Animal Models of PTSD: A Critical Review

Elizabeth I. Flandreau and Mate Toth

Abstract The goals of animal research in post-traumatic stress disorder (PTSD) include better understanding the neurophysiological etiology of PTSD, identifying potential targets for novel pharmacotherapies, and screening drugs for their potential use as PTSD treatment in humans. Diagnosis of PTSD relies on a patient interview and, as evidenced by changes to the diagnostic criteria in the DSM-5, an adequate description of this disorder in humans is a moving target. Therefore, it may seem insurmountable to model the construct of PTSD in animals such as rodents. Fortunately, the neural circuitry involved in fear and anxiety, thought to be essential to the etiology of PTSD in humans, is highly conserved throughout evolution. Furthermore, many symptoms can be modeled using behavioral tests that have face, construct, and predictive validity. Because PTSD is precipitated by a definite traumatic experience, animal models can simulate the induction of PTSD, and test causal factors with longitudinal designs. Accordingly, several animal models of physical and psychological trauma have been established. This review discusses the widely used animal models of PTSD in rodents, and overviews their strengths and weaknesses in terms of face, construct, and predictive validity.

Keywords Animal model • Anxiety • Fear learning • Predator stress • PTSD • Social defeat

E.I. Flandreau (✉) and M. Toth
Grand Valley State University, 1 Campus Drive, Allendale, MI 49401, USA
Department of Behavioral Neurobiology, Hungarian Academy of Sciences, Institute of Experimental Medicine, 43 Szigony Street, Budapest 1083, Hungary
e-mail: flandree@gvsu.edu; toth.mate@koki.mta.hu

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1 Animal Models of Traumatic Stress

Diagnostic criteria for PTSD according to DSM-5 include expression – for over 1 month – of debilitating symptoms from four clusters following exposure to traumatic events such as threat of death, or actual or threatened serious injury or violence:

- Cluster B: Intrusion (e.g., nightmares, flashbacks, intrusive thoughts, and physiological reactions to trauma reminders).
- Cluster C: Avoidance (intentionally avoiding trauma-related people, places, or activities).
- Cluster D: Negative alterations in cognitions and mood (e.g., dissociative amnesia, negative perception of self and world, anhedonia, social withdrawal).
- Cluster E: Alterations in arousal and reactivity (e.g., irritability, aggression, problems concentrating, sleep disturbances, and hypervigilance).

The goal of this chapter is to critically review current animal models of trauma exposure and how the behavioral and physiological outcomes of these models translate to these symptom clusters.

For models of PTSD to reach high translational value, essential requirements have been defined (Siegmund and Wotjak 2006): (1) the trauma must be severe, (2) a relatively short duration should be sufficient to provoke PTSD-like symptoms, (3) the intensity of the trauma should predict the severity of outcome, and (4) the stressor should induce persistent or progressive PTSD-like alterations with (5) significant interindividual variability in outcomes. Existing models apply stressors of a physical, psychological, social, or combined nature.

1.1 Physical Stressors

Restraint stress, underwater holding, and electric shock are all stressors of a physical nature. However, it is generally accepted that these lead to a combined physical and psychological stress from the rodents' perspective. Application of these stressors varies greatly between laboratories in terms of duration and severity. Although most of these can also be applied as chronic stressors, this discussion focuses on acute stressors in keeping with the above second criteria for an animal model of PTSD.

1.1.1 Restraint/Immobilization Stress

Both restraint and immobilization stress involve placing rodents in an enclosed chamber allowing for minimal or no movement. Total immobilization can be considered the most severe of the restraint methods. Two hours of complete immobilization has been shown to increase anxiety-like behavior in the elevated plus maze and open-field tests (Andero et al. 2013; Mitra et al. 2005), increase compulsive-like behavior in the marble-burying test (Kedia and Chattarji 2014), reduce declarative memory performance in the water maze task, increase fear learning (Andero et al. 2013), and increase REM sleep (Meerlo et al. 2001). Importantly, avoidance and morphological changes mostly manifested after a significant delay (i.e., 10 days) (Andero et al. 2013; Mitra et al. 2005; Kedia and Chattarji 2014). In line with endocrine data from PTSD patients (Yehuda et al. 2006), when immobilization stress was followed 1, 7, or 13 days later by a 20-min reminder restraint, significant hypoactivity of the hypothalamic pituitary adrenal (HPA) axis was induced, as measured by decreased concentrations of ACTH and corticosterone (Harvey et al. 2006).

1.1.2 Underwater Holding

In rats, underwater trauma typically involves 40 s of forced swimming followed by a 20-s forced submersion (Richter-Levin 1998). Rats exposed to this stress exhibit increased startle reactivity and anxiety-like behavior in the elevated plus maze both immediately, 7, and 30 days later with reduced corticosterone levels 7 days after stress (Richter-Levin 1998; Cohen et al. 2004; Moore et al. 2012). Additionally, contextual fear with altered limbic activity persists for over a month in this model (Ritov et al. 2016).

1.1.3 Single Prolonged Stress

Single prolonged stress (SPS) paradigms typically involve three stressors: 2-h restraint followed by forced swim, followed by ether anesthesia. These stressors induce psychological, physiological, and endocrine stress, respectively. Rats exposed to this SPS procedure exhibit increased anxiety-like behavior in the elevated plus maze, enhanced fear acquisition, and reduced fear extinction learning (Imanaka et al. 2006; Knox et al. 2012; Takahashi et al. 2006; Wang et al. 2008; Yamamoto et al. 2009). Halothane-combined SPS exposure also increased anxiety-like behavior; corticosterone concentration was elevated 1 day following SPS but normalized by day 7 (Harvey et al. 2006). Interestingly, when rats were exposed to a reminder restraint, increased anxiety-like behavior was associated with a decreased corticosterone response, suggesting that the initial trauma is insufficient to produce PTSD-like symptoms in the absence of a reminder (Harvey et al. 2006). Increased anxiety-like behavior in the open field and increased immobility in the forced swim test 1 and 7 (but not 4) days

were also apparent following SPS (Wu et al. 2016). The authors suggest that relevant compensatory changes occur in the first week following SPS exposure explain the timing of these effects (Wu et al. 2016).

Similar effects have been observed in mice: restraint, group swim, rat bedding (as a predator exposure) followed by ether exposure until unconsciousness led to enhanced cue-induced freezing, reduced extinction, and increased HPA axis negative feedback in the dexamethasone-suppression test, presumably due to elevated hippocampal expression of the glucocorticoid receptor (Perrine et al. 2016).

PTSD-like disruptions in sleep have also been observed in rats exposed to SPS. EEG recordings revealed that on the day of SPS exposure, rats exhibited increased REM sleep and decreased wakefulness (Vanderheyden et al. 2015). While these changes were not sustained over time, a decrease in non-REM sleep was observed during the active phase up to 7 days following trauma (Vanderheyden et al. 2015).

Morphological changes were also observed in SPS models including apoptotic volume loss in the hippocampus and the dorsal raphe nucleus (Han et al. 2013; Liu et al. 2012a) that may correspond with human findings reporting volumetric decreases in these brain regions in PTSD (Carrion et al. 2009; Gilbertson et al. 2002).

Selective serotonin reuptake inhibitors (SSRIs), a common treatment in PTSD patients (Steckler and Risbrough 2012), have been shown to reduce or prevent PTSD-like behavioral and endocrine symptoms in some SPS paradigms (Wang et al. 2008; Perrine et al. 2016) but not others (Takahashi et al. 2006). This difference could be due to laboratory differences in SPS design, SSRI administration, or behavioral testing. Of note, SSRIs are also ineffective in a portion of human PTSD patients (Stein et al. 2002); therefore successful symptom alleviation with SSRIs may not represent a meaningful concern from a validity perspective. Another possibility is that while SSRIs successfully treat comorbid anxiety and depression in PTSD patients, they may not truly address the core symptoms. As evidence for this hypothesis, a recent study demonstrated that the SSRI escitalopram reduced depressive-like and avoidance symptoms, but not fear extinction deficits in mice exposed to SPS (Lin et al. 2016).

1.1.4 Electric Shock

Uncontrollable and unpredictable foot shock is the most common method of stress exposure and even a single exposure can lead to long-lasting behavioral changes (reviewed in Bali and Jaggi 2015). Significant advantages of electric shock are (1) reliable delivery and precise control of the number and amperage of the shocks, length of the session, and inter-shock intervals; (2) well-defined and reproducible context and environmental cues by using standard boxes with lights and speakers; and (3) adjustable changes in contextual modalities, cues, and reminders. Another potential advantage of using electric shock is that, compared to restraint stress, there is less habituation (Siegmund and Wotjak 2007a). A potential limitation of electric shock as a model of PTSD-like trauma is that compared to human subjects and other animal

trauma models, there is relatively little individual variability in terms of sensitivity and resiliency; the vast majority of subjects exposed to electric shock will go on to display behavioral consequences to stress (reviewed in Bali and Jaggi 2015).

The underlying vulnerability, progressive development, and persistence of PTSD symptoms can only be modeled using long-term testing covering multiple symptom domains. Consistent with clinical criteria, several rodent studies reveal progressive development and persistence (3–5 weeks following single or multiple shocks, with or without subsequent reminders) of PTSD-like symptoms, including social withdrawal or avoidance, defensive behavior, hypervigilance, sleep disturbances, and generalization of fear (Siegmund and Wotjak 2007a; Cullen et al. 2015; Louvart et al. 2005; Mikics et al. 2008a, b; Philbert et al. 2011; Pynoos et al. 1996). Importantly, these studies applied somewhat higher amperage (0.8–3.0 mA) compared to most conditioned fear paradigms focusing on fear learning characteristics on a short term (~0.5–1.5 mA). Increased fear response over time in vulnerable individuals has been conceptualized as "incubation of fear" (Siegmund and Wotjak 2007a; Elharrar et al. 2013; Eysenck 1968; Milad et al. 2009) that refers to recurrent recalls leading to sensitization of fear and stress responses, and reconsolidation of traumatic memories, in contrast with the progressive habituation and extinction of traumatic memories in well-adjusting (aka resistant) individuals. Because exposure therapies in clinical settings correspond well with reexposures of animals to shock context or cues, animal models will likely provide important mechanistic information about how cue-based, out-of-context (i.e., reminders in therapy office), and "in-the-context" (virtual reality) exposure therapies are mediated and may be enhanced effectively.

It is important to note some confusion between paradigms that use electric shock as a *single excessive traumatic stressor* to induce lasting symptoms of PTSD and those that induce *conditioned fear* to understand the elements of fear learning. Electric shock as a trauma exposure, as described above, focuses on *long-term* outcomes on multiple PTSD domains (e.g., freezing, startle, avoidance, endocrine, and neuromorphological outcomes), and as such, it is more specific to PTSD. In contrast, fear conditioning, described below, investigates neurobiological mechanisms underlying learning processes relevant to fear and anxiety disorders, uses *short-term* protocols, and measures conditioned fear response (i.e., freezing) or enhanced unconditioned fear response (i.e., fear-potentiated startle).

Shock-Induced Conditioned Fear

Conditioned fear models are based on the classical findings of Pavlov, who originally described that an association is formed between a neutral "conditioned" stimulus and an unconditioned stimulus following repeated co-presentations. Fear conditioning investigates elements of fear learning: fear acquisition, recall, extinction, and reinstatement and renewal of fear memories (Quirk et al. 2010; VanElzakker et al. 2014).

Most conditioned fear studies deliver scrambled electric shocks via metal grid floor in acoustically isolated plastic boxes with no escape. Major parameters (e.g.,

conditioned stimulus intensity, number of acquisition trials, intensity of shock) can be modified to optimize phenotypic outcome to avoid ceiling/floor effects. These modifications are essential given the significant differences across species (mice vs. rats) and strains in fear learning, presumably due to differences in nociception, exploratory activity, or other background characteristics (Bolivar et al. 2001; Keeley et al. 2015; Keum et al. 2016; Schaap et al. 2013; Stiedl et al. 1999). Shock exposure typically ranges from one to ten 0.3–3 mA shocks lasting between 1 and 2 s each, administered during a 5- to 30-min session (Mikics et al. 2008b; Aliczki and Haller 2015; Davis and Gould 2007; Lehner et al. 2010; Roozendaal et al. 2006; Sanford et al. 2010; Shoji et al. 2014; Siegmund and Wotjak 2007b). For cued associations, shocks are commonly co-presented with novel and salient cues such as sound, light, or odor. These specific cues can be used to dissect context- and cue-dependent fear recall, which are mediated in part by unique neural circuits (Marschner et al. 2008; Phillips and LeDoux 1992). Importantly, these neural circuits are highly conserved across species such that morphological and functional changes in animal models are analogous to those in PTSD patients (Acheson et al. 2012). An overview of conditioned fear literature is beyond the limits of this review; for more detailed reviews see Herry and Johansen (2014); Milad and Quirk (2012); and VanElzakker et al. (2014).

There are two major arguments in support of simple conditioned fear models in PTSD research. First, its face validity is high given that a single shock session as traumatic experience induces fear response with strong associative memory. Second, several reports show alterations of fear conditioning characteristics in PTSD including enhanced fear acquisition, blunted extinction, and relapse (Acheson et al. 2015; Jovanovic et al. 2012; Norrholm et al. 2011). Indeed, conditioned fear paradigms are translatable between animal and human laboratories by using similar procedural protocols (Parsons and Ressler 2013; Risbrough et al. 2016; Singewald et al. 2015). Accordingly, fruitful interactions between animal and human conditioning models have been reported, particularly in endeavors to develop effective pharmacological enhancers of extinction learning (Bowers and Ressler 2015; Johnson et al. 2012). Animal studies also advanced our understanding of how associative components of fear memories are acquired, consolidated, and extinguished spontaneously or following repeated exposures to trauma-related stimuli. There exists a growing body of evidence mapping the specific prefrontal cortex, hippocampal, and amygdala neurocircuits that modulate fear learning and inhibition via distinct neurotransmitter systems (Haubensak et al. 2010; Liu et al. 2012b; Rozeske et al. 2015). In support of translational efforts, these studies suggest that fear circuitry is comparable between rodents and humans (Mahan and Ressler 2012).

Despite the advantages listed above, there exist major concerns about short-term conditioned fear paradigms as models of PTSD. Namely, most models ignore the *temporal and differential diagnostic characteristics* of PTSD. According to the 5th edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association 2013), acute symptoms must be separated from long-term, chronic symptomatology. The former is defined as “acute stress disorder” and may be an adaptive stress-coping response in well-adjusting individuals, whereas

in vulnerable individuals, PTSD symptoms are progressive and persistent and develop after a delay (DSM-5). Moreover, PTSD is no longer categorized as an "anxiety disorder" in DSM-5; rather, it is defined as "trauma- and stressor-related disorder." Accordingly, differential diagnostic criteria of PTSD require multiple-domain testing, especially given the heterogeneity of PTSD.

Fear-based reexperiencing (or intrusive memories) is a core symptom and is thought to be best modeled by fear conditioning in animals and humans. Acute and chronic phases of fear learning may have differential mechanisms and potential treatment targets; there is differential neuronal activation in the prefrontal cortex, amygdala, hippocampus, and monoaminergic systems during acute (24 h post-trauma) vs. remote (3–5 weeks) cued fear recall (Cullen et al. 2015; Mikics et al. 2008b; Frankland et al. 2004; Goshen et al. 2011; Tulogdi et al. 2012). Models focusing exclusively on acute phenotypic changes, short-term fear learning characteristics, and associative memory components of PTSD may have questionable construct validity and specificity. Considering the long-lasting behavioral consequences following a single shock session in several studies (Siegmund and Wotjak 2007a), conditioned fear models could potentially detect long-term PTSD-like outcomes if testing were extended.

1.2 Social and Psychological Stressors

1.2.1 Social Defeat Stress (SDS)

Social defeat stress (SDS) has been used as a robust stressor in both rat (Koolhaas et al. 2013) and mouse models (Golden et al. 2011) to induce depression (reviewed in Krishnan and Nestler 2011) and PTSD-like symptoms (reviewed in Daskalakis and Yehuda 2014). Evidence for SDS as a valid model for PTSD includes the following: a single defeat can lead to anxiety-like behavior, and behavioral abnormalities continue long after initial defeat (Pulliam et al. 2010).

As with other stress models, the intensity and duration of SDS can be modified. Most mouse SDS models follow a protocol in which experimental subjects as "intruders" are exposed to aggressive resident mice for 5–10 min, followed by 24 h of sensory (but not physical) exposure (Golden et al. 2011). This procedure is repeated for 5–10 days during which the intruder is exposed to different aggressor (Golden et al. 2011). PTSD-like symptoms following this social defeat exposure include decreased social exploration, anhedonia, and increased anxiety-like behavior, which can be reduced with chronic but not acute treatment with antidepressant drugs (Berton et al. 2006; Der-Avakian et al. 2014). Importantly, the PTSD-like symptoms are expressed by only 60–70% of exposed subjects, providing an inherent model of sensitivity and resiliency (Golden et al. 2011). Similar rates of sensitivity and resiliency have been achieved by 10 days of "witnessed social defeat stress." In this modification, the test mouse witnesses a peer exposed to an aggressive resident and then spends 24 h in sensory contact with that aggressor (Warren et al. 2013).

Another modified SDS model employs 6 h of indirect exposure of a partially restrained intruder mouse to an aggressive resident; within those 6 h, researchers randomly allow 1–3 direct exposures of 1 min each (Hammamieh et al. 2012). The authors argue that this model increases the unpredictability and uncontrollability of the stress exposure; this hypothesis is supported by data showing increased freezing behavior 28 days following a 10-day exposure, and increased grooming behavior 42 days following a 10-day exposure (Hammamieh et al. 2012; Muhie et al. 2015).

Although most SDS models violate the criteria of a single traumatic exposure (Yehuda and Antelman 1993), SDS may be relevant for socially induced or combat-related PTSD, which typically do involve multiple exposures. SDS may also be particularly relevant for comorbidity with depression. An important limitation of the SDS model is the lack of protocol for social stress in females. It has been suggested that ovariectomized female mice from an aggressive strain could be primed with male steroids and used as an appropriate resident for an SDS model in females (Daskalakis and Yehuda 2014), indicating that future studies are needed to establish models in females.

1.2.2 Predator Stress

In predator stress models, rodents are exposed to their natural predators or to their odor. In live predator exposure, the predator is well fed and habituated to rodents; therefore no physical injuries occur. In contrast to the physical stressors, which apply well-defined and precise traumatic experience, predator stress models focus on ecological validity to increase translatability.

Predator exposure immediately elicits unconditioned fear and marked stress responses in rodents (Adamec et al. 2006; Adamec and Shallow 1993; Blanchard and Blanchard 1989; Blanchard et al. 1998; Cohen et al. 2003; Dielenberg and McGregor 2001; Zoladz et al. 2008). A single predator exposure (1) increases avoidance behavior of both novel and predator-related stimuli (cluster C in DSM-5; Cohen et al. 2004; Toth et al. 2016), (2) induces cognitive impairments and negative mood-like states as measured by spatial learning in the Morris water maze (cluster D; Diamond et al. 2006), and (3) increases hyperarousal and hypervigilance as measured by startle reactivity (cluster E; Adamec et al. 2010). Depending on the specific predator stress model employed, enduring behavioral changes can last up to a month or more after exposure, replicating the chronic nature of PTSD (Adamec and Shallow 1993; Zoladz et al. 2012). These behavioral changes coincide with long-term structural changes of the hippocampus and the amygdala that are in line with circuit changes observed in PTSD patients (Diamond et al. 2006; Adamec et al. 2012; Mitra et al. 2009).

In a modified predator exposure, rodents are in a porous container to allow sensory but not physical exposure to live cats (Diamond et al. 1999; Mesches et al. 1999). The authors argue that besides offering physical protection, the container can enhance fear by increasing uncontrollability and helplessness. The exposure in this model is 30–75 min long. To maximize the predator interest across the session, food is placed on top of the container in which the rodent is held. This single exposure can induce

long-term memory and cognitive deficits in rats (Diamond et al. 1999; Mesches et al. 1999; Woodson et al. 2003). In a version of this model, rodents are also reexposed to the same procedure 10 days later. Because the reexposure is identical to the traumatic event itself, it is not clear if this modification simulates "flashback-like memory retrieval" as the authors indicate or simply increases the "dose" of trauma. According to a recent study, a single reexposure instigated PTSD-like symptoms while a second reexposure was not able to further increase predator-induced effects and actually caused a slight decrease in PTSD-like symptoms (Zoladz et al. 2015), potentially due to habituation.

Social instability after the initial trauma has been used as a chronic stressor to exacerbate PTSD-like behaviors (Zoladz et al. 2008). Three weeks after this procedure, increased anxiety-like behavior, startle hyperreactivity, and spatial memory deficits are observed (Zoladz et al. 2008, 2012). This combined paradigm also results in reduced thymus and adrenal weights, reduced basal glucocorticoid levels, and increased dexamethasone suppression of the HPA axis (Zoladz et al. 2008, 2012), physiological and endocrine changes corresponding with HPA abnormalities in PTSD (Yehuda et al. 1993). This paradigm was also shown to impair spatial memory, presumably secondary to structural and functional plasticity changes in the hippocampus (Diamond et al. 2006; Mesches et al. 1999; Sandi et al. 2005). These symptoms and structural changes are similar to observations in PTSD patients (O'Doherty et al. 2015).

Exposure only to olfactory cues from a predator can also induce behavioral signs of fear in rats and mice, and activate brain regions mediating defensive, fear, and anxiety-like responses, such as the lateral septum, extended amygdala, paraventricular nucleus of the hypothalamus, hypothalamic circuits mediating defensive reaction, and periaqueductal gray (Dielenberg and McGregor 2001; Canteras et al. 2015; Takahashi 2014). Predator odor methods use litter or bedding from a predator, fur and/or urine, a cat-worn collar, or trimethylthiazoline, a synthetic component of fox feces. Based on data from field and laboratory studies, the fear-eliciting efficacy of predator odor exposure is highly variable; fur and used litter appear to be the most effective odor stimuli (Takahashi 2014; Apfelbach et al. 2005).

In support of the predictive validity of predator stress paradigms, benzodiazepines, SSRIs, and the tricyclic antidepressant tianeptine have all been shown to efficiently reduce some PTSD-like symptoms of predator exposure (Dielenberg and McGregor 2001; Vouimba et al. 2006; Zoladz et al. 2013).

2 Operationalizing PTSD-Like Symptoms in Animal Models

The DSM-5 divides PTSD symptoms into four major symptom clusters: intrusion, avoidance, negative alterations in cognitions and mood, and alterations in arousal and reactivity. There exist numerous validated behavioral tests in rodents designed to

operationalize these constructs. These behavioral tests can be used to assess sensitivity or resiliency to trauma exposure and to screen novel pharmaceutical treatments.

Assessing intrusive (i.e., reexperiencing) symptoms (Cluster B) is not possible in animal models; however measuring physiological reactions to trauma-related cues can operationalize fear memory processes thought to underlie reexperiencing symptoms. Approach-avoidance conflict tests such as the elevated plus maze, elevated zero maze, open-field arena, and light-dark box are designed to measure avoidance behavior (Cluster C) and generalized-like anxiety in different contexts, and they are widely used in preclinical research with reasonable predictive validity, although there are limitations in their use (Adamec 1997; Bailey and Crawley 2009; Belzung and Griebel 2001; File and Seth 2003; McCormick and Green 2013; Olson et al. 2011). Trauma-specific avoidance can also be measured via introduction of trauma-related cues, e.g., predator scent in the case of predator stress, novel conspecific in the case of social defeat stress, and training context in the case of active and passive avoidance.

Negative alterations in mood (depression-like symptoms; Cluster D) are commonly measured by tests of "behavioral despair," i.e., reduced activity in forced swim and tail suspension tests or decreased preference of sucrose as a sign of anhedonia, or reduced intracranial self-stimulation. Predominantly, these tests are based on their predictive validity for antidepressant medications which are also somewhat effective in PTSD (Cryan et al. 2005). Developing additional measures of negative mood in rodents should be an active area of continued research to expand reliability and validity and minimize locomotion and memory confounds.

Modeling negative alterations in cognition (Cluster D) is highly limited in animals as these symptoms are manifested in distorted, negatively tuned self- and environment interpretations. However, more general cognitive deficits are commonly measured, i.e., attention, spatial learning, and social recognition. For spatial learning and memory, Morris water maze became a golden standard test (Vorhees and Williams 2006), although less stressful versions, i.e., Barnes maze, T-maze, or radial maze (Sharma et al. 2010), are also widely used and may be advantageous in certain paradigms where testing with minimal stress is crucial. Attention and more complex learning characteristics can be reliably assessed in rodents using the five-choice paradigm (Chudasama and Robbins 2004); there is no data available on this specific symptom domain in the most common models of PTSD (see Table 1). As specific neurocircuits are involved in these tasks with documented changes in PTSD (Gilbertson et al. 2002; Moser and Moser 1998), identification of neurobiological changes including structural and functional alterations of the prefrontal and hippocampal networks can increase the construct validity of these models.

Trauma-induced hyperarousal and hyperreactivity can be assessed by startle amplitude, its habituation, and prepulse inhibition (Adamec et al. 2010). Hypervigilance can be measured using object-burying paradigms or locomotor changes (Mikics et al. 2008a; Philbert et al. 2011). Irritability-impulsivity can be approximated using the resident-intruder test, and delayed discounting paradigm (Fodor et al. 2014; Mar and Robbins 2007); however, none of the described models have been shown to result in such externalizing-like symptoms. Sleep disturbances (altered structure or quantitative changes in stages) can be measured using EEG signals (Pawlyk et al. 2008).

Table 1 Summary of symptoms induced in animal models of PTSD

	Cluster B: intrusive symptoms	Cluster C: avoidance symptoms	Cluster D: negative alterations in mood and cognition		Cluster E: hyperarousal symptoms					Criterion F: lasting symptoms
	"Signs of" intrusive memories	Increased avoidance	Anhedonia/ decreased activity	Cognitive dysfunctions	Irritability/ aggression	Hyper-vigilance	Exaggerated startle	Concentration problems	Sleep disturbance	Symptoms present
Restraint/ immobilization stress	Freezing (Andero et al. 2013)	Plus-maze (Andero et al. 2013; Mitra et al. 2005)		Spatial memory (Andero et al. 2013)		Marble burying (Kedia and Chattarji 2014)			Increased REM (Meerlo et al. 2001)	>10 Days
Underwater trauma	Freezing (Richter-Levin 1998)	Plus-maze (Moore et al. 2012)		Spatial memory (Richter-Levin 1998)			Enhanced reactivity (Cohen et al. 2004)			>Month
Single prolonged stress	Freezing (Takahashi et al. 2006; Yamamoto et al. 2009)	Plus-maze (Harvey et al. 2006; Imanaka et al. 2006)	Forced swim (Wu et al. 2016)	Disrupted extinction learning (Knox et al. 2012)			Enhanced reactivity (Khan and Liberzon 2004)		Increased REM (Vanderheyden et al. 2015)	>2 Weeks
Electric shock	Freezing, fear generalization (Siegmund and Wotjak 2007a; Cullen et al. 2015; Louvart et al. 2005; Mikics et al. 2008b)	Plus maze, social interaction, novelty- and social avoidance (Siegmund and Wotjak 2007a; Louvart et al. 2005; Mikics et al. 2008a; Philbert et al. 2011)	Forced swim (Siegmund and Wotjak 2007a)			Object burying, hyperlocomotion (Mikics et al. 2008a; Philbert et al. 2011)	Enhanced reactivity, fear-potentiated startle (Pynoos et al. 1996; Cassella and Davis 1985; Davis 1989)		Sleep fragmentation (Philbert et al. 2011)	>Month
Social defeat	Fear generalization (Rygula et al. 2006)	Plus maze, social avoidance (Berton et al. 2006; Golden et al. 2011)	Self-stimulation and sucrose preference (Der-Avakian et al. 2014; Rygula et al. 2006)				Enhanced reactivity (Pulliam et al. 2010)		Sleep fragmentation	>Month
Predator stress	Freezing (Zoladz et al. 2015)	Plus maze (Zoladz et al. 2015)		Spatial and working memory (Diamond et al. 1999; Woodson et al. 2003; Zoladz et al. 2015)			Enhanced reactivity (Zoladz et al. 2008; Adamec et al. 2010)			>Month

Examples/references are from representative studies showing replicated findings in validated tests

For instance, sleep disturbances in trauma-exposed rats correlated with other behavioral symptoms; rats with the greatest increase in REM sleep following SPS exposure also had the largest percent of freezing behavior in a fear conditioning paradigm (Vanderheyden et al. 2015).

To outline a current status of preclinical models of PTSD, Table 1 summarizes which clusters and symptoms have been induced in particular models, also indicating symptoms, which need to be addressed as data are not available. Noteworthy, we did not include models and symptoms, where repeated stress exposure is required to induce phenotypic changes, e.g., anhedonia or exaggerated startle following repeated exposures, to be more specific to PTSD, and eliminate mixed (e.g., depression-like) models.

3 The "Cutoff Behavioral Criteria" Model of PTSD: Focus on Vulnerability and Resiliency Factors

Large epidemiological studies estimated the incidence of PTSD following traumatic events around 10–20% with significant sex differences (i.e., 8–13% for men and 20–30% for women), although these rates are highly dependent on the type of the trauma (Breslau et al. 1991, 1997; Kessler et al. 1995). Accordingly, animal models have sought to use individual variability as a means to identify the neurobiological risk factors and underpinnings of PTSD.

The "cutoff behavioral criteria" established by Cohen and colleagues (2006a, 2012) used predator stress as the trauma; according to predefined behavioral "cutoff" criteria, traumatized animals are assigned to "extreme behavioral response" (EBR; aka vulnerable) and "minimal behavioral response" (MBR; aka resilient) groups based on their post-stress startle reactivity and performance on the elevated plus maze. Although potentially other behavioral, endocrine, or physiological measurements could be used as cutoff criteria, startle reactivity and avoidance on the elevated plus maze detect changes in measures of hyper-alertness and overall behavioral avoidance/anxiety, modeling two main symptom clusters of PTSD. To maximize effect size and interpretability of the data, the group of rodents between EBR and MBR is considered to have a "partial behavioral response," and not investigated (Cohen et al. 2006a).

The cutoff criteria described by Cohen and colleagues are fairly conservative, yet the rate of EBR varies substantially between strains. For rodents to meet EBR criteria they must spend the entire 5-min elevated plus session in the closed arms, and exhibit >800 units startle amplitude without habituation. MBR criteria required rodents to spend less than 1 of 5 min in closed arms of the elevated plus, and to exhibit <700 units startle amplitude with significant habituation over time (Matar et al. 2013). Using these criteria with Sprague-Dawley rats, EBR rates 24 h following predator stress may be as high as 90%. However, similar to humans, acute-anxiety symptoms fade in well-adapted animals; the EBR rate drops sharply in the days

immediately following predator stress (Cohen et al. 2004). For Sprague-Dawley rats, EBR incidence settles at around 25% (Cohen et al. 2003), which is comparable with human epidemiological data (Breslau et al. 1991; Kessler et al. 1995). The EBR phenotype can persist for more than 3 weeks in the 25% of experimental subjects classified as EBR (Cohen et al. 2004), which is again compatible with the temporal characteristic of human PTSD (Cohen et al. 2004). However, EBR rates vary significantly between strains in rats (10% and 50% in Lewis and in Fisher rats, respectively) (Cohen et al. 2006a, b), and mice (from 6% to 55% in the most widely used strains, DBA/2J and C57Bl/6J, as two extremes of the spectrum, respectively) (Cohen et al. 2008). Importantly, extreme behavioral response at baseline (without stress exposure) is hardly detectable in experimental populations, i.e., 1.3% (Cohen et al. 2012), confirming that EBR is clearly induced by trauma exposure, and lasts for weeks only in a vulnerable subpopulation (i.e., 25%).

Studies using these behavioral cutoff criteria reported enhanced stress reactivity in EBR subjects as measured by increased heart rate and sympathetic activity, and higher plasma corticosterone and adrenocorticotropic hormone concentrations (Cohen et al. 2003, 2005). EBR subjects also exhibited additional plasticity-related changes in the hippocampus, such as reduced expression of BDNF, synaptophysin, and ERK1/2 pathway elements, and elevated expression of glucocorticoid receptor and postsynaptic density protein-95 mRNA (Matar et al. 2013; Cohen et al. 2014; Kozlovsky et al. 2007). Importantly, acute and 7-day-long treatment with the SSRI sertraline was able to decrease anxiety, reduce startle reactivity, and significantly decrease the number of rodents meeting EBR criteria (Matar et al. 2006).

Importantly, this cutoff criteria approach can be applied to other PTSD models to compare vulnerable and resilient subjects. An underwater trauma model found that EBR rats exhibited lasting anxiety and hyperarousal (Cohen et al. 2004). In another study, mice were exposed to electric shock (14× 1-s-long shocks of 1 mA with variable intervals over 85-min period) followed 24 h later by a shock "trigger" (5× 1-s-long shocks of 0.7 mA with fixed intervals over 5 min) and tested behavioral and endocrine outcomes 7–16 days later. Based on a cumulative 5-test criteria system (upper quintiles in the marble-burying test, acoustic startle, pre-pulse inhibition, light-dark box, and home cage locomotor activity), researchers categorized subjects as "resilient" or "PTSD-like" (20–20% of the population). Compared to resilient subjects, mice with PTSD-like phenotype exhibited blunted corticosterone response to acute restraint stress with significant upregulation of hippocampal glucocorticoid receptors (Lebow et al. 2012). Moreover, PTSD-like phenotype was associated with altered expression of a number of "PTSD candidate" genes (i.e., previously reported as risk polymorphisms in human studies) in the bed nucleus of stria terminalis (Lebow et al. 2012).

In summary, despite low genetic variability, animal models of PTSD using inbred strains provide substantial individual variability for application of cutoff criteria. Models that can reliably and robustly identify resistant and vulnerable populations may be highly valuable to test the causal contribution of developmental risk factors and candidate mechanisms identified by human studies (e.g., single polymorphisms).

4 Special Challenges

While the animal models described above have strong face and predictive validity, reaching full diagnosis (based on human DSM-5 criteria) in a single animal model may not be feasible. Most of the symptoms operationalized in rodent PTSD models such as associative fear, avoidance, anhedonia, or hypervigilance are also described in other anxiety or mood disorders; thus specificity of a given model for PTSD is a challenge. Additionally, some symptoms are difficult (if not impossible) to model in animals, e.g., intrusive memories and flashbacks, feeling emotional numb, or detachment. These challenges might mean that we cannot create a rat or mouse model that is comprehensive and specific to PTSD. However, it is relevant to note that the symptoms of PTSD have significant overlap with mood and anxiety disorders and, furthermore, PTSD is frequently found comorbid with mood, anxiety, and substance-use disorders (Sareen 2014). PTSD patients also show extensive heterogeneity in symptom presentation, with over 20 possible symptoms described in DSM-5. To account for this heterogeneity, preclinical and clinical characterizations have shifted focus from diagnosis to domain-based conception of disorders based on biological mechanisms and their domain-like organization (i.e., Research Domain Criteria-RDoC proposed by NIMH), e.g., distinct and definite mechanisms underlying reexperiencing and hyperarousal in PTSD (Cuthbert 2014; Cuthbert and Insel 2013). This approach may increase translatability of animal findings in the clinics in the future, and benefit for our understanding of the pathogenesis of PTSD.

Although the above-described models have been found to successfully model some aspects of PTSD, lack of data on particular symptoms in particular models (e.g., externalizing or attention symptoms), negative findings (likely significant amount unpublished), and inconsistencies between laboratories still exist, which is difficult to interpret as experimental procedures show high variability between laboratories. Each laboratory has a unique method of trauma exposure and optimized behavioral test battery and specific optimized protocols to assess symptoms. For example "incubation time" periods between trauma and testing (~1–5 weeks), testing time (light or dark cycle), or the order of tests in a battery, and the specific tests included in a battery, all vary between laboratories.

5 Conclusions and Future Perspectives

Development of valid animal models with high translational values is a constant challenge for preclinical research and needs recurrent updates to maximize efficacy. On the one hand, diagnostic criteria are constantly shifting targets as clinical and epidemiological data are accumulating. Additionally, identification and interpretation of measured behaviors in animal models is a complex task as variables or behavioral symptoms must be ecologically valid in the behavioral repertoire of the experimental species and still translatable to humans.

To better understand the complex etiology and mechanisms of PTSD and specific contributions of different symptom domains will require wider screening of phenotypic changes in different models. From a translational standpoint, it would be particularly important to identify pathways linked to vulnerability and resilience against traumatic stress either causally or as robust and measurable predictive markers (e.g., blood-based markers). In this respect, the "cutoff behavioral criteria" strategy and comparative studies contrasting biological characteristics of resilient and vulnerable populations provide an essential approach either to clarify the causal role of candidate mechanisms identified by humans studies (e.g., polymorphisms) or to implicate new targets to test them in human studies.

Although large animal numbers and extensive screening are necessary to produce behavioral extremes with the necessary statistical power for reliable and reproducible results, the highly variable outcomes observed after trauma exposure in humans clearly warrant the application of such responder/nonresponder classification approaches based on the fact that only a portion of trauma-exposed individuals develop PTSD symptoms.

As a final comment, the past decade has seen a substantial increase in PTSD research in both humans and animal models. Building upon this literature is essential to developing tools that can actually improve the human experience. Although sex differences were beyond the scope of this review, to effectively study this topic will require expanding the subject pool more consistently to include females. The NIMH mandate to include female cells, tissue, and subjects in preclinical research will certainly push the field towards addressing this gap.

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