of five unsignaled 1-s footshocks separated by 5-min blocks between an initial and a final 5-min block. Context tests were conducted 1 day after conditioning. In our laboratory, the above conditioning and testing parameters yield reliable and marked fear conditioning to the context in cannulated rats, which have a tendency to be less fearful than unoperated rats (Bast et al., 2001d; Zhang et al., 2001). As to the analysis of experiment 1, it is important to note that, before conducting an overall analysis, the test data of the NMDA and VEH rats were analyzed to ensure that the different experimental history of the rats (naive vs used in previous experiments) did not interact with the infusion ($P > 0.69$).

Experiment 2: fear conditioning to a tone after dorsal hippocampal NMDA or MK-801 infusion

A specific involvement of dorsal hippocampal NMDA-receptor signaling in fear conditioning to context, but not tone, is a central assumption in current views on the hippocampal role in fear conditioning (Young et al., 1994; Anagnostaras et al., 2001; Gale et al., 2001). However, only in mice has intact fear conditioning to a tone actually been demonstrated after blockade of dorsal hippocampal NMDA receptors by local APV infusion (Stiedl et al., 2000). Experiment 2 further examined the effects of altered NMDA receptor-mediated signaling in the dorsal hippocampus on simple fear conditioning to tone. There were three infusion groups to receive VEH (n = 16), NMDA (n = 16), or MK-801 (n = 8) in the dorsal hippocampus before conditioning. As in experiment 1, the rats were experimentally naive, except for eight rats each of the VEH as well as the NMDA group, which received three infusions, one or two of them with NMDA (0.1, 0.25, or 0.7 μg/side), in prepulse-inhibition and open-field experiments finished 1 week before. Conditioning sessions lasted a total of 27 min and consisted of 10 tone-shock pairings separated by 2-min blocks between an initial and a final 2-min block. The context-test sessions, to test for freezing to the background context, were conducted 1 day after conditioning. Two days after conditioning, rats were subjected to tone-test sessions. Tone-test sessions lasted a total of 11 min. After 3 min, the tone was presented for the remaining 8 min. In our laboratory, these conditioning and testing parameters yield reliable and marked fear conditioning to the tone, but sometimes, in particular in cannulated rats, only a little or no freezing to the background context (Richmond et al., 1998; Bast et al., 2001d; Murphy et al., 2001; Zhang et al., 2001). As in experiment 1, the test data of the NMDA and VEH rats in experiment 2 were analyzed before the overall analysis in order to verify that the different experimental history of the rats (naive vs used in previous experiments) did not interact with the infusion ($P > 0.37$).

Experiment 3: different effects of dorsal hippocampal MK-801 infusion on fear conditioning to tone and context—a further within-subject comparison

Experiments 1 and 2 indicated that MK-801 infusion into the dorsal hippocampus specifically impaired fear conditioning to context, but not tone. The aim of experiment 3 was to corroborate this by demonstrating a dissociation of the effects of MK-801 on fear

conditioning to context and tone within the same animals. Experiment 2 had already yielded some evidence for this dissociation, but the overall level of freezing to the background context in the context test was very low, and differences between infusion groups were not very pronounced. Experiment 3 included two infusion groups to receive either VEH (n = 10) or MK-801 (n = 10) in the dorsal hippocampus before conditioning, and all consisting of experimentally naive animals. Conditioning sessions lasted a total of 35 min. The first 20 min consisted of five unsignaled 1-s footshocks at 2.5-min intervals in between two initial and one final 2.5-min intervals. The last 15 min consisted of five tone-shock pairings at 2.5-min intervals between an initial and a final 2.5-min interval. At 1 day and 2 days after conditioning, the rats were subjected to 8-min context-test sessions. Three days after conditioning, tone-test sessions of 11 min were conducted, with the tone being presented after 3-min for the remaining 8 min as in experiment 2. Except for the additional second context test, the conditioning and testing parameters in experiment 3 were the same as applied in a previous experiment (Zhang et al., 2001), where we obtained marked fear conditioning to both context and tone. The additional context test was included in the present experiment to further support extinction of contextual fear in order to minimize a possible contribution of contextual fear, which might have generalized from the shock to the no-shock boxes, to freezing during the tone test.

Experiment 4: impairment of fear conditioning by dorsal hippocampal NMDA infusion—the role of state dependency

Experiments 1 and 2 indicated that fear conditioning to both context and tone was impaired by dorsal hippocampal NMDA infusion. Experiment 4 aimed to confirm and further examine this impairment. In particular, we tested whether the reduced conditioned freezing during testing might merely have reflected state dependency, i.e., that associations formed in an altered brain-state may subsequently be retrieved only in a similar brain state (Overton, 1964), rather than an impairment of the specific processes underlying the formation of conditioned fear. Using freezing as measure of conditioned fear, a test for state dependency, involving drug infusions before conditioning and testing, is difficult when the infusions affect activity and thereby may affect the performance of the conditioned fear response (see Bast et al., 2001d; Zhang et al., 2001). NMDA infusions into the dorsal hippocampus, in contrast to dorsal hippocampal MK-801 infusion (Zhang et al., 2000) and ventral hippocampal drug infusions, whose effects on fear conditioning were studied previously (Bast et al., 2001d; Zhang et al., 2001), did not cause marked alteration of activity in previous open-field experiments (Zhang et al., 2002). Thus, while the contribution of state dependency to impaired fear conditioning after hippocampal manipulations is in many cases difficult to examine, it was possible to address this important issue in the case of dorsal hippocampal NMDA infusion. Given that experiment 4 involved infusions before testing, the results are also relevant with respect to the role of the dorsal hippocampus during retrieval/expression and extinction of conditioned fear. Forty experimentally naive rats were included in experiment 4. Before conditioning, rats received either VEH or NMDA in the dorsal hippocampus (each n = 20), resulting in two groups during conditioning and first context test. Before the first tone test, all rats received a second infusion of VEH or NMDA. One-half of the rats received the same infusion as before conditioning, and one-half received a different infusion; the result was four groups that differed with respect to the combinations of infusions (before conditioning-before first tone test): VEH-VEH, VEH-NMDA, NMDA-VEH, NMDA-NMDA (each group n = 10). The four groups were matched with respect to the behavioral measurements taken before the first tone test. Based on the observation in experiments 1 and 2 that dorsal hippocampal NMDA infusion may result in movement inhibition lasting a few minutes, infusions in experiment 4 were applied 4 min before the experimental sessions. Conditioning sessions lasted a total of 13 min and consisted of eight tone-shock pairings separated by 1-min blocks between an initial and a final 1-min block. The first context-test sessions were conducted 6 days after conditioning. Seven days after conditioning, rats were subjected to the first tone-test sessions. Tone-test sessions lasted a total of 10 min. After 2 min, the tone was presented for the remaining 8 min. Additional context- and tone-test sessions without the preceding infusions were conducted 10 and 13 days or 11 and 14 days, respectively, after conditioning to examine possible effects of the infusions on the extinction of conditioned fear, and to corroborate that the effects of NMDA in the dorsal hippocampus are temporary. The sessions after the infusions were planned to be as short as possible because the observation in experiments 1 and 2, that freezing during conditioning was reduced in the NMDA rats only until about the fifth shock, indicated that action of the drug might fade within ~15 min. The time span between conditioning and first test sessions was chosen so that repeated infusions were 1 week apart, in order to allow for recovery of the brain tissue from possible disturbances resulting from the first intracerebral infusion (Routtenberg, 1972). In order to reduce the number of repeated infusions to a minimum, infusions were only applied before conditioning and the first tone test, but not before the other tests.

RESULTS

Histology

In all rats included in experiments 1–4, the tips of the infusion cannulae were located within or around the borders of the dorsal hippocampus (Fig. 1). Therefore, the behavioral data of all rats were included in the analysis. Damage to hippocampal neuron layers and interspersed neurons, indicated by interruption in NeuN staining, was mainly restricted to the tracks of the infusion cannulae and the immediately surrounding areas, and may have been slightly more expanded in rats infused with NMDA. Overall, however, neuronal damage in the dorsal hippocampus did not differ markedly between animals infused with VEH, MK-801, or NMDA (Fig. 2).

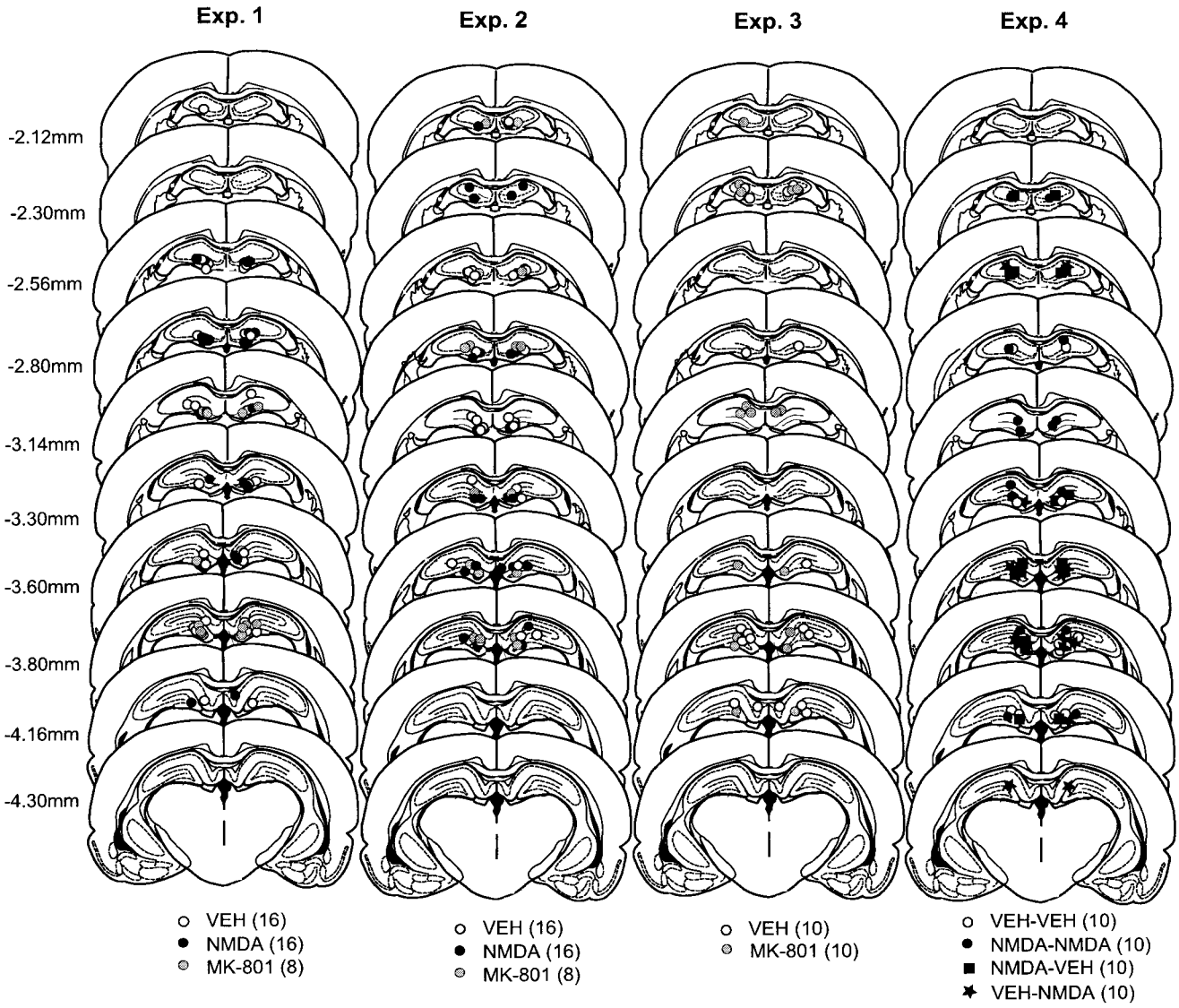

FIGURE 1. Approximate locations of the tips of the infusion cannulae in the different infusions groups of experiments 1–4, depicted on coronal sections. Drawn after Paxinos and Watson (1998). Numbers of animals per infusion group are indicated in parentheses. Values on the left represent distance from bregma.

Effects of MK-801 and NMDA Infusions on Unconditioned Behavior

While dorsal hippocampal MK-801 infusion did not induce any behavioral abnormalities that were detectable by mere visual inspection of the rat, dorsal hippocampal NMDA infusion induced an easily visible short-lasting movement inhibition in some rats. These rats, when put into their cages or the test boxes after infusion and left untouched, stood rigidly on their slightly outward-set four paws and stared in one direction. A few minutes (~5 min) after infusion, behavioral abnormalities were no longer detectable by mere visual inspection of the rats infused with NMDA.

Figure 3 depicts unconditioned activity before the first tone or shock in experiments 1–4 (Fig. 3A), and the alterations of unconditioned activity in response to the first tone in experiments 2 and 4 (Fig. 3B) for the different infusion groups.

Due to the short-lasting movement inhibition induced by dorsal hippocampal NMDA infusion in some rats (see above) average activity throughout the periods preceding the first tone or shock was decreased in the NMDA group as compared to the other groups in experiments 1 and 2, where infusions were given immediately before conditioning. Rats that received MK-801 infusions (experiments 1–3) or rats that received NMDA infusions 4 min before conditioning (experiment 4) exhibited similar activity levels as VEH rats (Fig. 3A). In experiments 1 and 2, ANOVA yielded a significant effect of infusion on the average activity throughout the 5 min before the first shock ($F_{2,37} = 3.40$, $P < 0.05$) or the 2 min before the first tone ($F_{2,37} = 5.33$, $P < 0.01$), respectively. Post hoc comparisons indicated significant differences between the NMDA and MK-801 groups in experiment 1 ($P < 0.05$), and between the NMDA and VEH group in experiment 2 ($P <$

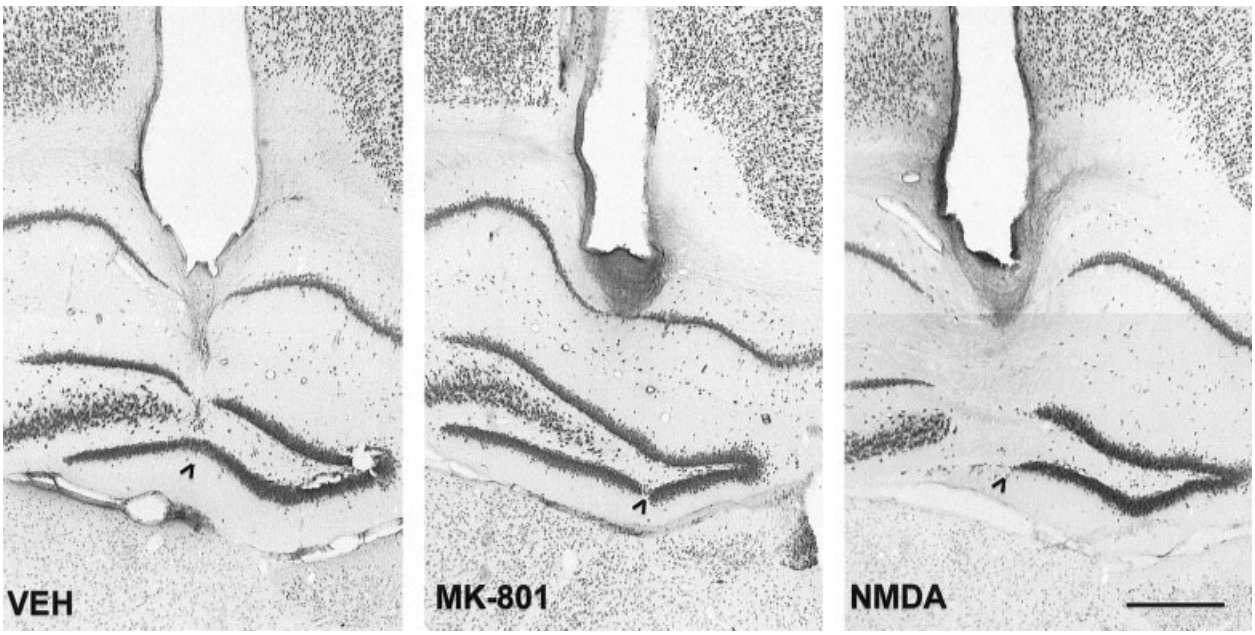

FIGURE 2. Photomicrographs of coronal sections through the dorsal hippocampus immediately around the infusion sites from rats that received one infusion of vehicle (VEH) (0.5 μl saline), MK-801 (6.25 μg/0.5 μl), or N-methyl-D-aspartate (NMDA) (0.7 μg/0.5 μl). Neurons are visualized by immunostaining of the neuronal marker protein NeuN. Arrowheads indicate the approximate location of the tips of the infusion cannulae. Scale bar = 500 μm.

0.0025). Moreover, the difference between VEH and NMDA rats in experiment 1 closely approached significance ($P = 0.050$). VEH and MK-801 rats did not differ in experiment 1 ($P > 0.50$) or 2 ($P > 0.18$). ANOVA of activity during the 5 min before the first shock in experiment 3 ($F_{1,18} = 0.00$, $P > 0.97$) or the 1 min before the first tone in experiment 4 ($F_{1,38} = 1.46$, $P > 0.23$) did not yield a significant effect of the infusion given before conditioning. Analysis of activity levels in the different 1-min blocks of the periods before the first tone or shock (data not shown) did not reveal a decrease of activity over time, which would have reflected habituation of exploratory activity, in any group.

In experiments 2 and 4, in which the tone was the first salient stimulus presented, all infusion groups were more active during the tone presentation than during the immediately preceding period (Fig. 3B), indicating that the tone induced similar behavioral arousal in all groups. For both experiments, ANOVA of the average activity in the 30-s periods both before and during the tone yielded an effect of period (experiment 2: $F_{1,37} = 20.19$, $P < 0.0001$; experiment 4: $F_{1,38} = 9.39$, $P < 0.005$) without an interaction of infusion group and period (experiment 2: $F_{2,37} = 2.60$, $P > 0.08$; experiment 4: $F_{2,38} = 1.56$, $P > 0.21$). Moreover, in both experiments there was a significant main effect of group,

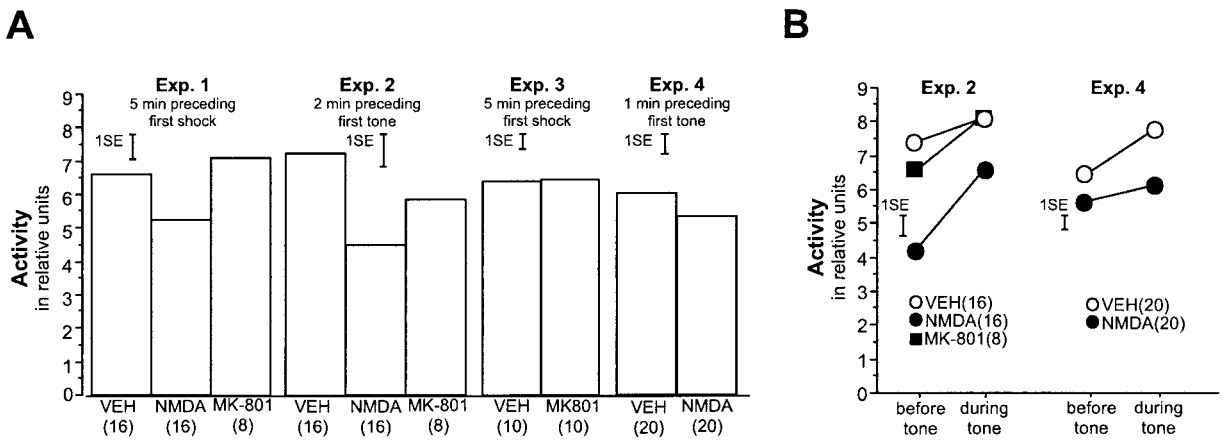

FIGURE 3. Effects of the different infusions on (A) unconditioned activity before the first tone or shock in experiments 1–4, and (B) on the increase of unconditioned activity in response to the first tone in experiments 2 and 4. In experiments 1–3, infusions were applied immediately, in experiment 4, 4 min before behavioral testing. Numbers of animals per infusion group are indicated in parentheses. Presented values are means. Bars = 1 standard error (SE) derived from the appropriate mean square of ANOVA. The SE provides an estimate of population variance.

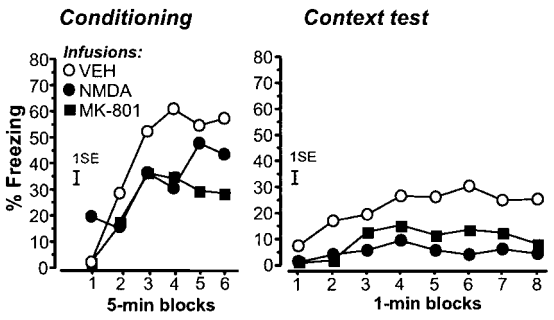

FIGURE 4. Freezing during conditioning and context-test sessions of experiment 1. Vehicle (VEH) (n = 16), N-methyl-D-aspartate (NMDA) (n = 16), or MK-801 (n = 8) was infused into the dorsal hippocampus immediately before conditioning with five unsignaled footshocks. Mean percentage of time spent freezing is depicted for the six 5-min blocks both preceding and following the five unsignaled footshocks during conditioning and for the eight 1-min blocks of the context test. Bars = 1 standard error (SE) derived from the appropriate mean square of ANOVA.

reflecting movement inhibition in some of the NMDA rats (experiment 2: $F_{2,37} = 4.09$, $P < 0.025$; experiment 4: $F_{1,38} = 4.58$, $P < 0.05$).

Inspection of the video images from the conditioning sessions of all four experiments yielded that all infusion groups exhibited similar vigorous twitching or jumping as immediate response to the footshock, indicating that NMDA or MK-801 infusions did not affect shock sensitivity.

Effects of MK-801 and NMDA Infusions on Conditioned Freezing

Experiment 1: foreground contextual fear conditioning after dorsal hippocampal NMDA or MK-801 infusion

The freezing data of the conditioning and the context-test session of experiment 1 are depicted in Figure 4. During conditioning, freezing resulting from the inescapable footshocks was decreased in MK-801 as compared with VEH rats until the end of the session. NMDA rats exhibited similarly low freezing as MK-801 rats during the 5-min blocks after the first three shocks, but showed freezing comparable to the VEH rats in the last two 5-min blocks. Moreover, as compared with the other two groups, NMDA rats spent more time immobile in the 5-min block before the first shock, reflecting the short-lasting movement inhibition induced by the dorsal hippocampal NMDA infusion. ANOVA of freezing throughout all six 5-min blocks both before and after the shocks in the conditioning session yielded an effect of infusion ($F_{2,37} = 4.39$, $P < 0.025$) and 5-min block ($F_{5,185} = 23.21$, $P < 0.0001$), as well as an interaction of these two factors ($F_{10,185} = 3.23$, $P < 0.001$), reflecting that differences between the groups changed throughout the session. During the first 5-min block ($F_{2,37} = 5.32$, $P < 0.01$), NMDA rats exhibited increased immobility as compared with the VEH ($P < 0.001$) and MK-801 groups ($P < 0.025$), which did not differ ($P > 0.99$). In the following three 5-min blocks ($F_{2,37} = 5.81$, $P < 0.01$), average freezing levels were lower in MK-801 ($P < 0.05$) and NMDA ($P < 0.005$) rats, which did not differ ($P > 0.76$), than in the VEH group. Finally, in the last two 5-min blocks ($F_{2,37} = 5.65$, $P < 0.01$), freezing was decreased in the MK-801 group as compared with VEH ($P < 0.0025$) and NMDA ($P < 0.05$) rats, which no longer differed ($P > 0.13$).

During the context test, both NMDA and MK-801 rats exhibited lower conditioned freezing than the VEH group. ANOVA of freezing values throughout the eight 1-min blocks of the context test yielded a significant effect of 1-min block ($F_{7,259} = 5.61$, $P < 0.0001$), reflecting a gradual increase of freezing throughout the first three 1-min blocks, as well as an effect of infusion ($F_{2,37} = 5.12$, $P < 0.025$). Post hoc comparisons demonstrated that average freezing levels throughout the eight 1-min blocks were lower in NMDA than in VEH rats ($P < 0.005$). There was a strong tendency ($P = 0.068$) for MK-801 rats, which exhibited similarly low freezing levels as the NMDA rats ($P > 0.50$), to show less freezing than the VEH group.

Experiment 2: fear conditioning to a tone after dorsal hippocampal NMDA or MK-801 infusion

The freezing data for the different sessions of experiment 2 are depicted in Figure 5. During conditioning, freezing in response to the footshocks was decreased in MK-801 as compared with VEH rats until the end of the session. NMDA rats exhibited similarly low freezing as MK-801 rats during the first half of the session, but showed freezing comparable to the VEH rats throughout the second half. Before the first shock, NMDA rats spent more time immobile than did the two other groups, reflecting movement inhibition induced by the dorsal hippocampal NMDA infusion. ANOVA of freezing throughout the 11 2-min blocks both before and after the tone-shock pairings, as well as of freezing during the 10 30-s blocks of tone presentation, yielded an effect of infusion ($F_{2,37} = 4.64$, $P < 0.025$; $F_{2,37} = 12.32$, $P < 0.0001$) and time block ($F_{10,370} = 9.85$, $P < 0.0001$; $F_{9,333} = 4.84$, $P < 0.0001$), as well as an interaction of these two factors ($F_{20,370} = 2.90$, $P < 0.001$; $F_{18,333} = 2.46$, $P < 0.001$), reflecting that differences between the groups changed throughout the session. During the first 2-min block ($F_{2,37} = 5.32$, $P < 0.01$), NMDA rats spent or tended to spend, respectively, more time immobile than the VEH ($P < 0.005$) or MK-801 group ($P = 0.070$), which did not differ ($P > 0.4$). Similar differences appeared to exist throughout the subsequent 30-s tone presentation of the first tone-shock pairing, although ANOVA only yielded a strong tendency toward an effect of infusion on the proportion of time spent immobile during this period ($F_{2,37} = 2.99$, $P = 0.063$). During the following five 2-min blocks ($F_{2,37} = 8.08$, $P < 0.0025$), as well as the adjacent 30-s tone presentations ($F_{2,37} = 12.82$, $P < 0.0001$), of the second to sixth tone-shock pairings, average freezing levels were lower in MK-801 ($P < 0.0025$; $P < 0.0001$) and NMDA ($P < 0.0025$; $P < 0.001$) rats, which did not differ ($P > 0.43$; $P > 0.08$), than in VEH rats. Finally, the effect of infusion on average freezing levels during the last five 2-min blocks ($F_{2,37} = 3.20$, $P = 0.052$) and the last four tone presentations ($F_{2,37} = 7.96$, $P < 0.0025$) closely approached significance or was significant, respectively. Post hoc comparisons

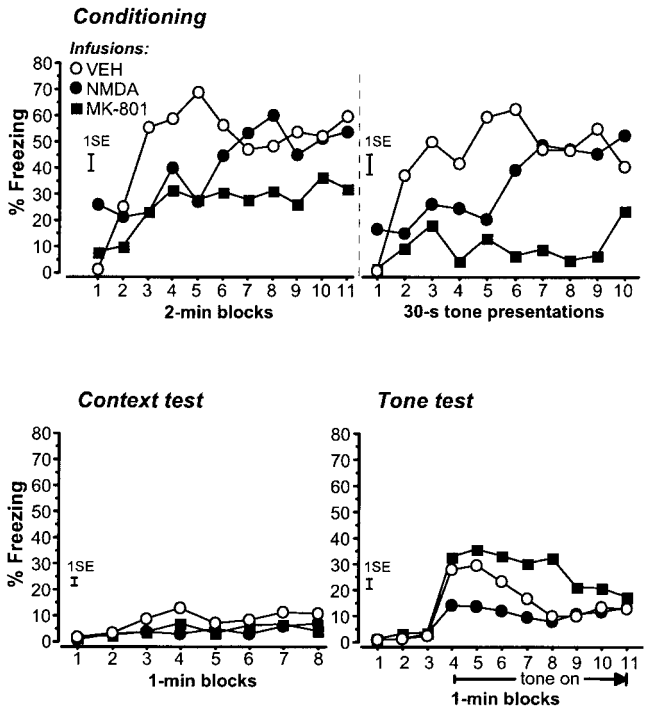

FIGURE 5. Freezing during conditioning, context-, and tone-test sessions of experiment 2. Vehicle (VEH) (n = 16), N-methyl-D-aspartate (NMDA) (n = 16), or MK-801 (n = 8) was infused into the dorsal hippocampus immediately before conditioning with 10 tone-shock pairings. Mean percentage of time spent freezing during conditioning is depicted for the 11 2-min blocks both preceding and following the tone-shock pairings as well as for the 10 30-s tone presentations of the pairings. Mean percentage of time spent freezing during context- and tone test is depicted for each of the eight or 11, respectively, 1-min blocks of the sessions. Bars = 1 standard error (SE) derived from the appropriate mean square of ANOVA.

indicated that average freezing was decreased in the MK-801 group as compared with VEH ($P < 0.05$; $P < 0.001$) and NMDA ($P < 0.025$; $P < 0.001$) rats, which did not differ anymore ($P > 0.93$; $P > 0.89$).

During the context test, all groups exhibited relatively low levels of freezing (<14%) throughout all eight 1-min blocks, indicating that the conditioning procedure did not result in marked conditioned fear to the context. Nevertheless, ANOVA yielded an effect of infusion on freezing levels ($F_{2,37} = 3.1$, $P < 0.05$), and post hoc comparisons indicated that, as compared with the VEH group, freezing was or tended to be reduced, respectively, in the NMDA ($P < 0.025$) and the MK-801 ($P = 0.090$) group, which exhibited very similar freezing ($P > 0.77$).

During the tone test, conditioned freezing to the tone appeared to be decreased in the NMDA group as compared with both VEH and MK-801 rats. During the three 1-min blocks before tone onset, rats virtually did not exhibit conditioned fear, as evidenced by low levels of immobility (<4%), which did not differ between the infusion groups (main effect of infusion: $F_{2,37} = 1.31$, $P > 0.28$; interaction infusion × 1-min block: $F_{4,74} = 4.51$, $P > 0.60$). ANOVA of freezing levels throughout the eight 1-min blocks after tone onset yielded a trend toward an effect of infusion ($F_{2,37} = 2.67$, $P = 0.082$), indicating higher freezing levels in VEH and MK-801 rats as compared with the NMDA group, a significant main effect of 1-min block ($F_{10,370} = 18.82$, $P < 0.0001$), and an interaction of infusion and 1-min block ($F_{10,370} = 2.15$, $P < 0.005$). The interaction reflected that, throughout the first two to three 1-min blocks after tone onset, VEH and MK-801 rats exhibited similarly marked freezing (~30%), which then appeared to decrease faster in the VEH than in the MK-801 group toward the end of the session, while NMDA rats exhibited little freezing (~10%) throughout all eight 1-min blocks of tone presentation.

Experiment 3: different effects of dorsal hippocampal MK-801 infusion on fear conditioning to tone and context—a further within-subject comparison

Figure 6 depicts the freezing data for the different sessions of experiment 3. During conditioning, freezing in response to the footshocks was decreased in MK-801 as compared with VEH rats. ANOVA of freezing during the 12 2.5-min blocks both preceding and following the unsignaled shocks and tone-shock pairings yielded an effect of infusion ($F_{1,18} = 28.10$, $P < 0.0001$), 2.5-min block ($F_{11,198} = 25.53$, $P < 0.0001$), as well as an interaction of these two factors ($F_{11,198} = 6.16$, $P < 0.0001$). The interaction reflected that, while both infusion groups exhibited virtually no freezing during the two 2.5-min blocks before the first shock, freezing levels increased to a level of ~60% in the VEH, but only to ~20%, in the MK-801 group within the 2.5-min blocks after the first two shocks. ANOVA of freezing levels during the 30-s tone presentations of the five tone-shock pairings presented in the second half of the conditioning session revealed only an effect of infusion ($F_{1,18} = 41.13$, $P < 0.0001$), but not an effect of tone presentation ($F_{4,72} = 0.25$, $P > 0.91$) or an interaction of infusion and tone presentation ($F_{4,72} = 0.33$, $P > 0.86$). Thus, during conditioning, MK-801 rats exhibited less freezing than VEH rats, in absence, as well as in presence, of the tone.

During the first context test, VEH, but not MK-801 rats, exhibited marked conditioned freezing. ANOVA of freezing levels throughout the eight 1-min blocks yielded an effect of infusion ($F_{1,18} = 6.23$, $P < 0.025$). Although it appeared that conditioned freezing in the VEH rats developed gradually throughout the first three 1-min blocks, and that toward the end of the session VEH rats exhibited slight extinction of conditioned freezing, ANOVA did not show an effect of 1-min block ($F_{7,126} = 1.74$, $P > 0.10$) or an interaction of infusion and 1-min block ($F_{7,126} = 1.07$, $P > 0.38$). During the second context test, both VEH and MK-801 rats exhibited similarly low levels of immobility ($F_{1,18} = 0.11$, $P > 0.74$). ANOVA on the time spent immobile throughout the eight 1-min blocks only yielded an effect of 1-min block ($F_{7,126} = 4.10$, $P < 0.0005$) as immobility levels slightly increased toward the end of the session, probably reflecting a decrease in activity due to habituation to the context.

In the tone test, MK-801 and VEH rats exhibited similar marked conditioned freezing to the tone. During the three 1-min blocks before tone onset, rats exhibited virtually no conditioned fear, as evidenced by low levels of immobility (<6%) which did

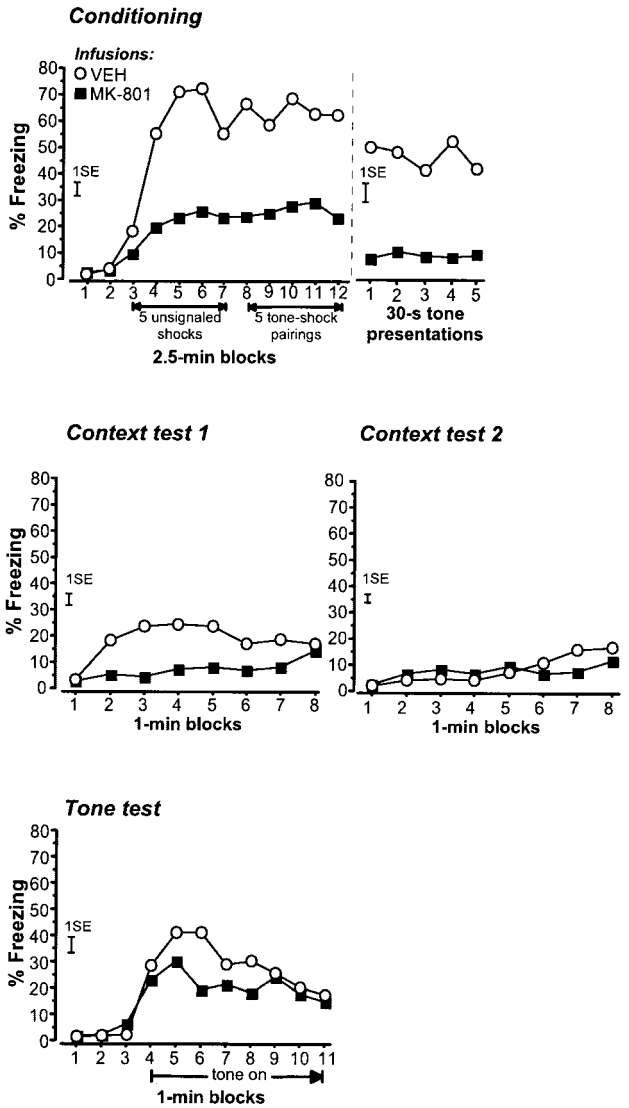

FIGURE 6. Freezing during conditioning, context-, and tone-test sessions of experiment 3. Vehicle (VEH) (n = 10) or MK-801 (n = 10) was infused into the dorsal hippocampus immediately before conditioning with five unsignaled footshocks and five tone-shock pairings. Mean percentage of time spent freezing during conditioning is depicted for the two 2.5-min blocks preceding the shocks, for the five 2.5-min blocks following the unsignaled shocks and the tone-shock pairings, as well as for the five 30-s tone presentations of the pairings. Mean percentage of time spent freezing during the context and tone tests is depicted for each of the eight or 11, respectively, 1-min blocks of the sessions. Bars = 1 standard error (SE) derived from the appropriate mean square of ANOVA.

not differ between groups. ANOVA on freezing levels during the eight 1-min blocks of tone presentation did not reveal an effect of infusion ($F_{1,18} = 0.68$, $P > 0.42$) or an interaction of infusion and 1-min block ($F_{7,126} = 0.87$, $P > 0.53$). There was only a significant effect of 1-min block ($F_{7,126} = 2.79$, $P < 0.01$), reflecting that freezing levels, which had increased steeply throughout the first 1-min block of tone presentation, reached peak levels in the second 1-min block of tone presentation and then gradually decreased toward the end of the session.

Experiment 4: impairment of fear conditioning by dorsal hippocampal NMDA infusion—the role of state dependency

The freezing data for experiment 4, in which rats received infusions before conditioning, as well as before the first tone test, are depicted in Figure 7. During the conditioning session, the development of conditioned freezing in response to the inescapable footshocks was retarded in the rats that received NMDA infusion before conditioning, as compared with the rats that received VEH infusion. Throughout the first part of the session, NMDA rats exhibited less freezing than did VEH rats, while toward the end of the session both groups exhibited similar levels of conditioned freezing. ANOVA of freezing levels during the nine 1.5-min blocks both preceding and following the footshocks yielded no effect of infusion ($F_{1,38} = 2.26$, $P > 0.14$), but an effect of time block ($F_{8,304} = 22.96$, $P < 0.0001$), as well as an interaction of infusion and time block ($F_{8,304} = 9.99$, $P < 0.0001$). ANOVA of freezing levels during the 30-s tone presentations of the eight tone-shock pairings yielded an effect of infusion ($F_{1,38} = 15.08$, $P < 0.0005$) and time block ($F_{7,266} = 38.28$, $P < 0.0001$), as well as an interaction of tone presentation and infusion ($F_{7,266} = 10.30$, $P < 0.0001$). The interaction between time block or tone presentation and infusion reflected that conditioned freezing was lower in the NMDA than in the VEH group until administration of about the fifth shock, i.e., during the second to fifth 1.5 min block ($F_{1,38} = 25.10$, $P < 0.0001$) and the second to fifth tone presentation ($F_{1,38} = 45.10$, $P < 0.0001$), while afterward, i.e., during the last four 1.5-min blocks ($F_{1,38} = 2.59$, $P > 0.11$) and the last three tone presentations ($F_{1,38} = 0.10$, $P > 0.75$), NMDA and VEH rats showed similar freezing levels. Moreover, levels of immobility before the first shock, i.e., during the first 1.5-min block ($F_{1,38} = 5.05$, $P < 0.05$) and the 30-s tone of the first tone-shock pairing ($F_{1,38} = 4.34$, $P < 0.05$), were slightly higher in NMDA than in VEH rats, reflecting that some of the rats that received NMDA 4 min before conditioning still exhibited a slight movement inhibition at the beginning of the session.

During the first context test, VEH, but not NMDA, rats exhibited marked conditioned freezing. ANOVA of freezing levels during the eight 1-min blocks yielded an effect of group ($F_{1,38} = 9.77$, $P < 0.005$) and of 1-min block ($F_{7,266} = 2.83$, $P < 0.01$), as well as an interaction of these two factors ($F_{7,266} = 2.90$, $P < 0.01$). The interaction reflected that conditioned freezing in the VEH group increased from ~10% in the first 1-min block to ~25% in the third and fourth 1-min blocks, and then decreased toward the end of the session, reflecting extinction of conditioned fear to the context, while NMDA rats exhibited little freezing (<5%) throughout the whole session.

During the first tone test, all three groups that received NMDA before conditioning or the first tone test, or at both occasions, exhibited markedly reduced conditioned freezing to the tone as compared with the group that had received VEH infusion at both occasions. ANOVA of freezing levels during the two 1-min blocks before tone onset yielded an effect of the infusion ($F_{1,36} = 6.01$, $P < 0.025$) given 4 min before the tone test, reflecting movement inhibition in some rats that received NMDA before the tone test

session. Moreover, there was also an effect of the infusion received before conditioning ($F_{1,36} = 4.13$, $P < 0.05$), but no interaction between the two infusions ($F_{1,36} = 1.87$, $P > 0.18$). This reflected that average levels of immobility were higher in rats that had received VEH before conditioning than in those that had received NMDA before conditioning. First, VEH-VEH rats exhibited higher freezing than the NMDA-VEH rats in the second 1-min block, possibly reflecting that in the rats that had received VEH before conditioning there was still some fear to the conditioning context that generalized to the test context. Second, immobility levels, reflecting movement inhibition induced by the NMDA infusion before conditioning, were higher in VEH-NMDA than in NMDA-NMDA rats. This might reflect tolerance to the effects of NMDA due to the previous NMDA infusion. However, NMDA-induced movement inhibition was only observed in some rats, and it is striking that VEH-NMDA rats exhibited twice as much immobility before tone onset in the first tone test as displayed by rats that received NMDA before conditioning, before the first shock during conditioning. Thus, the higher immobility levels in VEH-NMDA as compared with NMDA-NMDA rats before tone onset in the first tone test are most likely reflecting a sampling error. For the eight 1-min blocks of tone presentation, an effect of time ($F_{7,252} = 10.52$, $P < 0.0001$) was the only simple main effect revealed by ANOVA of freezing. However, all possible interactions of between-subjects (infusion before conditioning, infusion before first tone test) and repeated-measures (1-min block) factors were significant. Most importantly, there was a three-way interaction of infusion before conditioning, infusion before first tone test, and time block ($F_{7,252} = 3.48$, $P < 0.0025$). This reflected that freezing in the rats receiving two VEH infusions was higher as compared with all other groups during the beginning of the tone presentation, and that this difference was gradually decreasing, indicating extinction of conditioned fear.

Freezing levels throughout the context test 2 and 3 were relatively low, indicating extinction of conditioned fear to the context, and did not differ between the groups (all main effects and interactions involving infusion before conditioning or first tone test: $F < 3.53$, $P > 0.06$). During tone test 2, however, considerable conditioned freezing was still exhibited by the groups that received VEH before conditioning. ANOVA of freezing during the eight 1-min blocks of tone presentation yielded an interaction of infusion before conditioning and 1-min block ($F_{7,252} = 3.92$, $P < 0.0005$). This reflected that freezing in the rats which had received VEH before conditioning was higher as compared with the other groups only during the first four 1-min blocks of tone presentation ($F_{1,36} = 7.36$, $P < 0.025$). Although the VEH-NMDA group appeared to exhibit higher levels of conditioned freezing than the VEH-VEH group, indicating that NMDA infusion before tone test 1 may have impaired extinction of conditioned fear to the tone,

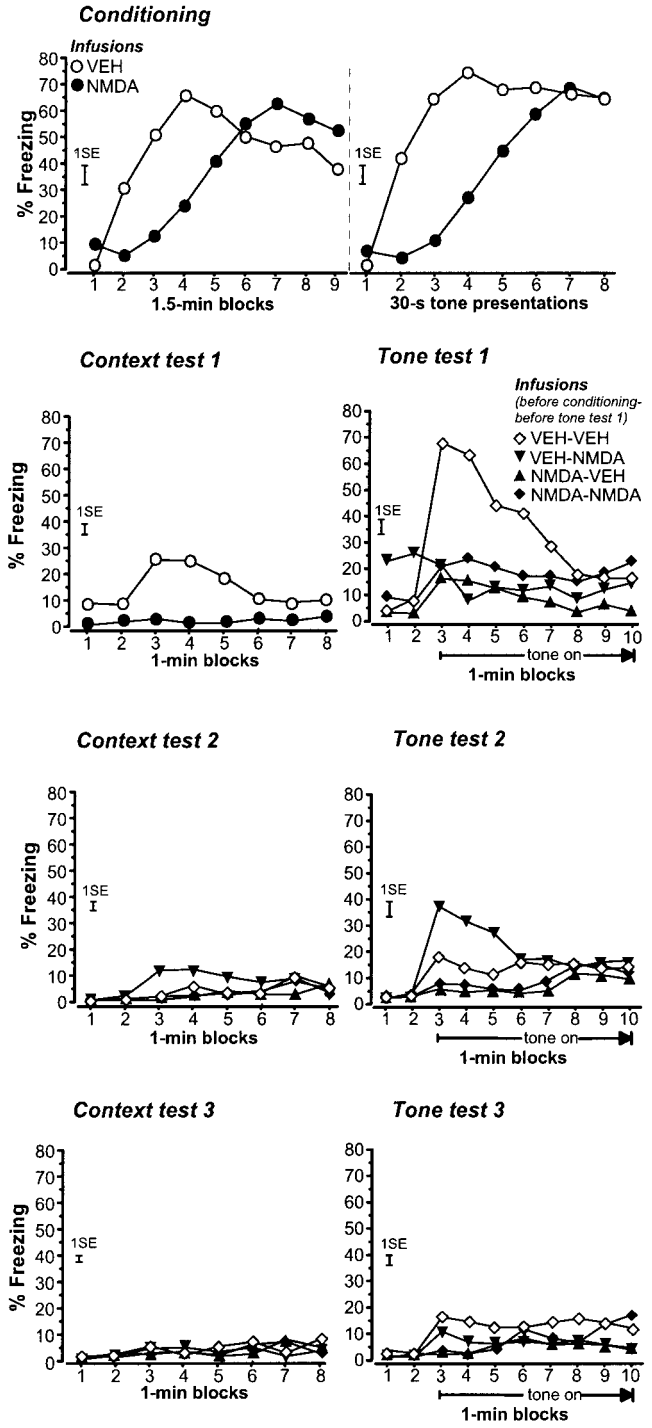

FIGURE 7. Freezing during conditioning, context-, and tone-test sessions of experiment 4. Conditioning was conducted with eight tone-shock pairings. A first infusion of vehicle (VEH) or N-methyl-D-aspartate (NMDA) was given 4 min before conditioning, resulting in two infusion groups (each n = 20) for conditioning and context test 1. A second infusion of VEH or NMDA was given 4 min before tone test 1, so that four infusion groups (each n = 10), differing with respect to the combination of infusions received before conditioning and tone test 1, resulted for tone test 1 and the following test sessions. Mean percentage of time spent freezing during conditioning is depicted for the nine 1.5-min blocks both preceding and following the tone-shock pairings, as well as for the eight 30-s tone presentations of the pairings. Mean percentage of time spent freezing during the context and tone tests is depicted for each of the eight or 10, respectively, 1-min blocks of the sessions. Bars = 1 standard error (SE) derived from the appropriate mean square of ANOVA.

ANOVA on freezing during the eight 1-min blocks of tone presentation did not yield significant interactions involving both infusions ($P > 0.11$). During tone test 3, the tone onset still appeared to induce weak conditioned freezing in the two groups that received VEH infusions before conditioning. ANOVA on freezing during the eight 1-min blocks of tone presentation yielded an interaction of infusion before conditioning and 1-min block ($F_{7,252} = 2.86$, $P < 0.01$). In the first 1-min block after tone onset, rats that had received VEH before conditioning exhibited higher conditioned freezing than rats that had received NMDA ($F_{1,36} = 4.28$, $P < 0.05$).

DISCUSSION

Altogether, the four experiments of the present study yielded two major findings. Dorsal hippocampal MK-801 infusion before conditioning to context or tone resulted in reduced freezing to the context, but not the tone, in subsequent test sessions (experiments 1–3). Freezing to both context and tone, however, was reduced by dorsal hippocampal NMDA infusion before conditioning (experiments 1, 2, and 4).

Dorsal Hippocampal Infusion of MK-801 and NMDA

In view of the neurotoxic potential of MK-801 (Olney et al., 1989) and NMDA (Hajos et al., 1986), we examined infusion-induced neuronal damage, using selective visualization of neurons by immunostaining of the neuronal marker protein NeuN (Wolf et al., 1996). This examination demonstrated that the neuronal damage in the dorsal hippocampus was restricted mainly to the cannula tracks and the immediately surrounding areas. Even though NMDA may have induced slight additional damage, overall neuronal damage in the dorsal hippocampus did not differ markedly between rats infused with VEH, MK-801, or NMDA. That NMDA in the dorsal hippocampus exerts mainly temporary effects is further corroborated by the results of experiment 4, where rats infused with NMDA only before tone test 1 exhibited reduced freezing as compared with VEH rats during tone test 1, but not 2. Moreover, with the small infusion volume (0.5 μl/side) and fine infusion cannulae (34 gauge) used in the present study, the estimated spread of the infused substances, occurring preferentially dorsally along the external wall of the infusion cannula, is <1 mm (Myers, 1966; Myers et al., 1971; Routtenberg, 1972). Thus, it can be assumed that differences observed between rats infused with VEH as compared to those infused with MK-801 or NMDA reflected a temporary alteration of neuronal activity by blockade or stimulation of dorsal hippocampal NMDA receptors.

MK-801 inhibits or shuts off any NMDA receptor-mediated signaling. NMDA tonically stimulates NMDA receptor-mediated mechanisms, disrupting the time and synapse specificity of NMDA receptor-mediated signaling. Thus, disruption of the same behavioral process by both MK-801 and NMDA in the dorsal hippocampus indicates that this process depends on time- and synapse-specific local NMDA receptor-mediated mechanisms, such as synaptic plasticity (Martin et al., 2000). Although MK-801 disrupts NMDA receptor-mediated signaling generally, whereas NMDA only interferes with the specificity of this signaling, it is possible that some processes, like fear conditioning to tone in the present study, are spared by dorsal hippocampal MK-801 infusion, but are disrupted by local NMDA infusion. NMDA induces strong local neuronal excitation and thereby likely interferes also with local coordinated signaling not primarily mediated by NMDA receptors. Furthermore, NMDA may induce aberrant stimulation of dorsal hippocampal efferents, thereby disrupting normal processing in projection sites of dorsal hippocampal neurons. Which of the two possibilities accounts for the effects of NMDA in the dorsal hippocampus on fear conditioning will be discussed below.

Conditioned Freezing Resulting From the Different Conditioning Procedures

Freezing levels observed during conditioning and test sessions of experiments 1–3 in the VEH rats are comparable to those obtained under very similar experimental conditions in previous studies (Bast et al., 2001d; Zhang et al., 2001). Freezing during test sessions, though marked, reached peak levels of at most 45%. Such levels are low enough to largely rule out the possibility of ceiling effects occluding treatment-induced reductions in conditioned fear. Thus, the specific reduction of freezing during context-, but not tone-test, sessions in MK-801 rats cannot result from contextual conditioning being weaker than conditioning to the tone. This is also supported by the fact that MK-801 rats showed reduced freezing in the context test of experiment 1, but not in the tone test of experiment 2, despite the VEH rats exhibiting similar peak levels (~30%) of freezing in both cases.

Interestingly, freezing during the first tone test in experiment 4 (Fig. 7) was stronger than freezing during the tone test in experiment 2 (Fig. 5), although rats received more tone shock pairings in experiment 2. The different levels of conditioned freezing were possibly due to the longer time span between conditioning and testing in experiment 4 (7 days) as compared with experiment 2 (1 day), suggesting an enhancement of fear memory over periods of several days. While it is well accepted that memory enhances, i.e., consolidates, over time (McGaugh, 2000), only a few studies have reported proceeding enhancement in the expression of memory over periods longer than 24 h (cf. Martí et al., 2001). It may therefore be of general interest for the concept of memory consolidation to further examine the possibility that consolidation of fear memory is proceeding over several days.

Unspecific Infusion Effects

Alterations in sensorimotor functions

Observations in the present study did not indicate effects of the infusions on US or CS processing, as infusion groups did not considerably differ in the unconditioned immediate shock response or the unconditioned activity response to the first tone presentation. Altered startle reactivity found after dorsal hip-

pocampal infusion of NMDA (Zhang et al., 2002) and MK-801 (Zhang et al., 2000) may indicate that these infusions affect the perception or evaluation of US and CS, since alterations in startle reactivity have been related to changes in attentional or emotional states (Koch, 1999). Given, however, that startle reactivity was decreased by NMDA, but increased by MK-801, in the dorsal hippocampus, the reduced conditioned freezing observed in the present study appears not to be linked to the infusion-induced alterations in startle reactivity.

The measure of unconditioned activity before the first tone or shock at the beginning of conditioning was not altered by MK-801, while NMDA induced short-lasting movement inhibition in some rats. In our previous studies, MK-801 in the dorsal hippocampus markedly increased activity for ~30 min (Zhang et al., 2000), while NMDA did not alter activity (Zhang et al., 2002) in rats habituated to their environment. This suggests that, during conditioning in the present study, unconditioned activity was increased in the MK-801, but not altered in the NMDA group, except for the first few minutes of the session. High levels of unconditioned activity at the beginning of the conditioning sessions probably masked the increase in activity induced by the dorsal hippocampal MK-801 infusion while favoring detection of the short-lasting movement inhibition induced by the NMDA infusion. Short-lasting movement inhibition may occur after subconvulsive stimulation of the dorsal as well as ventral hippocampus (Hallak et al., 1993; Zhang et al., 2001; present study), even though, overall, the latter induces hyperactivity (Bast et al., 2001e; Zhang et al., 2002). This may reflect an initial strong disruption of coordinated hippocampal electrical activity related to movement initiation (Leung, 2000). The short-lasting movement inhibition, or the underlying mechanisms, were not linked to the freezing deficits observed in the present study. Movement inhibition only occurred in some of the rats infused with NMDA, and inspection of the data revealed freezing deficits were not related to whether or not a rat exhibited movement inhibition. Reduced freezing has been proposed to reflect, in some cases, hyperactivity interfering with the performance of the freezing response, for example, in rats with hippocampal lesions (e.g., Richmond et al., 1999; Gewirtz et al., 2000). In view of the hyperactivity induced by MK-801 in the dorsal hippocampus (Zhang et al., 2000), a performance deficit is likely to account for reduced freezing observed in the presence of MK-801 in the dorsal hippocampus during conditioning. This also explains why, during conditioning, MK-801 rats exhibited reduced freezing also during the tone although exhibiting unimpaired conditioned fear to the tone during later testing without drug. Furthermore, this account is consistent with the finding that freezing during conditioning was not reduced by the NMDA receptor antagonist APV in the dorsal hippocampus (Young et al., 1994) at a dose not affecting activity (Kawabe et al., 1998). Rats with NMDA in the dorsal hippocampus are not hyperactive (Zhang et al., 2002) and performed a normal freezing response after about four to six shock administrations during conditioning. The latter effect was not due to fading of drug activity given that it was observed regardless of conditioning lasting ~30 min (experiments 1 and 2) or only 10 min (experiment 4). Thus, NMDA in the dorsal hippocampus does not interfere with performance of the freezing response.

State dependency

Learning can be state dependent, i.e., information learned in a particular brain state induced by systemic treatment (Overton, 1964) or specific manipulations of single brain sites, such as electrical stimulation of the dorsal hippocampus (McIntyre et al., 1985), can in some cases only be retrieved with the same brain state prevailing. If reduced conditioned fear is demonstrated during testing in rats conditioned and tested with drugs, the reduction in conditioned fear cannot merely be due to state dependency. With respect to dorsal hippocampal MK-801 infusion, which induces hyperactivity and thus is likely to disrupt the performance of freezing (see above), this demonstration is not possible using freezing as a measure of conditioned fear (see Bast et al., 2001d; Zhang et al., 2001). However, when tested without drug, rats that received MK-801 infusion before conditioning, only exhibited reduced conditioned freezing to context, but normal freezing in response to the tone. This specific reduction in fear to context argues against state dependency, unless fear conditioning to tone and context are differently state dependent (compare Gale et al., 2001). Moreover, it was reported that infusions of NMDA receptor antagonists into the amygdala (Kim and McGaugh, 1992) and even systemic injection of MK-801 (Nakagawa and Iwasaki, 1996) did not induce state dependency of inhibitory avoidance learning, another form of classical aversive conditioning. The virtual absence of conditioned fear during tone test in rats conditioned and tested with NMDA in the dorsal hippocampus (experiment 4) is a direct demonstration that the effects of NMDA in the dorsal hippocampus on fear conditioning do not merely reflect state dependency. A similar pattern of results has been obtained with muscimol infusion into the amygdala (Helmstetter and Bellgowan, 1994; Muller et al., 1997). Finally, normal conditioned fear during testing was found in rats that received behaviorally effective infusions of a protein kinase inhibitor or a dopamine antagonist before testing, but not conditioning, into the amygdala (Goosens et al., 2000; Guarraci et al., 2000); of the local anesthetic bupivacaine before conditioning, but not testing, into the nucleus accumbens (Haralambous and Westbrook, 1999); or of a dopamine agonist or antagonist before conditioning, but not testing, into the medial prefrontal cortex (Pezze et al., 2002a; 2003). Thus, even though generalization over different drugs (Castellano and McGaugh, 1990) and brain regions (Phillips and LePiane, 1981) has to be made with caution, there is no clear evidence of drug infusions into single brain sites inducing state dependency of conditioned fear, while several findings argue against this possibility.

Specific Infusion Effects on Fear Conditioning

Freezing emerging after the first shock during conditioning is widely considered to reflect, at least in part, a conditioned fear response and, thus, short-term memory of fear (e.g., Kim et al., 1992, 1993; Young et al., 1994; Fanselow, 2000). As discussed above, reduced freezing observed in MK-801 rats during conditioning probably resulted from infusion-induced hyperactivity,

and the data of the present study do not therefore allow one to decide whether MK-801 in the dorsal hippocampus impairs formation of short-term conditioned fear. The retarded development of freezing during conditioning in the NMDA rats, however, probably reflected an interference with the formation of short-term fear memory.

The reduced freezing during test sessions in rats that had received dorsal hippocampal MK-801 or NMDA infusions before conditioning indicated a genuine impairment of specific memory processes involved in the formation of long-term conditioned fear to either tone or context, or both. The formation of long-term memory comprises the initial acquisition, short-term consolidation, completed within seconds or tens of minutes, and the subsequent long-term consolidation (Nadel and Moscovitch, 1997; McGaugh, 2000). Our data do not allow one to decide which of the several stages contributing to long-term memory formation the drug infusions actually affected. The drug infusions may have interfered with initial acquisition and short-term consolidation, which is believed to occur within seconds or tens of minutes after the acquisition, and, given that the drugs may have been active in the dorsal hippocampus beyond the conditioning sessions, the early phase of the subsequent long-term consolidation. Finally, reduced freezing during the tone test in rats that received dorsal hippocampal NMDA infusion only before the tone test (experiment 4) indicates a disruption of retrieval/expression of long-term conditioned fear to tone by NMDA in the dorsal hippocampus. In experiment 4, rats that had NMDA in the dorsal hippocampus only during tone test 1 appeared to exhibit increased freezing during tone test 2, indicating reduced extinction of conditioned fear. Effects of dorsal hippocampal manipulations on extinction of conditioned fear may be the subject of future studies.

At the single doses used in the present study, MK-801 (6.25μg/0.5 μl/side) infusion into the dorsal hippocampus only impaired fear conditioning to context, while NMDA (0.7μg/0.5μl/side) infusion impaired fear conditioning to both tone and context. It is possible that other drug doses would have had other effects. For example, while the observed effects of MK-801 indicate that fear conditioning to context is more susceptible to NMDA receptor blockade in the dorsal hippocampus than fear conditioning to tone, higher doses of MK-801 may also have affected fear conditioning to tone. It is important to note, however, that the MK-801 solution used in the present study was nearly saturated and, thus, MK-801 can hardly be infused into the dorsal hippocampus at doses higher than that used in the present study. In contrast, while the effects of NMDA observed in the present study demonstrate that NMDA in the dorsal hippocampus can strongly impair fear conditioning to both tone and context, lower doses of NMDA could have preferentially interfered with fear conditioning to context. Altogether, the infusion effects on fear conditioning observed in the present study are consistent with the notion that fear conditioning to context is more susceptible to dorsal hippocampal manipulations than simple fear conditioning to tone (Anagnostaras et al., 2001), but that, nevertheless, dorsal hippocampal manipulations may also affect fear conditioning to tone (Maren et al., 1997).

Role of the Dorsal Hippocampus and Local NMDA Receptor-Mediated Processes in Fear Conditioning: Comparison With the Ventral Hippocampus

Fear conditioning to context

Several studies indicated fear conditioning to context to be more sensitive to lesions of the rat dorsal hippocampus than fear conditioning to tone (Selden et al., 1991; Kim and Fanselow, 1992; Phillips and LeDoux, 1992, 1994; Anagnostaras et al., 1999a), even though impaired fear conditioning to a tone (Maren et al., 1997) and intact contextual fear conditioning (Phillips and LeDoux, 1994; Maren et al., 1997; Richmond et al., 1999) were also found after such lesions. Moreover, infusion of the competitive NMDA receptor antagonist APV into the rat dorsal hippocampus resulted in anterograde amnesia of foreground contextual fear conditioning, while this manipulation's effects on fear conditioning to a tone have not been examined (Young et al., 1994). Based on the above data, NMDA receptor-mediated processes in the rat dorsal hippocampus have been suggested to be required for fear conditioning to context, but not tone (Young et al., 1994; Anagnostaras et al., 2001; Gale et al., 2001). This suggestion is confirmed directly by the present finding that blockade of NMDA receptors in the rat dorsal hippocampus by local infusion of the noncompetitive antagonist MK-801 impaired the formation of foreground and background contextual fear conditioning, while leaving fear conditioning to a tone intact. Similar results were obtained in a recent study after infusion of APV into the dorsal hippocampus of mice (Stiedl et al., 2000). In our previous study, MK-801 infusion into the ventral hippocampus of rats also induced such selective effects on fear conditioning to a context (Zhang et al., 2001). Thus, in the dorsal as well as ventral hippocampus, NMDA receptor signaling is only required for fear conditioning to context, but not tone. This is consistent with a specific role of NMDA receptor-mediated synaptic plasticity (Martin et al., 2000) in the ventral as well as dorsal hippocampus in contextual fear conditioning, even though NMDA receptors may contribute to several aspects of hippocampal synaptic transmission (e.g., Rosenblum et al., 1999). In addition to NMDA receptors, acetylcholine receptors in the rat dorsal hippocampus have recently been indicated to be involved in fear conditioning to context, but not tone (Gale et al., 2001; Wallenstein and Vago, 2001). Interestingly, this has been related to a possible modulation of NMDA receptor-mediated synaptic plasticity in the dorsal hippocampus by cholinergic transmission (Gale et al., 2001).

NMDA receptor activation in the dorsal and ventral hippocampus may be required solely to form a unified representation of the single elements making up a context or also for the association between context and US. Dorsal hippocampal mechanisms have been suggested to solely support the formation of a unified context representation, which afterward becomes independent of the dorsal hippocampus and is possibly stored in the neocortex. This suggestion is supported by the finding that exposure to the conditioning context several weeks before lesioning the dorsal hippocampus protected rats from anterograde and retrograde amnesia

of contextual fear conditioning (Young et al., 1994; Anagnostaras et al., 2001). Moreover, it is consistent with the dependence of spatial learning upon the dorsal hippocampus (Moser and Moser, 1998) and, in particular, local NMDA receptor-mediated processes (Morris et al., 1989; Steele and Morris, 1999; Lee and Kesner, 2002), given that spatial learning involves the formation of a unified representation of the environment (Nadel and Willner, 1980; Anagnostaras et al., 2001). The ventral hippocampus is commonly believed to be less important for spatial learning than the dorsal hippocampus (Moser and Moser, 1998), and thus it may also be less important in forming a context representation. Rather, in contextual fear conditioning, ventral hippocampal processes may complement the role of the dorsal hippocampus by supporting formation and, possibly, also further processing and expression of the context-US association. This would also be plausible based on the anatomy of the hippocampus (Amaral and Witter, 1995). Although the dorsal hippocampus is well equipped with projections from the sensory cortices, providing contextual information, only the ventral hippocampus has direct connections with extrahippocampal structures implicated in formation, processing, and expression of conditioned fear, such as amygdala (e.g., Cahill et al., 1999; Fanselow and LeDoux, 1999), nucleus accumbens (e.g., Riedel et al., 1997; Haralambus and Westbrook, 1999; Murphy et al., 2000; Pezze et al., 2001b, 2002b), and prefrontal cortex (e.g., Lacroix et al., 2000; Feenstra et al., 2001; Pezze et al., 2001a, 2002a; 2003). For example, it has been proposed that context and US are associated via synaptic plasticity in the projection from the ventral hippocampus to the basolateral amygdala (Maren and Fanselow, 1995; Anagnostaras et al., 2001). Information about the contextual representation formed with participation of dorsal hippocampal mechanisms can reach the ventral hippocampus via direct intrahippocampal connections between dorsal and ventral hippocampus or via the parahippocampal cortices, in particular the entorhinal cortex.

Fear conditioning to tone

In contrast to the specific impairments in contextual fear conditioning after blockade of dorsal hippocampal NMDA receptors, tonic stimulation of these receptors by NMDA disrupted formation of short-term and long-term fear to both tone and context, as well as the retrieval/expression of long-term fear to the tone. This disruption of basic mechanisms of fear conditioning may reflect interference with ordered neuronal processing within the dorsal hippocampus or tonic stimulation of dorsal hippocampal projections resulting in interference with neuronal processing outside the dorsal hippocampus. For example, ventral hippocampal NMDA stimulation results in complete anterograde amnesia of fear (Zhang et al., 2001), and the effects of dorsal hippocampal NMDA stimulation may reflect concomitant stimulation of the ventral hippocampus via intrahippocampal connections. This is, however, unlikely, given that dorsal hippocampal NMDA stimulation hardly affects behavioral processes other than fear conditioning (sensorimotor gating, locomotor activity) that are markedly altered by ventral hippocampal stimulation (Bast et al., 2001c,e; Zhang et al., 2002). Moreover, the nucleus accumbens core, which receives direct dorsal hippocampal projections (Groenewegen et al., 1987), has been implicated in fear conditioning. However, processing in the nucleus accumbens core may mainly contribute to contextual fear conditioning (see Pezze et al., 2001b). Finally, examination of Fos-protein production after electrical stimulation of the dorsal hippocampus suggested that subconvulsive dorsal hippocampal stimulation does not propagate out of the dorsal hippocampus (Sato et al., 1998). Thus, the complete anterograde amnesia of fear induced by NMDA in the dorsal hippocampus likely reflects disturbed neuronal processing within the dorsal hippocampus. This interpretation is in contrast to prevalent concepts, emphasizing the specific role of the dorsal hippocampus in contextual fear conditioning (Fanselow, 2000; Anagnostaras et al., 2001; Rudy and O'Reilly, 2001). Evidence supporting this interpretation was, however, provided by two experiments, in which electrolytic as well as cytotoxic lesions of the dorsal hippocampus impaired fear conditioning to a tone (Maren et al., 1997), and by preliminary results indicating deficits in fear conditioning to tone after temporary inhibition of dorsal hippocampal neurons by the $GABA_A$ agonist muscimol (Bellgowan and Helmstetter, 1995). Connections between dorsal and ventral hippocampus may link dorsal hippocampal processes to other structures contributing to formation, processing, and expression of conditioned fear to a tone, such as amygdala, nucleus accumbens, and prefrontal cortex (see above). A contribution of the ventral hippocampus to formation of conditioned fear to tone has clearly been suggested by several recent studies (Maren, 1999; Richmond et al., 1999; Bast et al., 2001b,d; Zhang et al., 2001).

Intact fear conditioning after dorsal hippocampal lesions

There is an apparent discrepancy between the suggested involvement of the dorsal hippocampus in fear conditioning to context and, possibly also tone, and intact fear conditioning found after permanent dorsal hippocampal lesions by several studies (see above). It has long been recognized that the loss of function induced by permanent lesions of a brain structure may be compensated for by other structures due to redundancy of neural structures and connections. Consequently, a behavioral function may be performed even after permanent lesion of a structure that normally serves this function (Bures and Buresova, 1990; Lomber, 1999). Functional compensation has recently been proposed to account for intact fear conditioning after permanent dorsal hippocampal lesions (Anagnostaras et al., 2001; Bast et al., 2001b). However, it is not clear why compensation after dorsal hippocampal lesions occurs in some studies, but not in others. Thus, a review of the literature does not yield a clear relation between a particular experimental parameter (time between lesion and conditioning/testing, time between conditioning and testing, conditioning and testing procedures) and the effect of dorsal hippocampal lesions on fear conditioning.

CONCLUSIONS

The present study demonstrates that NMDA receptor-mediated processes in the rat dorsal hippocampus are required for formation of fear to a context, possibly to form a context representation, but

not for fear conditioning to tone. Given that NMDA receptor-mediated processes in the dorsal hippocampus are also required for formation of spatial and episodic-like memory (Morris et al., 1989; Steele and Morris, 1999; Lee and Kesner, 2002), these processes may generally contribute to form representations of more complex relationships among stimuli, and, thus, also participate in the formation of human declarative memory (Eichenbaum, 1996). The impairments in formation and retrieval/expression of fear conditioning to tone by NMDA in the dorsal hippocampus indicated that some processes in the dorsal hippocampus, as in the ventral hippocampus (Maren, 1999; Richmond et al., 1999; Bast et al., 2001b,d; Zhang et al., 2001), may be involved in simple fear conditioning to a tone. This suggestion may be substantiated by future studies examining the effects of temporary dorsal hippocampal inactivation (e.g., by local tetrodotoxin infusion) on classical fear conditioning. Altogether, the present data are in line with the view that some hippocampal mechanisms selectively contribute to particular learning and memory functions while collectively the role of the hippocampus in learning and memory may be more general than previously assumed (see Bast et al., 2001b).

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