Prefrontal microcircuit underlies contextual learning after hippocampal loss

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Edited by Richard F. Thompson, University of Southern California, Los Angeles, CA, and approved April 17, 2013 (received for review January 25, 2013)

Specific brain circuits have been classically linked to dedicated functions. However, compensation following brain damage suggests that these circuits are capable of dynamic adaptation. Such compensation is exemplified by Pavlovian fear conditioning following damage to the dorsal hippocampus (DH). Although the DH normally underlies contextual fear and fear renewal after extinction, both can be learned in the absence of the DH, although the mechanisms and nature of this compensation are currently unknown. Here, we report that recruitment of alternate structures, specifically the infralimbic and prelimbic prefrontal cortices, is required for compensation following damage to the hippocampus. Disconnection of these cortices in DH-compromised animals and immediate early gene induction profiles for amygdala-projecting prefrontal cells revealed that communication and dynamic rebalancing within this prefrontal microcircuit is critical. Additionally, the infralimbic cortex normally plays a role in limiting generalization of contextual fear. These discoveries reveal that plasticity through recruitment of alternate circuits allows the brain to compensate following damage, offering promise for targeted treatment of memory disorders.

anxiety | recovery of function | amnesia | medial prefrontal cortex

A widely accepted view is that the brain is comprised of multiple independent circuits dedicated to performing specific functions and encoding specific information. This view is exemplified in the field of learning and memory, where it is held that different circuits specialize in integrating and storing different classes of memories (1). However, studies looking at learning following brain damage clearly demonstrate that the brain can also behave dynamically. For example, in Pavlovian fear conditioning, contextual memories can be formed in the absence of circuits classically thought to underpin integration and storage of information about an animal's environment or context (2).

In fear conditioning, contexts play two key roles in controlling fear learning and expression. First, a context can act as a conditional stimulus (CS), to which fear can be directly conditioned when an aversive experience, such as a foot shock [unconditional stimulus (US)] is signaled by the context. Second, contexts can modulate responding to a discrete cue, such as a tone-CS, which has acquired multiple meanings. For example, in extinction, a tone previously conditioned to elicit fear is presented in the absence of the aversive foot shock US, such that the conditional fear response begins to extinguish. However, because extinction is not an erasure of the original fear but rather new learning that interferes with retrieval of the original fear memory, an extinguished cue has multiple meanings (shock/no shock), which compete for behavioral expression (3). This competition is mediated by context, as fear renewal occurs when an extinguished stimulus is presented outside of the extinction context (4).

These context-sensitive effects provide excellent models to test the flexibility of learning and memory systems following damage, as both have been shown to require the dorsal hippocampus (DH). Specifically, the DH is thought to normally underlie contextual fear memories, as posttraining DH damage results in retrograde amnesia for recently acquired contextual fear memories (5). Importantly, however, animals that suffer damage to the hippocampus before conditioning show a striking ability to overcome hippocampal loss provided they are given adequate training (2). This ability to overcome anterograde amnesia suggests that plasticity in the absence of the hippocampus allows the brain to compensate for contextual fear memory formation (6). This pattern of findings is paralleled by fear renewal after extinction, as posttraining lesions of the DH cause a deficit in fear renewal (7, 8), whereas impairments due to pretraining lesions can be overcome (7, 9).

One possibility is that rats recruit alternate circuitry to compensate following damage to primary memory structures, such as the DH. Although compensation for DH damage has been well documented, the identity of compensatory structures is unknown. This question is of vital importance, as it has the potential to generate a broader understanding of how different anatomical structures within the fear circuit normally interact and how such dynamic interplay could allow for compensation following brain damage. Successful identification of regions responsible for hippocampal compensation could provide sites to target for the treatment of memory loss-related disorders.

We hypothesized that the medial prefrontal cortex (mPFC) could potentially be a site of compensation in the absence of the DH for several reasons. First, the mPFC has already been identified as a key structure underlying the long-term storage of remote contextual fear memories, suggesting that under normal conditions, contextual fear is comprised and controlled by a hippocampal-prefrontal-amygdala circuit (10, 11). Moreover, an mPFC-hippocampus-amygdala circuit has been implicated in controlling fear extinction (12–14), indicating that this region has the ability to modulate fear responses generated in the amygdala. Second, the mPFC has been implicated in spatially sensitive, hippocampus-dependent working memory tasks (15), and neuronal ensembles in the mPFC are thought to encode rich contextual representations (16, 17), suggesting that the mPFC is capable of processing spatial information that would otherwise be encoded by the hippocampus. These findings, combined with studies showing that the mPFC is phase-locked to hippocampal theta (18) and that the two are synchronized during spatial tasks (19), suggest that the mPFC and hippocampus are intimately connected with regard to context-sensitive learning and memory.

Importantly, the mPFC sits in an anatomical position that might allow it to mediate context-sensitive fear following loss of the

Author contributions: M.Z., S.B., B.V., and M.S.F. designed research; M.Z., T.A.H., R.Z.B., and A.A. performed research; A.A. and B.V. contributed new reagents/analytic tools; M.Z. analyzed data; and M.Z., S.B., B.V., and M.S.F. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1301691110/-/DCSupplemental.

hippocampus. Both the infralimbic (IL) and prelimbic (PL) subregions of the mPFC normally receive input from the ventral hippocampal formation (20, 21), as well as from extrahippocampal regions involved in processing information about the environment [e.g., entorhinal cortex (22); retrosplenial cortex; and perirhinal cortex (20)]. Finally, any region compensating for the DH in contextual fear conditioning must be able to control fear responses. IL and PL both send projections to the amygdala complex (23–25).

To test the role of the mPFC in context-sensitive fear formed in the absence of the DH, we developed a unique behavioral paradigm that allowed us to assess both context fear and fear renewal within the same animal. We found that the compensatory context fear and fear renewal found in DH-damaged animals was abolished in animals that suffered additional bilateral damage to either the PL or IL. Blocking communication between the IL and PL cortices using a disconnection approach replicated the profound deficit in hippocampal compensation. Immediate early gene and tract tracing analyses in DH-lesioned animals revealed that compensatory context fear correlated with a dramatic rearrangement in the balance of activity between the PL and IL cortices in amygdala-projecting cells. Additionally, we discovered that IL lesions on their own enhanced generalization of contextual fear. Collectively, these data identify a microcircuit within mPFC that is required for context-sensitive fear in the absence of the DH.

Results

DH Lesions Produce Retrograde but Not Anterograde Amnesia. To investigate the structures underlying context-sensitive fear formed in the absence of the DH, we first sought to replicate the compensation effect (2). Rats ($n = 8$) were tone fear conditioned, given excitotoxic lesions of the DH or sham lesions, and tested for context fear (Fig. 1*A*). Consistent with previous findings (2), we found that rats showed a significant retrograde amnesia for context fear when DH lesions where made after training ($t = 4.050$; $P < 0.01$; Fig. 1*B*). Following this initial context test, rats were fear conditioned again (retraining) and retested such that their prior "post"training lesions were now "pre"training lesions with respect to retraining (Fig. 1*A*). Differences in context fear between sham and DH rats were attenuated, indicating that DH-lesioned animals were able to overcome anterograde amnesia for context fear ($t = 1.654$; $P > 0.05$; Fig. 1*B*).

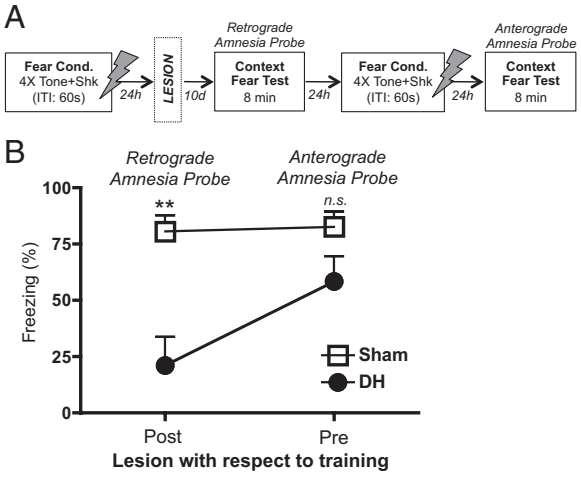

Fig. 1. Retrograde but not anterograde amnesia for the same contextual information. (*A*) Experimental design. (*B*) Lesions made to the DH posttraining resulted in a significant reduction of context fear compared with sham controls (retrograde amnesia). When rats were retrained in the absence of the DH, they demonstrated contextual fear expression at test that was equivalent to their sham counterparts (attenuated anterograde amnesia). Results are presented as mean ± SEM percent freezing.**$P < 0.01$; n.s., not significant; shk, shock.

Context Fear in the Absence of the DH Requires IL and PL. To test whether the IL or PL could be potential sites of compensation, animals were given double lesions of either the IL+DH ($n = 15$) or PL+DH ($n = 15$) and compared to animals with lesions to only the DH ($n = 16$), IL ($n = 20$), PL ($n = 17$), or sham controls ($n = 24$; lesions depicted in Fig. S1). We chose to separately examine IL and PL contributions to DH compensation because these regions have been shown to play opposing roles in modulating fear expression (13, 26). We assessed compensation using lesions of DH as opposed to ventral hippocampus (VH) because DH lesions produce the same compensatory effects as lesions to the entire hippocampal formation (2), and the DH has been directly implicated in spatial and episodic memory (27). Additionally, the VH, but not the DH, projects monosynaptically to mPFC, and we wanted to avoid changes that could be a result of direct deafferentation. We hypothesized that if either the IL or PL was recruited to compensate in the absence of the DH, then pretraining damage to either would result in a failure to express context fear in DH-compromised animals.

Following surgery, rats underwent tone fear conditioning, context testing, tone extinction, and tone fear renewal testing using a protocol we developed to test for direct context fear and context-modulated fear renewal within the same animal (Fig. 2*A*). All animals acquired significant tone fear (indexed by percent freezing; $F_{(3,303)} = 239.9$; $P < 0.0001$; Fig. S2*A*), regardless of their lesion condition ($F_{(5,303)} = 1.294$; $P > 0.05$). Importantly, baseline (BL) freezing to the context before the start of fear conditioning was <1%, demonstrating that the lesions alone did not result in nonspecific freezing to novel environments (Fig. S2*A*).

The following day, rats were tested for context fear in the training context or fear generalization in a novel context (Fig. 2*B*). Two-way ANOVA revealed a main effect of lesion ($F_{(5,95)} = 8.669$; $P < 0.0001$), context ($F_{(1,95)} = 18.58$; $P < 0.0001$), and their interaction ($F_{(5,95)} = 4.179$; $P < 0.01$). As previously demonstrated, context fear in DH-lesioned animals was not significantly different then shams ($t = 1.629$; $P > 0.05$) (2, 28). DH-lesioned rats froze more in the training compared with novel context ($t = 3.525$; $P < 0.01$), which deviates from studies showing that the DH is required for context discrimination (29). However, in those studies, the contextual discrimination was more difficult than that used here. Rodents with hippocampal impairments easily discriminate very different contexts even though they have trouble discriminating very similar ones (5, 29, 30). Crucially, the compensatory context fear seen in DH animals was lost in double-lesioned animals (DHIL vs. DH: $t = 3.229$; $P < 0.01$; DHPL vs. DH: $t = 3.604$; $P < 0.01$), suggesting that both regions are required for context fear in the absence of the DH. This reduction in freezing could not be explained by a failure to freeze, as animals with double lesions showed normal acquisition (Fig. S2*B*).

Interestingly, rats with IL lesions were unable to differentiate between a context they were shocked in and a novel context, such that they froze no differently between the two ($t < 1$). This effect was due to a combination of reduced freezing in the training context (IL vs. sham: $t = 2.665$; $P < 0.05$) and enhanced freezing in the novel context. This failure to distinguish between contexts in IL-lesioned rats is surprising given that these animals have an intact hippocampus and thus might be expected to discriminate between two contexts (29). Low baseline freezing to the context before fear conditioning (Fig. S2*B*) suggests that an IL lesion on its own does not cause freezing to a novel context. Additionally, the failure for IL-lesioned rats to exhibit an anxiety-like phenotype, as indexed by elevated plus maze (EPM) testing (Fig. S3), suggests that their inability to discriminate was not caused by a general increase in anxiety. This failure to appropriately and selectively freeze in a shocked context rather than a novel

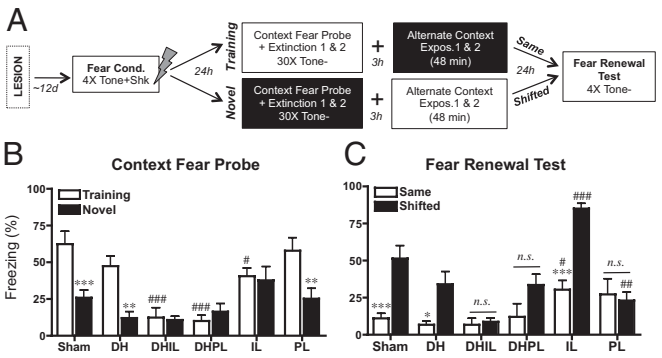

Fig. 2. Context fear and renewal in lesioned rats. (*A*) Experimental design aimed at probing both the direct (Context Test) and indirect (Fear Renewal Test) contribution of context to fear learning and memory. Black and unfilled rectangles refer to different contexts; physical contexts were counterbalanced. Same and shifted refer to whether extinction occurred in the same or novel context with respect to the training/test context. Acquisition and extinction data are displayed in Fig. S2. (*B*) Test for context fear probed during the 180-s baseline (BL) period before extinction 1. Context fear for DH rats was not reliably different then sham rats indicating hippocampal compensation. Context fear was abolished in animals with double DHIL and DHPL lesions. Sham, DH, and PL lesion groups showed significantly reduced freezing when shifted to a novel context, whereas IL groups froze similarly when tested in the same context as acquisition or shifted to a novel context. (*C*) Test for fear renewal after extinction. Fear renewal was observed in the sham, DH, and IL groups but lost in the DHIL and DHPL groups. IL lesions produced poor extinction retention in the same and shifted condition compared with shams, and PL lesions resulted in no renewal and a deficit in fear responding in the shifted condition. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, $^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$, $^{\#}P < 0.05$; n.s., not significant. Results are presented as mean ± SEM percent freezing. ITI, intertrial interval; trial, tone-shock pairing.

context was seen in IL- but not PL-lesioned rats. PL animals, like sham controls, expressed appropriate levels of high contextual fear in the training context coupled with significantly lower fear in the novel context ($t = 3.344$; $P < 0.01$).

Context-Modulated Fear Renewal in the Absence of the DH Requires IL and PL. Following the probe test for contextual fear, animals underwent extinction training (Fig. S2*C*). DH-lesioned rats displayed enhanced rates of within-session extinction ($t = 6.759$; $P < 0.001$), and IL-lesioned rats showed a retarded rate of extinction ($t = 4.026$; $P < 0.01$). However, all groups reached similar low levels of fear by the end of extinction training (Fig. S2*D*). Following extinction, rats were tested for context-modulated fear renewal. Renewal was assessed by comparing animals tested in the same vs. shifted context (with respect to the extinction context), thereby controlling for any differences due to surgical condition (e.g., baseline fear, spontaneous recovery).

A two-way ANOVA found a main effect of context ($F_{(1,95)} = 35.20$; $P < 0.0001$), lesion ($F_{(5,95)} = 12.05$; $P < 0.0001$), and their interaction ($F_{(5,95)} = 5.530$; $P < 0.001$; Fig. 2*C*). Significant fear renewal was displayed by sham controls ($t = 4.911$; $P < 0.001$), as well as rats with DH damage ($t = 2.710$; $P < 0.05$), replicating our previous results (7). However, compensatory fear renewal in the absence of the DH was lost for animals with additional lesions to the IL or PL (DHIL: $t = 0.1904$; $P > 0.05$; DHPL: $t = 2.059$; $P > 0.05$), mimicking the pattern of findings observed for context fear. It should be noted, however, that the loss of compensation for DHIL animals was more pronounced then that observed in DHPL rats. Importantly, baseline was <5% and did not differ between groups (Fig. S4), showing that alternate context exposure reduces baseline context fear before test. Rats with IL-only lesions demonstrated significant fear renewal ($t = 5.955$; $P < 0.001$). However, IL rats also showed impaired extinction memory recall, as they froze significantly more to the tone presented in the extinction context compared with shams (i.e., the contrast between Same-Sham and Same-IL is reliable, $t = 2.339$; $P < 0.05$; Fig. 2*C*). These results are consistent with findings by Quirk and colleagues (31, 32).

Last, animals with PL-only damage failed to mount a renewal response ($t = 4265$; $P > 0.05$), which can be partially explained by reduced freezing in the shifted condition compared with sham controls ($t = 3.095$; $P < 0.01$), consistent with studies implicating the PL in fear expression (33). A summary of the various behavioral effects obtained with each lesion condition relative to sham controls is provided in Table S1.

Compensatory Context Fear Is Characterized by Changes in mPFC cfos Induction. Our behavioral results revealed that in the absence of the DH, both the IL and PL are required for rats to express compensatory context fear and fear renewal. That is, loss of only the IL or only the PL prevented DH-lesioned rats from expressing context-sensitive fear. To investigate whether this role for the IL and PL corresponds to alterations in neuronal activity within these regions, we examined cfos expression as an indirect marker of neuronal activation.

Rodents with pretraining DH lesions ($n = 5$) or sham controls ($n = 5$) were fear conditioned and tested for context fear (Fig. 3*A*). Animals with DH lesions and shams froze similarly during testing ($t < 1$; Fig. 3*B*). Brains were extracted and sections were processed for cfos. The total number of cells positive for cfos (cfos+) in the IL and PL cortex was quantified using rigorous unbiased stereological methods (34). Additional home cage (HC+) control rats were added to the cfos analyses to provide a baseline level of cfos expression in the IL and PL for each surgical condition ($n = 8$). HC+ rats were trained identically to experimental animals except the

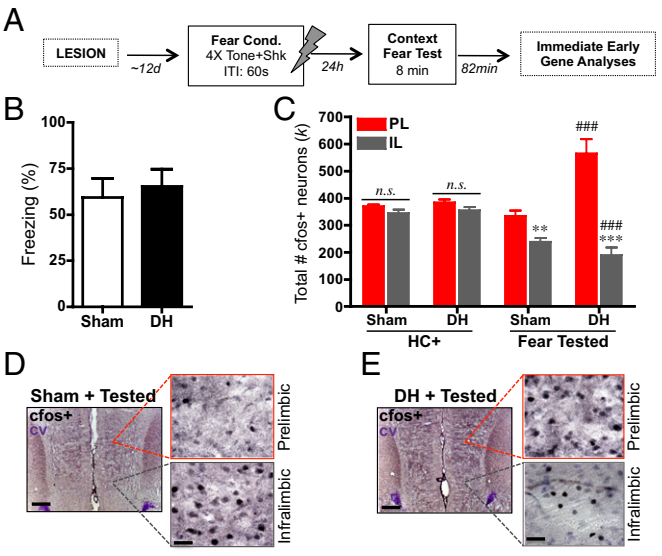

Fig. 3. Cfos expression in the IL and PL regions following compensatory context fear expression. (*A*) Experimental design. (*B*) Context fear test. DH-lesioned rats showed similar context fear as their sham counterparts (compensation). (*C*) Total number of cells immunoreactive for cfos (estimated using unbiased stereology) in the PL (red bars) and IL (gray) of Sham (*Left*) and DH (*Right*) rats that were either homecaged controls (HC+) or tested for context fear (Fear Tested). Fear testing disrupted the balance between PL and IL activity, with reduced IL activation for both shams and DH animals. Compensation and rebalancing in DH rats was additionally driven by increased activation of PL compared with shams and HC+ rats. (*D*) Representative micrograph and magnified insets showing cfos expression in both the IL and PL cortices in sham (*D*) and DH lesioned animals (*E*). (Scale bars, 50 μm for the mPFC overviews and 15 μm for inset images.) Results are presented as mean ± SEM, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{\#\#\#}P < 0.001$; n.s., not significant.

context test was omitted, therein controlling for any changes in cfos expression due to prior conditioning.

Analyses of cfos expression revealed an effect of lesion ($F_{(1, 28)} = 7.55$; $P < 0.05$) and the mPFC subregion analyzed ($F_{(1, 28)} = 48.64$; $P < 0.0001$; Fig. 3*C*). Post hoc analyses found that HC+ controls showed similar levels of cfos activation in the PL and IL ($t < 1$), demonstrating that the mPFC normally maintains a balance of activity between its subregions. This balance of activity between PL and IL was disrupted in experimental animals tested for context fear regardless of whether their DH was intact ($t = 4.56$; $P < 0.01$) or damaged ($t = 10.90$; $P < 0.001$). Thus, the retrieval and/or expression of a context fear memory induces and suppresses cfos activation in the PL and IL, respectively.

Animals trained in the absence of the DH and tested for compensatory context fear showed significant increase in PL activity ($t = 4.78$; $P < 0.001$) coupled with a decrease in IL activity ($t = 4.38$; $P < 0.001$) compared with their HC+ surgical counterparts. Moreover, PL activity in fear conditioned rats was elevated in DH compared with sham rats ($t = 5.062$; $P < 0.001$), revealing that only in a brain-damaged animal does fear conditioning drive activation of PL neurons above their baseline level. Shams tested for context fear failed to show any difference in activation of PL compared with their HC+ counterparts ($t < 1$). Collectively, these results imply that under normal conditions, context fear expression disrupts the balance of PL-IL activity because of decreased activation of IL cortex (Fig. 3*D*), but that under compensating conditions, this difference becomes exaggerated such that an additional significant induction of cfos in PL occurs (Fig. 3*E*).

Compensation-Induced Changes in mPFC cfos Occurs in BLA-Projecting Cells. Unbiased stereology provides a very accurate population estimate of cfos-expressing neurons; however, it does not tell us whether changes in mPFC cfos expression occurred in basolateral amygdala (BLA)-projecting neurons that regulate fear. To address this issue, sham ($n = 4$) or DH-lesioned ($n = 4$) animals were given intra-BLA injections of the retrograde tracer fast blue (FB) and subjected to fear conditioning and testing that was otherwise identical to the initial cfos study (Fig. 4 *A*–*C*). Sections were processed for cfos (Fig. 4*D*). Analyses of the total number of BLA-projecting cells (FB+) that were also positive for cfos revealed that only animals that had engaged in compensatory context fear in the absence of the DH showed a significant difference between double labeled cells in the PL and IL (Fig. 4*E*; $t = 12.00$; $P < 0.001$). This rearrangement in mPFC cfos expression was comprised of a significant increase in PL activity ($t = 6.524$; $P < 0.001$) coupled with a decrease in IL activity ($t = 3.511$; $P < 0.01$). A reduction in the activation of IL cells projecting to GABAergic intercallated (ITC) cell clusters flanking the BLA (see Fig. S5) could potentially provide an additional contribution to compensation following hippocampal damage.

Cross-Talk Between the IL and PL Is Required for Compensation. Our behavioral results demonstrate that the IL and PL are both required for context-sensitive fear expression in the absence of the DH. However, our immediate early gene analyses and tract-tracing results demonstrate that compensatory context fear correlates with an increase in PL activity and a decrease in IL activity. The IL data seem somewhat paradoxical: if a decrease in IL activity underlies compensation, then why do DHIL-lesioned animals show no compensatory context fear, as a lesion should model a severe loss in activity? One possibility is that compensation requires IL and PL to interact, rather than behave independently, to influence BLA function and fear expression. Therefore, we tested whether cross-talk between the IL and PL was required for DH compensation using a disconnection design (Fig. 5*A*). Because the IL and PL are reciprocally connected (23, 24), we hypothesized that contralateral damage to these areas would disrupt inter–IL-PL communication but leave the ability for each structure to communicate with the amygdala intact. Thus, if the two structures work independently, then a disconnection between the two should permit compensation; however, if they operate in conjunction, then a disconnection would abolish compensatory context fear.

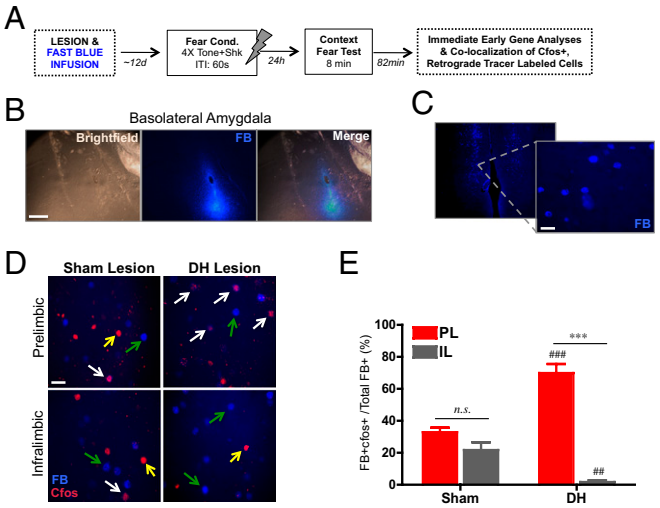

Fig. 4. Colocalization of cfos immunoreactivity with BLA-projecting neurons. (*A*) Experimental design. (*B*) Representative low-magnification bright field, fluorescent, and merged images of a successful FB injection hit (blue) in the BLA. (Scale bar, 200 μm.) (*C*) Representative low and high magnification of retrogradely labeled cell bodies (blue) in the IL and PL regions. (Scale bar, 20 μm.) (*D*) Representative high-magnification fluorescent frames of cells positive for FB (green arrows), cfos (red, yellow arrows), or both (white arrows) in the PL (*Upper*) and IL (*Lower*) cortex for a sham and DH rat. (Scale bar, 20 μm.) (*E*) Quantification of percent FB+ cells also positive for cfos in sham or DH conditions. Rebalancing of PL and IL activity in BLA-projecting was only found in DH-lesioned rats. DH rats also showed an increase in PL activation and decrease in IL activity compared with shams. Results are presented as mean ± SEM, $^{###}P < 0.001$, $^{##}P < 0.01$, $***P < 0.001$; n.s., not significant.

Animals with sham lesions ($n = 8$) were compared to animals with DH lesions plus contralateral lesions of the IL and PL ($n = 9$, DH+Contra; disconnected animals; Fig. 5*B*) or control ipsilateral lesions of the IL and PL ($n = 8$, DH+Ipsi; Fig. 5*C*) (counterbalanced across hemispheres) using the protocol that allows measurement of both context fear and fear renewal (Fig. 5*A*). The results from the context fear probe test (Fig. 5*D*) showed appropriate, discriminatory context fear in shams ($t = 6.60$; $P < 0.001$). Contextual fear was maintained in DH-lesioned rats with ipsilateral lesions of the IL-PL ($t = 4.34$; $P < 0.01$). However, context fear in the absence of the DH was lost in rats when damage to the IL and PL was contralateral to each other ($t = 5.31$; $P < 0.01$).

The subsequent test for tone fear (Fig. 5*E*) revealed that fear renewal was maintained in sham controls ($t = 4.23$; $P < 0.01$) and compensated for in DH+ipsi rats ($t = 3.83$; $P < 0.01$) but lost in DH+Contra animals ($t < 1$). These findings are consistent with those obtained for animals with double lesions in our original findings (Fig. 2), suggesting that compensatory context fear and context-modulated fear renewal requires an interaction between the IL and PL. Additionally, intact compensation in DH+Ipsi rats serves to control for the loss of context fear and renewal as a result of total brain damage, as ipsi- and contralateral surgeries produced the same amount of total tissue loss.

Discussion

Following extensive DH damage, the brain can invoke alternate circuitry to compensate (2, 7, 35), restoring some of the essential elements of context-sensitive learning and memory. In the present study, we identified the IL cortex, PL cortex, and cross-talk between the two as essential for this compensation. We also

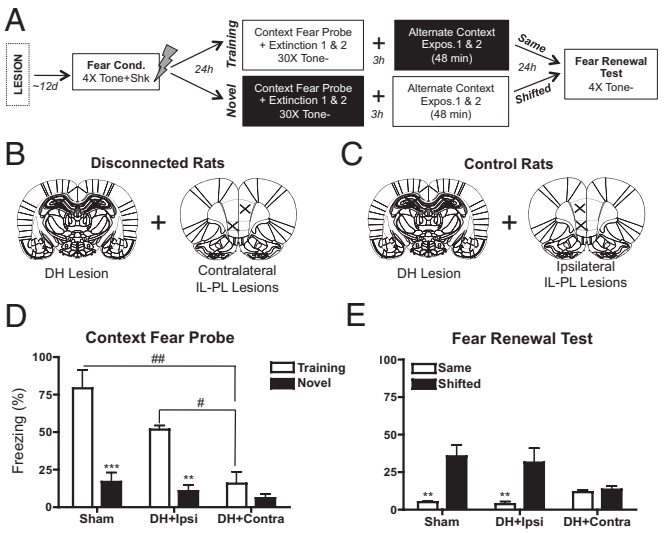

Fig. 5. Disconnecting the IL and PL in DH-lesioned animals blocks compensation. (*A*) Behavioral design of ipsilateral or contralateral IL and PL lesions given in addition to DH lesions. (*B*) Schematic diagram of IL-PL disconnection in a DH-lesioned rat. (*C*) Schematic diagram of a control rat with DH lesions and ipsilateral damage to IL-PL. (*D*) Test of direct context fear confirming that disconnected animals failed to compensate for DH damage, whereas ipsi animals show intact compensatory context fear. (*E*) Test for context-modulated fear renewal also showed a loss of fear renewal in lesioned animals with the IL-PL disconnected. Renewal was significant in sham and ipsi controls. Results are presented as mean ± SEM percent freezing, $***P < 0.001$, $**P < 0.01$, $^{\#}P < 0.5$, $^{\#\#}P < 0.01$.

uncovered the neural signature of this compensation: a silencing of BLA-projecting IL neurons complemented by an increase in the activation of PL neurons projecting to the BLA.

The focus on the mPFC in fear learning and memory has revolved largely around the role of the PL and the IL cortex in extinction, wherein these structures play opposing roles in fear excitation and inhibition, respectively (13, 26). In the present set of findings, we found that the IL and PL cortices are required for compensatory context-sensitive fear learning and memory following damage to the hippocampus for both fear renewal after extinction and context fear. This suggests that perhaps the IL and PL cortices play a role in fear that is not specific to extinction, but rather, to any context-sensitive form of fear. Accordingly, the role of the IL and PL cortex in fear expression after extinction would not be driven solely by the power of inhibitory processes but rather by the contextual encoding that occurs during extinction learning and context conditioning. This view expands the role of the mPFC to context-sensitive learning and memory systems in general rather than fear inhibition in particular. Such a role is supported by various behavioral paradigms testing other forms of context-sensitive learning and memory (36–39). This more general role for the mPFC in contextual processing is also parsimonious with its role in remote long-term contextual fear memories (10, 11) and the idea that the mPFC and hippocampus are continuously in communication to allow for the systems consolidation of contextual memories as they move from a recent to remote state (15, 40).

The loss of compensation when DH-lesioned animals have a disconnected IL-PL shows that these two mPFC regions do not behave independently but instead form an integrated micronetwork that works to produce behavior in a cohesive, unified manner. Indeed, compensation for hippocampal damage correlates with a rebalancing of activity within the mPFC that could not occur without communication between the IL and PL. Thus, the deficits in context-sensitive fear resulting from double lesions of the DH and either the IL or PL occur because these structures comprise a dynamic flexible mPFC microcircuit capable of compensation.

Our finding that in the absence of the IL, animals fail to distinguish between a dangerous, fear-conditioned context and a novel context suggests that this area is integral to the ability to determine whether fear should be expressed or inhibited in a particular environment, even when the DH is intact. The EPM data suggest that this is not due to exaggerated anxiety in IL rats following fear conditioning. Whether the IL functions specifically within the fear circuitry to control context-elicited fear expression and inhibition or whether the IL is required for the general ability of the hippocampus to form adequate contextual representations remains an open question. The finding that IL damage alone causes a loss of contextual discrimination supports a general role for the IL in context processing.

These findings also have important implications for a more general set of rules regarding compensation following brain damage (6). Previous findings have found that in the absence of the BLA, animals are able to form compensatory fear memories, provided they are given adequate training (41, 42). This compensation was shown to require the bed nuclei of the stria terminalis (43), a structure already implicated in fear-related behavior (44, 45) and positioned in an ideal neuroanatomical location to mediate contextual processing in the DH and midbrain regions controlling freezing (46, 47). The findings presented here share a striking similarity with these BLA-based effects. Collectively, these findings support the idea that the fear system is comprised of interconnected, highly parallel circuits that provide compensatory plasticity in the event that one structure is compromised (6).

Last, these findings have powerful clinical implications. The extent of recovery of brain function following injury such as stroke can be remarkable in some cases (48, 49), and there is potential for significant compensation during neurodegenerative diseases such as Alzheimer's (50, 51). Although several mechanisms of compensation have been proposed, the mechanisms underlying such compensation are unclear. Our study reveals that recruitment of alternate circuits provides one mechanism of brain compensation. Most views of compensation following tissue loss (e.g., stroke) think of compensation occurring in adjacent tissue (52, 53). A unique finding here is that compensation can occur in regions that are not directly proximal to the site of injury. By identifying the mPFC as a region of compensation following hippocampal damage and uncovering the neural signature of this compensation, these results open up the doors to the development of targeted approaches for the treatment of memory loss–related disorders due to brain damage, disease, or aging.

Materials and Methods

Animals. Subjects were naïve, adult male Long-Evans rats, initially weighing 270–300 g, purchased from Harlan. All procedures were in accordance with policy set and approved by the Institutional Animal Care and Use Committee of the University of California, Los Angeles, CA.

Surgery. NMDA-induced excitotoxic lesions were made to the IL, PL, DH, or a combination of the three. Rats were given 10–14 d of recovery before behavioral training.

Behavioral Testing. Animals received fear conditioning, extinction, and testing using two distinct physical "contexts" (Med Associates). Percent time freezing was scored by an automated motion tracking system calibrated to a trained observer (Med Associates).

Histology, Immunohistochemistry, and Retrograde Labeling. Standard procedures were used to verify the extent of lesion damage, stain for cfos expression, image, and count using unbiased stereology (Stereoinvestigator 7). Standard immunofluorescence techniques were used to colocalize cells positive for the retrograde tracer FB and cfos.

Statistical Analyses. Data were analyzed using between-subjects ANOVAs and repeated-measures (RM) ANOVAs where appropriate. Bonferroni-corrected pairwise comparisons were performed following significant findings ($P < 0.05$). Additional methodological details can be found in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank Mareike Kuypers for technical assistance and the laboratory of Dr. Marie F. Chesselet for generously providing the use of their unbiased stereology suite. We also thank Patricia A Quick, Bill Gruy, Geoffrey Towner, and Nicholas Kell for help and support. This work was supported by National Institutes of Health Grant R01 MH62122 (to M.S.F.), the National Science Foundation East Asia and Pacific Summer Institutes award (to M.Z.), the Australian Academy of Science (M.Z.), National Alliance for Research on Schizophrenia and Depression Young investigator Award 17532 (to S.B.), the Henry Roth Foundation (B.V.), Iain S. Gray Foundation (B.V.), Walter & Edith Sheldon (B.V.), and Tony & Vivian Howland-Rose (B.V.).

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