6 Toward Animal Models of Post-Traumatic Stress Disorder

Hagit Cohen and Gal Richter-Levin

Contents

Abstract

The development of animal models for PTSD and other traumatic stress related brain changes is an important part of advancing our neurobiological understanding of the disease process as well as recovery, resilience, and possible therapeutic targets.

Although animal models for PTSD are limited to the assessment of measurable and observable behavioral parameters and cannot assess complex psychological symptoms such as intrusive thoughts, meaning and dreams, valid and reliable animal models offer a means for researching biomolecular, pathophysiological, and pharmacological features of the disorder in ways that are not feasible in human studies.

Trauma/stress-based Models were developed in an attempt to induce in the animal a state similar to PTSD by exposing animals to an equivalent of a traumatic experience.

Mechanism-based models were developed considering potential brain mechanisms that may underlay the disorder. The most studied are enhanced fear conditioning, impaired extinction and more recently, impaired contextualization.

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Another important line of research addresses the question of additional factors that contribute to the susceptibility to develop PTSD. Genetic background and environmental factors have been studied and have led to the recognition of the importance of individual differences in susceptibility to develop the disorder.

This chapter presents and discusses findings from various animal models, with the understanding that no single model encompasses in full the complexity of the disorder but that each of these models contributes to our understanding of PTSD.

Key Words: Amygdala, animal models, corticotrophin-releasing hormone, HPA axis.

INTRODUCTION

Animal models of psychiatric disorders offer a complementary research modality that supports clinical research. To achieve a satisfactory degree of validity and reliability, animal models of complex and intricate psychiatric disorders must fulfill certain criteria. For example, the behavioral responses must be observable and measurable and must reliably reflect clinical symptomatology; pharmacological agents that are known to affect symptoms in human subjects should correct measurable parameters that model symptoms of the disorder with equal efficacy.

Developing an animal model for post-traumatic stress disorder (PTSD) is not a trivial issue. Diagnosis in human patients relies heavily on personal reports of thoughts, dreams, and images, which cannot be studied in rats. Furthermore, several of the typical symptoms of PTSD may be unique to humans and thus not be found in rats. Likewise, an important factor of the trauma in humans is the perception of the life-threatening potential of the situation. It is not clear whether rats can make this judgment or which stressors will be most effective for rats. In addition, there is as yet no clearly effective pharmacological treatment for PTSD. It is thus difficult to test a potential rodent model for its pharmacological predictability in relation to PTSD or other traumatic stress-related disorders.

Nevertheless, using animals to study PTSD holds advantages for several reasons. First, unlike many other mental disorders, the diagnostic criteria for PTSD specify an etiological factor, which is an exposure to a life-threatening, traumatic event (*6*). In a model for PTSD, variables such as the quality and intensity of the stressor and the degree of exposure to it can be carefully controlled, and the behavioral and concomitant physiological responses to a (valid) threatening stimulus could be studied. Second, little is known about pretrauma etiological aspects of the disorder since, naturally, the studies so far have focused on retrospective assessments of the patients after the onset of PTSD. An animal model will enable a prospective follow-up design, in which the disorder is triggered at a specified time and in a uniform manner, in controllable and statistically sound population samples, and enable the assessment of behavioral and gross physiological parameters. Moreover, unlike studies in human subjects, animal model studies enable the assessment of concomitant biomolecular

changes in dissected brain areas and the experimentation with pharmacological agents with potential therapeutic effects.

This chapter presents and discusses findings from various animal models of PTSD, which differ from one another in the rationale for their development. These models use different paradigms but show a range of behavioral and physiological manifestations seen in PTSD patients.

TRAUMA-/STRESS-BASED MODELS

Stress paradigms in animals studies aim to model criterion A of the DSM diagnostic criteria (*2*). They consist of extremely stressful experiences aimed to engender a sense of threat and helplessness in the animal. Some of these have focused more on the intensity of the experience, whereas others have combined this with an attempt to design an ethologically valid experience, one that an animal might encounter in its natural environment.

Exposure of rodents to predator stimuli (cat, cat odor, fox odor or trimethylthiazoline, a synthetic compound isolated from fox feces) is fear provoking and stressful and produces long-lasting behavioral and physiological responses. Blanchard et al. (*3–8*), Adamec et al. (*9–14*), and others (*15–20*) have established the validity of this paradigm, in which adult rodents are exposed to feline predators for 5–10 min in a closed environment (i.e., inescapable exposure). The resultant freezing response mode is ethologically adaptive for animals when both "fight" and "flight" options are ineffective. Predator stress has ecological validity in that it mimics brief, intense threatening experiences with lasting affective consequences (*12–13*). The predator stress paradigm has proven to be effective in inducing the expected range of behavioral and physiological responses (*1,9–14*). These include freezing, avoidance, increased secretion of stress hormones, and changes in transmission from hippocampus via the ventral angular bundle to the basolateral amygdala and from central amygdala to lateral column of the periaqueductal gray (*3,4,10,11,13,15–19,21–29*). These pathways are of interest because neuroplastic changes within them are associated with aversive learning. Predator stimuli potency is comparable to that of a variety of paradigms in which the threat is more tangible and immediate, such as paradigms based on inescapable pain or electric shock, swimming and near-drowning, a small raised platform, and even direct proximity to a kitten or a car (separated by a mesh divide or a solid divide with an opening large enough for the rodent to slip through).

Richter-Levin (*30*) developed an interesting stress model, the underwater trauma. Although rats naturally swim well and are able to dive and to cope with exposure to water, brief (30–45 s) uncontrollable restraint under water establishes an ethologically relevant traumatic experience. Exposure of rats to underwater trauma resulted in long-lasting heightened anxiety and context-specific spatial memory deficits (*30–32*). Underwater trauma in a different (out-of-context) water container had no effects on the ability of rats to perform a spatial memory task in the water maze (*30*). These results may explain

the lack of effect of inescapable tail shock procedure on spatial performance reported by others (*33*) because in their study the stressor was not associated with the context of the maze. Moreover, underwater trauma resulted in both behavioral and electrophysiological aversive effects. At 20 min after the trauma, the traumatized rats performed poorly in the spatial memory task in the water maze, and 40 min after the tetanic stimulation (100 min after the underwater trauma) they showed a reduced level of long-term potentiation (LTP). Thus, the underwater trauma induced electrophysiological alterations that resembled those observed in other models of stress (*34–37*). In addition, the impaired performance in the water maze was significantly correlated with the reduced ability to induce LTP. These findings of a strong correlation between LTP and spatial learning suggest that these two phenomena are related. However, it is possible that the trauma impairs performance not by affecting memory but by affecting memory-related processes such as attention. It was suggested that the underwater trauma could provide an important and potentially powerful model for understanding the mechanisms underlying the relationship among stress, cognition, and learning.

MECHANISM-BASED MODELS

Another approach in developing animal models of PTSD was to consider potential brain mechanisms that could underlay the disorder and to develop behavioral protocols that would mimic the activation of such mechanisms.

Enhanced Fear Conditioning

The persistence of the psychological and biological fear responses could not be satisfactorily explained by the stress theory, leading some to suggest that fear conditioning might underlay the phenomenon (*38*). In certain respects, fear conditioning resembles PTSD (*39*). During Pavlovian fear conditioning, a neutral conditioned stimulus (CS; usually a tone or light) is repeatedly paired with a stressful unconditioned stimulus (US; usually a foot shock). Once the CS-US association has been formed, the CS produces a conditioned fear response (CR; such as freezing [or movement arrest], enhancement of musculature [startle] reflexes, autonomic changes, analgesia and behavioral response suppression) in anticipation of the US (*40,41*). A CR is also evoked when the animal is placed in the environment in which the experiment took place. Translating to PTSD, the traumatic event (US) triggers an Unconditioned Response (UR) which is characterized by strong arousal and intense fear. This UR becomes associated with cues, such as smells, voices, or sights (CSs), that were present during the traumatic event. As a result of this pairing, these cues can trigger similar responses (CRs) even in the absence of the US (*38,42*). Thus, given theassociation between traumatic recall and seemingly unrelated stimuli and the ensuing fearful response, the mechanism of enhanced fear conditioning has often been suggested as a model for the reexperiencing phenomena in PTSD (*43–47*).

Impaired Extinction

Conditioned fear responses can be extinguished by repeatedly presenting the CS without the US (*39*). Pavlov, in his classic investigation of appetitive conditioning in dogs, observed that extinguished responses spontaneously recovered with the passage of time (*39*). This suggested that extinction suppresses, rather than erases, the original CS-US association. Thus, extinction is an important behavioral phenomenon that allows the organism to adapt its behavior to a changing environment (*48*). Moreover, experimental extinction is a behavioral technique that leads to suppression of the acquired fear, that is, a decrease in the amplitude and frequency of a CR as a function of nonreinforced CS presentations (*49*). More recently, impaired extinction learning has been proposed as an alternative mechanism for the formation of PTSD symptoms (*42,50–52*).

Part of the attraction of fear conditioning was that much was concurrently being learned about the neurobiology of this animal paradigm. A large body of evidence from lesion, pharmacological, and neurophysiological studies indicate that the amygdala (corpus amygdaloideum) is involved in the acquisition and extinction of fear memory (*53*) and seems to have a pivotal role in the extinction of learned conditioning fear (*54*). The hypothesis that lateral amygdala (LA) neurons encode fear memories, and conditional stimulus-elicited LA firing is contextually modulated after extinction has been demonstrated to require a functional hippocampus (*55*). Based on this assertion, it has been proposed that contextual modulation of CS-evoked spike firing could be implemented by hippocampal modulation of medial prefrontal cortex (mPFC) control over the amygdala (*56*). Alternatively, direct projections from the hippocampus to the amygdala may regulate fear expression after extinction (*57*). Because the hippocampus is connected with many brain areas (including the mPFC and the amygdala), it is yet unclear which of these connections is important for the contextual modulation of extinction. This model proposes that the hippocampus performs an executive role in the balance of excitation and inhibition in fear circuits, in which the mPFC may come to inhibit LA neuronal activity during fear extinction that would otherwise excite the fear response (*56*). Furthermore, the regulation of this fear is dependent on the context in which fear stimuli are encountered (*56*). When animals are tested in contexts associated with extinction, the hippocampus drives mPFC inhibition of the LA (*56*). However, if animals are presented with an extinguished CS outside the extinction context, the hippocampus may inhibit mPFC activation and thus promote excitation in the LA to renew the previously extinguished fear under these conditions (*56*). In support of this, lesions of rat infralimbic (IL) cortex (analogous to the mPFC in humans) enhance renewal of extinguished appetitive Pavlovian responding when tested in the acquisition context following extinction in an alternative context (*58*). These results parallel previous observations of increased spontaneous recovery and reinstatement in animals with damage to the IL region (*59*). Moreover, they are consistent with previous structural and functional neuroimaging studies in PTSD patients, indicating a hyperresponsive amygdala accompanied by hypoactivation of the PFC (*39,60–72*).

However, PTSD is a complex disorder that involves far more than a fear response and cannot be explained by a simple conditioning model.

Impaired Contextualization

A different mechanism that may contribute to the development of PTSD symptoms is the inability to appropriately "contextualize" the traumatic events in autobiographic memory. Clinically, PTSD patients relive their traumatic experiences repeatedly, unable to assimilate them as time- and context-limited events without negative implications for their future. For example, for a combat veteran, the sound of a passing helicopter in the current, objectively safe environment can evoke the traumatic experience of combat that took place years earlier. Deficient embedding or contextualization of the traumatic events in autobiographic memory is thought to be one of the main problems in PTSD (*73*). Indeed, suggestion of contextual memory deficits has been reported in the single prolonged stress (SPS) animal model of PTSD (*74–76*). However, direct testing of contextual cue processing is required to reliably demonstrate inability to contextualize memory in PTSD animal models.

We recently tested the hypothesis that exposure to a traumatic/stressful experience could impair contextual odor discrimination, and that this impairment is associated with PTSD-like behavioral responses. To support this study, a novel experimental paradigm, differential contextual-odor conditioning (DCOC), was devised to examine the animals' abilities to discriminate between the significance of an odor cue acquired in either safe or dangerous contextual environments when encountered in a novel, neutral environment. The odor cue consists of a cinnamon smell that could signal either reward or punishment (safety or threat signal) depending on the contextual cues that are present. Each of the conditions was learned in a different chamber. Animals were tested in a third, new chamber, so all other contextual cues were controlled for, and the only previously encountered cue that was present was the cinnamon odor (*77*).

Our findings demonstrated that, in this novel experimental paradigm, animals trained in the DCOC paradigm acquired the ability to discriminate between contextual cues signaling safe versus dangerous contextual environments, validating the DCOC paradigm for the assessment of contextualization. Exposure to severe traumatic stress (predator scent stress, PSS) interfered with processes related to subsequent adequate and flexible application of contextualization. Traumatized animals were unable to acquire the ability to accurately evaluate the contextual relevance of an odor stimulus or lost this ability after having effectively acquired it (Fig. 1). Thus, the DCOC paradigm is suggested as an effective animal model that would enable the study of the neurobiology of contextualization and of related pathology (*77*).

Other animal models focus on modeling specific neurobiological sequelae or specific behavior findings reported in PTSD.

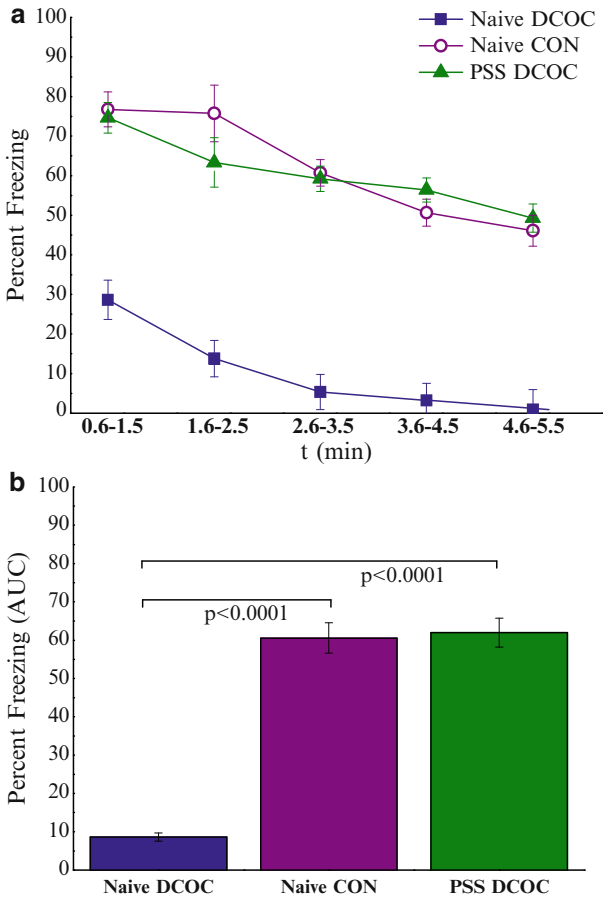

Fig. 1. Percent freezing in the neutral arenas for control and DCOC animals and the effects of pretraining stress-exposure: **a** Percent free zingin five blocks of 1 min each. **b** The area under the curve (AUC) during all training. The DCOC (differential contextual odor conditioning) rats displayed significantly less immobility than control (CON) or stress-exposed DCOC animals in the neutral arena. *PSS* predator scent stress

ANIMAL MODELS BASED ON CHANGES IN NEUROBIOLOGICAL SYSTEMS

Hypothalamic-Pituitary-Adrenal Axis Response

The SPS model introduced by Liberzon et al (*75,76*), was developed to mimic specific hypothalamic-pituitary-adrenal (HPA) abnormalities and enhanced acoustic startle (*74–6*). In the SPS paradigm, rats are exposed sequentially to 2 h of restraint, 20 min of swimming, and ether exposure until loss of consciousness. One week after the experience, rats show increased startle responses

to 50-ms, 108-dB tones, both when compared to a nonstressed control group and compared to their own startle responses before the SPS session (*74*). Most important, the SPS model has been found to induce long-term alteration of the expression of glucocorticoid receptors in the hippocampal formation (*6*). Whereas a long-term decrease was observed in type I (mineralocorticoid) receptor, the type II (glucocorticoid) receptors showed a transient decrease (24 h), followed by enhanced expression at 7 days post-SPS.

Another study assessed aspects of the HPA axis response in strains with deficient and excessive HPA axis responsiveness compared to normal rats (*78*). Stress responses were also examined in populations of inbred Lewis and Fischer rats and compared to outbred Sprague-Dawley rats. Lewis rats exhibit a reduced synthesis and secretion of corticotropin-releasing factor (CRF), leading to reduced plasma adrenocorticotropic hormone (ACTH) and reduced Corticosterone (CORT) release from the adrenal cortex, whereas Fischer rats possess a hyperresponsive HPA axis. Prevalence rates of extreme behavioral response (EBR) individuals were significantly higher in Lewis (50%) than in Fischer rats (10%) or controls (25%) (*78*). However, exogenous administration of cortisol to Lewis rats before applying the stressor decreased the prevalence of EBR significantly (8%). These results suggest that blunted HPA axis response to stress may play a role in the susceptibility to experimentally induced PTSD-like behavioral changes, especially as these effects may be reversed by preexposure administration of corticosterone (*78*).

MODELING ADDITIONAL FACTORS

Individual Differences in Response to an Exposure to a Traumatic Experience

It is important to note that, while PTSD requires exposure to a traumatic experience, the trauma alone is not sufficient for PTSD to develop since most individuals exposed to a traumatic event will not develop PTSD.

Identifying the Affected Ones: The Cutoff Behavioral Criteria Approach

The clinical diagnosis of PTSD, one of the most severe outcomes, is made only if an individual exhibits a certain number of symptoms from each of three quite well-defined symptom clusters over a certain period of time (*2*). Irrespective of the study design or of the stress paradigm, animal studies have generally included the entire stress-exposed population as the study population, and the results discussed and conceptualized as involving this population versus "others," although in practice, just as with humans, the exposed animals display heterogeneous responses. To more closely approximate the approach to understanding animal behavioral models to contemporary understanding of the clinical condition, Cohen and Zohar (*79*) conceived an approach to understanding the consequences of exposure to a variety of stress paradigms (exposure to a predator or its scent on soiled cat litter,

underwater trauma, and elevated platform) in a manner that would enable us to segregate the study animals into groups according to the degree of their response to the stressor, that is, the degree to which their behavior is altered or disrupted. To achieve this, behavioral criteria that would reflect something akin to clinical symptoms needed to be defined and then complemented by the definition of a series of cutoff behavioral criteria (CBC) reflecting severity of response, paralleling clinical inclusion and exclusion criteria applied to clinical research. The idea was to set apart the most clearly affected, that is, the EBR group from their minimal behavioral response (MBR) counterparts (*26–28,32,78–88*).

The CBC method has been applied in a series of studies and has repeatedly enabled a greater degree of resolution in viewing data, a means to reflect them in starker contrast. First, there was highly significant overlap between animals showing EBRs and those with extreme biophysiological measures (i.e., HPA axis assays and heart rate variability [reflecting autonomic nervous system activity]), much more clearly so than when the exposed group was analyzed as a whole (*26–28*),(*79,87*). Different types of traumatic stress paradigm could be seen to cause different proportions of EBR versus MBR animals, not unlike the "dose-response" phenomenon in the human condition, by which different forms of stressor are known to be associated with different incidence rates of PTSD. Serial assessments in the period after exposure to the stressor elicited a curve reflecting the incidence of EBR that parallels that seen in studies of acute stress reaction and subsequent development of chronic disorders: Initially, almost all animals responded "extremely" severely, and over the next 30 days the incidence dropped to an unvarying 25%. This rate of incidence has recurred in all the studies and parallels rates of incidence of PTSD in the general population exposed to trauma (estimated to be between 15% and 35% for most types of trauma throughout the Western Hemisphere).

Modeling Early Life Stress as a Risk Factor for Developing PTSD

Separating out the more clearly affected animals also elicited significantly different effects on the incidence of chronically disordered behavior when recurrent exposure to trauma occurred in early childhood or later life, both compared to single exposure: In both cases earlier exposure had a greater effect, as is seen in many studies of human subjects exposed to trauma in childhood and youth (*81*).

An important characteristic of stress paradigms is the age at which the animals are exposed to the stressor (*89*). There are many indications that across the life span there are specific windows of vulnerability when high levels of stress have an increased impact on further development (*89–94*). Recent years have witnessed growing interest in effectively modeling in animals the long-term effects of childhood emotional trauma on stress responses in adulthood. Most studies concerned with the impact of early life stress on subsequent stress responses in adulthood in rodents have focused on the postnatal preweaning period (i.e., 3–14 days) and involve some form of maternal deprivation or

maternal separation producing acute and long-term effects that vary with the pups' age at exposure to stress (*95–97*). However, marked differences exist between neonate rats and infants' stress response mechanisms (*98*). For example, rat pups' HPA axis is characterized by a silent hyporesponsive period (*99*), while in humans there is no conclusive evidence of a hyporesponsive period in the HPA axis course of development (*100*). Indeed, it has been suggested that the ages of 3 to 14 days in the rat roughly correspond to the 23rd week of gestation in humans. Furthermore, psychiatric studies often refer to human childhood rather than infancy when investigating the traumatic history of stress-related psychopathologies in patients (*101,102*). Thus, Richter-Levin and colleagues (*81,103–107*) have started to examine the consequences of stress exposure at a later early life period: the juvenile or the early adolescent period. The authors reported that the combination of juvenile and adulthood exposures to stress increased anxiety levels, in comparison not only with control unstressed rats but also with rats exposed to stress twice in adulthood. Tsoory and Richter-Levin (*107*) showed that exposure to stress during juvenility (27–29 days) has a stronger long-term deleterious effect on learning under stressful conditions in adulthood than exposure to the same stressor during "adolescence" (33–35 days). The physiological changes associated with juvenile stress have also been reported and include increases of Dehydroepiandrosterone-sulphate (DHEA-S) concentrations in both the hypothalamus and the entorhinal cortex (*103*), reduced levels of corticosterone (*108*), altered autonomic nervous system responses (heart rate and heart rate variability) (*81*), and downregulation of brain-derived neurotrophic factor (BDNF) messenger ribonucleic acid (mRNA) in the hippocampal CA1 subregion (*108*). These physiologic changes presumably mediate clinical manifestations of PTSD.

The Contribution of Genetic Background

Twin and family studies of PTSD patients raised questions regarding a possible genetic predisposition to PTSD, although the relative contributions of genotype and environment to endophenotypic expression are unclear (*109*).

Post-Traumatic Stress Behavioral Responses in Inbred Mouse Strains

To examine the importance of the genetic background, six inbred strains of mice frequently employed in transgenic research were assessed at baseline and 7 days after PSS exposure (*84*). Inbred strains are expected to demonstrate about 97.5% homozygosity of loci as the result of at least 20 generations of sibling matings. The results, however, revealed an unexpectedly high degree of within-strain individual heterogeneity at baseline and in the degree of response to stress. This within-strain phenotypic heterogeneity most likely implies that environmental factors play a significant role in characterizing individual responses in spite of the significant strain-related (i.e., genetic) underpinnings. The authors thus suggested that heritable factors may be involved only in part

of the endophenotypes associated with the PTSD-like phenotype and may be influenced through a highly indirect route with considerable potential for interaction with environmental variables (*110,111*).

These data imply that the attempt to identify "genetic" versus "environmental" causality as independent main effects is probably logically and procedurally flawed. The evaluation of genetic effects on behavioral phenotypes should consider interactions among genes as well as interactions between genes and environment (*84*).

CONCLUSION

The development of animal models for PTSD and other traumatic stress-related brain changes is an important part of advancing our neurobiological understanding of the disease process as well as recovery, resilience, and possible therapeutic targets. Ultimately, the "optimal" animal model should incorporate trauma-like exposure, will mimic pathophysiological and behavioral findings present in PTSD, and will presumably involve neurobiological mechanisms that participate in PTSD pathophysiology. However, no single widely accepted animal model of PTSD has been established to date, and there is an ongoing debate over what constitutes a valid animal model for this disorder.

Although animal models for PTSD are limited to the assessment of measurable and observable behavioral parameters and cannot assess complex psychological symptoms such as thought, meaning and dreams, valid and reliable animal models offer a means for researching biomolecular, pathophysiological, and pharmacological features of the disorder in ways that are not feasible in human studies.

REFERENCES

1. Nutt D, Davidson J . Post-Traumatic Stress Disorder Diagnosis, Management and Treatment. London: Taylor and Francis; 2000.
2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th ed. Washington, DC: American Psychiatric Association; 1994.
3. Blanchard DC, Griebel G, Blanchard RJ. Conditioning and residual emotionality effects of predator stimuli: some reflections on stress and emotion. Prog Neuropsychopharmacol Biol Psychiatry 2003;27(8):1177–85.
4. Blanchard RJ, Blanchard DC. Anti-predator defense as models of fear and anxiety.In: RJBlanchard and SParmigiani, ed. Brain. London: Harwood Academic; 1990.
5. Blanchard RJ, Blanchard DC, Rodgers J, Weiss SM. The characterization and modelling of antipredator defensive behavior. Neurosci Biobehav Rev 1990;14(4):463–72.
6. Blanchard RJ, Griebel G, Henrie JA, Blanchard DC. Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries. Neurosci Biobehav Rev 1997;21(6):783–89.
7. Blanchard RJ, Nikulina JN, Sakai RR, McKittrick C, McEwen B, Blanchard DC. Behavioral and endocrine change following chronic predatory stress. Physiol Behav 1998;63(4):561–69.
8. Blanchard RJ, Yang M, Li CI, Gervacio A, Blanchard DC. Cue and context conditioning of defensive behaviors to cat odor stimuli. Neurosci Biobehav Rev 2001;25(7–8):587–95.

9. Adamec R. Transmitter systems involved in neural plasticity underlying increased anxiety and defense—implications for understanding anxiety following traumatic stress. Neurosci Biobehav Rev 1997;21(6):755–65.
10. Adamec R, Head D, Blundell J, Burton P, Berton O. Lasting anxiogenic effects of feline predator stress in mice: sex differences in vulnerability to stress and predicting severity of anxiogenic response from the stress experience. Physiol Behav 2006;88(1–2):12–29. Epub 2006 Apr 19.
11. Adamec R, Muir C, Grimes M, Pearcey K. Involvement of noradrenergic and corticoid receptors in the consolidation of the lasting anxiogenic effects of predator stress. Behav Brain Res 2007;179(2):192–207. Epub 2007 Feb 6.
12. Adamec R, Strasser K, Blundell J, Burton P, McKay DW. Protein synthesis and the mechanisms of lasting change in anxiety induced by severe stress. Behav Brain Res 2006;167(2):270–86.
13. Adamec RE, Blundell J, Burton P. Relationship of the predatory attack experience to neural plasticity, pCREB expression and neuroendocrine response. Neurosci Biobehav Rev 2006;30(3):356–75. Epub 2005 Aug 22.
14. Adamec RE, Shallow T. Lasting effects on rodent anxiety of a single exposure to a cat. Physiol Behav 1993;54(1):101–9.
15. Cohen H, Benjamin J, Kaplan Z, Kotler M. Administration of high-dose ketoconazole, an inhibitor of steroid synthesis, prevents posttraumatic anxiety in an animal model. Eur Neuropsychopharmacol 2000;10:429–35.
16. Cohen H, Friedberg S, Michael M, Kotler M, Zeev K. Interaction of CCK-4 induced anxiety and post-cat exposure anxiety in rats. Depress Anxiety 1996;4(3):144–45.
17. Cohen H, Kaplan Z, Kotler M. CCK-antagonists in a rat exposed to acute stress: implication for anxiety associated with post-traumatic stress disorder. Depress Anxiety 1999;10(1):8–17.
18. Diamond DM, Campbell AM, Park CR, et al. Influence of predator stress on the consolidation versus retrieval of long-term spatial memory and hippocampal spinogenesis. Hippocampus 2006;16(7):571–76.
19. File SE, Zangrossi H Jr, Sanders FL, Mabbutt PS. Dissociation between behavioral and corticosterone responses on repeated exposures to cat odor. Physiol Behav 1993;54:1109–11.
20. Griebel G, Blanchard DC, Jung A, Lee JC, Masuda CK, Blanchard RJ. Further evidence that the mouse defense test battery is useful for screening anxiolytic and panicolytic drugs: effects of acute and chronic treatment with alprazolam. Neuropharmacology 1995;34(12):1625–33.
21. Sullivan M, Gratton A. Relationships between stress-induced increases in medial prefrontal cortical dopamine and plasma corticosterone levels in rats: role of cerebral laterality. Neuroscience 1998;83:81–91.
22. Apfelbach R, Blanchard CD, Blanchard RJ, Hayes RA, McGregor IS. The effects of predator odors in mammalian prey species: a review of field and laboratory studies. Neurosci Biobehav Rev 2005;29(8):1123–44. Epub 2005 Aug 8.
23. Blundell J, Adamec R, Burton P. Role of NMDA receptors in the syndrome of behavioral changes produced by predator stress. Physiol Behav 2005;86(1–2):233–43.
24. Endres T, Apfelbach R, Fendt M. Behavioral changes induced in rats by exposure to trimethylthiazoline, a component of fox odor. Behav Neurosci 2005;119(4):1004–10.
25. Takahashi LK, Nakashima BR, Hong H, Watanabe K. The smell of danger: a behavioral and neural analysis of predator odor-induced fear. Neurosci Biobehav Rev 2005;29(8): 1157–67. Epub 2005 Aug 10.

26. Cohen H, Maayan R, Touati-Werner D, et al. Decreased circulatory levels of neuroactive steroids in behaviorally more extremely affected rats subsequent to exposure to a potentially traumatic experience. Int J Neuropsychopharmacol 2007;10(2):203–9.
27. Kozlovsky N, Matar MA, Kaplan Z, Kotler M, Zohar J, Cohen H. The immediate early gene Arc is associated with behavioral resilience to stress exposure in an animal model of posttraumatic stress disorder. Eur Neuropsychopharmacol 2007;2:2.
28. Mazor A, Matar M, Kozlovsky N, Zohar J, Kaplan Z, Cohen H. Gender-related qualitative differences in baseline and post stress anxiety responses are not reflected in the incidence of criterion-based PTSD-like behavior patterns. World J Biol Psychiatry 2007;13:1–14.
29. Roseboom PH, Nanda SA, Bakshi VP, Trentani A, Newman SM, Kalin NH. Predator threat induces behavioral inhibition, pituitary-adrenal activation and changes in amygdala CRF-binding protein gene expression. Psychoneuroendocrinology 2007;32(1):44–55. Epub 2006 Nov 20.
30. Richter-Levin G. Acute and long-term behavioral correlates of underwater trauma—potential relevance to stress and post-stress syndromes. Psychiatry Res 1998;79(1):73–83.
31. Wang J, Akirav I, Richter-Levin G. Short-term behavioral and electrophysiological consequences of underwater trauma. Physiol Behav 2000;70(3–4):327–32.
32. Cohen H, Zohar J. Animal models of post traumatic stress disorder: the use of cut off behavioral criteria.Ann N Y Acad Sci 2004;1032:167–78.
33. Warren DA, Castro CA, Rudy JW, Maier SF. No spatial learning impairment following exposure to inescapable shock. Psychobiology 1991;19:127–34.
34. Diamond DM, Rose GM. Stress impairs LTP and hippocampal-dependent memory. Ann N Y Acad Sci 1994;746:411–14.
35. Foy MR, Stanton ME, Levine S, Thompson RF. Behavioral stress impairs long-term potentiation in rodent hippocampus. Behav Neural Biol 1987;48(1):138–49.
36. Shors TJ, Seib TB, Levine S, Thompson RF. Inescapable versus escapable shock modulates long-term potentiation in the rat hippocampus. Science 1989;244(4901):224–26.
37. Xu L, Holscher C, Anwyl R, Rowan MJ. Glucocorticoid receptor and protein/RNA synthesis-dependent mechanisms underlie the control of synaptic plasticity by stress. Proc Natl Acad Sci U S A 1998;95(6):3204–8.
38. Yehuda R, LeDoux J. Response variation following trauma: a translational neuroscience approach to understanding PTSD. Neuron 2007;56(1):19–32.
39. Milad MR, Rauch SL, Pitman RK, Quirk GJ. Fear extinction in rats: implications for human brain imaging and anxiety disorders. Biol Psychol 2006;73(1):61–71. Epub 2006 Feb 13.
40. Dunsmoor JE, Bandettini PA, Knight DC. Neural correlates of unconditioned response diminution during Pavlovian conditioning. Neuroimage 2008;40:811–17.
41. LeDoux J. Emotional networks and motor control: a fearful view. Prog Brain Res 1996;107:437–46.
42. Blechert J, Michael T, Vriends N, Margraf J, Wilhelm FH. Fear conditioning in posttraumatic stress disorder: evidence for delayed extinction of autonomic, experiential, and behavioural responses. Behav Res Ther 2007;45(9):2019–33.
43. Bonne O, Grillon C, Vythilingam M, Neumeister A, Charney DS. Adaptive and maladaptive psychobiological responses to severe psychological stress: implications for the discovery of novel pharmacotherapy. Neurosci Biobehav Rev 2004;28(1):65–94.
44. Foa EB, Kozak MJ. Emotional processing of fear: exposure to corrective information. Psychol Bull 1986;99(1):20–35.

45. Kolb LC. A neuropsychological hypothesis explaining posttraumatic stress disorders. Am J Psychiatry 1987;144(8):989–95.
46. LeDoux JE. Emotion circuits in the brain. Annu Rev Neurosci 2000;23:155–84.
47. Maren S. Neurobiology of Pavlovian fear conditioning. Annu Rev Neurosci 2001;24, 897–931.
48. Bouton ME. Context and behavioral processes in extinction. Learn Mem 2004;11(5):485–94.
49. Akirav I, Maroun M. The role of the medial prefrontal cortex-amygdala circuit in stress effects on the extinction of fear. Neural Plast 2007;2007:30873.
50. Guthrie RM, Bryant RA. Extinction learning before trauma and subsequent posttraumatic stress. Psychosom Med 2006;68(2):307–11.
51. Maren S, Chang CH. Recent fear is resistant to extinction. Proc Natl Acad Sci U S A 2006;103(47):18020–25. Epub 2006 Nov 7.
52. Myers KM, Davis M. Behavioral and neural analysis of extinction. Neuron 2002;36:567–84.
53. Maren S, Aharonov G, Stote DL, Fanselow MS. N-Methyl-Daspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. Behav Neurosci Biobehav Rev 1996;110:1365–74.
54. Walker DL, Davis M. The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. Pharmacol Biochem Behav 2002;71:379–92.
55. Maren S. Auditory fear conditioning increases CS-elicited spike firing in lateral amygdala neurons even after extensive overtraining. Eur J Neurosci 2000;12:4047–54.
56. Goosens KA, Hobin JA, Maren S. Auditory-evoked spike firing in the lateral amygdala and Pavlovian fear conditioning: mnemonic code or fear bias? Neuron 2003;40:1013–22.
57. Maren S, Quirk GJ. Neuronal signalling of fear memory. Nat Rev Neurosci 2004;5:844–52.
58. Rhodes SE, Killcross AS. Lesions of rat infralimbic cortex enhance renewal of extinguished appetitive Pavlovian responding. Eur J Neurosci 2007;25(8):2498–503.
59. Quirk GJ, Russo GK, Barron JL, Lebron K. The role of ventromedial prefrontal cortex in the recovery of extinguished fear. J Neurosci 2000;20:6225–31.
60. Bremner JD, Innis RB, Ng CK, et al. Positron emission tomography measurement of cerebral metabolic correlates of yohimbine administration in combat-related posttraumatic stress disorder. Arch Gen Psychiatry 1997;54(3):246–54.
61. Semple WE, Goyer PF, McCormick R, et al. Higher brain blood flow at amygdala and lower frontal cortex blood flow in PTSD patients with comorbid cocaine and alcohol abuse compared with normals. Psychiatry 2000;63(1):65–74.
62. Semple WE, Goyer PF, McCormick R, et al. Attention and regional cerebral blood flow in posttraumatic stress disorder patients with substance abuse histories. Psychiatry Res 1996;67(1):17–28.
63. Rauch SL, Shin LM. Functional neuroimaging studies in posttraumatic stress disorder. Ann N Y Acad Sci 1997;821:83–98.
64. Shin LM, McNally RJ, Kosslyn SM, et al. Regional cerebral blood flow during script-driven imagery in childhood sexual abuse-related PTSD: a PET investigation. Am J Psychiatry 1999;156(4):575–84.
65. Rauch SL, van der Kolk BA, Fisler RE, et al. A symptom provocation study of posttraumatic stress disorder using positron emission tomography and script-driven imagery. Arch Gen Psychiatry 1996;53(5):380–87.
66. Rauch SL, Whalen PJ, Shin LM, et al. Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. Biol Psychiatry 2000;47(9):769–76.
67. Bremner JD. Alterations in brain structure and function associated with post-traumatic stress disorder. Semin Clin Neuropsychiatry 1999;4(4):249–55.

68. Bremner JD, Randall P, Scott TM, et al. MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. Am J Psychiatry 1995; 152(7):973–81.
69. Freeman TW, Cardwell D, Karson CN, Komoroski RA. In vivo proton magnetic resonance spectroscopy of the medial temporal lobes of subjects with combat-related posttraumatic stress disorder. Magn Reson Med 1998;40(1):66–71.
70. De Bellis MD, Keshavan MS, Spencer S, Hall J. N-Acetylaspartate concentration in the anterior cingulate of maltreated children and adolescents with PTSD. Am J Psychiatry 2000;157(7):1175–77.
71. Lanius RA, Williamson PC, Densmore M, et al. Neural correlates of traumatic memories in posttraumatic stress disorder: a functional MRI investigation. Am J Psychiatry 2001;158(11):1920–22.
72. Etkin A, Wager TD. Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am J Psychiatry 2007;164(10):1476–88.
73. Ehlers A, Clark DM. A cognitive model of posttraumatic stress disorder. Behav Res Ther 2000;38(4):319–45.
74. Khan S, Liberzon I. Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD. Psychopharmacology (Berl) 2004;172(2):225–29. Epub 2003 Oct 30.
75. Liberzon I, Lopez JF, Flagel SB, Vazquez DM, Young EA. Differential regulation of hippocampal glucocorticoid receptors mRNA and fast feedback: relevance to post-traumatic stress disorder. J Neuroendocrinol 1999;11(1):11–17.
76. Liberzon I, Krstov M, Young EA. Stress-restress: effects on ACTH and fast feedback. Psychoneuroendocrinology 1997;22(6):443–53.
77. Cohen H, Liberzon I, Richter-Levin G. Exposure to extreme stress impairs contextual odor discrimination in an animal model of PTSD. In J Neuropsychopharmacol 2008;Aug 13:1–13 [Epub ahead of print].
78. Cohen H, Zohar J, Gidron Y, et al. Blunted HPA axis response to stress influences susceptibility to posttraumatic stress response in rats. Biol Psychiatry 2006;59(12):1208–18.
79. Cohen H, Zohar J, Matar M. The relevance of differential response to trauma in ananimal model of post-traumatic stress disorder. Biol Psychiatry 2003;53(6):463–73.
80. Cohen H, Kaplan Z, Matar M, Loewenthal U, Kozlovsky N, Zohar J. Anisomycin, a protein synthesis inhibitor, disrupts traumatic memory consolidation and attenuates post traumatic stress response in rats. Biol Psychiatry 2006;60(7):767–76.
81. Cohen H, Kaplan Z, Matar MA, Loewenthal U, Zohar J, Richter-Levin G. Long-lasting behavioral effects of juvenile trauma in an animal model of PTSD associated with a failure of the autonomic nervous system to recover. Eur Neuropsychopharmacol 2007;17(6–7): 464–77.
82. Cohen H, Matar MA, Richter-Levin G, Zohar J. The contribution of an animal model toward uncovering biological risk factors for PTSD. Ann N Y Acad Sci 2006;1071: 335–50.
83. Cohen H, Ziv Y, Cardon M, et al. Maladaptation to mental stress mitigated by the adaptive immune system via depletion of naturally occurring regulatory CD4+CD25+cells. J Neurobiol 2006;66(6):552–63.
84. Cohen H, Zohar J, Matar M, Loewenthal U, Kaplan Z. The impact of environment factors in determining post-exposure responses in isogenic strains of mice:Can genetic predisposition explain phenotypic vulnerability? Int J Neuropsychopharmacol 2008;11:331–49.
85. Cohen H, Zohar J, Matar MA, Kaplan Z, Geva AB. Unsupervised fuzzy clustering analysis supports behavioral cutoff criteria in an animal model of posttraumatic stress disorder. Biol Psychiatry 2005;58(8):640–50.

86. Cohen H, Zohar J, Matar MA, Zeev K, Loewenthal U, Richter-Levin G.Setting apart the affected:the use of behavioral criteria in animal models of post traumatic stress disorder. Neuropsychopharmacology 2004;29(11):1962–70.
87. Kozlovsky N, Matar MA, Kaplan Z, Kotler M, Zohar J, Cohen H. Long-term down-regulation of BDNF mRNA in rat hippocampal CA1 subregion correlates with PTSD-like behavioural stress response. Int J Neuropsychopharmacol 2007, 1–18.
88. Matar MA, Cohen H, Kaplan Z, Zohar J. The effect of early poststressor intervention with sertraline on behavioral responses in an animal model of post-traumatic stress disorder. Neuropsychopharmacology 2006;31(12):2610–18.
89. Schmidt MV, Sterlemann V, Ganea K, et al. Persistent neuroendocrine and behavioral effects of a novel, etiologically relevant mouse paradigm for chronic social stress during adolescence. Psychoneuroendocrinology 2007;32(5):417–29.
90. Ford JD, Kidd P. Early childhood trauma and disorders of extreme stress as predictors of treatment outcome with chronic posttraumatic stress disorder. J Trauma Stress 1998; 11(4):743–61.
91. Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders:preclinical and clinical studies. Biol Psychiatry 2001;49(12): 1023–39.
92. Heim C, Newport DJ, Wagner D, Wilcox MM, Miller AH, Nemeroff CB. The role of early adverse experience and adulthood stress in the prediction of neuroendocrine stress reactivity in women:amultiple regression analysis. Depress Anxiety 2002;15(3):117–25.
93. Breslau N. Psychiatric morbidity in adult survivors of childhood trauma. Semin Clin Neuropsychiatry 2002;7(2):80–88.
94. Shea A, Walsh C, Macmillan H, Steiner M. Child maltreatment and HPA axis dysregulation: relationship to major depressive disorder and post traumatic stress disorder in females. Psychoneuroendocrinology 2005;30(2):162–78.
95. Andersen SL, Teicher MH. Delayed effects of early stress on hippocampal development. Neuropsychopharmacology 2004;29(11):1988–93.
96. Schmidt MV, Enthoven L, vander Mark M, Levine S, deKloet ER, Oitzl MS. The postnatal development of the hypothalamic-pituitary-adrenal axis in the mouse. Int J Dev Neurosci 2003;21(3):125–32.
97. Vazquez DM, Bailey C, Dent GW, et al. Brain corticotropin-releasing hormone(CRH) circuits in the developing rat:effect of maternal deprivation.Brain Res 2006;1121(1): 83–94.
98. Vazquez DM. Stress and the developing limbic-hypothalamic-pituitary-adrenal axis. Psychoneuroendocrinology 1998;23(7):663–700.
99. Vazquez DM, VanOers H, Levine S, Akil H. Regulation of glucocorticoid and mineralocorticoid receptor mRNAs in the hippocampus of the maternally deprived infant rat. Brain Res 1996;731(1–2):79–90.
100. Gunnar MR, Donzella B. Social regulation of the cortisol levels in early human development. Psychoneuroendocrinology 2002;27(1–2):199–220.
101. DeBellis MD, Keshavan MS, Shifflett H, etal.Brain structures in pediatric maltreatment-related posttraumatic stress disorder: a sociodemographically matched study. Biol Psychiatry 2002;52(11):1066–78.
102. Nemeroff CB. Neurobiological consequences of childhood trauma. J Clin Psychiatry 2004;65(suppl1):18–28.
103. Avital A, Ram E, Maayan R, Weizman A, Richter-Levin G. Effects of early-life stress on behavior and neurosteroid levels in the rat hypothalamus and entorhinal cortex. Brain Res Bull 2006;68(6):419–24.

104. Avital A, Richter-Levin G. Exposure to juvenile stress exacerbates the behavioural consequences of exposure to stress in the adult rat. Int J Neuropsychopharmacol 2005;8(2):163–73.
105. Tsoory M, Cohen H, Richter-Levin G. Juvenile stress induces a predisposition to either anxiety or depressive-like symptoms following stress in adulthood. Eur Neuropsychopharmacol 2007;17(4):245–56.
106. Tsoory M, Guterman A, Richter-Levin G. Exposure to stressors during juvenility disrupts development-related alterations in thePSA-NCAM to NCAM expression ratio:potential relevance for mood and anxiety disorders. Neuropsychopharmacology 2008;33(2):378–93.
107. Tsoory M, Richter-Levin G. Learning under stress in the adult rat is differentially affected by"juvenile"or"adolescent"stress. Int J Neuropsychopharmacol 2006;9(6):713–28.
108. Bazak N, Kozlovsky N, Kaplan Z, et al. Pre-pubertal stress-exposure affects adult stress-response in correlation with changes in circulating corticosterone and brain-derived neurotrophic factor. In press (Phychoneuroendocrinology).
109. Stein MB, Jang KL, Taylor S, Vernon PA, Livesley WJ. Genetic and environmental influences on trauma exposure and posttraumatic stress disorder symptoms:a twin study. Am J Psychiatry 2002;159(10):1675–81.
110. Caspi A, Moffitt T. Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci 2006;7:583–90.
111. Moffitt T, Caspi A, Rutter M. Measured gene-environment interactions in psychopathology. Perspect Psychol Sci 2006;1:5–27.