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Inactivation of the ventromedial prefrontal cortex reduces expression of conditioned fear and impairs subsequent recall of extinction

Demetrio Sierra-Mercado Jr, Kevin A. Corcoran, Kelimer Lebrón-Milad and Gregory J. Quirk
Department of Physiology, Ponce School of Medicine, PO Box 7004, Ponce, PR 00732-7004, Puerto Rico

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Abstract

Anxiety disorders are thought to reflect deficits in the regulation of fear expression. Evidence from rodent studies implicates the ventromedial prefrontal cortex (vmPFC) in the regulation of conditioned fear. Lesions of the vmPFC have had differing effects on the acquisition and expression of conditioned fear, as well as on recall of extinction. The use of permanent lesions, however, makes it difficult to assess the phase of training in which the vmPFC is acting and can trigger recruitment of other structures, thereby masking lesion deficits. To overcome these problems, we temporarily inactivated the vmPFC of rats with tetrodotoxin (10 ng in a 0.5-μl midline infusion) at one of four time points: prior to conditioning, prior to extinction, immediately after extinction or prior to recall of extinction. Consistent with lesion findings, inactivation of the vmPFC prior to acquisition had no effect but inactivation prior to extinction led to impaired recall of extinction the following day. In contrast to lesion findings, inactivation of the vmPFC decreased freezing at all time points, suggesting that some component of the vmPFC facilitates the expression of conditioned fear. These findings suggest that inactivation of the vmPFC can have opposite effects depending on the phase of training. The vmPFC appears to be involved both in stimulating the expression of conditioned fear and in serving as a site of extinction-related plasticity that inhibits fear during recall of extinction.

Introduction

The neural circuitry underlying the acquisition and extinction of Pavlovian fear conditioning has received a great deal of attention. Mounting evidence suggests that the ventromedial prefrontal cortex (vmPFC) plays an important role in regulating fear responses after extinction. Rats with vmPFC lesions can acquire and extinguish conditioned freezing responses normally but show increased freezing when tested after a delay (Morgan *et al.*, 1993; Morgan & LeDoux, 1995; Morrow *et al.*, 1999; Quirk *et al.*, 2000; Weible *et al.*, 2000; Lebron *et al.*, 2004). Additional findings from studies employing electrophysiological recording, protein synthesis inhibition, protein kinase inhibition and electrical stimulation support a role for the vmPFC in the suppression of fear after extinction (Herry & Garcia, 2002; Milad & Quirk, 2002; Hugues *et al.*, 2004; Milad *et al.*, 2004; Santini *et al.*, 2004).

In contrast to these findings, separate evidence suggests a role for the vmPFC in the acquisition and expression of conditioned fear. vmPFC lesions decrease conditioning-induced changes in heart rate, respiration, ultrasonic vocalizations and freezing in response to a conditioned stimulus (CS) (Frysztak & Neafsey, 1991, 1994). Infusion of dopamine receptor antagonists into the vmPFC prevents the acquisition of olfactory fear conditioning (Laviolette *et al.*, 2005). Still other studies have found no effect of vmPFC lesions on fear conditioning, extinction or extinction recall (Gewirtz *et al.*, 1997; Garcia *et al.*, 2006). Thus, the use of permanent lesions has led to conflicting results, and highlights the need to clarify the role of the vmPFC in fear conditioning and extinction.

The interpretation of vmPFC lesion findings is complicated by the fact that the vmPFC is absent during all phases of the experiment. Moreover, lesions can lead to the recruitment of other structures and compensatory mechanisms (Maren *et al.*, 1997; Rudy & O'Reilly, 2001; Anglada-Figueroa & Quirk, 2005). To overcome these problems, pharmacological inactivation has been used to test the roles of various structures in fear acquisition, expression and extinction (Helmstetter & Bellgowan, 1994; Muller *et al.*, 1997; Wilensky *et al.*, 1999; Corcoran & Maren, 2001; Corcoran *et al.*, 2005). In the present experiments, we used the sodium channel blocker tetrodotoxin (TTX) to mimic electrolytic lesions of the vmPFC at one of four time points: prior to conditioning (day 1), prior to extinction training (day 2), immediately after extinction training or prior to recall of extinction (day 3). We found that pre-extinction inactivation of the vmPFC led to impaired recall of extinction the following day, consistent with our lesion findings (Quirk *et al.*, 2000). We also found that inactivation of the vmPFC decreased freezing whenever the drug was present, suggesting an additional role for the vmPFC in facilitating the expression of conditioned fear.

Materials and methods

Subjects

The procedures were approved by the Institutional Animal Care and Use Committee of the Ponce School of Medicine in compliance with National Institutes of Health guidelines for the care and use of

Correspondence: Dr Gregory J. Quirk, as above.
E-mail: gjquirk@yahoo.com

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laboratory animals (publication no. DHHS NIH 86-23). Male Sprague-Dawley rats (270–320 g) were housed and handled as described previously (Quirk *et al.*, 2000). Food was restricted to 18 g/day of standard laboratory rat chow until rats reached 85% of their free-feeding weight. They were then trained to press a bar for food on a variable interval schedule of reinforcement (VI-60) in order to maintain a constant level of activity against which freezing could be reliably measured (Quirk *et al.*, 2000).

Surgery

After bar-press training, rats were anaesthetized with ketamine (80–90 mg/kg, i.p.)-xylazine (5–10 mg/kg, i.p.) and implanted with a single stainless steel guide cannula as described previously (Santini *et al.*, 2004). Cannulas (26 ga; Plastics One, Roanoke, VA, USA) were aimed at the midline so that infusion would reach both sides with minimal damage to overlying cortical areas. The coordinates were 2.8 mm anterior, 1.0 mm lateral and 4.1 mm ventral to bregma, and the cannulas were angled 11° toward the midline in the coronal plane. Stainless steel obturators were inserted into the guides to maintain patency until infusions were made. Rats were allowed 7 days to recover from surgery prior to behavioural testing.

Fear conditioning

All rats were submitted to three experimental phases: fear conditioning, extinction and testing. All procedures were carried out in four identical operant conditioning chambers (Coulbourn Instruments, Allentown, PA, USA) situated inside sound-attenuating cubicles (Medassociates, St Albans, VT, USA) in an isolated testing room (Quirk *et al.*, 2000). Experiments took place between 08:00 and 13:00 h. On day 1, rats received five habituation tones (30 s, 4 kHz, 75 dB) in order to reduce novelty-induced fear responses to the tone. The habituation phase was immediately followed by fear conditioning, which consisted of seven coterminating tone/footshock (0.5 s, 0.50 mA) pairings. The interval between tone presentations was variable with an average of 3 min. The habituation-conditioning session lasted for 50 min. On day 2, rats were returned to the chambers for extinction training, which consisted of 15 tones in the absence of footshock, with the same intertrial interval (session duration 60 min). On day 3, rats were placed in the same chambers and presented with two tones to test for extinction recall. Food was available on a VI-60 schedule throughout the experiment. Between sessions, shock grids were cleaned with soap and water, and walls were wiped clean. Behaviour was recorded with digital video cameras (Micro Video Products, Bobcaygeon, Ontario, Canada) and stored as video files on a computer.

Intracranial infusions of tetrodotoxin

Inactivation of the vmPFC was achieved via intracranial infusion of the sodium channel blocker TTX (10 ng/0.5 μL; Sigma, St Louis, MO, USA) dissolved in artificial cerebrospinal fluid (vehicle). The dose used was similar to that used in previous studies examining the effects of local inactivation on conditioned fear (Capriles *et al.*, 2003; McLaughlin & See, 2003; Sacchetti *et al.*, 2003). Infusions were performed at one of four time points: 30 min prior to fear conditioning, 30 min prior to extinction learning, immediately after extinction learning or 30 min prior to extinction recall. Obturators were removed from the guide cannulas and replaced with stainless steel injection cannulas (33 ga; Plastics One), which were connected by polyethylene tubing (PE-20; Small Parts Inc., Miami Lakes, FL, USA) to 10-μL Hamilton syringes mounted in an infusion pump (Harvard Apparatus, South Natick, MA, USA). Injection cannulas extended 1 mm past the end of the guide cannulas. Drugs were infused at a rate of 0.2 μL/min for 2.5 min, for a total volume of 0.5 μL. After the infusion pumps were turned off, injection cannulas remained in place for at least 1 min to allow for diffusion of the drug. The injection cannulas were then removed, obturators replaced and the rats were returned to their home cages.

Time course of behavioural effects of tetrodotoxin

To determine the duration of the effects of TTX infused into the vmPFC, a subset of the rats that had been conditioned and extinguished were reconditioned using three tone/footshock pairings. Twenty-four hours later, the rats were infused with either TTX or vehicle prior to a series of tests for fear of the tone. These tests consisted of single tone presentations given at 2, 4, 8, 12 and 24 h post-infusion.

Histology

At the conclusion of each experiment, rats were given an overdose of sodium pentobarbital (150 mg/kg) and perfused intracardially with 0.9% saline, followed by 10% buffered formalin. The brains were then removed and placed in 10% buffered formalin with 30% sucrose. Frozen sections (40 μm thick) were cut with a microtome and stained with cresyl violet, and cannula tip placements were drawn.

Data collection and analysis

Freezing behaviour was measured during all experimental sessions as the dependent measure of conditioned fear. Freezing was quantified from digitized video images using commercial software (FREEZESCAN, Clever Systems, Reston, VA, USA), which analysed digitized video images. Freezing was calculated as the percent of the 30-s tone the rat was motionless. Spontaneous recovery of freezing was calculated by dividing the average freezing in the first two recall trials (day 3) by the average of the last two conditioning trials (day 1). Suppression of bar pressing was expressed as a ratio as follows:

$$\text{Suppression ratio} = (\text{pre-tone rate} - \text{tone rate})/(\text{pre-tone rate} + \text{tone rate})$$

A ratio of 0 indicates no suppression and 1 indicates maximal suppression. Data were averaged into blocks of two trials. Because we used an odd number of trials, the last trial was excluded. Group comparisons of trial blocks were made using ANOVA or Student's *t*-tests (STATISTICA, Statsoft, Tulsa, OK, USA). All *t*-tests were two-tailed. Following significant main effects, post-hoc comparisons using Tukey's HSD test were performed.

Results

Histology

Figure 1 shows a photomicrograph of a representative cannula tract, as well as guide cannula placements for each experiment. Guide cannulas were centred on the border between the prelimbic (PL) and infralimbic (IL) subdivisions of the medial prefrontal cortex (mPFC). Injector tips extended 1 mm beyond the guide cannulas, reaching the ventral IL. A

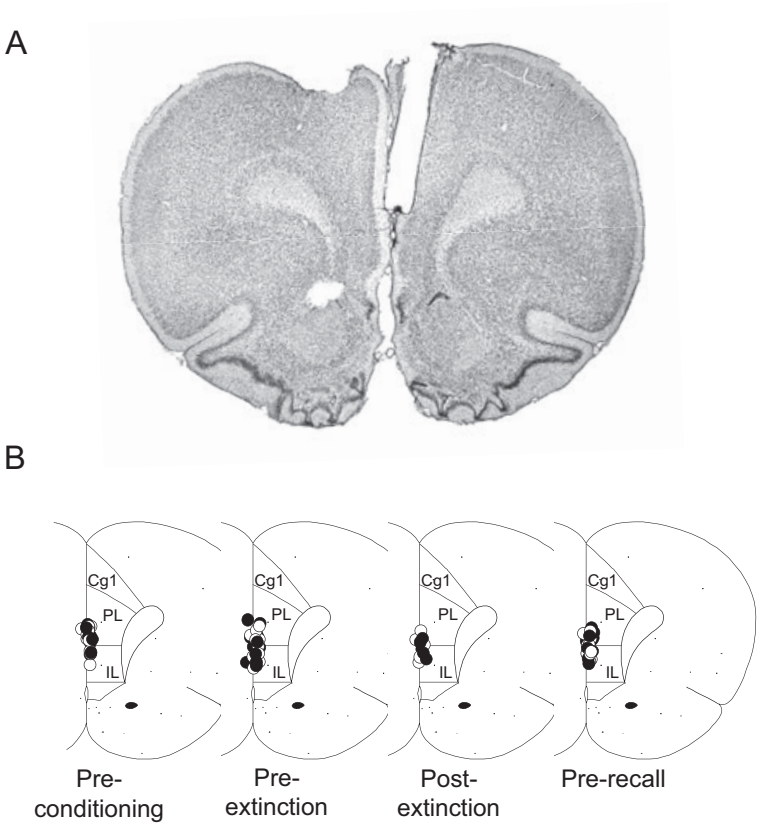

FIG. 1. Cannula placements in the ventromedial prefrontal cortex (vmPFC). (A) Photomicrograph of a nissl-stained section showing a representative midline cannula placement in the vmPFC. (B) Location of guide cannulas in the vmPFC for tetrodotoxin-infused (●) and vehicle-infused (○) groups. Injector tips extended 1 mm below guide cannulas. The time of infusion for each experiment is indicated. Drawings adapted from Paxinos & Watson (1998). Cg1, cingulate cortex; IL, infralimbic; PL, prelimbic.

total of 76 rats were used, which were infused with either TTX or vehicle in four experiments: (i) pre-conditioning infusion (TTX, $n = 9$; vehicle, $n = 10$); (ii) pre-extinction infusion (TTX, $n = 11$; vehicle, $n = 11$); (iii) post-extinction infusion (TTX, $n = 5$; vehicle, $n = 5$) and (iv) pre-recall infusion (TTX, $n = 13$; vehicle, $n = 12$).

Inactivation of the ventromedial prefrontal cortex prior to conditioning does not impair the acquisition of fear conditioning or its subsequent extinction

To determine if the vmPFC serves a critical role in fear acquisition, rats were infused with TTX or vehicle 30 min prior to conditioning (Fig. 2A). TTX-infused rats exhibited somewhat less freezing than controls during conditioning. In the last block of conditioning trials, the freezing levels were 21% and 37% in TTX and vehicle rats, respectively; however, this difference did not reach significance ($t_{17} = 1.48$; $P = 0.16$). The next day, the groups exhibited equally robust freezing (TTX, 63%; ACSF, 66%; $t_{17} = 0.21$; $P = 0.83$) which extinguished fully by the end of the session. There was no difference between TTX and vehicle groups in their ability to recall extinction on day 3 (TTX, 27%; vehicle, 19%; $t_{17} = 0.59$; $P = 0.56$).

Data from suppression of bar pressing mirrored the freezing data (Fig. 2B). TTX-infused rats exhibited somewhat less suppression at the end of conditioning (TTX, 0.47; vehicle, 0.86; $t_{17} = 1.68$; $P = 0.11$). No differences between groups were observed on day 2 at the start of extinction (TTX, 0.81; vehicle, 0.86; $t_{17} = 0.73$; $P = 0.35$) or during extinction recall on day 3 (TTX, 0.58; vehicle, 0.58; $t_{17} = 0.13$; $P = 0.99$). Therefore, vmPFC activity during the conditioning phase is not required for acquisition, retention or extinction of auditory fear conditioning.

Inactivation of the vmPFC also had no effect on the rat's motivation to press for food. Rates of spontaneous bar pressing did not differ significantly between TTX- and vehicle-infused groups (TTX, 22/min; vehicle, 24/min; $t_{17} = 0.56$; $P = 0.58$; Fig. 2C). Therefore, any effects of inactivation on freezing cannot be attributed to altered motivation to press for food.

Inactivation of the ventromedial prefrontal cortex prior to extinction disrupts subsequent recall of extinction

To determine if vmPFC activity is necessary for extinction, rats were fear conditioned on day 1 and infused with TTX or vehicle 30 min prior to extinction training on day 2. Inactivation of the vmPFC decreased freezing at the start of the extinction session (Fig. 3A). Two-way ANOVA of freezing data during the extinction session revealed a significant interaction of infusion and trial block ($F_{6,120} = 4.09$; $P < 0.001$) but no main effect of infusion ($F_{1,20} = 0.24$; $P = 0.63$). The interaction effect was driven by a trend toward decreased freezing in TTX-infused rats during the first block of extinction trials ($t_{20} = 1.90$; $P = 0.07$). The day after extinction, however, TTX-infused rats froze significantly more than vehicle controls (53% and 20%, respectively; $t_{20} = 2.28$; $P = 0.034$). These freezing values corresponded to spontaneous recovery values of 67% and 26% for the TTX and vehicle groups, respectively ($t_{20} = 2.37$; $P = 0.028$). Thus, inactivation of the vmPFC prior to extinction impaired the subsequent recall of extinction.

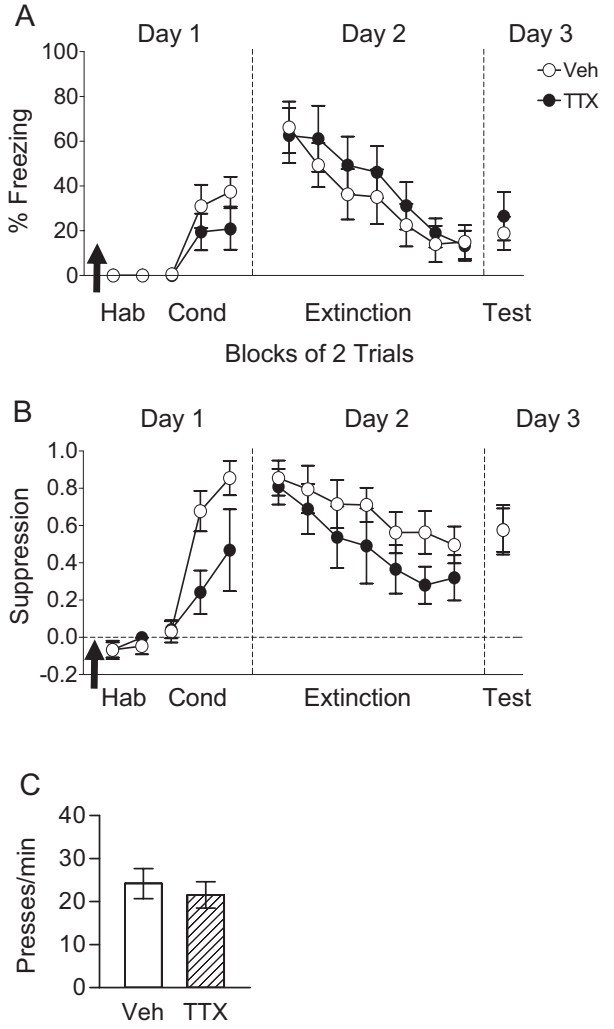

FIG. 2. Ventromedial prefrontal cortex (vmPFC) inactivation prior to fear conditioning does not impair fear acquisition or extinction. (A) Infusion of the sodium channel blocker tetrodotoxin (TTX) or vehicle (Veh) 30 min prior to conditioning had no effect on acquisition of conditioned freezing. (B) Acquisition of conditioned suppression was also unaffected. (C) Rates of spontaneous bar pressing in TTX and vehicle-infused rats. vmPFC inactivation did not alter the motivation to press for food. Cond, conditioning; Hab, habituation.

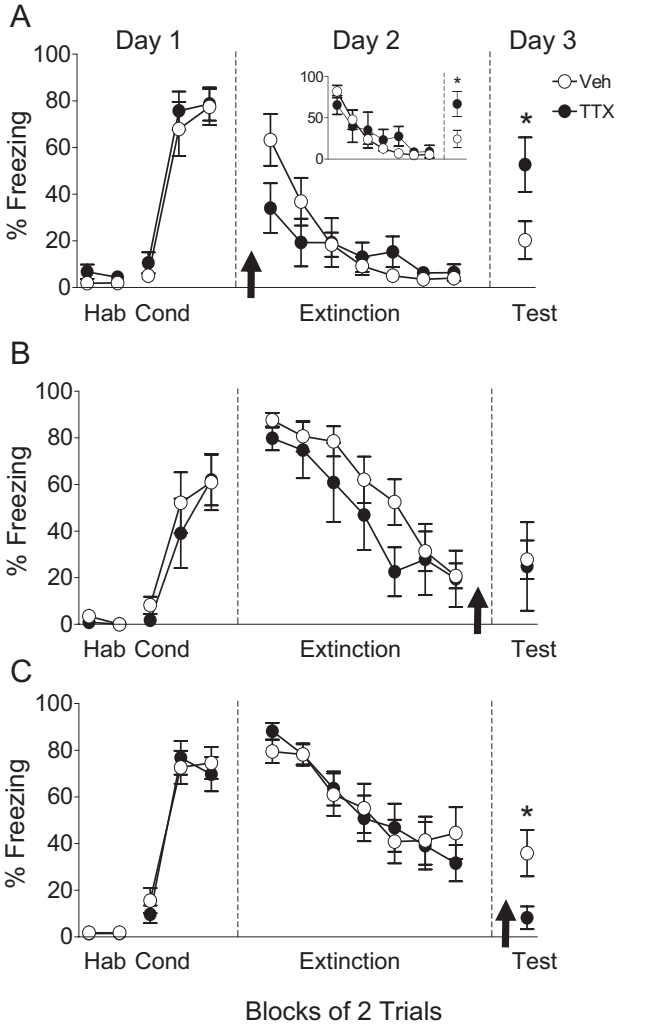

FIG. 3. The effects of ventromedial prefrontal cortex (vmPFC) inactivation on extinction of conditioned freezing. (A) vmPFC inactivation prior to extinction on day 2 did not prevent extinction learning but led to impaired recall of extinction on day 3 (*$P = 0.033$). (A inset) A subset of rats from A were chosen to match for freezing at the start of extinction. Despite matched freezing levels, tetrodotoxin (TTX) rats still froze more than vehicle (Veh)-infused rats at test (*$P = 0.037$). (B) vmPFC inactivation immediately after extinction had no effect on freezing at test. (C) Inactivation of the vmPFC prior to test on day 3 reduced freezing (*$P = 0.017$), indicating that vmPFC activity is necessary for expression of fear. Cond, conditioning; Hab, habituation.

Data for suppression of bar pressing for pre-extinction infusions were similar to freezing (see Fig. 4 for suppression data). Two-way ANOVA revealed a significant interaction of infusion and trial block ($F_{1,20} = 4.18$; $P < 0.001$) but no main effect of infusion ($F_{1,20} = 2.97$; $P = 0.10$). The interaction effect was driven by the decreased suppression in TTX-infused rats during the first block of extinction trials (TTX, 0.41; vehicle, 0.93; $t_{20} = 3.34$; $P = 0.003$). On day 3, the TTX group showed somewhat higher levels of suppression than the vehicle group (0.61 and 0.43, respectively) but, unlike the group differences in freezing, this difference was not statistically significant ($t_{20} = 0.94$; $P = 0.36$). The TTX group did, however, show significantly less bar pressing than the vehicle group prior to the first test tone (8.8/min and 19.8/min, respectively; $t_{20} = 3.38$, $P = 0.003$), which had the effect of reducing tone-induced suppression. Reduced pressing is consistent with higher fear in TTX-infused rats, in agreement with the freezing data.

One possible explanation for the impaired recall of extinction in TTX-infused rats is that they were impaired in their initial expression of fear. It has previously been demonstrated that reduced responding during extinction leads to diminished recall of extinction the following day (Rescorla, 2003). To address this issue, we matched for freezing levels post-hoc by selecting subsets of rats with freezing levels greater than 50% during the first tone presentation of the extinction session (TTX, $n = 5$; vehicle, $n = 8$; Fig. 3A inset). After matching for freezing on day 2, TTX-infused rats still showed significantly higher freezing on day 3 ($t_{12} = 2.34$; $P = 0.037$). Thus, impaired recall of extinction cannot be attributed to decreased expression of freezing during the extinction session.

The physiological effects of TTX are thought to last for several hours (Kilpatrick & Cahill, 2003). Therefore, our results suggest that recall of extinction requires activity in the vmPFC either during or shortly after extinction training. To dissociate these possibilities, we ran a separate experiment in which rats were infused with TTX or

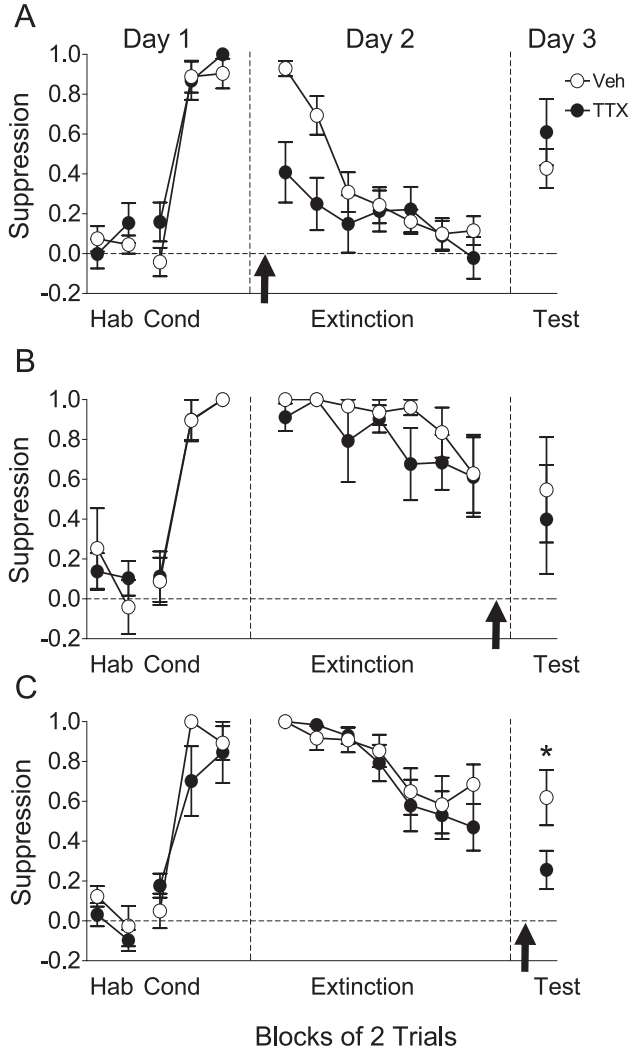

FIG. 4. The effects of ventromedial prefrontal cortex (vmPFC) inactivation on extinction of conditioned suppression. (A) vmPFC inactivation prior to extinction on day 2 reduced suppression at the start of extinction but did not impede extinction. On day 3, there was no significant difference between groups. (B) vmPFC inactivation immediately after extinction had no effect on suppression at test. (C) Inactivation of the vmPFC prior to test on day 3 reduced suppression (*$P = 0.04$). TTX, tetrodotoxin. Cond, conditioning; Hab, habituation.

vehicle immediately after extinction training (Fig. 3B). In contrast to pre-extinction infusions, post-extinction infusions had no effect on recall of extinction according to freezing (TTX, 25%; vehicle, 28%; $t_8 = 0.13$; $P = 0.90$) and suppression (TTX, 0.40; vehicle, 0.55; $t_8 = 0.39$; $P = 0.71$). This suggests that vmPFC activity during the extinction session is sufficient to support recall of extinction the following day.

Inactivation of the ventromedial prefrontal cortex prior to the recall test reduces fear expression

To determine the role of the vmPFC in extinction recall, rats were conditioned and extinguished drug-free, and infused with TTX or vehicle 30 min prior to the recall test. As illustrated in Fig. 3C, inactivation of the vmPFC significantly reduced freezing to the test tones (TTX, 8%; vehicle, 36%; $t_{23} = 2.57$; $P = 0.017$) and suppression (TTX, 0.19; vehicle, 0.62; $t_{23} = 2.17$; $P = 0.04$). Thus, vmPFC activity supports the expression of conditioned fear during the recall test.

Figure 5 summarizes, for all experiments, the effects of vmPFC inactivation on subsequent extinction recall. Inactivation prior to conditioning had no effect, inactivation prior to extinction increased freezing and inactivation prior to test decreased freezing. This suggests that the vmPFC is capable of both increasing and decreasing the expression of conditioned fear.

The behavioural effects of tetrodotoxin in the ventromedial prefrontal cortex last for at least 4 h

The behavioural effects of TTX infusions reportedly last for 4 h or more, according to studies in the midbrain (Rothfeld *et al.*, 1986), amygdala (Kilpatrick & Cahill, 2003) and hippocampus (Klement *et al.*, 2005). To verify this for the vmPFC, a subset of rats from the above experiments was reconditioned to the tone CS. The following day, rats were infused with TTX or vehicle ($n = 20$ rats/group) and given single test tones from 2 to 24 h post-infusion (Fig. 6). ANOVA revealed significant main effects of infusion ($F_{1,38} = 5.50$; $P = 0.024$) and trial ($F_{4,152} = 4.37$; $P = 0.002$), and a significant interaction ($F_{4,152} = 7.87$; $P < 0.0001$). Post-hoc tests indicated that TTX-infused rats showed significant decreases in fear expression at 2 and 4 h ($P < 0.001$) but not at 8, 12 or 24 h ($P > 0.30$). Thus, similar to other structures, the effects of TTX in the vmPFC last at least 4 h.

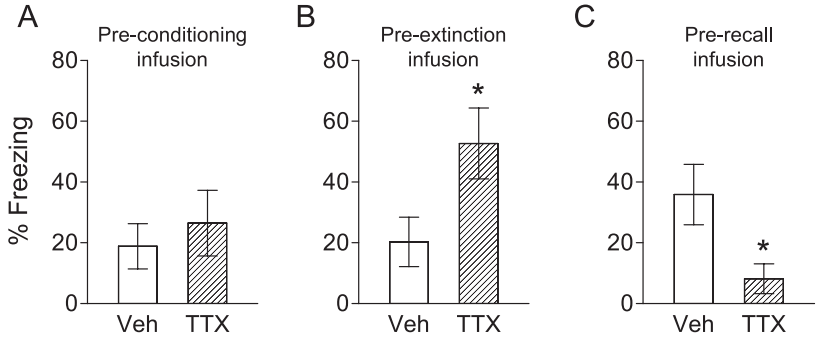

FIG. 5. Summary of the effects of ventromedial prefrontal cortex (vmPFC) inactivation on extinction recall. Pre-conditioning inactivation had no effect (A), pre-extinction inactivation increased freezing (B) and pre-test inactivation decreased freezing (C). Thus, vmPFC inactivation can enhance or impair recall of conditioned fear, depending on when inactivation occurs. TTX, tetrodotoxin; Veh, vehicle. *$P < 0.05$.

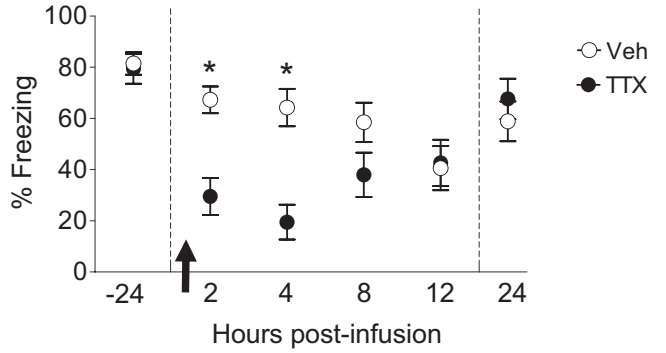

FIG. 6. The duration of tetrodotoxin (TTX) effects in the ventromedial prefrontal cortex. Rats were reconditioned and infused with TTX to assess the time-course of the effects of TTX on freezing. After infusion, all rats were given single extinction tones at 2, 4, 8, 12 and 24 h. Freezing was significantly reduced at 2 and 4 h (*$P < 0.001$) but not at 8, 12 or 24 h. Veh, vehicle.

Discussion

Previous studies using permanent lesions of the vmPFC have led to conflicting results regarding the role of the vmPFC in conditioned fear. We used temporary inactivation to probe the role of the vmPFC in the acquisition and expression of conditioned fear and its subsequent extinction. Our main findings were: (i) inactivation of the vmPFC did not affect the acquisition of conditioned fear; (ii) inactivation of the vmPFC reduced the expression of conditioned fear and (iii) inactivation of the vmPFC prior to extinction impaired extinction recall the following day. Thus, processes in the vmPFC are important for both the expression of fear and the recall of extinction.

Inactivating the vmPFC prior to conditioning did not prevent acquisition of conditioned fear. This agrees with previous studies in which pre-training lesions of the vmPFC (Morgan *et al.*, 1993; Morrow *et al.*, 1999; Quirk *et al.*, 2000; Lebron *et al.*, 2004) or other cortical areas (LeDoux *et al.*, 1989; Romanski & LeDoux, 1992; Campeau & Davis, 1995) had no effect on acquisition of classical fear conditioning. Negative lesion data such as these support the idea that the critical site of plasticity in auditory fear conditioning lies outside the vmPFC, in the lateral and basolateral amygdala (LeDoux, 2000; Davis, 2000; Maren, 2001; Anglada-Figueroa & Quirk, 2005). However, acquisition of more complex forms of fear conditioning may require the vmPFC. Manipulations of the vmPFC impair discriminative (CS+, CS–) fear conditioning (Frysztak & Neafsey, 1991, 1994) and trace fear conditioning (Runyan & Dash, 2004), in which a temporal gap is inserted between the CS and unconditioned stimulus. Thus, the vmPFC may be necessary for learning tasks with increased cognitive demands, such as those involving complex discriminations and working memory.

Although vmPFC inactivation did not block fear acquisition, it reduced fear expression during both extinction training and the recall test. This disagrees with our earlier hypothesis that the output of the vmPFC is purely inhibitory (Quirk *et al.*, 2000). This also disagrees with previous studies that found no effect of pre-conditioning lesions (Morrow *et al.*, 1999; Quirk *et al.*, 2000; Weible *et al.*, 2000; Lebron *et al.*, 2004) or post-conditioning lesions of the vmPFC (Gewirtz *et al.*, 1997; Morgan *et al.*, 2003; Garcia *et al.*, 2006) on expression of Pavlovian fear conditioning. Permanent lesions allow for recovery of function, however, which can mask lesion deficits (Anglada-Figueroa & Quirk, 2005). For this reason, prior lesion studies may have underestimated the role of the vmPFC in expression of conditioned fear. Consistent with our findings, it was recently shown that pharmacological inactivation of the vmPFC reduced expression of conditioned freezing to a tone CS (Blum *et al.*, 2006) and reduced anxiety (Shah *et al.*, 2004). Given that pre-training inactivation of the vmPFC did not prevent acquisition (present study), it is likely that the vmPFC is an essential site of expression of conditioned fear, rather than plasticity.

Importantly, inactivation of the vmPFC prior to extinction training led to poor extinction recall the following day, suggesting that TTX blocked extinction-related plasticity in the vmPFC. This result confirms and extends lesion studies demonstrating a role for the vmPFC in extinction recall (Morgan *et al.*, 1993; Morgan & LeDoux, 1995; Quirk *et al.*, 2000; Lebron *et al.*, 2004). Recent studies using other techniques support the idea that the vmPFC is a site of extinction-related plasticity. Intra-vmPFC infusion of an *N*-methyl-D-aspartate receptor antagonist (Burgos-Robles *et al.*, 2004), MAPK inhibitor (Hugues *et al.*, 2004, 2006) or protein synthesis inhibitor (Santini *et al.*, 2004) prior to extinction did not impair extinction learning but led to poor recall of extinction the following day. Moreover, tone-evoked responses of IL mPFC neurones are increased during recall of extinction (Milad & Quirk, 2002). Interestingly, post-extinction inactivation of the vmPFC did not impair subsequent recall of extinction, suggesting that post-training spiking in mPFC may not be necessary for consolidation of extinction. If true, this would differ from structures such as the hippocampus, in which post-training infusion of TTX prevents consolidation of inhibitory avoidance (Ambrogi Lorenzini *et al.*, 1997; Quiroz *et al.*, 2003). Another possibility, however, is that consolidation of extinction in the vmPFC resumed after TTX wore off, consistent with delayed consolidation of extinction (Santini *et al.*, 2001).

A recent study has challenged the idea that the mPFC is necessary for recall of fear extinction. Garcia *et al.* (2006) found no effect of pre-training vmPFC lesions on extinction learning or recall of extinction. The discrepancy with our current findings could be due to recovery of function in lesioned animals. However, given that we previously observed effects of electrolytic lesions in this task (Quirk *et al.*, 2000; Lebron *et al.*, 2004), it is likely that some procedural difference may be responsible for the discrepancy between laboratories. For example, Garcia *et al.* (2006) used 45 extinction trials compared with 15 trials in the present study, which may have resulted in overtraining and recruitment of other structures. Another possibility is that the vmPFC mediates behavioural competition between freezing and bar pressing for food, which was not used in the study of Garcia *et al.* (2006). Yet another possibility is the differences in the contextual designs of the two experiments. Garcia *et al.* (2006) extinguished rats in a unique context (ABB) and we extinguished rats in the conditioning context (AAA). Additional experiments are underway to determine the potential role of each of these variables in modulating vmPFC involvement in extinction of conditioned fear.

It is possible that the vmPFC contains separate modules for reducing and augmenting the expression of conditioned fear. This concept, although speculative, is supported by neuroanatomical and physiological studies of prefrontal–amygdala connections. For example, the IL projects to intercalated cell masses and the capsular division of the amygdala (McDonald *et al.*, 1996; Vertes, 2004), which inhibits central nucleus output neurones (Royer *et al.*, 1999). The PL, on the other hand, projects mostly to the basal nucleus of the amygdala (McDonald *et al.*, 1996; Vertes, 2004), which is critical for the expression of conditioned fear (Anglada-Figueroa & Quirk, 2005). Further experiments are needed to determine whether the PL and IL have opposite effects on fear expression.

The pattern of effects that we observed with vmPFC inactivation resembles the effects of hippocampal inactivation on extinction of auditory fear conditioning. Inactivating the hippocampus prior to

extinction learning causes increased freezing the following day (Corcoran *et al.*, 2005) but inactivating prior to extinction recall decreases freezing (Corcoran & Maren, 2001, 2004). It should be noted, however, that these experiments did not use the AAA design used in the present study. Nonetheless, the hippocampus sends dense projections to the vmPFC (Swanson, 1981; Tierney *et al.*, 2004), which are potentiated by extinction training (Hugues *et al.*, 2006), suggesting that these structures interact to mediate extinction recall (Hobin *et al.*, 2003; Maren & Quirk, 2004). We suggest that activity in the IL and prelimbic may be modulated by the hippocampus in order to gate the expression of fear under a variety of circumstances.

In summary, our findings suggest that the vmPFC is not a critical site of plasticity for acquisition of fear conditioning but is a critical site of fear expression. Furthermore, our findings support prior evidence from lesion and infusion studies indicating that the vmPFC is a site of extinction-related plasticity necessary for recall of extinction after a delay.

Note added in proof

A recently published study showed that inactivation of vmPFC with a low-dose of the $GABA_A$ agonist, muscimol, resulted in reduced freezing during extinction training (Akirav *et al.*, 2006), similar to present findings.

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Abbreviations

CS, conditioned stimulus; IL, infralimbic; TTX, tetrodotoxin; vmPFC, ventromedial prefrontal cortex; VI-60, variable interval schedule of reinforcement.

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