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Prefrontal infralimbic cortex mediates competition between excitation versus inhibition of body movements during Pavlovian fear conditioning

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Abstract

The infralimbic (IL) subregion of prefrontal cortex is broadly involved in behavioral flexibility, risk assessment, and outcome reinforcement. In aversive conditioning tasks, IL has been implicated in fear extinction, and in mediating transitions between Pavlovian versus instrumental responses. Here, we examined the role of IL in mediating transitions between two competing Pavlovian fear responses: conditioned motor inhibition (CMI) versus conditioned motor excitation (CME). Rats were trained to fear an auditory conditioned stimulus (CS) by pairing it with periorbital shock to one eyelid (the unconditioned stimulus, US). Trained animals exhibited CMI responses (movement suppression) to the CS when they had not recently encountered the US (>24 hr), but after recent encounters with the US (<5 min), the CS evoked CME responses (turning in circles away from anticipated shock). Animals then received bilateral infusions of muscimol or picrotoxin to inactivate or hyperactive IL, respectively. Neither drug reliably affected CMI responses, but there was a bidirectional effect upon CME responding: inactivation of IL attenuated CME responses, while hyperactivation potentiated CME responses. These results provide evidence that activation of IL may promote behavioral strategies that involve mobilizing the body, and suppress strategies that involve immobilizing the body.

Graphical Abstract

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Both authors take full responsibility for the integrity and accuracy of the data and analyses. Study concept and design: LRH and HTB. Acquisition of data: LRH. Analysis and interpretation of data: LRH and HTB. Drafting and revision of the manuscript: LRH and HTB. Statistical analysis: LRH and HTB. Obtained funding: LRH and HTB. Study supervision: LRH and HTB.

Prefrontal infralimbic cortex (IL) may mediate transitions between competing Pavlovian fear responses. As threat increases, IL activation may promote defensive behavioral strategies that involve mobilizing the body, and suppress strategies that involve immobilizing the body.

Keywords

fear conditioning; freezing; flight; fear expression; defensive; RGD ID 2308852

INTRODUCTION

The ability to select appropriate defensive behaviors is an important survival skill. For example, an animal may exhibit immobilization behavior, such as freezing, to avoid detection by a nearby predator. But if the animal has already been detected by the predator, then freezing may no longer be the most appropriate defensive response for avoiding danger; the animal may then engage in mobilization behaviors, such as flight (Bolles, 1970; Fanselow and Lester, 1988). Circuitry within the medial prefrontal cortex (mPFC) mediates shifting between different behavioral strategies in many kinds of tasks (Miller and Cohen, 2001; Rich and Shapiro, 2009), and may also mediate selection among different defensive response strategies. Supporting this, findings from both human and rodent studies suggest that mPFC can modulate behavioral strategies for responding to threat (Wall et al., 2004; Mobbs et al., 2007; Moscarello and LeDoux, 2013; Bravo-Rivera et al., 2014; Harnett et al., 2015). Evidence suggests that mPFC mediates defensive responding by way of its connections with the amygdala (Sierra-Mercado et al., 2011; Sotres-Bayon and Quirk, 2011; Orsini and Maren, 2012) and periaqueductal gray (PAG) (LeDoux et al., 1988; Bandler and DePaulis, 1988; Fanselow, 1991; Davis et al., 1992; Killcross et al., 1997; De Oca et al., 1998; Amorapanth et al., 2000; Mobbs et al., 2007, 2009; Gozzi et al., 2010), but the exact neural mechanisms for selection among competing defensive responses are not well understood.

The infralimbic (IL) subregion of mPFC is involved in the detection of aversive behavioral contingencies (Amat et al., 2005), as well as aspects of risk assessment (Wall et al., 2004), so it seems likely that IL could participate in mediating transitions between different defensive response strategies. Supporting this, it has recently been reported that pharmacological inhibition of IL increased Pavlovian freezing responses, and concurrently decreased expression of instrumental avoidance responses (Moscarello and LeDoux, 2013), suggesting that IL may promote avoidance behavior and inhibit freezing. Another study also found that IL inactivation increased freezing, but avoidance was unaffected (Bravo-Rivera et al., 2014), suggesting that IL may not directly drive avoidance, but could indirectly facilitate avoidance by inhibiting competing behaviors like freezing. Although these findings point to a role for IL in defensive response selection, it remains unclear exactly what this role might be. Does IL inhibit Pavlovian responses while exciting instrumental responses? Or does it inhibit behaviors driven by fear while exciting behaviors driven by other motivational incentives? Or perhaps it simply promotes body mobilization and inhibits body immobilization? Any of these possibilities could be consistent with existing evidence.

To further investigate the role of IL in defensive strategy selection, here we trained rats by pairing an auditory CS (20 s train of white noise pips) with an aversive US (unilateral periorbital shock). As reported previously (Tarpley et al., 2010; Halladay and Blair, 2012, 2015), trained rats displayed either conditioned motor inhibition (CMI, i.e., movement suppression or freezing) or conditioned motor excitation (CME, i.e., turning in the direction away from anticipated shock) depending upon whether they had recently encountered the US. CS-evoked CMI and CME were both Pavlovian fear responses (not instrumental responses), since neither response was ever reinforced by omission of the US or CS. Using this paradigm, we pharmacologically manipulated the IL region of PFC to determine its role in the selection of defensive response strategies. We found that inactivation of IL with muscimol blocked CME responses, and hyperactivation of IL with PTX enhanced CME responses. Based upon these findings, we argue that one function of IL may be to promote behaviors that require body mobilization (or inhibit behaviors that require body immobilization).

MATERIALS & METHODS

Subjects and surgery

Adult male Long-Evans rats (Charles River Laboratories, Hollister, CA; RGD ID 2308852) weighing 350–400 g were housed singly and reduced to 85% of their free-feeding weight by daily limited feeding. During surgery, all rats were deeply anaesthetized with isoflurane and implanted with a pair of insulated stainless steel wires (75µm diameter) threaded into the skin of each eyelid for delivering the periorbital shock US. Rats (n=36, of which 30 were included in the study and 6 were excluded for misplaced infusion cannulae or faulty eyelid wires) were implanted with a pair of 26 gauge microinjector guide cannulae (Plastics One, Roanoke, VA) targeted bilaterally in IL (2.5 mm anterior, 0.5 mm lateral and 4.8 mm ventral to bregma) at an angle of 28 degrees to prevent drug diffusion into areas immediately dorsal to the target region. All experimental procedures were approved by the UCLA Animal Research Committee and were conducted in accordance with the NIH Guide for the Care

and Use of Laboratory Animals, and the Policies on the Use of Animals and Humans in Research.

Fear conditioning

After recovery from surgery, rats were habituated for 5 days (20 min/day) to the experimental context (which was a 70 × 70 cm elevated platform) before any fear conditioning sessions were conducted. To provide a baseline of motor activity against which stimulus-evoked movement and turning behavior could be measured, rats constantly foraged for 20 mg purified food pellets (Bioserv, Frenchtown, NJ) dropped from an overhead dispenser at ~30 s intervals throughout all pre-exposure and fear conditioning sessions. For 5 days following pre-exposure, rats received an identical regimen of fear conditioning trials: 6 CS-alone presentations (test trials) immediately followed by 16 CS-US pairings (training trials). The CS was a train of 70 dB white noise pips, each lasting 250 ms, delivered at 1 Hz for 20 s through an overhead speaker. The US was a train of 2.0 mA shock pulses, each lasting 2.0 ms, delivered to one eyelid at a rate of 6.66 Hz for 2 s. During CS-US pairing trials, the first shock pulse was always delivered 300 ms after the offset of the final (20th) CS pip. The intertrial interval was uniformly random between 180 and 240 s.

Behavioral data acquisition and analysis

The rat's position on the experimental platform was sampled at 30 Hz by an overhead video tracking system (Neuralynx Corporation, Bozeman, MT), which monitored the location of three light-emitting diodes (LEDs) of different colors (red, blue, and green) attached to the animal's headstage for automated measurement of movement speed and turning behavior (see Tarpley et al., 2010; Halladay and Blair, 2012). Conditioned fear responses were assessed by comparing the animal's behavior during the context (CX) period, defined as the 20 s immediately preceding CS onset, versus the CS period, the 19 s immediately following CS onset (the 20th pip was omitted from the CS period because the first shock pulse was delivered <1 s after the offset of this pip).

CS suppression ratio (R_{CS})—Suppression of movement (CMI) during the CS was measured for each experimental trial by measuring the ratio of average movement speeds during the CX versus CS periods. The formula for computing the CS suppression ratio was $R_{CS} = S_{CS} / (S_{CX} + S_{CS})$, where S_{CS} and S_{CX} are the mean movements speeds during the CS versus CX periods, respectively.

US suppression ratio (R_{US})—Suppression of movement (CMI) following delivery of the first shock US (beginning of the post-shock portion of each session) was measured for each experimental session by computing the ratio of average movement speeds during the CX periods of pre- versus post-shock trials. The US suppression ratio was computed as $R_{US} = S_{POST} / (S_{POST} + S_{PRE})$, where S_{POST} and S_{PRE} are the mean movements speeds during the CX period of the session's post-versus pre-shock trials, respectively.

Intracranial drug infusions

Rats were given unilateral or bilateral injections of the GABA agonist muscimol (MUS) to inactivate mPFC, or the GABA antagonist picrotoxin (PTX) to hyperactivate mPFC. After 5

days of training in the fear conditioning task, rats received pre-session infusions of different drugs on different days, in a counterbalanced repeated measures design. A drug-free retraining session was always given in between each infusion session. Of the 30 rats that were included in the data analysis (see above, “Subjects and Surgery”), 14 rats received infusions of MUS only (into the left, right, or both hemispheres on different days in counterbalanced order), 13 received infusions of PTX only (bilaterally on the first day following the 5th training session), and 3 received infusions of both drugs (these three rats received bilateral MUS infusions on the first day following the 5th training session, then a drug free retraining day, followed by bilateral PTX infusions). Hence, data was analyzed from a total of 17 bilateral MUS infusions, 16 unilateral MUS infusions (because one of the MUS rats received only bilateral infusions), and 16 bilateral PTX infusions, in a total of 30 rats, with no rat receiving the same infusion twice. MUS infusions were carried out both uni- and bilaterally to determine whether IL’s role in mediating CME is hemispherically lateralized, since previous experiments in our lab (Tarpley et al., 2010) have found unilateral effects upon CME due to MUS infusions in both AMG and PAG. PTX was only infused bilaterally since there were no pronounced or interesting effects of unilateral MUS.

Both drugs were dissolved in 0.9% sterile saline, and a total volume of 0.4 μ l per hemisphere was infused into mPFC through 26 gauge injectors at a rate of 0.2 μ l/min. MUS was dissolved at a concentration of 0.25 mg/mL. PTX was dissolved at a concentration of 0.20 mg/mL. Prior to drug infusion, dummy cannulae (which were in place at all times except during infusions to prevent clogging of the guide cannulae) were removed and injector cannulae were inserted in their place. After drug infusions, the injectors were left in place for an additional 2 min to allow diffusion of the drug away from the cannulae tip, after which the injectors were removed and replaced with dummy cannulae. Throughout the infusion process, the animal was held gently on the experimenter’s lap. After the infusion was complete, the rat was returned to its home cage for 15 min to allow time for the drug to take effect before the experiment resumed.

Injector placements and drug diffusion

Cannula guides were bilaterally implanted into mPFC at a lateral-to-medial angle of 28 degrees (Fig. 1A), with injector tips targeted at IL (Fig. 1B). Analysis of fluorescence-tagged MUS diffusion (see Histological Procedures) indicated that drug concentrations were highest surrounding the injector tip in IL. We assume that PTX diffusion patterns were similar to those observed for MUS, but we cannot rule out the possibility that PTX diffusion patterns may have differed because its molecular properties are not identical to those of MUS.

Inferential statistics

Statistical analyses were performed using the Statistica^{TM} software package running on a Microsoft Windows^{TM} PC. For consistency, posthoc comparisons for all ANOVAs were made using the Newman-Keuls method, unless otherwise noted.

Histological procedures

At the end of the experiment, rats were intraperitoneally injected with an overdose of pentobarbital (100 mg/kg) and perfused intracardially. Brains were extracted and fixed in a formalin sucrose solution. Tissue was later sectioned into 40 μm slices and mounted on slides for cannula placement verification. To assess the extent of drug diffusion, cannula-implanted rats that had previously received MUS injections were given a final infusion of fluorescent MUS (tagged with Bodipy® TMR-X fluorophore, Invitrogen product #M2400) into IL, at the same volume, concentration, and rate that had been used for infusions of non-fluorescent MUS in behavioural experiments (see above, 'Intracranial drug infusions'). Fluorescent MUS was infused 30 min prior to the pentobarbital injection.

RESULTS

Pharmacological inactivation of either AMG or PAG has previously been shown to impair CMI and CME responses in the fear conditioning task used here (Tarpley et al., 2010). To investigate the contribution of IL to CMI and CME behaviors, we pharmacologically inactivated (with MUS) or hyperactivated (with PTX) the IL subregion of PFC.

Movement suppression as a measure of CMI responses

To quantify CMI responses evoked by the CS, a movement suppression ratio denoted R_{CS} was computed to compare movement speed during the CS relative to the CX on each experimental trial; $R_{CS} < 0.5$ indicates suppression of movement during the CS, $R_{CS} > 0.5$ indicates enhancement of movement during the CS, and $R_{CS} = 0.5$ indicates that the CS has no effect upon movement (see Materials & Methods). Fig. 2A shows mean R_{CS} values on each trial for all rats ($n=30$), averaged on the day prior to the rat's first bilateral drug infusion of either MUS or PTX (whichever came first). Z-tests revealed that R_{CS} was significantly less than 0.5 for every individual pre-shock trial, as well as for the mean of all pre-shock trials ($z_{29}=3.83$, $p=.0006$). However, R_{CS} did not differ significantly from 0.5 for any individual trial subsequent to the first post-shock trial, and consequently, the mean of R_{CS} averaged across post-shock trials for all rats did not differ significantly from 0.5 ($z_{29}=0.56$, $p=.58$). A paired t-test revealed that R_{CS} differed significantly between pre-versus post-shock trials ($t_{29}=3.14$, $p=.004$), indicating that CMI responses were evoked by the CS during pre-shock but not post-shock trials. For this reason, analyses presented below shall evaluate drug effects upon CMI responses only during pre-shock trials.

Turning bias as a measure of CME responses

One reason why the CS failed to evoke CMI responses (as measured by R_{CS}) during post-shock trials (Fig. 1A) was that after recent encounters with shock US, rats began responding to the CS by expressing a CME response on some trials, which often included turning in the direction away from the eyelid where shock was anticipated (Tarpley et al. 2010; Halladay and Blair 2012, 2015; see Fig. 2B). To quantify this CME response, we computed the rat's mean turning velocity in the direction away from the anticipated eyelid shock. Fig. 2B shows mean angular velocities for the drug-free condition, with positive velocities indicating a bias for turning away from the trained eyelid, and negative velocities indicating a bias for turning toward the trained eyelid. Turning bias data for pre-shock and post-shock trials were

analyzed using a 2x2 ANOVA with stimulus (CX versus CS) and trial type (pre-shock versus post-shock) as repeated factors. There was a significant interaction between stimulus and trial type ($F_{1,29}=21.94$, $p<.0001$), and posthoc comparisons revealed that turning bias did not differ during the CS versus CX ($p=.34$) for pre-shock trials (because the CS did not evoke CME responses during pre-shock trials), but for post-shock trials the CS evoked robust turning behavior compared to both the post-shock CX ($p=.0001$) and the pre-shock CS ($p=.0002$). These results indicate that CME responses (as measured by turning bias) were evoked by the CS during post-shock but not pre-shock trials, as reported previously (Tarpley et al., 2010; Halladay and Blair 2012, 2015) and in experiments reported above (Fig. 2B). For this reason, analyses presented below shall evaluate drug effects only during post-shock trials.

Post-shock movement suppression as a measure of US aversiveness

A main reason why the CS failed to evoke CMI responses during post-shock trials (as measured by R_{CS} in Fig. 2A) is that after shock delivery, movement speed was tonically suppressed during the CX period (stimulus x trial type, $F_{1,29}=21.70$, $p<.0001$). Post hoc comparisons showed that during pre-shock trials, rats exhibited suppression of their movement speed during the CS when compared against the CX ($p=.0002$), but during post-shock trials, movement speeds during the CX were lower ($p=.0002$), and there was no longer a significant difference between CX and CS movement speeds during post-shock trials ($p=.37$). This floor effect on movement speed made it difficult to observe CS-evoked CMI during post-shock trials (Tarpley et al., 2010; Halladay and Blair 2012, 2015).

To quantify this suppression of movement speed during the CX period of post-shock trials, a suppression ratio denoted R_{US} was computed to compare mean movement speeds during the CX for pre- versus post-shock trials in each session (see Materials & Methods). Fig. 2A shows that R_{US} was significantly less than 0.5 when averaged across rats ($z_{29}=3.33$, $p=.002$). Hence, movement speed was suppressed during the CX period of post-shock trials compared to pre-shock trials. Presumably, this was a consequence of post-shock fear induced by the aversive US. Assuming that post-shock fear is proportional to the aversiveness of the US, then R_{US} can be regarded as a quantitative (albeit indirect) measure for the aversive valence of the US. We thus analyzed R_{US} , along with other US-evoked movement speed, to assess how US aversiveness was affected by pharmacological manipulations (see below).

Drug effects upon CMI responses

Analysis of CX movement speeds during the pre-shock trials showed no significant differences in movement speed for drug-free (DF) baseline sessions compared with bilateral MUS (paired $t_{16}=1.04$, $p=.31$) or PTX (paired $t_{15}=0.71$, $p=.49$) infusion sessions. This is consistent with prior studies reporting that intra-IL infusions administered in a novel context (non-fear conditioned animals) had no locomotor effects for either MUS (Slattery et al., 2011; Jiang et al., 2014) or PTX (Ronzoni et al., 2015). Thus, any effects due to MUS or PTX infusions are assumed to be attributed to drug-induced changes in defensive behavior.

Fig. 3A shows how R_{CS} was affected by MUS or PTX infusions that inactivated or hyperactivated IL, respectively. All PTX infusions ($n=16$) were bilateral, whereas MUS

infusions were given into different hemispheres on different days (bilateral: $n=17$; contralateral to trained eyelid: $n=16$; ipsilateral to trained eyelid: $n=16$) in a repeated measures design (see Materials & Methods). Unilateral infusions of MUS were given to test whether IL's role in regulating CME might be hemispherically lateralized, because prior evidence indicates that MUS infusions into AMG and PAG can produce lateralized effects upon turning behavior (Tarpley et al., 2010). R_{CS} values were averaged separately for each infusion type during pre- versus post-shock trials, and compared against a DF baseline session given on the day immediately prior to each infusion. Z-tests revealed significant CMI responses ($R_{CS} < 0.5$) during pre-shock trials for all DF conditions (Fig. 3A). Z-tests revealed that significant CMI responses ($R_{CS} < 0.5$) persisted during pre-shock trials following all MUS infusions, regardless of hemisphere (Fig. 3A). Posthoc comparisons revealed that R_{CS} was unchanged from the DF condition following unilateral MUS infusions into either hemisphere (CONTRA: $p=.66$; IPSI: $p=.43$; see Fig. 2A). This pattern of results indicates that MUS infusions into IL never significantly impaired CMI responses to the CS during pre-shock trials, regardless of whether MUS was infused bilaterally or unilaterally, which is consistent with prior evidence that bilateral inactivation of IL does not impair expression of conditioned freezing to an auditory CS (Sierra-Mercado et al., 2011).

CS-evoked CMI responses were no longer significant (that is, R_{CS} did not differ from 0.5) following PTX infusions ($z_{1,16}=0.11$, $p=.92$), but this was largely attributable to greatly increased variance of R_{CS} after PTX infusions (Fig. 3A). Some of this variability resulted from two outlying animals that exhibited $R_{CS} > .7$ after PTX, which was far outside the range observed for any other rat under any other condition. When these two outlying rats were removed from the analysis (Fig. 3A), the mean R_{CS} value was quite similar to the pre-drug condition, but the variance was still considerably higher, and consequently, R_{CS} did not differ significantly from 0.5 after PTX. These findings suggest that PTX may have had a disruptive influence upon CMI behavior in some rats, consistent with prior data showing that expression of context freezing can be impaired by infusions of PTX into IL (Thompson et al., 2010). However, the high variability in R_{CS} after PTX makes it difficult to draw firm conclusions about reliable effects of PTX upon CMI behavior during pre-shock test trials.

Drug effects upon CME responses

Z-tests revealed that significant turning away from the shocked eyelid was observed during post-shock trials under all conditions except after bilateral MUS (Fig. 3B). Hence, bilateral MUS was the only drug infusion that appeared to block CME responses to the CS during post-shock trials. Effects of MUS and PTX infusions upon CME responses were more closely analyzed by performing 2×2 ANOVAs upon turning bias scores with drug (DF versus infusion) and trial type (pre versus post-shock) as repeated measures factors. As expected, CME responses were preferentially expressed during post-shock trials, yielding a significant main effect of trial type for bilateral ($F_{1,16}=8.05$, $p=.01$) and unilateral (CONTRA: $F_{1,15}=17.29$, $p=.0008$, IPSI: $F_{1,15}=25.77$, $p=.0001$) MUS infusions, as well as for PTX infusions ($F_{1,15}=14.85$, $p=.002$). The drug main effect of MUS was only significant following bilateral ($F_{1,16}=8.71$, $p=.009$) but not unilateral (CONTRA: $F_{1,15}=1.01$, $p=.33$, IPSI: $F_{1,15}=3.03$, $p=.10$) infusions.

Since both factors were treated as repeated measures in the ANOVA, the significance of Posthoc comparisons depended upon within-subject variance (rather than the between subject variance plotted in Fig 3B). Fig. 4 shows within-subject means and standard errors for the pre- versus post-drug change in turning bias during the CS of post-shock trials. As shown in Fig. 4, posthoc comparisons revealed that animals exhibited significantly less turning ($p=.002$) during the CS of post-shock trials after bilateral MUS than in the DF baseline condition. CS-evoked turning was also attenuated by unilateral MUS infusions, but posthoc comparisons did not quite reach significance for either hemisphere (CONTRA: $p=.06$, IPSI: $p=.06$). The effect size of this trend was similar in both hemispheres, implying that CS-evoked turning was partially impaired after unilateral MUS infusions into either hemisphere. Hence, there did not appear to be a hemispherically lateralized effect of IL inactivation upon turning responses, in contrast with laterality effects that have previously been observed following inactivation of AMG or PAG (Tarpley et al., 2010). However, given the close anatomical proximity of IL to the midline, it is possible that unilateral infusions may have diffused across the midline to affect both hemispheres. If so, then the reduced impairment of turning after unilateral infusions might simply reflect a decrease in the dose of MUS delivered to both hemispheres.

Bilateral infusions of PTX had opposite effects from MUS, enhancing rather than impairing turning responses (Fig. 4). The 2×2 ANOVA yielded a significant main effect of PTX ($F_{1,16}=8.14$, $p=.01$) but no interaction ($F_{1,16}=.26$, $p=.63$). Posthoc comparisons revealed that animals exhibited significantly more CS-evoked turning during post-shock trials after PTX compared to the DF condition ($p=.009$); on average, PTX increased a rat's turning bias by 5.5 ± 2.4 deg/s compared to the DF condition (Fig. 4), suggesting that PTX infusions not only failed to disrupt CS-evoked turning, but may even have enhanced it. One possible confound for this interpretation is that turning for the pre-PTX DF condition was lower than the DF condition preceding other infusions, raising concerns that the effect may have arisen from a change in the control condition rather than the experimental condition. However, a one-way repeated measures ANOVA (including only the rats that were given all four drug treatments) indicated no significant difference in post-shock CS-evoked turning scores across the four DF sessions preceding drug infusions ($F_{1,3}=1.73$, $p=.18$). Moreover when the two outlying rats who responded differently to PTX were excluded from the analysis, the DF turning scores became more similar to those in other conditions (Fig. 2B), and the significant increase in CS-evoked turning after PTX persisted ($p=.008$). In summary, hyperactivation of IL by PTX did not impair CME responses, but instead enhanced these responses during post-shock trials.

US-evoked reflexes and movement suppression

CS-evoked turning responses emerged only during post-shock trials (after delivery of the shock US) on each experiment day, so it is important to consider whether observed effects of MUS and PTX upon turning behavior might have resulted from drug-induced alterations in processing of the shock US. To assess whether US processing was altered by drug infusions, we performed analyses upon unconditioned reflex responses to the shock, and shock-induced suppression of movement (R_{US}) during the CX period of post-shock trials.

Effects of MUS and PTX upon unconditioned reflex responses were assessed by performing separate 2×2 ANOVAs (one for each infusion condition) of US-evoked movement speed with drug (DF versus infusions) and stimulus (CX versus US) as repeated factors (Fig. 3C). The main effect of stimulus was significant for all MUS (BI: $F_{1,16}=35.11$, $p<.0001$; CONTRA: $F_{1,15}=29.54$, $p=.00007$; IPSI: $F_{1,15}=33.52$, $p<.00001$) and PTX ($F_{1,16}=17.96$, $p=.0007$) infusions, so US-evoked reflex responses were never abolished. There was no main effect of drug ($F_{1,16}=2.12$, $p=.16$) or drug-by-stimulus interaction ($F_{1,16}=2.12$, $p=.16$) for bilateral MUS. There also was no main effect of drug for ipsilateral MUS ($F_{1,15}=1.46$, $p=.25$), but the interaction approached significance ($F_{1,15}=4.09$, $p=.06$), and posthoc comparisons revealed that this was entirely accounted for by an increase in movement speed during the CX ($p=.03$) rather than a decrease in movement speed during the US ($p=.67$), so US-evoked responding was not altered by ipsilateral MUS. The drug main effect was significant for contralateral MUS ($F_{1,15}=5.9$, $p=.03$), but the interaction was not ($F_{1,15}=0.1$, $p=.92$), because movement speed increased by the same amount during the CX and US, implying no major effects of contralateral MUS upon US-evoked reflexes. There was no main effect of drug for PTX infusions ($F_{1,16}=0.2$, $p=.66$), and although the interaction was significant ($F_{1,16}=4.46$, $p=.05$), posthoc comparisons showed that following PTX, movement speed was non-significantly increased during the CX ($p=.26$) and non-significantly decreased during the US ($p=.09$), implying no major effects of PTX upon US processing.

Effects of MUS and PTX upon post-shock movement suppression were assessed by analyzing R_{US} using a 4×2 ANOVA with drug (DF versus infusion) as a repeated factor and infusion type (bilat MUS, contra MUS, ipsi MUS, and bilat PTX) as an independent factor (Fig. 3D). Neither drug ($F_{1,62}=0.79$, $p=.38$) nor infusion type ($F_{3,62}=0.52$, $p=.67$) were found to significantly affect R_{US} , and the interaction also was not significant ($F_{3,62}=0.08$, $p=.97$), suggesting that US delivery during post-shock trials reduced CX movement speeds similarly for all conditions. This pattern of results indicates that neither MUS nor PTX had any effect upon the general aversiveness of the US, as assessed by its ability to suppress movement during subsequent CX periods. In summary, it appears that sensory processing and aversiveness of the US were not greatly altered by infusions of MUS or PTX.

DISCUSSION

Consistent with theories of predatory imminence (Bolles, 1970; Fanselow and Lester, 1988), as well as our past data (Tarpley et al., 2010; Halladay and Blair, 2012, 2015), rats in this study exhibited either CMI or CME behaviors in response to the CS, which were dependent upon whether the animal had recently encountered the US. As previously reported by others (Laurent and Westbrook, 2009; Sotres-Bayon et al., 2009; Sierra-Mercado et al., 2011), manipulations of IL had no effect upon CMI responses. However, pharmacological manipulation of IL affected the expression of CME behaviors in a bidirectional manner; bilateral inactivation of IL attenuated conditioned turning responses, while hyperactivation of IL potentiated defensive turning, suggesting that activity in IL may help to drive the transition from reactive to active defensive responding. This is consistent with the idea that IL is essential for some forms of risk assessment (Wall et al., 2004), behavioral flexibility (Ragozzino et al., 1999, 2003; Delatour and Gisquet-Verrier, 2000; Dias and Aggleton, 2000), and the integration of cognition and behavior (Sullivan and Gratton, 2002).

IL regulates body mobilization

Several lines of evidence suggest that IL may play a role active avoidance tasks, where a CS that is initially trained to evoke Pavlovian freezing responses (a CMI behavior) can later come to evoke instrumental avoidance responses (a CME behavior), after the avoidance responses have been reinforced by the absence of the US that was previously predicted by the CS (Mowrer and Lamoreaux, 1946). Active avoidance induces c-fos expression in IL that correlates with the extent of active avoidance expressed (Martinez et al., 2013). Moscarello and LeDoux (2013) found that pharmacological inhibition of IL increased freezing, and concurrently decreased expression of active avoidance responses, suggesting that IL may promote CME defenses and inhibit CMI defenses. However, another study found that IL inactivation increased freezing but left active avoidance unaffected (Bravo-Rivera et al., 2014), which suggests that IL may not directly drive expression of CME defenses (such as avoidance), but could instead indirectly facilitate CME behavior by inhibiting competition from CME responses (such as freezing). Here we found that IL inactivation impaired CME responses but spared CMI responses, but unlike prior studies, the CME responses measured here (defensive turning) were never reinforced by withholding shock, so acquisition of CME responses in our paradigm cannot be attributed to instrumental learning (Tarpley et al., 2010). This is an important difference with prior studies, especially since there has long been controversy about whether animals can really learn to express instrumental avoidance responses when they are in a fearful state (Bolles, 1970). Before instrumental avoidance responses can be learned, it may be necessary to first suppress fear, and if so, then the role of IL in avoidance learning may be to inhibit fear. This would be consistent with prior evidence that IL facilitates extinction learning (Bukalo et al., 2015; Do-Monte et al., 2015). But in our paradigm, it seems unlikely that rats were less afraid during CME responses than CMI responses (if anything, rats should have been more afraid since they had more recently been shocked), and it thus seems unlikely that IL promoted turning behavior by suppressing fear, or by suppressing Pavlovian responses in favor of instrumental responses. Instead, IL may have influenced defensive action selection by promoting CME behaviors (such as turning) that mobilize the body, while possibly also inhibiting CMI behaviors (such as freezing) that immobilize the body, independently of the animal's emotive state or of Pavlovian versus instrumental contingencies. This interpretation is consistent with recent evidence that neural activity in the amygdala may also be correlated with body movements, independently from motivational states (Amir et al., 2015).

IL drives contextually-appropriate defensive responding

Another possibility to consider is that IL plays a role in risk assessment processes that underlie context-dependent defensive action selection. PFC is thought to mediate contextually-driven action selection; not only does it seem to modulate initiation of contextually appropriate actions (Sierra-Mercado et al., 2011; Moorman and Aston-Jones, 2015), but also plays a role in the inhibition of contextually inappropriate behaviors (Orsini et al., 2011; Maren et al., 2013). Electrophysiological recording studies have shown that action-selective cells in PFC are modulated by contextual components, which may include spatial location, time, sensory attributes, and response-outcome contingencies (Halladay and Blair, 2015; Moorman and Aston-Jones, 2015). The experimental paradigm used here can be thought of as containing two distinct contexts, characterized by sequential order (time) as

well as the presence or absence of US reinforcement (sensory attribute). On any given day, the initial 'context' consisted of non-reinforced CS presentations, during which CMI expression was a contextually-appropriate, reactive behavior. But after the initial shock following the 7th CS presentation signaled the onset of a new, US-reinforced context, the change in cue-outcome contingency rendered CMI no longer the most appropriate action, and thus, active CME behaviors emerged. Following IL inactivation however, animals were incapable of adapting to the change in context, thereby resulting in a failure to execute contextually-appropriate CME defenses. This is consistent with prior evidence implicating PFC as a mediator of behavioral flexibility regarding risk assessment (Wall et al., 2004), reinforcement prediction (Maddux and Holland, 2011), and contextual control of action (Ashwell and Ito, 2014; Moorman and Aston-Jones, 2015).

IL modulates downstream structures

Recent electrophysiological recording experiments from our lab showed that PFC contains defensive strategy-specific neuronal populations whose tonic firing rates may mediate the expression of CMI and CME defenses (Halladay and Blair, 2015). These cells likely interact with downstream structures to regulate behavioral strategies (Miller and Cohen, 2001). These downstream structures include the AMG, which stores information about CS-US associations (Davis, 1992; Blair et al., 2003, 2005; Maren, 2003; Herry and Johansen, 2014) and modulates both reactive and active defensive strategies by way of its distinct pathways (Killcross et al., 1997; Amorapanth et al., 2000; Gozzi et al., 2010). While reactive defenses such as CMI are primarily mediated by the CeA, active responses may be mediated by the basal nucleus of AMG (BA) and BLA (Killcross et al., 1997; Amorapanth et al., 2000). Thus, it is possible that IL interacts with AMG in such a way to promote activity in BA and/or BLA, while inhibiting CeA. Traditionally, IL is thought to mediate fear extinction by inhibiting CeA output, which attenuates freezing behavior (Sotres-Bayon and Quirk, 2010; Orsini and Maren, 2012; but see Do-Monte et al., 2015). While many interpret this as evidence that IL inhibits *fear*, we posit that activity in PFC is correlated with specific defensive *action*, rather than affect (Halladay and Blair, 2015). Data presented here supports this, since neither inactivation nor hyperactivation of IL fully blocked the expression of fear (i.e., CMI or CME), but instead changed the nature of its expression.

Other downstream targets of IL include the ventral PAG (vPAG) (Floyd et al., 2000), which drives CMI defenses (De Oca et al., 1988; Fanselow, 1991; Vianna et al., 2001) and directly competes with the dorsal PAG (dPAG), which drives CME defenses (Bandler and Depaulis, 1988; Fanselow, 1991; Walker et al., 1997). It seems possible that PAG innervation from IL may modulate the competition between vPAG and dPAG by inhibiting vPAG output, thus enabling dPAG to drive CME behaviors. Alternatively, the ventral striatum (vSTR) has been implicated in avoidance expression, and receives inhibitory input from PFC that may mediate active avoidance (Bravo-Rivera et al., 2014, 2015; Lee et al., 2014) and escape behavior (Richard and Berridge, 2013). Interactions between IL and the vSTR might also influence the emergence of CME behaviors following recent encounter with shock. Although it is evident that IL plays a role in mediating defensive strategy selection, further investigation will be necessary to determine which other structures, if any, interact with IL in order to control the gating of reactive and active defenses.

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LITERATURE CITED

- Amat J, Baratta MV, Paul E, Bland ST, Watkins LR, Maier SF. Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. *Nat Neurosci.* 2005; 8:365–371. [PubMed: 15696163]
- Amir A, Lee SC, Headley DB, Herzallah MM, Pare D. Amygdala Signaling during foraging in a hazardous environment. *J Neurosci.* 2015; 35:12994–3005. [PubMed: 26400931]
- Amorapanth P, LeDoux JE, Nader K. Different lateral amygdala outputs mediate reactions and actions elicited by a fear-arousing stimulus. *Nat Neurosci.* 2000; 3:74–49. [PubMed: 10607398]
- Ashwell R, Ito R. Excitotoxic lesions of the infralimbic, but not prelimbic cortex facilitate reversal of appetitive discriminative context conditioning: the role of the infralimbic cortex in context generalization. *Front Behav Neurosci.* 2014; 8:63. [PubMed: 24616678]
- Bandler R, Depaulis A. Elicitation of intraspecific defense reactions in the rat from midbrain periaqueductal gray by microinjection of kainic acid, without neurotoxic effects. *Neurosci Lett.* 1988; 88:291–296. [PubMed: 3386876]
- Blair HT, Sotres-Bayon F, Moita MA, LeDoux JE. The lateral amygdala processes the value of conditioned and unconditioned aversive stimuli. *Neuroscience.* 2005; 133:561–9. [PubMed: 15878802]
- Blair HT, Tinkelman A, Moita MA, LeDoux JE. Associative plasticity in neurons of the lateral amygdala during auditory fear conditioning. *Ann N Y Acad Sci.* 2003; 985:485–7. [PubMed: 12724183]
- Bolles RC. Species-specific defense reactions and avoidance learning. *Psychol Rev.* 1970; 77:32–48.
- Bravo-Rivera C, Roman-Ortiz C, Brignoni-Perez E, Sotres-Bayon F, Quirk GJ. Neural structures mediating expression and extinction of platform-mediated avoidance. *J Neurosci.* 2014; 34:9736–42. [PubMed: 25031411]
- Bravo-Rivera C, Roman-Ortiz C, Montesinos-Cartagena M, Quirk GJ. Persistent active avoidance correlates with activity in prelimbic cortex and ventral striatum. *Front Behav Neurosci.* 2015; 9:184. [PubMed: 26236209]
- Bukalo O, Pinard CR, Silverstein S, Brehm C, Hartley ND, Whittle N, Colacicco G, Busch E, Patel S, Singewald N, Holmes A. Prefrontal inputs to the amygdala instruct fear extinction memory formation. *Sci Adv.* 2015:1.
- Choi JS, Cain CK, LeDoux JE. The role of amygdala nuclei in the expression of auditory signaled two-way active avoidance in rats. *Learn Mem.* 2010; 17:139–47. [PubMed: 20189958]
- Davis M. The role of the amygdala in fear and anxiety. *Annu Rev Neurosci.* 1992; 15:353–375. [PubMed: 1575447]
- Delatour B, Gisquet-Verrier P. Functional role of rat prelimbic-infralimbic cortices in spatial memory: evidence for their involvement in attention and behavioural flexibility. *Behav Brain Res.* 2000; 109:113–28. [PubMed: 10699663]
- De Oca BM, DeCola JP, Maren S, Fanselow MS. Distinct regions of the periaqueductal gray are involved in the acquisition and expression of defensive responses. *J Neurosci.* 1998; 18:3426–3432. [PubMed: 9547249]
- Dias R, Aggleton JP. Effects of selective excitotoxic prefrontal lesions on acquisition of nonmatching- and matching-to-place in the T-maze in the rat: differential involvement of the prelimbic-infralimbic and anterior cingulate cortices in providing behavioural flexibility. *Eur J Neurosci.* 2000; 12:4457–66. [PubMed: 11122356]
- Do-Monte FH, Manzano-Nieves G, Quinones-Laracuente K, Ramos-Medina L, Quirk GJ. Revisiting the role of infralimbic cortex in fear extinction with optogenetics. *J Neurosci.* 2015; 35:3607–15. [PubMed: 25716859]

- Fanselow, MS. The midbrain periaqueductal gray as a coordinator of action in response to fear and anxiety. In: Depaulis, A.; Bandler, R., editors. *The rat brain periaqueductal gray matter: functional, anatomical and neurochemical organization*. Plenum Press; New York: 1991. p. 1-8.
- Fanselow, MS.; Lester, LS. A functional behavioristic approach to aversively motivated behavior: Predatory imminence as a determinant of the topography of defensive behavior. In: Bolles, RC.; Beecher, MD., editors. *Evolution and Learning*. Earlbaum; Hillsdale NJ: 1998. p. 185-211.
- Floyd NS, Price JL, Ferry AT, Keay KA, Bandler R. Orbitomedial prefrontal cortical projections to distinct longitudinal columns of the periaqueductal gray in the rat. *J Comp Neurol*. 2000; 422:556–578. [PubMed: 10861526]
- Gozzi A, Jain A, Giovannelli A, Bertollini C, Crestan V, Schwartz AJ, Tsetsenis T, Ragozzino D, Gross CT, Bifone A. A neural switch for active and passive fear. *Neuron*. 2010; 67:656–666. [PubMed: 20797541]
- Halladay LR, Blair HT. The role of mu-opioid receptor signaling in the dorsolateral periaqueductal gray on conditional and unconditional responding to threatening and aversive stimuli. *Neurosci*. 2012; 216:82–93.
- Halladay LR, Blair HT. Distinct ensembles of medial prefrontal cortex neurons are activated by threatening stimuli that elicit excitation vs. inhibition of movement. *J Neurophysiol*. 2015; 114:793–807. [PubMed: 25972588]
- Harnett NG, Wheelock MD, Wood KH, Ladnier JC, Mrug S, Knight DC. Affective state and locus of control modulate the neural response to threat. *Neuroimage*. 2015; 121:217–226. [PubMed: 26196669]
- Jiang ZC, Pan Q, Zheng C, Deng XF, Wang JY, Luo F. Inactivation of the prelimbic rather than infralimbic cortex impairs acquisition and expression of formalin-induced conditioned place avoidance. *Neurosci Lett*. 2014; 569:89–93. [PubMed: 24726402]
- Johansen JP, Herry C. Encoding of fear learning and memory in distributed neuronal circuits. *Nat Neurosci*. 2014; 17:1644–54. [PubMed: 25413091]
- Killcross S, Robbins TW, Everitt BJ. Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. *Nature*. 1997; 388:377–380. [PubMed: 9237754]
- Lee AT, Vogt D, Rubenstein JL, Sohal VS. A class of GABAergic neurons in the prefrontal cortex sends long-range projections to the nucleus accumbens and elicits acute avoidance behavior. *J Neurosci*. 2014; 34:11519–25. [PubMed: 25164650]
- Laurent V, Westbrook RF. Inactivation of the infralimbic but not the prelimbic cortex impairs consolidation and retrieval of fear extinction. *Learn Mem*. 2009; 16:520–9. [PubMed: 19706835]
- Lazaro-Munoz G, LeDoux JE, Cain CK. Sidman instrumental avoidance initially depends on lateral and basal amygdala and is constrained by central amygdala-mediated Pavlovian processes. *Biol Psychiatry*. 2010; 67:1120–7. [PubMed: 20110085]
- LeDoux JE, Iwata J, Cicchetti P, Reis DJ. Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *J Neurosci*. 1988; 8:2517–2529. [PubMed: 2854842]
- Maddux JM, Holland PC. Effects of dorsal or ventral medial prefrontal cortical lesions on five-choice serial reaction time performance in rats. *Behav Brain Res*. 2011; 221:63–74. [PubMed: 21376088]
- Maren S. The amygdala, synaptic plasticity, and fear memory. *Ann N Acad Sci*. 2003; 985:106–13.
- Maren S, Phan KL, Liberzon I. The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat Rev Neurosci*. 2013; 14:417–28. [PubMed: 23635870]
- Martinez RC, Gupta N, Lazaro-Munoz G, Sears RM, Kim S, Moscarello JM, LeDoux JE, Cain CK. Active vs. reactive threat responding is associated with differential c-Fos expression in specific regions of amygdala and prefrontal cortex. *Learn Mem*. 2013; 20:446–52. [PubMed: 23869027]
- Miller EK, Cohen JD. An integrative theory of prefrontal cortex function. *Annu Rev Neurosci*. 2001; 24:167–202. [PubMed: 11283309]
- Mobbs D, Marchant JL, Hassabis D, Seymour B, Tan G, Gray M, Petrovic P, Dolan RJ, Frith CD. From threat to fear: the neural organization of defensive fear systems in humans. *J Neurosci*. 2009; 29:12236–12243. [PubMed: 19793982]

- Mobbs D, Petrovic P, Marchant JL, Hassabis D, Weiskopf N, Seymour B, Dolan RJ, Frith CD. When fear is near: threat imminence elicits prefrontal-periaqueductal gray shifts in humans. *Science*. 2007; 317:1079–1083. [PubMed: 17717184]
- Moorman DE, Aston-Jones G. Prefrontal neurons encode context-based response execution and inhibition in reward seeking and extinction. *Proc Natl Acad Sci USA*. 2015; 112:9472–7. [PubMed: 26170333]
- Moscarello JM, LeDoux JE. Active avoidance learning requires prefrontal suppression of amygdala-mediated defensive reactions. *J Neurosci*. 2013; 33:3815–23. [PubMed: 23447593]
- Mowrer OH, Lamoreaux RR. Fear as an intervening variable in avoidance conditioning. *J Comp Psychol*. 1946; 39:29–50. [PubMed: 21018303]
- Orsini CA, Kim JH, Knapska E, Maren S. Hippocampal and prefrontal projections to the basal amygdala mediate contextual regulation of fear after extinction. *J Neurosci*. 2011; 31:17269–77. [PubMed: 22114293]
- Orsini CA, Maren S. Neural and cellular mechanisms of fear and extinction memory formation. *Neurosci Biobehav Rev*. 2012; 36:1773–802. [PubMed: 22230704]
- Ragozzino ME, Detrick S, Kesner RP. Involvement of the prelimbic-infralimbic areas of the rodent prefrontal cortex in behavioral flexibility for place and response learning. *J Neurosci*. 1999; 19:4585–94. [PubMed: 10341256]
- Ragozzino ME, Kim J, Hassert D, Minniti N, Kiang C. The contribution of the rat prelimbic-infralimbic areas to different forms of task switching. *Behav Neurosci*. 2003; 117:1054–65. [PubMed: 14570554]
- Ramirez F, Moscarello JM, LeDoux JE, Sears RM. Active avoidance requires a serial basal amygdala to nucleus accumbens shell circuit. *J Neurosci*. 2015; 35:3470–7. [PubMed: 25716846]
- Rich EL, Shapiro M. Rat prefrontal cortical neurons selectively code strategy switches. *J Neurosci*. 2009; 29:7208–19. [PubMed: 19494143]
- Richard JM, Berridge KC. Prefrontal cortex modulates desire and dread generated by nucleus accumbens glutamate disruption. *Biol Psychiatry*. 2013; 73:360–70. [PubMed: 22981656]
- Ronzoni G, Anton M, Mora F, Segovia G, Del Arco A. Infralimbic cortex controls the activity of the hypothalamus-pituitary-adrenal axis and the formation of aversive memory: Effects of environmental enrichment. *Behav Brain Res*. 2016; 297:338–44. [PubMed: 26518332]
- Sierra-Mercado D, Padilla-Coreano N, Quirk GJ. Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology*. 2011; 36:529–38. [PubMed: 20962768]
- Slattery DA, Neumann ID, Cryan JF. Transient inactivation of the infralimbic cortex induces antidepressant-like effects in the rat. *J Psychopharmacol*. 2011; 25:1295–303. [PubMed: 20530589]
- Sotres-Bayon F, Diaz-Mataix L, Bush DE, LeDoux JE. Dissociable roles for the ventromedial prefrontal cortex and amygdala in fear extinction: NR2B contribution. *Cereb Cortex*. 2009; 19:474–82. [PubMed: 18562331]
- Sotres-Bayon F, Quirk GJ. Prefrontal control of fear: more than just extinction. *Curr Opin Neurobiol*. 2010; 20:231–235. [PubMed: 20303254]
- Sullivan RM, Gratton A. Behavioral effects of excitotoxic lesions of ventral medial prefrontal cortex in the rat are hemisphere-dependent. *Brain Res*. 2002; 927:69–79. [PubMed: 11814433]
- Tarpley JW, Shlifer IG, Halladay LR, Blair HT. Conditioned turning behavior: a Pavlovian fear response expressed during the post-encounter period following aversive stimulation. *Neurosci*. 2010; 169:1689–1704.
- Vianna DM, Landeira-Fernandez J, Brandao ML. Dorsolateral and ventral regions of the periaqueductal gray matter are involved in distinct types of fear. *Neurosci Biobehav Rev*. 2001; 25:711–719. [PubMed: 11801296]
- Walker DL, Cassella JV, Lee Y, De Lima TC, Davis M. Opposing roles of the amygdala and dorsolateral periaqueductal gray in fear-potentiated startle. *Neurosci Biobehav Rev*. 1997; 21:743–53. [PubMed: 9415899]

Wall PM, Blanchard RJ, Yang M, Blanchard DC. Differential effects of infralimbic vs. ventromedial orbital PFC lidocaine infusions in CD-1 mice on defensive responding in the mouse defense test battery and rat exposure test. *Brain Res.* 2004; 1020:73–85. [PubMed: 15312789]

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SIGNIFICANCE STATEMENT

Anxiety disorders such as post-traumatic stress disorder (PTSD) are characterized by excessive, and often inappropriate responses to aversive stimuli. By understanding the neural mechanisms underlying different responses to threat, and the way by which defensive behaviors are selected, we will gain insight into improved treatments for disorders like PTSD. This study provides evidence that the infralimbic cortex (IL) specifically mediates defensive behaviors elicited by imminent threat, such as flight, but not defensive behavior during more distant threat, like freezing. These results suggest that IL may serve as a potential therapeutic target for reducing excessive responding to aversive stimuli.

Figure 1. Histological reconstruction of intracranial infusion sites

A, Representative example of IL cannulation using fluorescent muscimol (red) against DAPI counterstain (blue) to visualize cannula tip location. **B**, Reconstructed cannula tip placements in mPFC (n=30 per hemisphere) at +2.5 mm anterior to bregma are overlaid on a coronal template from the atlas of Paxinos and Watson (1997); symbols indicate rats infused with MUS (●) or PTX (□). IL=infralimbic cortex; PL=prelimbic cortex; AC=Cingulate cortex.

Figure 2. Movement suppression and turning bias in drug-free animals

A, Left graph shows trial-by-trial averages of CS movement suppression ratio (R_{CS}) from pre- (PreSh) and post-shock (PostSh) trials for all rats ($n=30$) on the drug free day prior to bilateral MUS or PTX infusion; bar graphs at right show mean R_{CS} (black) during PreSh and PostSh trials, as well as mean US movement suppression ratio (R_{US} ; white) during the CX period of PreSh versus PostSh trials. **B**, Left, same as 'A' except that mean turning velocity is plotted on the ordinate (positive and negative velocities indicate turning away from or toward the trained eyelid, respectively); bar graphs at right show mean turning bias during CX and CS periods of PreSh and PostSh trials. In all graphs, symbols indicate significance levels for Newman-Keuls posthoc comparisons; symbols in 'A' denote comparisons to .50.

while symbols in '**B**' denote comparisons of CX versus CS in trial graphs at left, or of PreSh versus PostSh in bar graphs at right (***= $p < .001$, **= $p < .01$, *= $p < .05$, ns=not significant).

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Figure 3. Effects of MUS and PTX on CMI versus CME responses

In all graphs, DF data is plotted by open symbols and drug infusion data is plotted by filled symbols. **A**, The CS significantly suppressed movement ($R_{CS} < .5$) before and after MUS infusions, but not after PTX infusions; asterisks denote significance levels for z-test comparisons against $R_{CS} = .5$. **B**, Defensive turning bias during the CS period of pre- and post-shock trials following MUS versus PTX infusions; asterisks denote significance levels for z-test comparisons against zero turning bias. **C**, US-evoked reflex responses following MUS and PTX; carat symbols denote significance levels for paired Newman-Keuls tests comparing movement speed during CX versus US for post-shock trials ($^{***}p < .001$). **D**, Shock-induced movement suppression (R_{US}) scores following infusions of MUS and PTX; asterisks denote significance levels for z-test comparisons against $R_{US} = .5$. Z-score significance levels in graphs A, B, and D are denoted by $^{***}p < .001$, $^{**}p < .01$, $^{*}p < .05$.

Figure 4. Effects of MUS and PTX on CS-evoked turning during post-shock trials

Each bar shows the mean and standard error of the within-subject change in turning bias after drug infusion, compared to the drug-free session on the previous day; ** $p < .01$, † $p = .06$. Data for PTX includes $n = 16$ subjects (outliers included).