Dissociable contributions of the amygdala and ventral hippocampus to stress-induced changes in defensive behavior

Zachary T. Pennington[1], Alexa R. LaBanca[1], Patlapa Sompolpong[1], Zoe Christenson Wick[1], Yu Feng[1], Zhe Dong[1], Tristan Shuman[1], Denise J. Cai[1]

1. Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai

Correspondence: Denise.Cai@mssm.edu and Zachary.Pennington@mssm.edu

Abstract:

Severe stress can produce multiple persistent changes in defensive behavior. While much is known about the circuits supporting stress-induced associative fear responses, how circuit plasticity supports the broader changes in defensive behavior observed after severe stress remains unclear. Here, we find that stress-induced plasticity in the ventral hippocampus (vHC) and basolateral amygdala (BLA) support doubly dissociable defensive behavioral changes. Stress-induced protein synthesis in the BLA was found to support lasting enhancements in stress sensitivity but not enhancements in exploratory anxiety-related behaviors, whereas protein synthesis in the vHC was found to support enhancements in anxiety-related behavior but not enhancements in stress sensitivity. Like protein synthesis, neuronal activity of the BLA and vHC were found to differentially support the expression of these same defensive behaviors. Lastly, blockade of associative fear had no impact on stress-induced changes in anxiety-related behavior. These findings highlight that multiple memory-systems support stress-induced defensive behavior changes.

Keywords:

Basolateral amygdala, ventral hippocampus, stress, fear, anxiety, PTSD, memory, stress-enhanced fear learning, defensive behavior

INTRODUCTION

In immediate response to stressful and life-threatening events, animals display evolutionarily conserved defensive responses, including changes in heart rate and respiration, stress hormone release, as well as the behavioral initiation of fight, flight, and freezing [1-6]. If sufficiently strong, stressful events can also instantiate persistent changes in how animals interact with their environment. Perhaps most extensively studied are associative fear responses, in which animals engage in defensive behaviors such as freezing and/or flight when re-exposed to environmental cues present at the time of the initial stressful experience [1,7-12]. However, after severe stress, animals also display alterations in foraging and exploration in uncertain environments [3,5,13,14] (often referred to as anxiety-related behavior), as well as heightened responses to future stressful events [14-17]. These long-lasting defensive behavioral changes are fundamental to anxiety disorders, which include fear of stress-related cues, heightened stress responses, and reduced environmental engagement; and are frequently predated by the experience of severe stress [18-21].

It is often assumed that many of the defensive behavioral changes observed in the aftermath of stress are fundamentally associative in nature – animals could either be responding to cues that were directly present at the time of stress, or stimuli resembling these cues to some degree (i.e., stimulus generalization) [22-30]. For example, it is well-documented that startle responses are potentiated by the presence of associative fear cues [31,32], suggesting that associative stimuli may drive heightened responses to aversive events after stress. Moreover, it is possible that following stress, alterations in exploration in anxiety-related behavior tests such as the elevated-plus maze could be accounted for by shared features with the environment in which the stressor took place. Lastly, several reports document altered associative fear learning and generalization in humans with anxiety disorders [23,24,33-35]. In light of these findings, broad emphasis has been placed on associative learning processes governing the lasting consequences of stress. However, the explanatory reach of an associative framework has its limits. Pre-weanling rodents incapable of forming associative fear memories have been found to nevertheless display decreased exploration in exploratory anxiety-related behavior tests, as well as heightened responses to subsequent aversive stimuli following stressful experiences [14]. Moreover, extinguishing fear to stress-associated cues does not necessarily mitigate increased responses to new stressors [15,36,37]. These findings

highlight the persistence of some stress-induced behavioral phenotypes despite weak associative fear, indicating a potential dissociation. As such, it could be the case that multiple memory systems – associative and non-associative – support the enduring consequences of stress on defensive behavior. However, a direct biological dissociation of such memory systems has remained elusive. If discovered, this would have broad implications for the treatment of anxiety disorders, potentially explaining why treatments focused on associative processes are ineffective in some individuals [38-40].

Here, we explore the contributions of stress-induced plasticity within the ventral hippocampus (vHC) and basolateral amygdala (BLA) to the enduring impacts of stress on different defensive behaviors. Neuronal activity within both the BLA and vHC are well known to regulate defensive behaviors [8,41-48]. However, whether stress-induced plasticity within these structures act in concert to support a common defensive behavioral process, or whether they support distinct defensive behavior changes, is unclear. We find that plasticity within each brain region supports separate defensive behavior changes in response to stress, demonstrating unique functions of these structures, and supporting the view that multiple memory systems underly stress-induced defensive behavioral changes.

RESULTS

Acute stress produces multiple, lasting, changes in defensive behavior.

We first sought to establish a behavioral protocol in which a single acute stressor produces lasting changes in multiple defensive behaviors, adapting a prior model that has been used extensively in mice and rats [14,15,49-51] (Fig 1A). Animals were placed in a distinct environment where they received 10 foot-shocks during a 10-min period ('trauma', T), or were placed in the same environment but did not receive foot-shocks ('no-trauma', NT). A week later, multiple defensive behaviors were assessed. In the light-dark box, an exploratory test that captures rodents' natural avoidance of well-lit places and is sensitive to anxiolytics [52,53], trauma-exposed animals showed increased anxiety-related behavior, reflected in more time spent in the dark side of the light-dark box (Fig 1C. $t_{47.5}$=5.5, p<0.001). To assess associative fear, animals were returned to the trauma environment. As expected, trauma-exposed animals spent large amounts of time freezing (Fig 1D. $t_{40.7}$=11.5, <0.001). Lastly, we assessed the animals' stress sensitivity by placing the animals in a novel environment, in which they showed very little initial freezing at baseline (0.1% baseline freezing in no-trauma vs 1.3% in trauma; $t_{42.9}$=2.8, p<0.01). A loud auditory startle stimulus was then presented. When returned to this environment the next day, trauma animals showed substantially more freezing (Fig 1E. $t_{47.6}$=5.79, <0.001). Notably, although this phenomenon is often termed stress-enhanced fear learning, learning rate analysis suggests the enhanced learning likely reflects heightened sensitivity to the aversive stimulus as opposed to an increase in learning rate.

Next, as a preliminary means of establishing that these defensive behaviors convey information about unique biobehavioral processes, we correlated these phenotypes in a large group of trauma-exposed animals, as high inter-phenotype correlations would suggest shared biological origins. We found minimal to no relationship between behavioral tests (Fig 1F-H. trauma recall and light-dark: R^2=0.04, p=0.19; trauma recall and novel stressor: R^2=0.19, p<0.01; light-dark and novel stressor: R^2=0.13, p=0.32). Though each of these measures is likely subject to imperfect test-retest reliability, these findings nevertheless suggest that these phenotypes may be independently governed.

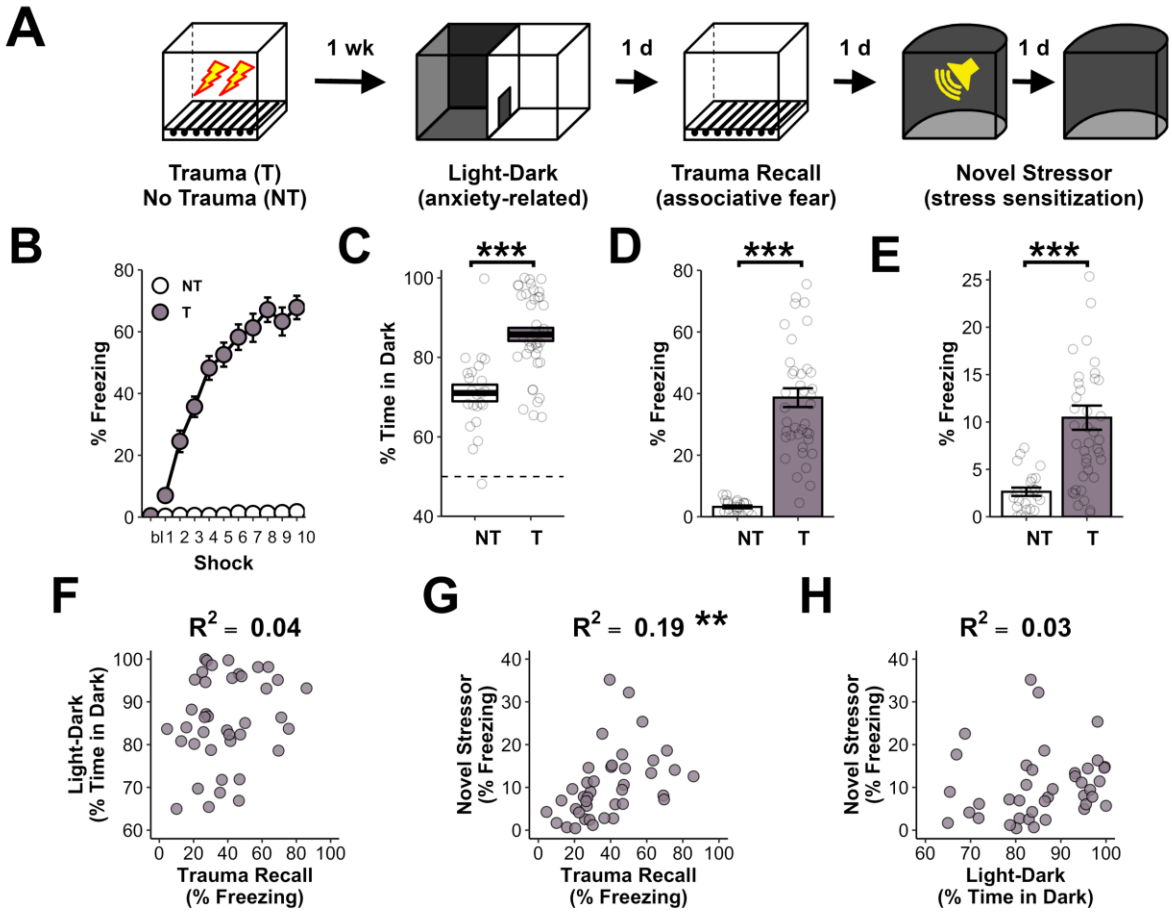

Figure 1: Acute stress produces multiple, lasting, changes in defensive behavior. Animals were exposed to a distinct environment in which they received 10 foot-shocks (trauma, T) or were placed in the same environment and received no foot-shocks (no-trauma, NT). A week later, they were tested in the light-dark test of anxiety-related behavior, associative fear of the trauma environment, and their response to a novel stressor in a new environment (A). Trauma-exposed animals displayed high levels of post-shock freezing during the trauma (B), increased time in the dark in the light-dark test (C), strong associative fear of the trauma environment (D), and increased fear of the novel stressor environment when placed in this environment on the final test day (E). Examining a large set of trauma-exposed animals, these post-trauma phenotypes were found to be poorly correlated, suggesting they reflect unique biological processes (F-H). NT=23 & T=40 mice. $p<.05$ (*), $p<0.01$ (**), $p<0.001$ (***).

Stress-induced protein synthesis in the BLA and vHC produce distinct changes in defensive behavior.

In order to assess how stress-induced plasticity supports persistent changes in defensive behavior, we utilized post-stress administration of the protein synthesis inhibitor anisomycin, as protein synthesis is known to support the consolidation of many forms of memory and to regulate synaptic plasticity [54-60]. Further, because manipulations of protein synthesis can be done after a learning experience, it provides a means of disrupting the consolidation of an experience without altering its initial encoding. To validate that the emergence of the observed defensive behavior changes are indeed supported by stress-induced protein synthesis, we first tested the effects of systemically administered anisomycin (Fig 2C-E). Animals underwent trauma and were immediately after given 3 injections of anisomycin or vehicle (at 0, 4, and 8 hours). Alternatively, animals were placed in the same environment but did not receive trauma and were treated with vehicle. A week later, relative to no-trauma controls, trauma-exposed animals treated with vehicle exhibited increases in anxiety-related behavior in the light dark-test (Fig 2C. $t_{12.1}$=4.9, p<0.001), associative fear in the trauma recall test (Fig 2D. $t_{8.4}$=7.2, p<0.001), and heightened fear of the novel stressor environment (Fig 2E. t_{14}=2.7, p=0.02). Post-trauma anisomycin administration reduced all of these stress-induced defensive behaviors relative to trauma-exposed animals given vehicle (Fig 2C-E. light-dark test: $t_{16.3}$=2.4, p=0.031; trauma recall: $t_{8.4}$=6.6, p<0.001; novel stressor: $t_{15.9}$=2.9, p<.01).

Next, we assessed the impacts of targeting trauma-induced protein synthesis specifically in the BLA or vHC, regions previously linked to regulating defensive behaviors. Mice had indwelling cannulas implanted above either the BLA or vHC (Fig 2B.). After surgical recovery, animals then underwent trauma and immediately thereafter received intracranial infusions of anisomycin or vehicle. Alternatively, they experienced no trauma and were treated immediately with vehicle. Animals treated with vehicle in the BLA or vHC showed no behavioral differences and are collapsed here. Moreover, prior to vehicle/anisomycin treatment, no differences were observed in freezing during the trauma session for animals that underwent trauma (Fig 2F. Group: $F_{2,76}$=1.1, p=0.33; Group x Time: $F_{20,760}$=0.5, p=0.96). As expected, relative to no-trauma controls, trauma-exposed animals treated with vehicle exhibited heightened anxiety-related behavior in the light-dark test (Fig 2G. $t_{47.5}$=5.5, p<0.001),

a strong associative trauma memory (Fig 2H. $t_{40.7}$=11.5, p<0.001), and heightened fear of the novel stressor (Fig 2I. $t_{47.6}$=5.8, p<0.001). These behaviors were differentially affected by blocking trauma-induced protein synthesis in the BLA and vHC. In the light-dark test, anisomycin in the vHC greatly attenuated trauma-induced increases in animals' preference for the dark (Fig 2G. $t_{28.2}$=3.1, p<0.01). However, anisomycin in the BLA was without effect ($t_{44.9}$=1.09, p=0.28). In the trauma recall test, anisomycin in either the BLA or vHC were effective at reducing associative freezing relative to trauma controls (Fig 2H. BLA: $t_{56.9}$=8.2, p<0.001, vHC: t_{44}=4.2, p<0.001), though the BLA appeared to contribute to a more sizable degree (Fig 2H. T:BLA-ani vs T:vHC-ani: $t_{30.5}$=2.4, p=0.02). Lastly, anisomycin in the BLA was able to block the enhanced sensitivity to a novel stressor, whereas anisomycin in the vHC was without effect (Fig 2I. BLA: $t_{56.4}$=4.1, p<0.001, vHC: $t_{46.2}$=1.6, p=0.11). These findings highlight that while stress-induced plasticity in the BLA is paramount for associative fear and heightened stress sensitivity, it is not necessary for alterations in exploratory anxiety-related behavior. Conversely, stress-induced plasticity in the vHC is essential for increased exploratory anxiety-related behavior, and to a lesser extent associative fear recall, but not heightened stress sensitivity. Importantly, the finding that blockade of protein synthesis in the BLA had a profound impairment on associative fear for the trauma environment but no detectable effect on the light-dark test further suggests a dissociation between exploratory anxiety-related behavior and associative fear.

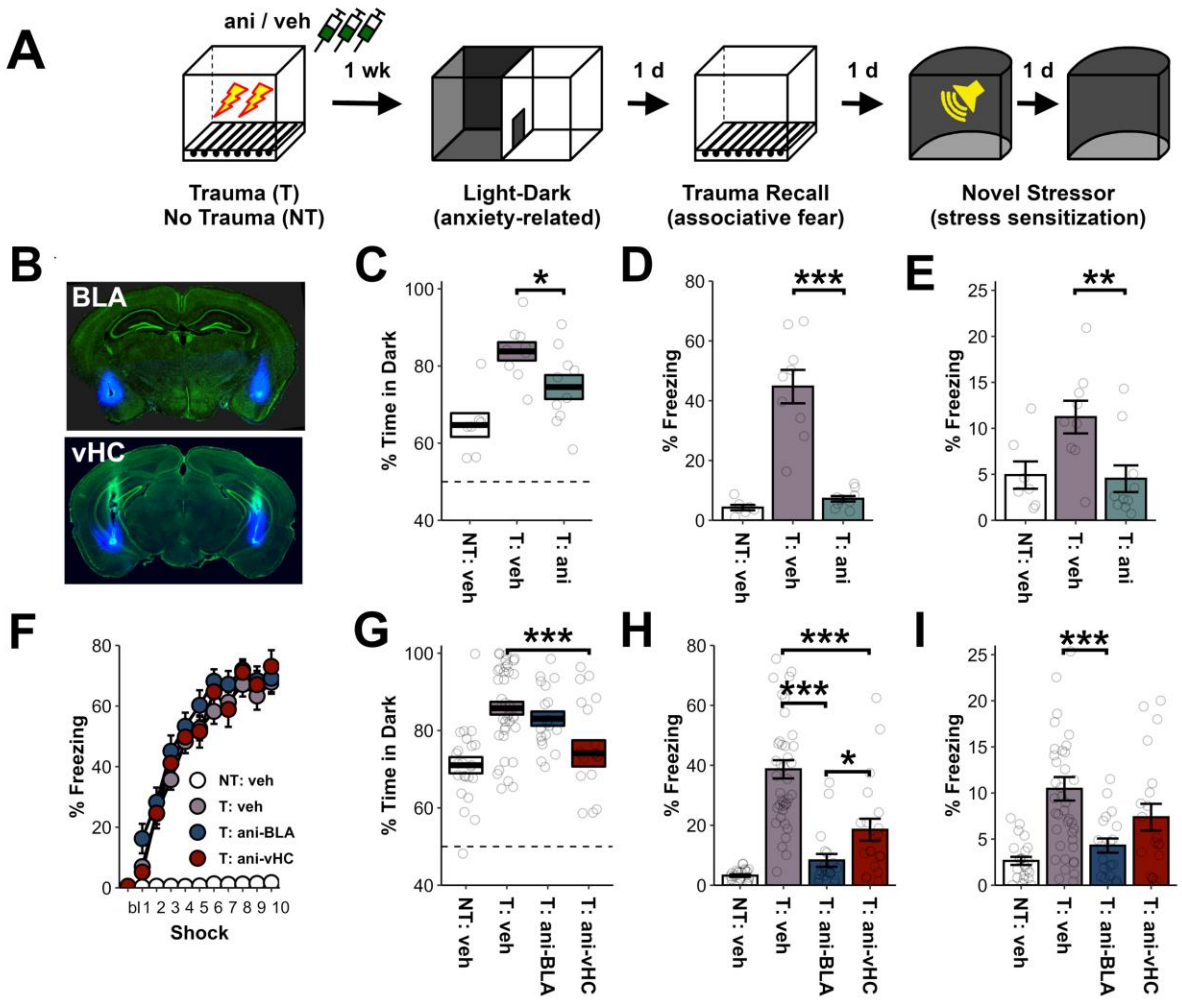

Figure 2: Stress-induced protein synthesis in the BLA and vHC support distinct changes in defensive behavior. Immediately after trauma (T) or no-trauma (NT), animals were administered 3 injections of anisomycin (ani) or vehicle (veh). A week later, they were tested in the light-dark test of anxiety-related behavior, associative fear of the trauma environment, and their response to a novel stressor in a new environment (A). Example placement of cannula injectors in the BLA (top) and vHC (bottom) (B). Systemic administration of anisomycin after trauma reduced time in the dark side in the light-dark test (C), associative freezing in the trauma environment (D), and reduced freezing in the novel stressor environment when placed in this environment on the final test day (E). Animals given trauma and intracranial infusions into the BLA or vHC showed no initial differences during trauma (F). Anisomycin in the vHC after trauma reduced subsequent time in the dark side of the light-dark test, whereas anisomycin in the BLA was without effect (G). Anisomycin in the BLA or vHC reduced associative fear of the trauma environment (H). Anisomycin in the BLA reduced freezing in the novel stressor test relative to controls, whereas anisomycin in the vHC was without effect (I). For systemic injections, NT: veh=7, T:veh=9, and T:ani=10 mice. For intracranial infusions, NT:veh=23, T:veh=40, T:ani-BLA=19 , and T:ani-vHC=20 mice. $p<.05$ (*), $p<0.01$ (**), $p<0.001$ (***).

Distinct stress-induced defensive behaviors require activity of the BLA and vHC

The prior findings indicate that stress-induced plasticity within the BLA and vHC support the induction of distinct post-stress phenotypes. We next sought to address whether activity within these structures also differentially contributes to the expression of these behaviors after trauma. To test this possibility, we utilized the inhibitory ionotropic designer receptor PSAM4-GlyR (PSAM) in conjunction with its activating ligand uPSEM-817-tartrate (uPSEM) [61].

To first verify that we could reversibly alter the activity of the BLA and vHC to change defensive behavior, we used the PSAM-uPSEM system to inactivate the BLA or vHC during trauma memory recall, as the activity of both regions seemed likely to influence this behavior [62]. A pan-neuronal virus expressing PSAM was infused into either of these two structures a month prior to behavioral testing (Fig 3A.). Then, animals received the trauma previously described and a week later were tested in a trauma recall test, once after receiving an injection of uPSEM and once after receiving an injection of vehicle. No differences were observed in freezing behavior during the trauma experience for animals with the PSAM receptor in the BLA versus the vHC (Region: $F_{1,14}$=2, p=0.18; Region x Time: $F_{10,140}$=0.5, p=0.91). However, in the recall test, inhibition of either the BLA or vHC via administration of the agonist uPSEM was effective at reducing associative freezing (Fig 3C. Drug: $F_{1,14=}$12.1, p<0.01; Drug X Region: $F_{1,14}$=0.2, p=0.67).

Next, to assess the contributions of BLA and vHC neural activity to the expression of enhanced anxiety-related behavior and stress sensitivity after trauma, PSAM-expressing virus was infused into either of these structures, or animals underwent a control surgery in which PBS was infused (CTRL) (Fig 3D.). A month later, all animals underwent the trauma procedure and were tested for anxiety-related behavior and stress sensitization. Notably, no behavioral differences were observed during the initial trauma, suggesting that expression of the receptor alone had no effect on the acquisition or expression of conditioned freezing (Fig 3E. Group: $F_{2,23}$=0.3, p=0.72; Group x Time: $F_{20,230}$=1.6, p=0.1). A week later, animals were tested in the light-dark test after administration of uPSEM. Consistent with our finding that stress-induced protein synthesis within these structures supports distinct behaviors, inhibition of the vHC produced a dramatic decrease in time spent on the dark side (Fig 3F. $t_{11.7}$=5, p<0.001), whereas inhibition of the BLA was without effect (Fig 3F. $t_{16.8}$=0.4, p=0.68). Next, we tested the animals drug-free in a trauma recall session in order to ensure that prior inactivation

during the light-dark test did not influence subsequent behavior, as well as to further confirm that receptor expression alone did not influence memory recall. As expected, there was no difference in freezing between groups (Fig 3G. $F_{2,23}$=0.5, p=0.6). Lastly, we inactivated either the BLA or the vHC via uPSEM administration prior to the novel stressor. The following day, when animals were returned to the novel stressor environment, BLA-inactivated animals froze less than controls (Fig 3H. t_{13}=2.4, p=0.04), whereas vHC inhibition did not alter freezing ($t_{13.8}$=1.3, p=0.23). In summary, inactivation of the BLA/vHC replicated the effects observed with protein synthesis inhibition, where the BLA supports heightened stress sensitivity, and the vHC supports enhanced anxiety-related behavior.

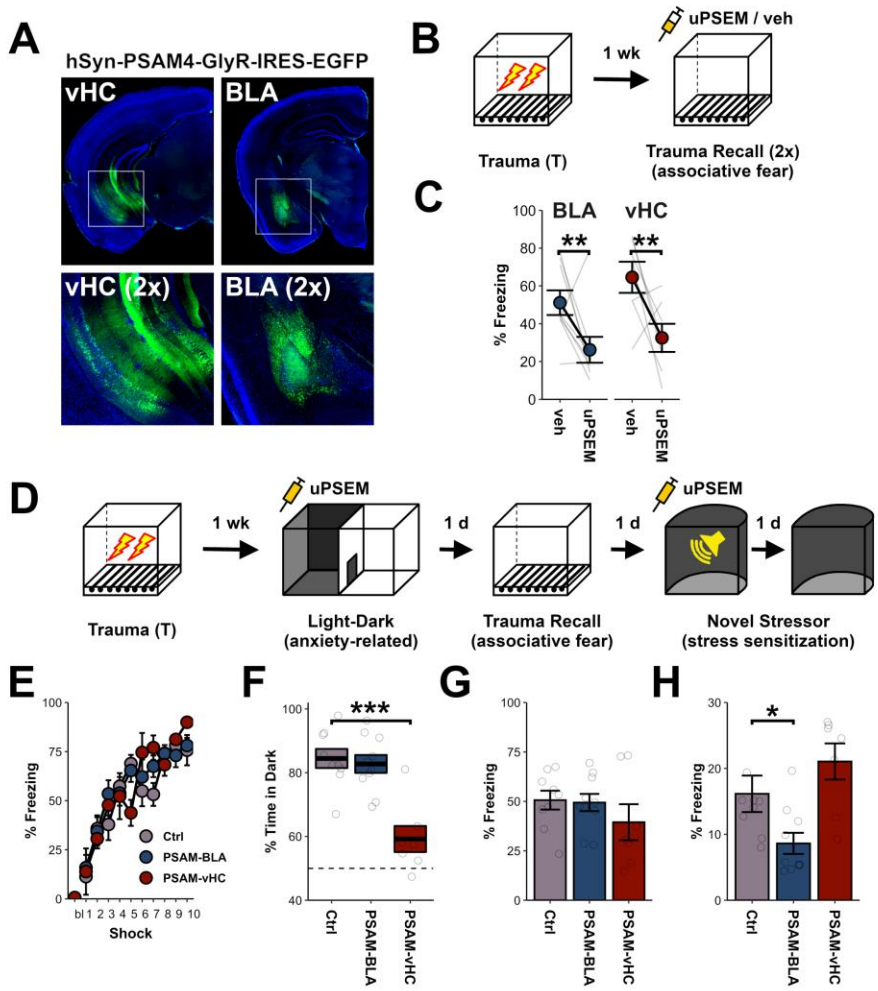

Figure 3. Distinct stress-induced defensive behaviors require activity of the BLA and vHC. A pan-neuronal virus expressing the inhibitory ionotropic receptor PSAM4-GlyR (PSAM) was expressed in either the BLA or vHC a month before behavior, or animals underwent a control surgery (Ctrl) in which PBS was infused (A). To assess efficacy of PSAM-mediated neuronal silencing on behavior, a group of animals expressing PSAM in either the BLA or vHC underwent trauma, and week later their associative recall of the trauma environment was tested in the presence or absence of the PSAM ligand uPSEM (B). Inhibition of either the BLA or vHC was able to reduce trauma memory recall (C). In a separate set of animals that underwent trauma, we then tested the effects of inhibiting the BLA or vHC during testing of anxiety-related behavior in the light-dark test and administration of the novel stressor. Animals had PSAM-expressing virus infused into the BLA, vHC, or underwent Ctrl surgery (D). No differences were observed in freezing during trauma, indicating no effect of receptor expression alone (E). Inhibition of the vHC, but not the BLA, was able to attenuate stress-induced increases in time spent in the dark side in the light-dark test (F). In a drug-free test, there were no differences in trauma memory recall (G). Inhibition of the BLA, but not the vHC, during the novel stressor was able to reduce subsequent freezing when placed back in this environment (H). For 2B/2C, BLA=9 and vHC=7 mice. For 2D-2H, Ctrl=9, PSAM-BLA=10, and PSAM-vHC=7 mice. $p<.05$ (*), $p<0.01$ (**), $p<0.001$ (***).

Activity of the vHC, but not BLA, is necessary for multiple anxiety-related behaviors.

We selected the light-dark test instead of other exploratory measures of anxiety-related behavior because initial pilot studies suggested behavior in this test was more reliably altered by prior stress. To assess whether our findings generalize to other measures of exploratory anxiety-related behavior, PSAM expressing virus was infused into the BLA or vHC a month before behavioral testing. Animals were then given the trauma and a week later were tested in the open field, elevated plus maze, and the light-dark tests, once with vehicle and once with uPSEM for each test (drug and testing order counterbalanced; see Fig S5 for placement). Again, inhibition of the vHC, but not the BLA, reduced time in the dark side of the light-dark test (Fig 4B. vHC: $F_{1,7}$=14.9, p<0.01, BLA: $F_{1,6}$=0.01, p=0.92). Additionally, inhibition of the vHC reduced time in the closed arms of the elevated plus maze (Fig 4C. $F_{1,7}$=55.6, p<0.001). Of some interest, inhibition of the BLA produced a slight increase in time in the closed arms ($F_{1,6}$=10.2, p=0.02), although this effect does not survive multiple comparisons across all exploratory anxiety-related measures. Lastly, neither inhibition of the BLA nor the vHC affected behavior in the open field test (Fig 4D. Drug: $F_{1,13}$=1.7, p=0.211; Drug X Region: $F_{1,13}$=0.003, p=0.96). These findings confirm that the effects of vHC inhibition generalize to at least one other commonly used measure of exploratory anxiety-related behavior, and further suggests that inhibition of the BLA does not alter these measures.

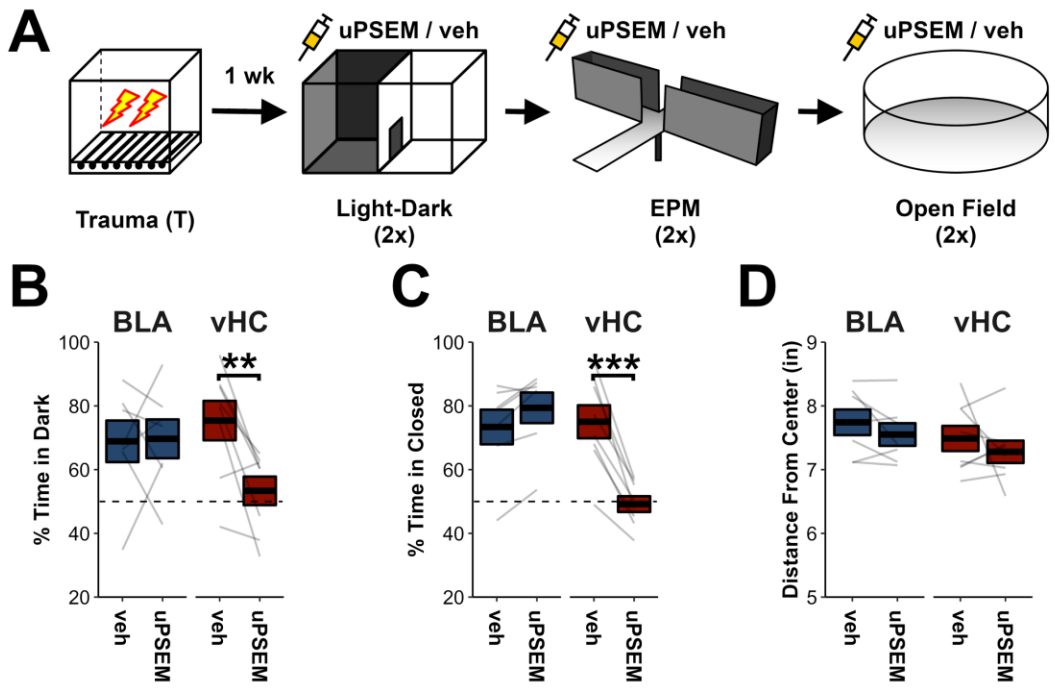

Figure 4. Activity of the vHC, but not BLA, is necessary for multiple anxiety-related behaviors. Animals expressing PSAM in either the BLA or vHC were subjected to trauma and then tested in multiple exploratory anxiety-related tests, both with and without uPSEM (A). Inhibition of the vHC reduced time in the dark side in the light-dark test (B), as well as time in the closed arms of the elevated plus maze. Neither inhibition of the BLA nor the vHC altered distance from the center in the open field test (C).

DISCUSSION

A wealth of literature supports the notion that the BLA and vHC regulate defensive behaviors [8,41-48]. In light of reciprocal connections between these structures [63-65], it would be reasonable to hypothesize that stress-induced plasticity within the BLA and vHC subserve a common defensive process (or processes). While the findings presented here do not negate this possibility, they nevertheless highlight that under certain conditions the BLA and vHC support dissociable defensive behavioral functions, both at the levels of stress-induced plasticity and neuronal activity. Stress-induced protein synthesis within the BLA was found to be critical to stress-evoked enhancements in stress sensitivity, whereas stress-induced protein synthesis within the vHC had no bearing on this defensive phenotype. Conversely, stress-induced protein synthesis within the vHC, but not the BLA, was found to support heightened exploratory anxiety-related behaviors. Suppressing neural activity within the BLA or vHC was of similar effect to protein synthesis blockade.

Notably, it could be argued that although stress-induced plasticity within each of these structures supports the induction of distinct defensive phenotypes, connectivity between the two structures could still be fundamental to the expression of both phenotypes. That is, stress-induced plasticity within one structure may support behavior changes via its connections with the other. Our results suggest otherwise. If plasticity within the vHC was supporting increased anxiety-related behaviors via its connections with the BLA, inhibition of either structure would be expected to have an effect on anxiety-related behavior. Similarly, if plasticity within the BLA regulated stress sensitivity through its connections with the vHC, inhibition of the vHC would be expected to alter this phenotype. However, blockade of activity within each of these structures produced dissociable impacts on behavior, mirroring the effects of protein synthesis blockade. As such, these structures appear to contain distinct systems through which stress produces lasting changes in behavior.

The dissociation of the contributions of the BLA and vHC to the defensive behaviors studied here is not entirely without precedent, although side by side comparisons of their functions are limited. For instance, despite the large literature on the role of the BLA in associative fear learning [9-11,41,42,66-69], several studies have reported that inhibition of the BLA is without effect on exploratory anxiety-related behaviors [70-73], although discrepancies also exist

[74]. Additionally, a recent report found that optogenetic stimulation of projections from the vHC to the BLA do not regulate exploratory anxiety-related behavior [75]. This corroborates the hypothesis that vHC regulates exploratory-anxiety related behavior through its connection with other down-stream structures such as the hypothalamus [75-77] or medial prefrontal cortex [44]. While stimulation of BLA terminal fibers in the vHC has been found to alter anxiety-related behavior [71], this may reflect a general effect of exciting the vHC, as opposed to the natural role served by BLA to vHC efferents. In concert with our findings, these results broadly suggest that the vHC may regulate exploratory anxiety-related behavior in a manner distinct from its connections with the BLA.

Relatively few studies have examined how stress sensitivity is altered as a function of prior experience. However, consistent with our findings, existing work supports a role for the BLA. Either inactivation of the BLA or blockade of glucocorticoid receptors in the BLA prior to an acute stressor have been found to reduce subsequent enhancements in responding to an aversive stimulus [49]. Further, antagonism of ghrelin receptors in the BLA during chronic stress is able to block subsequently enhanced responses to a new stressor[78]. Our results strengthen the proposition that plasticity within the BLA supports these changes by utilizing a post-stress manipulation, which does not interfere with the initial stress experience. By contrast, outside of the work presented here, little is known about the contributions of the vHC to stress-induced changes in stress sensitivity. Thus, the finding that enhancements in stress sensitivity following aversive experience are independent of plasticity and activity of the vHC is entirely novel.

Blockade of stress-induced plasticity in the BLA and vHC were both able to impair associative fear learning for the initial stressor, and blockade of activity within either structure was similarly able to impair associative memory recall. That said, blockade of stress-induced plasticity within the BLA had a much more pronounced effect than the same manipulation performed in the vHC. This discrepancy is perhaps in line with prior reports pointing to a more equivocal role of the vHC in associative fear. For instance, modulation of vHC granule cells have been found to modulate anxiety-related behaviors, but not associative fear learning [45]; another report indicates that inactivation of the vHC disrupts the acquisition of auditory, but not contextual, fear learning [79]; and newer findings suggest vHC to BLA projections are able to modulate the acquisition of contextual fear, although these effects are relatively small in size

[75,80]. While the precise role of the vHC in associative fear learning is yet to be determined, it is possible that connections between the vHC and BLA permit modulation of the more heavily BLA-mediated associative fear learning.

Plasticity within the BLA was necessary for the acquisition and expression of both heightened associative memories of a stressful event and the heightened sensitivity to novel stressors after stress. Furthermore, these two phenotypes were correlated, albeit weakly. Therefore, it may be argued that these defensive phenotypes are one in the same. Prior evidence, as well as data presented here, stands in opposition to this possibility. First, extinction of the associative memory for an initial stressor has been found to leave the enhanced response to a second stressor intact [15,36,37]. Second, early life stress at a time point when rodents are not able to form associative memories nevertheless leaves animals with heightened responses to subsequent stressors in adulthood [14]. Third, pharmacological blockade of NMDA receptors during initial stress that provides near-complete loss of associative memory for that event do not reduce the heightened responding to subsequent aversive events [15]. Lastly, despite the finding presented here that inactivation of the BLA and vHC were able to equivalently impair trauma memory recall, only inactivation of the BLA was able to alter the heightened sensitivity to a novel stressor. Therefore, despite both phenotypes' dependence upon the BLA, associative memory for a stressor and the enhanced responding to subsequent stress are dissociable. It could be that synapse- and ensemble-specific plasticity within the amygdala supports the associative memory for a specific stressor, whereas a broader form of non-associative plasticity within the amygdala supports sensitized stress responses. We are currently pursuing avenues to disentangle how plasticity within the amygdala supports these two forms of learning.

It is particularly striking that only manipulations of the vHC were able to alter stress induced-changes in exploratory anxiety-related behaviors despite both the BLA's and vHC's role in associative fear learning. If exploratory anxiety-related behaviors reflected generalization of the associative memory for a stressor, as is sometimes assumed, one would expect that manipulations that affect associative fear memories would also affect anxiety-related behaviors. Instead, manipulations of the BLA produced a profound impact on associative fear but no change in exploratory anxiety-related behavior, indicating that these behaviors are supported by distinct neural processes. Furthermore, we saw no evidence of a

correlation between measures of associative fear for an initial stressor and exploratory anxiety-related behavior. This is in line with prior reports where near-complete abolition of an associative fear memory did not reduce stress-induced changes in anxiety-related behavior[14,15], and indicate that these phenotypes are supported by unique biological processes. Here, we extend these results by showing where plasticity underlying stress-induced changes in exploratory anxiety-related behavior occur, in the vHC.

In closing, these results shed new light on how stress-induced plasticity within the BLA and vHC support the formation of defensive behavioral phenotypes. Furthermore, they highlight just how distinct memories for stressful events might be. Similar to the separate memory systems in the brain supporting episodic and procedural learning of the same event, we find that different defensive behaviors induced by stress are supported by distinct brain regions. This has important clinical implications for the treatment of anxiety disorders and other stress-associated mental health conditions. First, it suggests that clinically targeting one stress-induced defensive behavior, or the circuits that support that behavior, may leave others wholly unaffected. Perhaps some of the existing gaps in treatment result from a failure to adequately target the spectrum of defensive processes altered in these conditions. Second, by understanding the relationship between specific defensive behaviors and their biology, in combination with knowing the specific defensive behaviors altered in a particular mental health condition, we may make greater headway in the treatment of these conditions. For instance, associative learning processes are more likely affected in some mental health conditions, while anxiety-related behaviors might be more affected in others, and these differences undoubtedly covary with different neuronal patterns. Indeed, it is known that the different anxiety disorders are not only symptomatically different, but characterized by unique brain activity patterns[81]. By understanding these inter-relationships, we may more rapidly find the appropriate key for each lock as we try to advance treatments for stress-associated mental health conditions.

METHODS

Animals*:*

All animals were adult male C57BL/6J mice obtained from Jackson Laboratories, aged 2-6 months. Animals were housed in a temperature- and humidity-controlled vivarium on a 12/12 light-dark cycle (lights on at 7 a.m.). All experimental procedures were approved by the Icahn School of Medicine at Mount Sinai's IACUC.

Behavioral testing:

For all experiments, animals were singly housed beginning 1 week prior to the start of behavioral testing and were handled by the experimenters for approximately 1 min/day for 5 days during this time. When systemic injections were to be given, animals were additionally briefly habituated to restraint 2-3 times. Animals were habituated to being transported from the vivarium to the laboratory 2-3 times to mitigate transport serving as an associative cue.

Trauma and trauma recall: Animals were transported from the vivarium in their cages on a cart to the experimental testing room, which was well lit and had a fan providing ambient sound. Animals were then placed in a brightly lit experimental testing chamber with a grid floor (Med Associates), scented with 5% Simple Green solution. During trauma, after a 5 min period of baseline exploration, animals received 10, 1 sec, 1 mA, scrambled foot-shocks, with an inter-shock interval of 30 sec. Animals were taken out of the testing chamber 30 sec after the last shock and returned to the vivarium in their home cage. For trauma recall sessions, animals were transported to the same experimental testing chamber for an 8 min test session.

Novel stressor and novel stressor recall: Animals were transported from the vivarium in P1000 pipet boxes and carried in a dark box to the experimental testing room, which was dark except a dim red light. Animals were then placed inside of a dark testing chamber (Med Associates) with a flat plexiglass floor and a curved back wall. The chamber was scented with 1% acetic acid solution. After a 3 min baseline period, animals were exposed to a single loud auditory stimulus (3 sec, 130 dB white noise, 0 ms rise time) that was delivered by a speaker attached to the wall. Animals were removed 10 sec later and returned to the vivarium. For novel stressor recall sessions, animals were transported to the same experimental testing chamber for an 8 min test session.

Exploratory anxiety-related tests: The light-dark test was conducted using two interconnected square compartments with an open top (each compartment 7.5 in width x 11.25 in height), separated by a 1.5 in wide passageway that could be closed with an opaque sliding divider. One chamber was made of all white acrylic, while the walls of the other were covered in matte black wallpaper and had a red acrylic floor. Overhead lighting provided luminance of 50 lux on the light side. After a 1 min baseline period in which animals were confined to the dark side, the central divider was raised and the animals could freely explore both sides of the light-dark box. The open field test was conducted in a circular arena (19 in diameter; 10 in height) made of white acrylic. A circular field was utilized in order to avoid the need to define arbitrary center/outer areas. Animals were placed along one wall and allowed to explore for 5 min. Luminance was approximately 50 lux. For the elevated plus maze, each arm measured 2 3/8 in wide and 13.75 in long. The floor of the maze was made of white acrylic, and the enclosed arms had black walls (8 in high). Luminance of the open arms was 25 lux. Animals were placed at the end of a closed arm and then allowed to explore freely for 5 min. For all anxiety-related behavior tests, apparatus were cleaned with 70% ethanol between test sessions and behavior was captured with an overhead webcam. These sessions were conducted in an otherwise dark room with a fan providing ambient background noise.

Behavior quantification: For analysis of freezing and motion in conditioning chambers, Med Associates Video Freeze software was used to analyze videos acquired from a near infra-red camera located in the chamber [82]. For measuring distance travelled and time spent in regions of interest in exploratory anxiety-related behavior tests, ezTrack was used [83,84]. With the exception of freezing during the trauma and novel stressor session, all measures reflect the average across the entire session. For freezing during the trauma, time was binned into the 300 sec baseline and then 10 post-shock periods. Each post-shock period was 20 sec in length and begun 10 sec after shock offset.

Surgery:

For surgery, anesthesia was induced with 5% isoflurane and subsequently maintained at 1-2%. Body temperature was maintained during surgery and recovery with a heating pad below the animal, and ophthalmic ointment was applied to lubricate the eyes. All surgeries followed aseptic surgical technique. For viral surgeries, 100 nL of AAV5-hSyn-PSAM4-GlyR-

IRES-EGFP (2.4 x 10^{13} GC/mL; Addgene 119741) was infused into the BLA (AP: -1.4; ML: 3.3; DV: -5) or vHC (AP: -3; ML: 3.2; DV: -4.5) at 2 nL/sec via glass pipettes. Alternatively, an equivalent volume of sterile PBS was infused. 10 min was allowed for diffusion before removing the injector, irrigating the incision with saline, and suturing the incision site. For cannulation surgeries, 26 gauge guide cannula (P1 Technologies; 8IC315GMNSPC) were implanted overlying the BLA (AP: -1.4; ML: 3.2; DV: -3.5) or vHC (AP: -3; ML: 3.2; DV: -3), and affixed to the skull with dental cement and super glue. A skull screw was also implanted during surgery to help secure the head cap (P1 Technologies; 00-96X1/16). After surgery, dummy cannula that extended 1.5 mm below the guide cannula were inserted (P1 Technologies 8IC315DCMNSP). Following surgery, animals were given 20 mg/kg ampicillin and 5 mg/kg carprofen (s.c.) per day for 7 days and body weight and general disposition were monitored. All surgeries followed aseptic surgical technique.

Anisomycin experiments:

For experiments in which anisomycin (Sigma A9789) was administered systemically, we utilized a dose of 150 mg/kg (10 mL/kg, s.c.), consistent with prior literature [85,86]. Because numerous waves of protein synthesis have been found to support memory consolidation [59,87,88], we opted to administer anisomycin 3 times, once every 4 hours. In line with prior reports [89,90], this should maintain approximately 90% blockade of protein synthesis for 12 hours. For experiments in which anisomycin was administered intracranially, 33 gauge injectors (P1 Technologies; 8IC315IMNSPC) attached via PE-20 tubing (Instech) to a Harvard syringe pump (Harvard Apparatus, #55-2222) were utilized to infuse anisomycin (10 ng/nL) at a rate of 150 nL/min. 300 nL of anisomycin solution was administered per hemisphere in the vHC. 200 nL was administered per hemisphere in the BLA. Control animals were infused with an equivalent volume of 1X PBS. Following infusions, injectors were left in place for 1 min before removal. Again, anisomycin was infused 3 times, once every four hours. Prior to testing, animals were habituated to handling such that infusions could be done while mice were gently held by the experimenter. Additionally, all animals received a habituation infusion of 1X PBS 2-3 days prior to the trauma day. Anisomycin was first dissolved in a small volume of 0.1 N HCL (90% PBS, 10% 1 N HCL), brought near concentration with the addition of 1X PBS, and the pH was then normalized to 6-7 by the addition of 1 N NaOH.

PSAM experiments:

For PSAM experiments, actuation of PSAM4-GlyR was achieved through intraperitoneal administration of 1 mg/kg uPSEM-817-tartrate (Tocris), 15-20 minutes prior to behavior, at a volume of 10 mL/kg (dissolved in saline). For the study in which the effects of inhibiting the vHC or BLA were assessed on multiple measures of exploratory anxiety-related behavior, each animal underwent each of these tests twice, once with uPSEM and once with vehicle. Tests occurred in a fixed order across two weeks, with open-field on Monday, EPM on Wednesday, and Light-Dark on Friday. However, drug order was counterbalanced, such that half the animals that received uPSEM on the first open field test received saline on the first EPM, and so forth.

Histology:

At the end of behavioral testing, animals that underwent surgical manipulation were deeply anesthetized, and their brains were then extracted and placed in paraformaldehyde overnight at 4C. For animals with cannula implants, 100 nl of DAPI (0.5 mg/mL) was infused prior to brain extraction, but after anesthesia, to mark cannula placement. The next day, brains were transferred to 30% sucrose in 1X PBS and left at 4C to sink before being frozen and sectioned at 50 um on a cryostat. Tissue was then mounted on slides and either cover-slipped using mounting media with DAPI (Vector Laboratories, #H-1200-10) for checking viral placement or cover-slipped with non-fluorescent mounting media (Vector Laboratories, #H-1000-10) after a green nucleic acid stain. For green nucleic acid staining, slides were submerged in 50 mM Sytox Green (diluted in 1X PBS from 5 mM. Thermo Fisher #S7020) for 10 min and then washed 3x in 1X PBS. Tissue was then imaged on a Leica DM6 epifluorescent microscope.

Analysis:

All analyses were performed using RStudio. All data and statistical analysis are available at github.com/ZachPenn/BLAvHC_Dissoc. Briefly, omnibus ANOVA were conducted using the package ezANOVA with type 3 degrees of freedom. The white adjustment was implemented to correct for heterogeneity of variance and adjusted degrees of freedom are

presented. Groups sizes are listed in each figure legend. For repeated measures ANOVA, the Greenhouse-Geisser correction was implemented when the assumption of sphericity was not met. Planned comparisons and post-hoc t-tests (Welch unequal variance) were performed after omnibus significance was met. Post-hoc tests were evaluated against a modified criterion calculated using the Dunn-Sidak method in order to keep family-wise type 1 error at 0.05.

KEY RESOURCE TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Bacterial and virus strains		
AAV5-hSyn-PSAM4-GlyR-IRES-EGFP (2.4 x 10^{13} GC/mL)	Magnus et al, 2019[61]	Addgene: 119742
Biological samples		
Chemicals, peptides, and recombinant proteins		
Anisomycin from *Streptomyces griseolus*	Millipore Sigma	Millipore Sigma: A9789
uPSEM 817 tartrate	Tocris	Tocris: 6866
Critical commercial assays		
Deposited data		
Experimental models: Cell lines		
Experimental models: Organisms/strains		
Mouse: C57BL/6J	The Jackson Laboratory	JAX: 000664
Oligonucleotides		
Recombinant DNA		
Software and algorithms		
Med Associates Video Freeze	Med Associates[82]	Med Associates: SOF-843
ezTrack	Pennington et al, 2019[84]	www.github.com/denisecailab/eztrack
Other		

References and Literature Cited

1. Fendt, M., and Fanselow, M.S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. Neurosci Biobehav Rev *23*, 743-760.
2. Bolles, R.C. (1970). Species-specific defense reations and avoidance learning. Psychological Review *77*, 32-48.
3. Fanselow, M.S., and Lester, L.S. (1988). A functional behavioristic approach to aversively motivated behavior. In Evolution and Learning, R.C. Bolles, and M.C. Beecher, eds. (Erlbaum), pp. 185-211.
4. Cannon, W.B. (1915). Bodily changes in pain, hunger, fear and rage: An account of recent researches into the function of emotional excitement. (D. Appleton and Componay).
5. Blanchard, R.J., Blanchard, D.C., Rodgers, J., and Weiss, S.M. (1990). The characterization and modelling of antipredator defensive behavior. Neurosci Biobehav Rev *14*, 463-472. 10.1016/s0149-7634(05)80069-7.
6. Blanchard, R.J., and Blanchard, D.C. (1969). Passive and active reactions to fear-eliciting stimuli. J Comp Physiol Psychol *68*, 129-135. 10.1037/h0027676.
7. Bienvenu, T.C.M., Dejean, C., Jercog, D., Aouizerate, B., Lemoine, M., and Herry, C. (2021). The advent of fear conditioning as an animal model of post-traumatic stress disorder: Learning from the past to shape the future of PTSD research. Neuron *109*, 2380-2397. 10.1016/j.neuron.2021.05.017.
8. Maren, S. (2005). Synaptic mechanisms of associative memory in the amygdala. Neuron *47*, 783-786. 10.1016/j.neuron.2005.08.009.
9. Maren, S. (2003). The amygdala, synaptic plasticity, and fear memory. Ann N Y Acad Sci *985*, 106-113.
10. Fanselow, M.S., and LeDoux, J.E. (1999). Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. Neuron *23*, 229-232.
11. Davis, M. (1992). The role of the amygdala in fear and anxiety. Annu Rev Neurosci *15*, 353-375. 10.1146/annurev.ne.15.030192.002033.
12. Fadok, J.P., Krabbe, S., Markovic, M., Courtin, J., Xu, C., Massi, L., Botta, P., Bylund, K., Müller, C., Kovacevic, A., et al. (2017). A competitive inhibitory circuit for selection of active and passive fear responses. Nature *542*, 96-100. 10.1038/nature21047.
13. Adamec, R.E., and Shallow, T. (1993). Lasting effects on rodent anxiety of a single exposure to a cat. Physiol Behav *54*, 101-109. 10.1016/0031-9384(93)90050-p.
14. Poulos, A.M., Reger, M., Mehta, N., Zhuravka, I., Sterlace, S.S., Gannam, C., Hovda, D.A., Giza, C.C., and Fanselow, M.S. (2014). Amnesia for early life stress does not preclude the adult development of posttraumatic stress disorder symptoms in rats. Biol Psychiatry *76*, 306-314. 10.1016/j.biopsych.2013.10.007.
15. Rau, V., DeCola, J.P., and Fanselow, M.S. (2005). Stress-induced enhancement of fear learning: an animal model of posttraumatic stress disorder. Neurosci Biobehav Rev *29*, 1207-1223. 10.1016/j.neubiorev.2005.04.010.
16. Pynoos, R.S., Ritzmann, R.F., Steinberg, A.M., Goenjian, A., and Prisecaru, I. (1996). A behavioral animal model of posttraumatic stress disorder featuring repeated exposure to situational reminders. Biol Psychiatry *39*, 129-134. 10.1016/0006-3223(95)00088-7.

17. Servatius, R.J., Ottenweller, J.E., and Natelson, B.H. (1995). Delayed startle sensitization distinguishes rats exposed to one or three stress sessions: further evidence toward an animal model of PTSD. Biol Psychiatry *38*, 539-546. 10.1016/0006-3223(94)00369-E.
18. American Psychiatric Association. (2013). American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. (American Psychiatric Publishing).
19. Moreno-Peral, P., Conejo-Cerón, S., Motrico, E., Rodríguez-Morejón, A., Fernández, A., García-Campayo, J., Roca, M., Serrano-Blanco, A., Rubio-Valera, M., and Bellón, J. (2014). Risk factors for the onset of panic and generalised anxiety disorders in the general adult population: a systematic review of cohort studies. J Affect Disord *168*, 337-348. 10.1016/j.jad.2014.06.021.
20. Kessler, R.C., Gruber, M., Hettema, J.M., Hwang, I., Sampson, N., and Yonkers, K.A. (2008). Co-morbid major depression and generalized anxiety disorders in the National Comorbidity Survey follow-up. Psychol Med *38*, 365-374. 10.1017/S0033291707002012.
21. Blanco, C., Rubio, J., Wall, M., Wang, S., Jiu, C.J., and Kendler, K.S. (2014). Risk factors for anxiety disorders: common and specific effects in a national sample. Depress Anxiety *31*, 756-764. 10.1002/da.22247.
22. Keiser, A.A., Turnbull, L.M., Darian, M.A., Feldman, D.E., Song, I., and Tronson, N.C. (2017). Sex Differences in Context Fear Generalization and Recruitment of Hippocampus and Amygdala during Retrieval. Neuropsychopharmacology *42*, 397-407. 10.1038/npp.2016.174.
23. Lissek, S., Bradford, D.E., Alvarez, R.P., Burton, P., Espensen-Sturges, T., Reynolds, R.C., and Grillon, C. (2014). Neural substrates of classically conditioned fear-generalization in humans: a parametric fMRI study. Soc Cogn Affect Neurosci *9*, 1134-1142. 10.1093/scan/nst096.
24. Lissek, S., Kaczkurkin, A.N., Rabin, S., Geraci, M., Pine, D.S., and Grillon, C. (2014). Generalized anxiety disorder is associated with overgeneralization of classically conditioned fear. Biol Psychiatry *75*, 909-915. 10.1016/j.biopsych.2013.07.025.
25. Zelikowsky, M., Bissiere, S., Hast, T.A., Bennett, R.Z., Abdipranoto, A., Vissel, B., and Fanselow, M.S. (2013). Prefrontal microcircuit underlies contextual learning after hippocampal loss. Proc Natl Acad Sci U S A *110*, 9938-9943. 10.1073/pnas.1301691110.
26. Xu, W., and Südhof, T.C. (2013). A neural circuit for memory specificity and generalization. Science *339*, 1290-1295. 10.1126/science.1229534.
27. Wiltgen, B.J., Zhou, M., Cai, Y., Balaji, J., Karlsson, M.G., Parivash, S.N., Li, W., and Silva, A.J. (2010). The hippocampus plays a selective role in the retrieval of detailed contextual memories. Curr Biol *20*, 1336-1344. 10.1016/j.cub.2010.06.068.
28. Starita, F., Kroes, M.C.W., Davachi, L., Phelps, E.A., and Dunsmoor, J.E. (2019). Threat learning promotes generalization of episodic memory. J Exp Psychol Gen *148*, 1426-1434. 10.1037/xge0000551.
29. Dunsmoor, J.E., Kroes, M.C.W., Braren, S.H., and Phelps, E.A. (2017). Threat intensity widens fear generalization gradients. Behav Neurosci *131*, 168-175. 10.1037/bne0000186.
30. Dunsmoor, J.E., Otto, A.R., and Phelps, E.A. (2017). Stress promotes generalization of older but not recent threat memories. Proc Natl Acad Sci U S A *114*, 9218-9223. 10.1073/pnas.1704428114.
31. Davis, M. (1989). Neural systems involved in fear-potentiated startle. Ann N Y Acad Sci *563*, 165-183. 10.1111/j.1749-6632.1989.tb42197.x.
32. Brown, J.S., Kalish, H.I., and Farber, I.E. (1951). Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. J Exp Psychol *41*, 317-328. 10.1037/h0060166.

33. Kaczkurkin, A.N., Burton, P.C., Chazin, S.M., Manbeck, A.B., Espensen-Sturges, T., Cooper, S.E., Sponheim, S.R., and Lissek, S. (2017). Neural Substrates of Overgeneralized Conditioned Fear in PTSD. Am J Psychiatry *174*, 125-134. 10.1176/appi.ajp.2016.15121549.
34. Grillon, C., and Morgan, C.A. (1999). Fear-potentiated startle conditioning to explicit and contextual cues in Gulf War veterans with posttraumatic stress disorder. J Abnorm Psychol *108*, 134-142.
35. Acheson, D.T., Geyer, M.A., Baker, D.G., Nievergelt, C.M., Yurgil, K., Risbrough, V.B., and Team, M.-I. (2015). Conditioned fear and extinction learning performance and its association with psychiatric symptoms in active duty Marines. Psychoneuroendocrinology *51*, 495-505. 10.1016/j.psyneuen.2014.09.030.
36. Long, V.A., and Fanselow, M.S. (2012). Stress-enhanced fear learning in rats is resistant to the effects of immediate massed extinction. Stress *15*, 627-636. 10.3109/10253890.2011.650251.
37. Hassien, A.M., Shue, F., Bernier, B.E., and Drew, M.R. (2020). A mouse model of stress-enhanced fear learning demonstrates extinction-sensitive and extinction-resistant effects of footshock stress. Behav Brain Res *379*, 112391. 10.1016/j.bbr.2019.112391.
38. Difede, J., Olden, M., and Cukor, J. (2014). Evidence-based treatment of post-traumatic stress disorder. Annu Rev Med *65*, 319-332. 10.1146/annurev-med-051812-145438.
39. Bradley, R., Greene, J., Russ, E., Dutra, L., and Westen, D. (2005). A multidimensional meta-analysis of psychotherapy for PTSD. Am J Psychiatry *162*, 214-227. 10.1176/appi.ajp.162.2.214.
40. Cukor, J., Olden, M., Lee, F., and Difede, J. (2010). Evidence-based treatments for PTSD, new directions, and special challenges. Ann N Y Acad Sci *1208*, 82-89. 10.1111/j.1749-6632.2010.05793.x.
41. Maren, S., Aharonov, G., and Fanselow, M.S. (1996). Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal gradient. Behav Neurosci *110*, 718-726.
42. Blanchard, D.C., and Blanchard, R.J. (1972). Innate and conditioned reactions to threat in rats with amygdaloid lesions. J Comp Physiol Psychol *81*, 281-290.
43. Feinstein, J.S., Adolphs, R., Damasio, A., and Tranel, D. (2011). The human amygdala and the induction and experience of fear. Curr Biol *21*, 34-38. 10.1016/j.cub.2010.11.042.
44. Padilla-Coreano, N., Bolkan, S.S., Pierce, G.M., Blackman, D.R., Hardin, W.D., Garcia-Garcia, A.L., Spellman, T.J., and Gordon, J.A. (2016). Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Behavior. Neuron *89*, 857-866. 10.1016/j.neuron.2016.01.011.
45. Kheirbek, M.A., Drew, L.J., Burghardt, N.S., Costantini, D.O., Tannenholz, L., Ahmari, S.E., Zeng, H., Fenton, A.A., and Hen, R. (2013). Differential control of learning and anxiety along the dorsoventral axis of the dentate gyrus. Neuron *77*, 955-968. 10.1016/j.neuron.2012.12.038.
46. Adhikari, A., Topiwala, M.A., and Gordon, J.A. (2010). Synchronized activity between the ventral hippocampus and the medial prefrontal cortex during anxiety. Neuron *65*, 257-269. 10.1016/j.neuron.2009.12.002.
47. Fanselow, M.S., and Dong, H.W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures? Neuron *65*, 7-19. 10.1016/j.neuron.2009.11.031.
48. Bannerman, D.M., Yee, B.K., Good, M.A., Heupel, M.J., Iversen, S.D., and Rawlins, J.N. (1999). Double dissociation of function within the hippocampus: a comparison of dorsal, ventral, and

complete hippocampal cytotoxic lesions. Behav Neurosci *113*, 1170-1188. 10.1037//0735-7044.113.6.1170.

49. Perusini, J., Meyer, E., Rau, V., Avershal, J., Rajbhandari, A., Hoffman, A., Nocera, N., Condro, M., Waschek, J., Spigelman, I., and Fanselow, M. (2014). Mechanisms underlying the induction and expression of fear sensitization following acute traumatic stress. Pavlovian Society.
50. Rau, V., and Fanselow, M.S. (2009). Exposure to a stressor produces a long lasting enhancement of fear learning in rats. Stress *12*, 125-133. 10.1080/10253890802137320.
51. Pennington, Z.T., Trott, J.M., Rajbhandari, A.K., Li, K., Walwyn, W.M., Evans, C.J., and Fanselow, M.S. (2020). Chronic opioid pretreatment potentiates the sensitization of fear learning by trauma. Neuropsychopharmacology *45*, 482-490. 10.1038/s41386-019-0559-5.
52. Chaouloff, F., Durand, M., and Mormède, P. (1997). Anxiety- and activity-related effects of diazepam and chlordiazepoxide in the rat light/dark and dark/light tests. Behav Brain Res *85*, 27-35. 10.1016/s0166-4328(96)00160-x.
53. Crawley, J.N. (1981). Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines. Pharmacol Biochem Behav *15*, 695-699. 10.1016/0091-3057(81)90007-1.
54. Smith, A.C.W., Jonkman, S., Difeliceantonio, A.G., O'Connor, R.M., Ghoshal, S., Romano, M.F., Everitt, B.J., and Kenny, P.J. (2021). Opposing roles for striatonigral and striatopallidal neurons in dorsolateral striatum in consolidating new instrumental actions. Nat Commun *12*, 5121. 10.1038/s41467-021-25460-3.
55. Hernandez, P.J., Sadeghian, K., and Kelley, A.E. (2002). Early consolidation of instrumental learning requires protein synthesis in the nucleus accumbens. Nat Neurosci *5*, 1327-1331. 10.1038/nn973.
56. Santini, E., Ge, H., Ren, K., Peña de Ortiz, S., and Quirk, G.J. (2004). Consolidation of fear extinction requires protein synthesis in the medial prefrontal cortex. J Neurosci *24*, 5704-5710. 10.1523/JNEUROSCI.0786-04.2004.
57. Schafe, G.E., and LeDoux, J.E. (2000). Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. J Neurosci *20*, RC96.
58. Nader, K., Schafe, G.E., and Le Doux, J.E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature *406*, 722-726. 10.1038/35021052.
59. Bourtchouladze, R., Abel, T., Berman, N., Gordon, R., Lapidus, K., and Kandel, E.R. (1998). Different training procedures recruit either one or two critical periods for contextual memory consolidation, each of which requires protein synthesis and PKA. Learn Mem *5*, 365-374.
60. Kandel, E.R. (2001). The molecular biology of memory storage: a dialogue between genes and synapses. Science *294*, 1030-1038. 10.1126/science.1067020.
61. Magnus, C.J., Lee, P.H., Bonaventura, J., Zemla, R., Gomez, J.L., Ramirez, M.H., Hu, X., Galvan, A., Basu, J., Michaelides, M., and Sternson, S.M. (2019). Ultrapotent chemogenetics for research and potential clinical applications. Science *364*. 10.1126/science.aav5282.
62. Sierra-Mercado, D., Padilla-Coreano, N., and Quirk, G.J. (2011). Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacology *36*, 529-538. 10.1038/npp.2010.184.

63. Hintiryan, H., Bowman, I., Johnson, D.L., Korobkova, L., Zhu, M., Khanjani, N., Gou, L., Gao, L., Yamashita, S., Bienkowski, M.S., et al. (2021). Connectivity characterization of the mouse basolateral amygdalar complex. Nat Commun *12*, 2859. 10.1038/s41467-021-22915-5.
64. Canteras, N.S., Simerly, R.B., and Swanson, L.W. (1992). Connections of the posterior nucleus of the amygdala. J Comp Neurol *324*, 143-179. 10.1002/cne.903240203.
65. Canteras, N.S., and Swanson, L.W. (1992). Projections of the ventral subiculum to the amygdala, septum, and hypothalamus: a PHAL anterograde tract-tracing study in the rat. J Comp Neurol *324*, 180-194. 10.1002/cne.903240204.
66. Sprengelmeyer, R., Young, A.W., Schroeder, U., Grossenbacher, P.G., Federlein, J., Büttner, T., and Przuntek, H. (1999). Knowing no fear. Proc Biol Sci *266*, 2451-2456. 10.1098/rspb.1999.0945.
67. Fanselow, M.S., and Kim, J.J. (1994). Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid to the basolateral amygdala. Behav Neurosci *108*, 210-212.
68. Davis, M. (1992). The role of the amygdala in fear-potentiated startle: implications for animal models of anxiety. Trends Pharmacol Sci *13*, 35-41.
69. Rumpel, S., LeDoux, J., Zador, A., and Malinow, R. (2005). Postsynaptic receptor trafficking underlying a form of associative learning. Science *308*, 83-88. 10.1126/science.1103944.
70. Ribeiro, A.M., Barbosa, F.F., Munguba, H., Costa, M.S., Cavalcante, J.S., and Silva, R.H. (2011). Basolateral amygdala inactivation impairs learned (but not innate) fear response in rats. Neurobiol Learn Mem *95*, 433-440. 10.1016/j.nlm.2011.02.004.
71. Felix-Ortiz, A.C., Beyeler, A., Seo, C., Leppla, C.A., Wildes, C.P., and Tye, K.M. (2013). BLA to vHPC inputs modulate anxiety-related behaviors. Neuron *79*, 658-664. 10.1016/j.neuron.2013.06.016.
72. Tye, K.M., Prakash, R., Kim, S.Y., Fenno, L.E., Grosenick, L., Zarabi, H., Thompson, K.R., Gradinaru, V., Ramakrishnan, C., and Deisseroth, K. (2011). Amygdala circuitry mediating reversible and bidirectional control of anxiety. Nature *471*, 358-362. 10.1038/nature09820.
73. Moreira, C.M., Masson, S., Carvalho, M.C., and Brandão, M.L. (2007). Exploratory behaviour of rats in the elevated plus-maze is differentially sensitive to inactivation of the basolateral and central amygdaloid nuclei. Brain Res Bull *71*, 466-474. 10.1016/j.brainresbull.2006.10.004.
74. Bueno, C.H., Zangrossi, H., and Viana, M.B. (2005). The inactivation of the basolateral nucleus of the rat amygdala has an anxiolytic effect in the elevated T-maze and light/dark transition tests. Braz J Med Biol Res *38*, 1697-1701. 10.1590/s0100-879x2005001100019.
75. Jimenez, J.C., Su, K., Goldberg, A.R., Luna, V.M., Biane, J.S., Ordek, G., Zhou, P., Ong, S.K., Wright, M.A., Zweifel, L., et al. (2018). Anxiety Cells in a Hippocampal-Hypothalamic Circuit. Neuron *97*, 670-683.e676. 10.1016/j.neuron.2018.01.016.
76. Bang, J.Y., Sunstrum, J.K., Garand, D., Parfitt, G.M., Woodin, M., Inoue, W., and Kim, J. (2022). Hippocampal-hypothalamic circuit controls context-dependent innate defensive responses. Elife *11*. 10.7554/eLife.74736.
77. Yan, J.J., Ding, X.J., He, T., Chen, A.X., Zhang, W., Yu, Z.X., Cheng, X.Y., Wei, C.Y., Hu, Q.D., Liu, X.Y., et al. (2022). A circuit from the ventral subiculum to anterior hypothalamic nucleus GABAergic neurons essential for anxiety-like behavioral avoidance. Nat Commun *13*, 7464. 10.1038/s41467-022-35211-7.

78. Meyer, R.M., Burgos-Robles, A., Liu, E., Correia, S.S., and Goosens, K.A. (2014). A ghrelin-growth hormone axis drives stress-induced vulnerability to enhanced fear. Mol Psychiatry *19*, 1284-1294. 10.1038/mp.2013.135.
79. Maren, S., and Holt, W.G. (2004). Hippocampus and Pavlovian fear conditioning in rats: muscimol infusions into the ventral, but not dorsal, hippocampus impair the acquisition of conditional freezing to an auditory conditional stimulus. Behav Neurosci *118*, 97-110. 10.1037/0735-7044.118.1.97.
80. Jimenez, J.C., Berry, J.E., Lim, S.C., Ong, S.K., Kheirbek, M.A., and Hen, R. (2020). Contextual fear memory retrieval by correlated ensembles of ventral CA1 neurons. Nat Commun *11*, 3492. 10.1038/s41467-020-17270-w.
81. Etkin, A., and Wager, T.D. (2007). Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am J Psychiatry *164*, 1476-1488. 10.1176/appi.ajp.2007.07030504.
82. Anagnostaras, S.G., Wood, S.C., Shuman, T., Cai, D.J., Leduc, A.D., Zurn, K.R., Zurn, J.B., Sage, J.R., and Herrera, G.M. (2010). Automated assessment of pavlovian conditioned freezing and shock reactivity in mice using the video freeze system. Front Behav Neurosci *4*. 10.3389/fnbeh.2010.00158.
83. Pennington, Z.T., Diego, K.S., Francisco, T.R., LaBanca, A.R., Lamsifer, S.I., Liobimova, O., Shuman, T., and Cai, D.J. (2021). ezTrack-A Step-by-Step Guide to Behavior Tracking. Curr Protoc *1*, e255. 10.1002/cpz1.255.
84. Pennington, Z.T., Dong, Z., Feng, Y., Vetere, L.M., Page-Harley, L., Shuman, T., and Cai, D.J. (2019). ezTrack: An open-source video analysis pipeline for the investigation of animal behavior. Sci Rep *9*, 19979. 10.1038/s41598-019-56408-9.
85. Lattal, K.M., and Abel, T. (2001). Different requirements for protein synthesis in acquisition and extinction of spatial preferences and context-evoked fear. J Neurosci *21*, 5773-5780. 10.1523/JNEUROSCI.21-15-05773.2001.
86. Frankland, P.W., Ding, H.K., Takahashi, E., Suzuki, A., Kida, S., and Silva, A.J. (2006). Stability of recent and remote contextual fear memory. Learn Mem *13*, 451-457. 10.1101/lm.183406.
87. Lattal, K.M., and Abel, T. (2004). Behavioral impairments caused by injections of the protein synthesis inhibitor anisomycin after contextual retrieval reverse with time. Proc Natl Acad Sci U S A *101*, 4667-4672. 10.1073/pnas.0306546101.
88. Grecksch, G., and Matthies, H. (1980). Two sensitive periods for the amnesic effect of anisomycin. Pharmacol Biochem Behav *12*, 663-665. 10.1016/0091-3057(80)90145-8.
89. Wanisch, K., and Wotjak, C.T. (2008). Time course and efficiency of protein synthesis inhibition following intracerebral and systemic anisomycin treatment. Neurobiol Learn Mem *90*, 485-494. 10.1016/j.nlm.2008.02.007.
90. Flood, J.F., Rosenzweig, M.R., Bennett, E.L., and Orme, A.E. (1973). The influence of duration of protein synthesis inhibition on memory. Physiol Behav *10*, 555-562. 10.1016/0031-9384(73)90221-7.