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Acute Systemic Fibroblast Growth Factor-2 Enhances Long-Term Extinction of Fear and Reduces Reinstatement in Rats

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Despite having made substantial advances in the treatment of anxiety disorders over the past few decades it appears that we have now reached a 'therapeutic impasse'. Further clinical progress requires a greater understanding of the neural mechanisms underlying fear inhibition. In this study, we examined, for the first time, the effects of fibroblast growth factor-2 (FGF2), a mitogen involved in the molecular cascade of memory, on extinction and relapse in rats. In all experiments, rats were first trained to fear a white noise-conditioned stimulus, and then had this learned fear extinguished the following day. Extinction is the process underlying exposure-based therapy in humans. Experiments 1 and 2 demonstrated that FGF2 facilitated the loss of learned fear (ie, extinction) when given either prior to or immediately after extinction but not when given 4 h after extinction. This suggests that FGF2 must be present during the consolidation of the extinction memory to have an effect. Experiment 3 further supported this interpretation by showing that short-term extinction must occur for FGF2 to facilitate long-term extinction, suggesting that FGF2 is facilitating the translation of memory from short-term to long-term storage. In experiment 4 rats given FGF2 immediately after extinction exhibited less shock-induced reinstatement, which is a model preparation of relapse, than did vehicle-treated rats. Together, these experiments demonstrate that FGF2 facilitates extinction and attenuates relapse. Thus, FGF2 may be a novel pharmacological adjunct to exposure therapy.
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INTRODUCTION

Exposure therapy is considered among the most successful treatments for anxiety disorders, and involves repeatedly presenting the feared stimulus or outcome in the absence of any danger. However, the limitations of exposure therapy include a high dropout rate and a failure by many to maintain treatment gains in the long term (Choy *et al*, 2007). This has prompted the comment that a 'therapeutic impasse' has been reached, and an understanding of the neural mechanisms underlying fear inhibition is necessary for further clinical progress to be made (McNally, 2007). In recent years it has been demonstrated that d-cycloserine (DCS), a partial *N*-methyl-D-aspartate (NMDA) receptor agonist, can enhance exposure therapy. This has been demonstrated pre-clinically in rats using the animal model of exposure therapy (extinction; Walker *et al*, 2002; Ledgerwood *et al*, 2003), and in human clinical trials with a range of anxiety disorders (Ressler *et al*, 2004; Hofmann *et al*, 2006; Kushner *et al*, 2007; Guastella *et al*, 2008).

It has been proposed that DCS enhances extinction by facilitating the consolidation of the extinction memory. This is supported by the fact that DCS activates the NMDA receptor complex, which is critical for long-term memory formation, and that DCS has no intrinsic anxiolytic properties. It is further supported by the finding that short-term extinction must occur for DCS to enhance long-term extinction, suggesting that DCS is enhancing the conversion of the short-term extinction memory into long-term memory (Weber *et al*, 2007).

The use of DCS as an agent that enhances exposure therapy has been claimed to represent a 'new paradigm' in the treatment of anxiety disorders (Davis *et al*, 2006). Rather than identifying drugs that reduce the experience of anxiety (eg, the benzodiazepines), research has now turned to the investigation of other drugs that may enhance the effects of exposure therapy. If it is the case that DCS enhances exposure therapy by improving the consolidation of memory, then theoretically any drug that facilitates long-term memory may provide a useful adjunct to exposure therapy.

We have recently demonstrated that Fibroblast Growth Factor-2 (FGF2) facilitates long-term memory for contextual

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conditioning in developing rats (Graham and Richardson, 2009). FGF2 is a potent mitogen that regulates brain development, adulthood neurogenesis, and regenerative plasticity following brain damage. We hypothesized that an additional function of FGF2 is to regulate the neural plasticity underlying long-term memory formation, on the basis that FGF2 modulates (and is modulated by) several key molecules involved in the molecular storage of memory (Abe *et al*, 2001; Moffett *et al*, 1998; Shitaka *et al*, 1996). For a more comprehensive review of the relation between FGF2 and the molecular processes involved in the storage of memory see Graham and Richardson (2009). Given our recent demonstration that FGF2 facilitates long-term memory of contextual fear, the aim of this study was to determine whether FGF2 also enhances long-term memory for the extinction of learned fear. In experiments 1 and 2, we administered FGF2 prior to extinction, immediately after extinction, or following a delay to determine the effects of FGF2 on extinction when it is administered at different time points. In experiment 3, we examined whether FGF2 facilitates the consolidation of the extinction memory by examining the effects of FGF2 (administered immediately after extinction) on long-term extinction when no short-term extinction occurs. In this experiment half the rats received fewer extinction-training trials than normal, which prevented the expression of short-term extinction. If FGF2 facilitates the consolidation of the extinction memory, then FGF2 should only lead to enhanced long-term extinction in rats that exhibit short-term extinction. Finally, in experiment 4 we examined the effects of FGF2 (administered immediately after extinction) on reinstatement. In studies of reinstatement in fear conditioning preparations, the typical procedure is to present the unconditioned stimulus (US; eg, shock) by itself after extinction and then test for responding to the extinguished conditioned stimulus (CS; eg, Rescorla and Heth, 1975; Westbrook *et al*, 2002). Animals given an unsignalled shock exhibit a return of fear responding to the CS at the test (usually the following day). In humans, stress is a common precipitant to relapse, and as such, reinstatement is a useful animal model of stress-precipitated relapse.

MATERIALS AND METHODS

Subjects

Experimentally naive Sprague–Dawley-derived rats, bred and housed in the School of Psychology, The University of New South Wales, were used. Rats were 23 ($\pm$ 1) days old at the start of all experiments. Rats this age exhibit adult-like extinction behavior (eg, Kim and Richardson, 2008). All rats were male, and no more than one rat per litter was used per group. Rats were housed with their littermates and mother in plastic boxes (24.5 cm long $\times$ 37 cm wide $\times$ 27 cm high) covered by a wire lid. Animals were maintained on a 12 h light–dark cycle (lights on at 0600 hours) with food and water available *ad libitum*. Animals were treated according to the principles of animal use outlined in *The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (Seventh Edition), and all procedures were approved by the Animal Care and Ethics Committee at The University of New South Wales.

Drug

Rats were injected subcutaneously (s.c.) in the dorsal neck region with either vehicle or 20 ng/gm of body weight FGF2. FGF2 (Fibroblast Growth Factor-2; R&D Systems) was reconstituted at a concentration of 10 μg/ml in phosphate-buffered serum (PBS) containing 0.1% bovine serum albumin (BSA). This dose was chosen on the basis that we have previously shown that it facilitates long-term memory of contextual fear (Graham and Richardson, 2009), and because it has previously been shown to facilitate neurogenesis in rats (Cheng *et al*, 2002). This solution, or the vehicle (PBS containing 0.1% BSA), was administered in a volume of 0.002 ml/gm of body weight. Rats were administered FGF2 or vehicle 8 min prior to extinction in experiment 1, immediately after or 4 h after extinction in experiment 2, and immediately after extinction in experiments 3 and 4.

Apparatus

The chamber used for training in all experiments was rectangular (13.5 cm long $\times$ 9 cm wide $\times$ 9 cm high), with the front wall, rear wall, and ceiling constructed of clear Plexiglas. The floor and side walls consisted of 3 mm stainless steel rods set 1 cm apart. The chamber was housed within a wooden cabinet so that external noise and visual stimulation were minimized. An infrared light was the sole source of illumination in the conditioning chamber.

The chamber used for extinction and test in all experiments, and reinstatement in experiment 4, was rectangular (30 cm long $\times$ 30 cm wide $\times$ 23 cm high) and constructed of Plexiglas. The walls were transparent, except for two side walls that consisted of vertical black and white stripes (5 cm each). The floor consisted of a sheet of Plexiglas except during reinstatement in experiment 4, when a grid floor that consisted of 3-mm stainless steel rods set 1 cm apart was placed in the chamber. The chamber was housed in a wooden cabinet, so that external noise and visual stimulation were minimized. A white LED and an infrared light were the sole sources of illumination in this chamber.

The CS was a white-noise; noise level in the chambers was increased by 8 dB when the CS was presented. The US used during training was a 0.6 mA, 1.0 s footshock. A computer controlled all presentations of the CS and the US.

Procedure

Training. Training was identical in all experiments. On day 1, rats received three pairings of the white-noise CS and the footshock US. Rats were placed in the conditioning chamber, and after a 2 min adaptation period the CS was presented for 10 s and co-terminated with the shock US. The inter-trial interval (ITI) ranged from 85 to 135 s with a mean of 110 s. Thirty to sixty seconds after the last pairing rats were returned to their home cages.

Extinction. On day 2 in experiments 1 and 2 all rats received 15, non-reinforced presentations of the CS. After a 2 min adaptation period, the 10 s CS was presented with a 10 s ITI. Thirty to sixty seconds after the last trial, rats were

Table 1 Mean (±SEM) Pre-CS Freezing Prior to Extinction and Test for All Experiments

Experiment	**Group**			
Experiment 1	Vehicle	FGF2		
Extinction	4.7 (1.6)	7.3 (6.8)		
Test	5.5 (3.3)	2.5 (1.3)		
Experiment 2	Vehicle	FGF2-Imm	FGF2–4 h	
Extinction	16.1 (6.5)	13.2 (5.4)	6.4 (2.7)	
Test	3.1 (2.1)	5 (2.9)	10.6 (4.6)	
Experiment 3	Vehicle-short	FGF2-short	Vehicle-Long	FGF2-Long
Extinction	6.9 (4.6)	13.1 (7.6)	9.6 (4.3)	20.1 (10.1)
Test	0 (0)	15 (10.2)	1.4 (1.4)	8.6 (8.6)
Experiment 4	Vehicle-no reinstate	FGF2-no reinstate	Vehicle-Reinstate	FGF2-Reinstate
Extinction	7 (4.2)	8.6 (5)	7.9 (4.1)	18.9 (7.5)
Test	3.3 (1.9)	4.5 (2.5)	26.5 (8.2)[a]	31.9 (8)[a]

[a]Indicates a significant difference from other experimental groups.

returned to their home cages. Experiment 3 examined the effect of FGF2 on long-term extinction when short-term extinction is prevented. Therefore, during extinction on day 2, half of the rats received five non-reinforced CS presentations (10 s CS with a 10 s ITI) whereas the other half received 15 non-reinforced CS presentations. Half of the rats in each of these conditions were given vehicle immediately after extinction whereas the other half were given FGF2. In experiment 4 the levels of freezing at test between the non-reinstated vehicle and FGF2 rats had to be equated to allow for meaningful conclusions about the effect of FGF2 on reinstatement. Therefore, during extinction on day 2, the vehicle rats received 30 non-reinforced CS presentations (10 s CS with a 10 s ITI), whereas the FGF2 rats received 15 non-reinforced CS presentations.

Reinstatement. In experiment 4 only, on day 3 half of the rats received reinstatement. After a 2 min adaptation period, a single shock US (0.4 mA, 1 s) was administered. Thirty to sixty seconds after the shock, rats were returned to their home cages. Rats in the no reinstatement groups were given an equivalent amount of exposure to the reinstatement chamber, but not given any presentations of the US.

Test. On day 3 in experiments 1, 2, and 3, and on day 4 in experiment 4, rats were tested for their level of freezing in the presence of the CS. Their pre-CS level of freezing was recorded for 1 min and the CS was then presented for 2 min.

Scoring and Statistics. Each animal was scored for freezing during extinction training and test. Freezing was scored by a time sampling procedure whereby each rat was scored every 3 s as freezing or not freezing. Freezing was defined as the absence of all movement other than those required for respiration (Fanselow, 1980). A percentage score was calculated for each animal to indicate the proportion of total observations scored as freezing. Extinction data were collapsed across trials to produce blocks of extinction (each block consisting of three trials), with the exception of the short-extinction condition in experiment 3, in which only five trials were given. The scorer was unaware of the condition of the rats.

RESULTS

Experiment 1: Pre-Extinction Administration of FGF2

Table 1 presents the pre-CS freezing prior to extinction and prior to test for all experiments. A *t*-test revealed that there were no significant differences between groups in levels of pre-CS freezing prior to extinction ($t_{(15)} = 0.36$) or prior to test ($t_{(15)} = 0.86$). A group (FGF2 or vehicle) by extinction block mixed-design analysis of variance (ANOVA) of the extinction data (Figure 1a) yielded a significant effect of block ($F = 32.6_{(4,\ 60)}$, $p < 0.0001$), due to the level of freezing by both the vehicle- and the FGF2-treated rats decreasing across blocks. Furthermore, there was a significant between-groups effect ($F = 5.8_{(1,\ 15)}$, $p < 0.029$), because the rats administered FGF2 were freezing significantly less than the rats administered vehicle. There was no significant block-by-group interaction ($F < 1$), meaning that both groups exhibited similar rates of extinction. Freezing levels to the CS at test are shown in Figure 1b. A *t*-test revealed that FGF2 rats exhibited significantly lower levels of CS-elicited freezing at test in comparison to vehicle rats ($t_{(15)} = 2.77$, $p = 0.014$). Together, these results demonstrate that pre-extinction administration of FGF2 leads to reduced freezing during extinction training, and further, that pre-extinction administration of FGF2 facilitates long-term extinction.

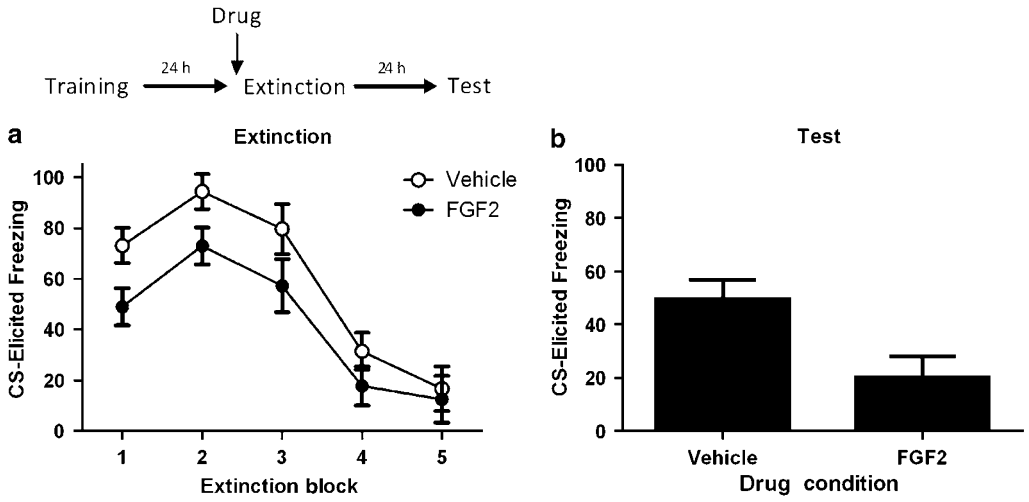

Figure 1 (a) Mean (±SEM) freezing by rats in response to the CS during extinction training in experiment 1. (b) Mean (±SEM) CS-elicited freezing by rats during test in experiment 1. Rats had been injected with vehicle ($n=9$) or FGF2 ($n=8$) prior to extinction training.

Experiment 2: Post-Extinction Administration of FGF2

A one-way ANOVA revealed no significant differences between groups in levels of pre-CS freezing prior to extinction ($F=1.01$) or prior to test ($F=1.37$). A group (vehicle, FGF2, or FGF2–4 h) by extinction block mixed-design ANOVA of the extinction data (Figure 2a) yielded a significant effect of block ($F=28.97_{(4, 80)}$, $p<0.0001$), due to the level of freezing by rats in all groups decreasing across blocks. Furthermore, there was no significant effect of group and no significant block-by-group interaction ($Fs<1$), meaning that all groups exhibited similar levels of conditioned fear and similar rates of extinction. This is as would be expected given that extinction occurred prior to any injections.

Freezing levels to the CS at test are shown in Figure 2b. A one-way ANOVA revealed a significant effect of group ($F=9.56_{(2, 22)}$, $p<0.001$). Subsequent *post hoc* comparisons using Tukey's Honestly Significantly Differences (HSD) Test revealed that rats administered FGF2 immediately after extinction exhibited significantly less CS-elicited freezing at test than the other two groups ($p<0.007$), which did not differ. These results demonstrate that post-extinction administration of FGF2 facilitates long-term extinction only when administered immediately after extinction, and not when administered 4 h post-extinction.

Experiment 3: Effect of Post-Extinction FGF2 on Long-Term Extinction when Short-Term Extinction Does Not Occur

A 2×2 ANOVA, where the first factor was drug (vehicle or FGF2) and the second factor was extinction condition (short or long) revealed no significant differences between groups in levels of pre-CS freezing prior to extinction ($Fs<1.42$) or prior to test ($Fs<2.72$). Freezing levels during the five trials of extinction are shown in Figure 3a for short-extinction rats and freezing levels during the five blocks of extinction (where each block consists of three extinction trials) are shown in Figure 3b for the long-extinction rats. As the short-extinction rats received five extinction trials and the long-extinction rats received 15 extinction trials, an initial analysis compared performance across the first five trials during extinction. There was a significant effect of trial ($F=12.04_{(4, 96)}$, $p<0.0001$), due to the level of freezing in all groups increasing across these first five trials. This demonstrates that five trials of extinction did not lead to any observable short-term extinction. Furthermore, there was no significant main effect of drug or extinction condition, and no significant drug-by-extinction condition interaction ($Fs<1.33$), meaning that all groups exhibited similar levels of conditioning and similar levels of freezing throughout the five extinction trials. To confirm that the long-extinction rats (receiving 15 extinction trials) showed short-term extinction, the five blocks of extinction were analyzed. There was a significant effect of block ($F=13.29_{(4, 48)}$, $p<0.0001$), due to the level of freezing by rats in the two long-extinction groups decreasing across blocks. Furthermore, there was no significant main effect of drug, and no significant block-by-drug interaction ($Fs<1.07$), meaning that both groups exhibited similar levels of conditioned fear and similar rates of extinction. The lack of a drug effect and the lack of a significant block-by-drug interaction is what would be expected given that extinction occurred prior to any injections. In summary, the data from this part of the experiment shows that the rats that received five extinction trials did not exhibit any observable short-term extinction whereas the rats that received 15 extinction trials exhibited substantial amounts of short-term extinction.

Freezing levels at test are shown in Figure 4. There was no significant main effect of drug or extinction condition ($Fs<3.8$). However, there was a significant interaction ($F=5.8_{(1, 24)}$, $p<0.024$). Subsequent *post hoc* comparisons using Tukey's HSD Test revealed that the interaction was due to the FGF2-treated rats that received 15 extinction trials exhibiting lower levels of CS-elicited freezing than all other groups ($p<0.024$). This demonstrates two things. Firstly, even though the long-extinction vehicle-treated rats exhibited substantial short-term extinction, this did not translate to long-term extinction as these rats exhibited substantial recovery of fear. Secondly, FGF2 only facilitated extinction in rats that received 15 extinction trials (and thus

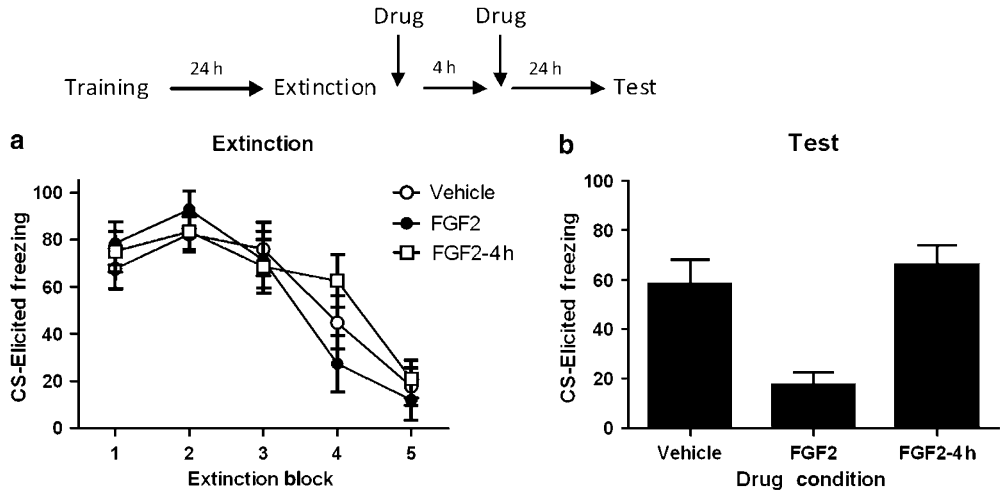

Figure 2 (a) Mean (±SEM) freezing by rats in response to the CS during extinction training in experiment 2. (b) Mean (±SEM) CS-elicited freezing by rats during test in experiment 2. Rats had been injected with vehicle ($n=8$) or FGF2 immediately after extinction training ($n=7$) or FGF2 4 h after extinction training ($n=8$).

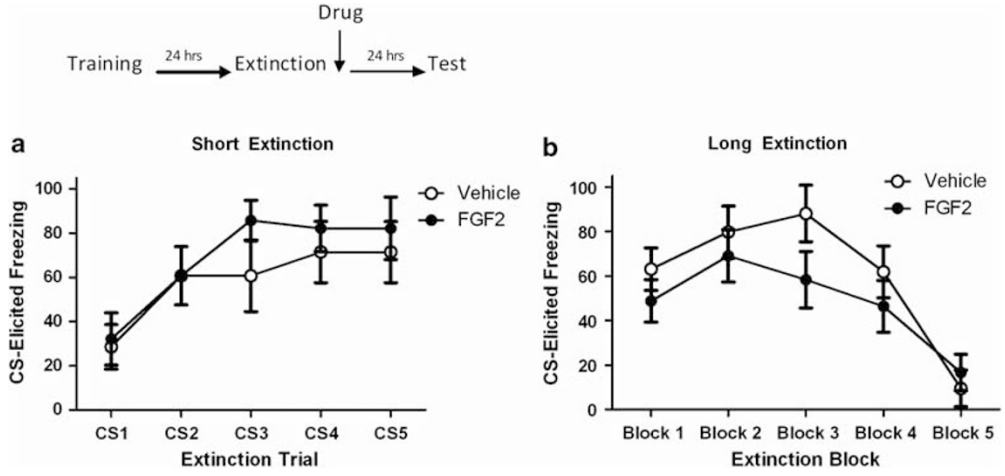

Figure 3 (a) Mean (±SEM) freezing by vehicle- and FGF2-treated rats in response to the CS during short-extinction training in experiment 3. (b) Mean (±SEM) freezing by vehicle- and FGF2-treated rats in response to the CS during long-extinction training in experiment 3. Rats were in four conditions: Vehicle-short ($n=7$), FGF2-short ($n=7$), Vehicle-long ($n=7$), and FGF2-long ($n=7$).

exhibited short-term extinction), suggesting that FGF2 facilitates the translation of the extinction memory from short-term to long-term storage. It should be noted that the short-extinction rats exhibited levels of freezing at test comparable to that observed in the vehicle-treated long-extinction rats. This may be due to several factors. Firstly, five extinction trials may have led to some extinction, but the amount of learning may have been too small to lead to observable short-term extinction, or to be facilitated by FGF2 treatment. It is interesting that our earlier study on FGF2 and contextual fear conditioning also reported that FGF2 only facilitated learning when some threshold level of learning had been exceeded. Secondly, as already noted, vehicle-treated rats receiving 15 extinction trials exhibit substantial recovery of fear after extinction at test 24 h later. Together, these factors may have contributed to the comparable levels of freezing at test between the short-extinction rats and the long-extinction vehicle-treated rats.

Experiment 4: Effect of Post-Extinction FGF2 on Reinstatement

A 2×2 ANOVA, where the first factor was drug (vehicle or FGF2) and the second factor was reinstatement condition (no reinstatement or reinstatement) revealed no significant effect of drug or reinstatement on levels of pre-CS freezing prior to extinction ($Fs < 1.45$). Freezing levels during extinction are presented in Figure 5a (for vehicle rats) and Figure 5b (for FGF2 rats). As the vehicle rats received 30 extinction trials and the FGF2 rats received 15 extinction trials, only the first fifteen trials during extinction were analyzed. There was a significant effect of trial ($F = 39.3_{(4, 132)}$, $p < 0.0001$), meaning that the level of freezing by rats in all groups decreased across trials. Furthermore, there was no significant main effect of drug or reinstatement ($Fs < 1$), and no significant drug-by-reinstatement interaction ($F < 1$), meaning that all groups exhibited similar levels of conditioned fear and similar rates of extinction. This is as

would be expected given that extinction occurred prior to any injections or the reinstatement procedure.

Freezing levels at test are shown in Figure 6. There was a significant effect of reinstatement condition on levels of pre-CS freezing prior to test ($F = 18.28_{(1, 33)}$, $p < 0.0001$). That is, reinstated rats exhibited significantly higher pre-CS levels of freezing prior to test than non-reinstated rats (29.19 *vs* 3.9%, respectively). There was no significant effect of drug and no significant drug-by-reinstatement interaction ($Fs < 1$) on pre-CS levels of freezing prior to test. To take account of the group differences in pre-CS freezing we conducted an analysis of covariance (ANCOVA) of the test data, using pre-CS freezing scores as the covariate. The ANCOVA revealed a significant main effect of reinstatement condition ($F = 18.48_{(1, 32)}$, $p < 0.0001$), due to reinstated rats showing significantly more CS-elicited freezing at test than the non-reinstated rats. There was also a significant main effect of drug ($F = 4.53_{(1, 32)}$, $p < 0.041$), due to the rats that received FGF2 exhibiting significantly less CS-elicited freezing at test than the rats that received vehicle. There was also a significant interaction ($F = 4.93_{(1, 32)}$, $p < 0.034$). This interaction was due to FGF2-treated rats exhibiting lower levels of freezing than vehicle-treated rats following the reinstatement shock ($t_{(15)} = 2.72$, $p < 0.019$) whereas non-reinstated rats exhibited comparable levels of freezing at test, regardless of drug condition ($t_{(17)} = 0.098$). Therefore, experiment 4 demonstrated that FGF2 significantly reduced reinstatement following extinction, even when the levels of freezing in the non-reinstated FGF2 and vehicle rats were equated.

DISCUSSION

These experiments demonstrate that FGF2 facilitates extinction, and further, that FGF2 attenuates reinstatement-induced recovery of fear. Experiment 1 demonstrated that when FGF2 was administered prior to extinction it facilitated long-term extinction, and that it also reduced the amount of freezing exhibited during extinction. Given that pre-extinction FGF2 led to reduced freezing during extinction it is possible that FGF2 suppresses freezing, or has anxiolytic properties, and that the reduction in freezing at test was caused by residual effects of the drug. However, this possibility is not supported by the results of experiment 2, which showed that FGF2 did not facilitate long-term

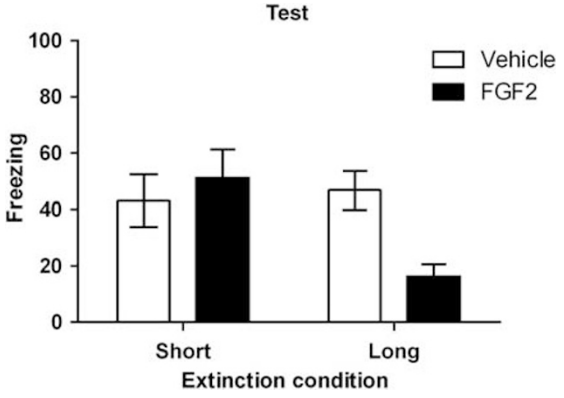

Figure 4 Mean (±SEM) freezing by rats in response to the CS during test in experiment 3.

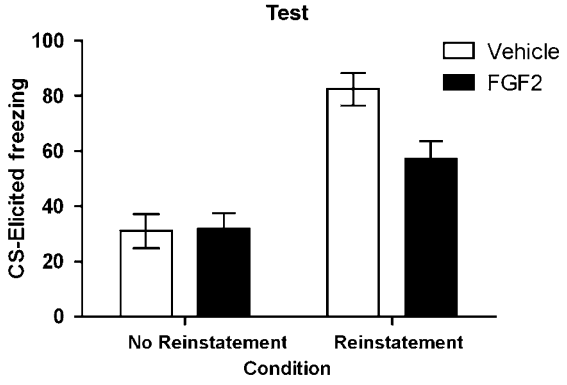

Figure 6 Mean (±SEM) freezing by rats in response to the CS during test in experiment 4.

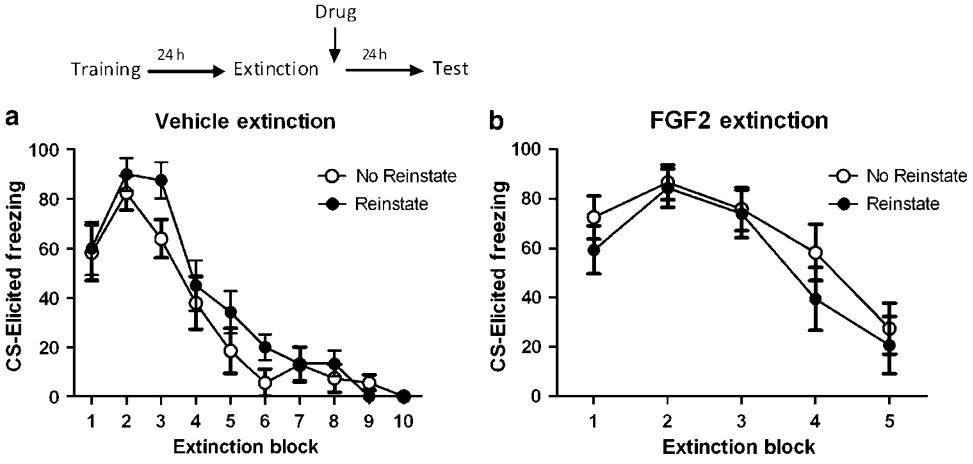

Figure 5 (a) Mean (±SEM) freezing by vehicle rats in response to the CS during extinction training in experiment 4. Rats were in two conditions: reinstate ($n = 10$) and no reinstate ($n = 10$). (b) Mean (±SEM) freezing by FGF2 rats in response to the CS during extinction training in experiment 4. Rats were in two conditions: no reinstate ($n = 10$) and reinstate ($n = 8$).

extinction when it was administered 4 h after extinction. This temporal gradient suggests that the most likely mechanism by which FGF2 reduced CS-elicited freezing at test is by enhancing consolidation of the extinction memory. The results of experiment 3 further support the interpretation that FGF2 facilitates the consolidation of the extinction memory as they demonstrated that FGF2 facilitated long-term extinction only if observable short-term extinction occurs. This suggests that FGF2 may facilitate the translation of the extinction memory from short-term to long-term storage. It should also be noted that the results from experiment 3 further refute the possibility that the FGF2-induced facilitation of long-term extinction was due to residual effects of the drug on locomotion or anxiety. That is, if it were the case that FGF2 merely suppresses freezing or is an anxiolytic, then FGF2-treated rats should always show low levels of freezing regardless of (a) the timing of administration, or (b) the number of extinction trials. However, together experiments 2 and 3 demonstrate that FGF2 facilitates long-term extinction only when FGF2 is administered immediately after extinction, and only when rats received sufficient training to produce observable short-term extinction.

Experiment 4 demonstrated that FGF2 reduced reinstatement-induced recovery of learned fear. Importantly, this finding cannot be attributed to FGF2-treated rats having lower levels of fear at test than vehicle rats because the levels of freezing at test were equated (ie, vehicle rats received double the amount of extinction). Given that stress-induced relapse is a significant problem in the treatment of anxiety disorders, the finding that FGF2 reduces reinstatement has potentially important clinical implications. Although FGF2 has been trialed in humans as a potential inducer of angiogenesis (Laham *et al*, 2000; Lederman *et al*, 2002; Simons *et al*, 2002), until now it has not been considered as a potential pharmacological adjunct to exposure therapy.

The results of these experiments parallel findings regarding the effects of DCS on extinction, which suggests that both drugs may enhance extinction through similar mechanisms (ie, consolidation of the extinction memory). Specifically, the effects of DCS on extinction are also dependent on the time of the administration, such that DCS only facilitates extinction when administered either prior to or shortly after extinction (Ledgerwood *et al*, 2003). Further, DCS-induced facilitation of extinction has also been shown to be dependent on the occurrence of short-term extinction (Weber *et al*, 2007). Finally, DCS has also been shown to reduce reinstatement in rats (Ledgerwood *et al*, 2004). Future research will need to further examine the similarities and differences between the effects of FGF2 and DCS on extinction, and in particular, to investigate whether FGF2 is subject to the same limitations as DCS. Specifically, one limitation to DCS is that multiple pre-exposures to DCS prevent its enhancement of extinction in rats (Parnas *et al*, 2005). Another limitation is that chronic pre-exposure to the antidepressant imipramine also prevents the enhancement of extinction by DCS, possibly because imipramine impairs the function of the NMDA receptor complex, rendering it less sensitive to DCS (Werner-Seidler and Richardson, 2007). These findings are clinically significant as exposure therapy in humans takes place over multiple sessions (and thus multiple administrations of DCS could be required), and individuals with anxiety disorders are often prescribed antidepressant medication. Future research will need to examine whether the efficacy of FGF2 is reduced following multiple administrations of FGF2, or chronic antidepressant administration.

In addition to being clinically relevant, the results of this study also contribute to our understanding of the molecular bases of memory in general. Although it is known that long-term memory relies on structural changes in the brain mediated by neural plasticity, little is known about the signals that give rise to this neural plasticity. The findings that FGF2 regulates neural plasticity during development and in response to brain injury in adulthood make FGF2 a likely candidate for the regulation of neural plasticity during learning and memory. We have now demonstrated that FGF2 enhances two kinds of long-term memory: contextual fear (Graham and Richardson, 2009) and the extinction of learned fear (present study). This suggests that FGF2 may be a critical molecule involved in memory in general.

In conclusion, we have demonstrated for the first time that FGF2 facilitates long-term extinction in rats when administered before or immediately after extinction training. In addition, we have demonstrated that FGF2-induced facilitation of long-term extinction requires the occurrence of short-term extinction, suggesting that FGF2 enhances the transfer of short-term memory to long-term memory (ie, memory consolidation). Finally, we have also shown that FGF2 significantly attenuates reinstatement. These findings are theoretically interesting as they provide further evidence that FGF2 may be involved in the formation of long-term memories. They are also clinically interesting as they point to a potential novel pharmacological adjunct to exposure therapy.

ACKNOWLEDGEMENTS

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DISCLOSURE

The authors have a patent in preparation for the use of FGF2 in the treatment of anxiety disorders. Neither author has any other financial conflicts.

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