Animal Models of Post-Traumatic Stress Disorder

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ABSTRACT

Animal behavioral studies have commonly regarded the entire group of animals subjected to the study conditions as homogeneous, disregarding individual differences in response patterns. The following discussion will focus on a method of analyzing data that aims to model clinical diagnostic criteria applied to individual patterns of response using data from behavioral measures, and employing cut-off scores to distinguish between extremes of response versus non-response and the sizeable proportion of study subjects in-between them. This protocol unit will present the concept of the model and its background, provide detailed protocols for each of its components, and present a selection of studies employing and examining the model, alongside the underlying translational rationale of each. *Curr. Protoc. Neurosci.* 64:9.45.1-9.45.18. © 2013 by John Wiley & Sons, Inc.

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INTRODUCTION

Developing an Animal Model of Post-Traumatic Stress Disorder (PTSD)

Developing an animal model for 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 rodents. Furthermore, several of the typical symptoms of PTSD may be unique to humans and thus not be found in rodents. For example, intrusive memories of the traumatic event, one of the core symptoms for PTSD in humans, cannot be translated in animal behavioral models. 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 rodents can make this judgment or which stressors will be most effective for animals. 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 predictive (from a pharmacological perspective) 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 (Nutt and Davidson, 2000). 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 can be studied. Second, little is known about pre-trauma etiological aspects of the disorder, since, naturally, the studies so far have focused on retrospective assessments of the patients after the onset of PTSD.

This unit presents protocols for the "cut-off behavioral criteria (CBC)" model of PTSD, which was originally motivated by the criteria for a clinical diagnosis of PTSD, which is

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made only if an individual exhibits a certain number of symptoms of sufficient severity from a number of well-defined symptom clusters over a specific period of time. Most animal studies relate to "global" groups, i.e., the entire exposed population versus control populations without distinction, whereas in practice, stress-exposed animals display a diverse range of responses. Hence, the analysis of data in the CBC model employs well-defined behavioral criteria, according to which individual study animals are retrospectively classified into groups according to the degree of their response to the trauma stimulus, as is detailed below.

In this manuscript, we present four basic protocols. Basic Protocol 1 outlines a standard PTSD induction method, the Predator-Scent-Stress (PSS) paradigm. Following induction, we present the Elevated Plus Maze (EPM) and Acoustic Startle Response (ASR) to assess exploratory behavior (Basic Protocol 2). In Basic Protocol 3, we utilize EPM and ASR data to classify test animals according to the Cut-off Behavioral Criteria (CBC) model. Finally, we validate animals that fulfill the CBC criteria by exposing them to traumatic cues in Basic Protocol 4.

Please refer to Critical Parameters and Troubleshooting for important considerations related to animal housing, handling, and experimental design.

The methods described in this manuscript have been applied mainly to rats based on the behavioral model for PTSD, although mice have been involved in a number of studies, to be detailed below. In general, the mice have tended to respond in a manner almost identical to rat subjects. The descriptions below may thus be taken to hold true for mice as well, overall.

NOTE: All protocols using live animals must first be reviewed and approved by an Institutional Animal Care and Use Committee and must follow officially approved procedures for the care and use of laboratory animals (File et al., 1993).

BASIC PROTOCOL 1

THE PREDATOR-SCENT STRESS (PSS) PARADIGM

Stress paradigms in animal studies aim to model criterion A (the individual experienced or was confronted with an event that involved actual or threatened death or serious injury and responded with intense fear, helplessness, or horror) of the DSM IV-TR diagnostic criteria (American Psychiatric Association, 1994). They thus use extremely stressful experiences aimed at engendering 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 intensity with an attempt to design an ethologically valid experience, one that an animal might encounter in its natural environment.

The standard stressor in the following studies consists of exposure of rodents to the scent of the urine of their prime predator—the cat. Blanchard et al. (1990, 1997, 1998, 2001, 2003), Adamec (1997); Adamec and Shallow (1993); Adamec et al. (2006a,b,c, 2007), and others (File et al., 1993; Griebel et al., 1995; Diamond et al., 2006) have established the validity of this paradigm, in which adult rodents are inescapably exposed to urine-soiled substrate (cat-litter) for 5 to 10 min in a closed environment, where both "fight" and "flight" options are ineffective. Predator stress has ecological validity in that it mimics brief intense threatening experiences inducing the expected range of behavioral and physiological responses. The potency of predator stimuli 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 cat (separated by a mesh divide or a solid divide with an opening large enough for the rodent to slip through).

Materials

Naïve experimental subject (rat or mouse) of appropriate strain, sex, and age: we routinely use male and female Sprague Dawley rats (180 to 350 g), but Lewis, Fischer (F344), and Wistar strains have also been proven to be suitable for this protocol

Well-soiled cat litter bedding (in use by the cat for 2 days, sifted for stools)

Fresh, unused cat litter bedding

Quiet yard or quiet test room away from disturbances

Quiet yard or quiet test room away from disturbances-for sham exposure

Predator scent stress (PSS) test apparatus: The apparatus consists of a 40 × 40 × 40–cm chamber of transparent Plexiglas with transparent roof (Fig. 9.45.1)

Plexiglas behavioral arena 40 × 40 × 40 with transparent roof (for sham exposure)

Timer

Initial considerations

1. Standardize the time of testing to minimize diurnal variation.
2. Randomly allocate animals to the test condition groups (exposed versus control).

For PSS exposure subjects

3a. Place the test chamber in the yard (or test room) and line it with a 5- to 8-cm depth of well-soiled cat litter bedding.

Select a quiet area (yard/patio/room) situated far enough from the behavioral rooms and other rodent facilities so as to prevent cat odor from being detectable (at temperature between 18° and 28°C, daytime and nighttime). A quiet and isolated yard/test room is needed because rodents are very sensitive to external noise and movement.

It is important that soiled litter not be allowed into the animal-house or the area devoted to behavioral experiments at any time.

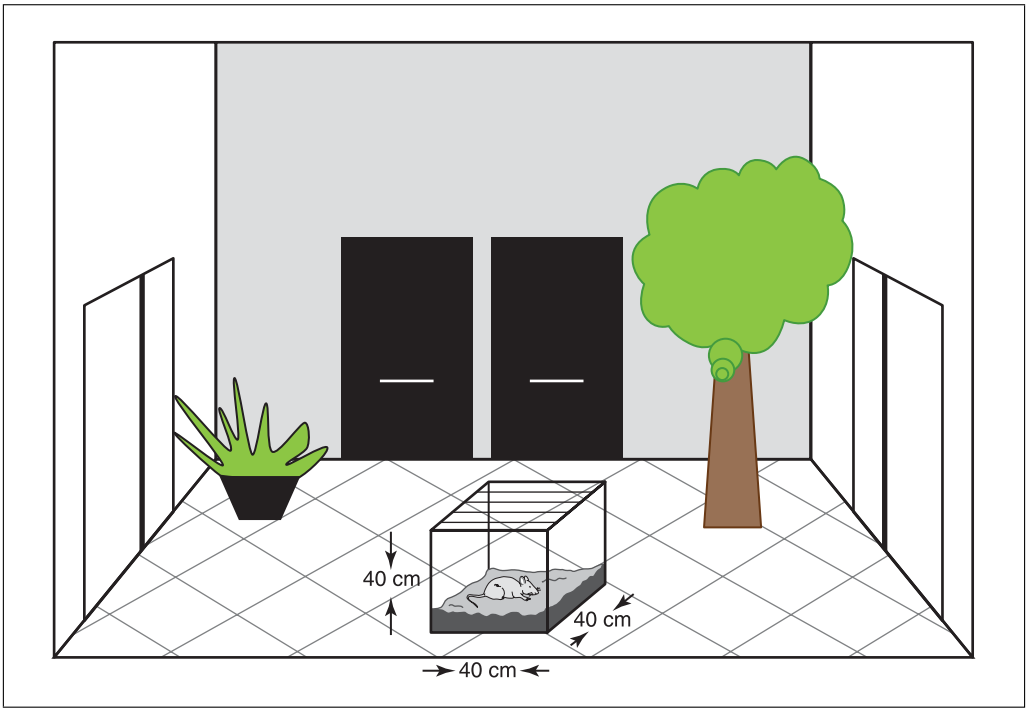

Figure 9.45.1 Predator scent stress (PSS) test apparatus. The apparatus consists of a 40 × 40 × 40–cm chamber of transparent Plexiglas with transparent roof. The floor is covered with a 5- to 6-cm layer of well soiled cat litter (in use by the cat for 2 days, sifted for stools). The apparatus is placed on a yard paving stone.

4a. Place the animals in the PSS test apparatus for 15 min.

5a. Remove the animal from the PSS test apparatus into the transporting cage and return to the home cage.

For control condition subjects

3b. Place the test chamber in another test room and line it with a 5- to 8-cm depth of fresh, unused cat litter bedding.

Annotations in step 3a apply here as well.

4b. Place the control subjects in the fresh, unused litter for 15 min.

5b. Remove the animal from the maze into the transporting cage and return to the home cage.

6. In order to minimize any trace scent left on the soiled litter by test animals, change batches of litter after every three rats and mix the litter well between rats.

BASIC PROTOCOL 2

ASSESSMENT OF OVERALL EXPLORATORY BEHAVIOR USING THE ELEVATED PLUS MAZE (EPM) AND QUANTIFICATION OF HYPER-ALERTNESS USING THE ACOUSTIC STARTLE RESPONSE (ASR)

A variety of mazes and open environments have been employed to assess changes in exploratory behavior resulting from stress exposure. These test environments assess behaviors whose disruption indicates anxiety-like fearful behaviors and behaviors reflecting avoidance. Various learning and memory tasks are employed in which both exploration and learned task performance can be assessed. Some studies have investigated social behavior in home cages and in challenge situations. The startle response, which characterizes many PTSD patients, has been employed as one of the more definitively measurable parameters for the hyper-vigilant/hyper-alert component of the behavioral responses (File et al., 1993). In the studies presented below, exploratory behavior on the elevated plus maze (EPM; steps 1 to 9) serves as the main platform for the assessment of overall behavior, and the acoustic startle response (ASR;, steps 10 to 21) paradigm provides a precise quantification of hyper-alertness, in terms of magnitude of response and habituation to the stimulus.

The EPM is a well-characterized behavioral paradigm to investigate anxiety in rodents. The test is based upon the conflict between an innate aversion to exposed spaces and a tendency to explore new environments (Pellow et al., 1985; File et al., 1993). The EPM was designed to provide measures of anxiety that are relatively uncontaminated by changes in overall motor activity and has been extensively validated (Pellow and File, 1986).

The adaptive response to a sudden and unexpected stimulus is to be startled by it, thus focusing attention and preparing the organism for a response should it be required. Repeated stimuli resulting in no actual danger ought normally to result in habituation. PTSD patients tend to display poor habituation, and the phenomenon has been included in diagnostic criteria (American Psychiatric Association, 1994). In rodents the startle response consists of rapid contraction of head, neck, trunk and leg muscles in addition to the arrest of ongoing activity (Graham, 1979; B'aszczyk, 2003). One of the most widely used stimuli is a series of repeated intense auditory signals eliciting an acoustic startle response (ASR; Landis and Hunt, 1939; Ekman et al., 1985).

Timing of behavioral assessments

A large number of studies performed in a range of research centers indicate quite clearly that behavioral changes that are observed in rodents at day 7 after stress exposure are

unlikely to change significantly over the next 30 days (Cohen et al., 2004). The average life expectancy for the domestic rat is 2.5 to 3 years. Hence, behavioral patterns observed at day 7 can reliably be taken to represent PTSD-like responses (i.e., "translating" a week for a rodent to a month for a human). Note that the EPM and ASR tests are performed sequentially on the same day and within the same diurnal phase for each batch of test and control subjects (usually one cannot perform more than about ten of each in this time).

Startle responses differ among strains of rats and mice and even among animals of the same strain obtained from different providers. Therefore, it is particularly important to consider this variable when replicating work from other laboratories and to maintain a consistent line of experimentation within a laboratory.

NOTE: Each batch of animals is brought to the laboratory at least 1 hr before testing begins, to diminish the immediate effects of stress associated with transport from the housing area to the test room, as well as the effects of novel environment. Animals must be of approximately equal weight and age.

Materials

Exposed/control experimental subject (rat or mouse) of appropriate strain, sex, and age
Pharmacological agents to be tested/appropriate drug vehicle for control injections (optional)
Elevated plus maze (EPM) apparatus (see Fig. 9.45.2)
Quiet test room away from disturbances, illuminated either brightly (300 radiometric lux) or dimly (30 radiometric lux)
Video camera and monitor (optional: television/monitor screen connected to the video camera, located in an adjacent room)
Transporting and home cages
Damp cloths (for cleaning cages)
Acoustic startle response (ASR) apparatus (see Fig. 9.45.3)
Vibration isolation platform (startle recording device): device that reflects and absorbs waves of oscillatory energy, extending from the working gear or electrical equipment, with the aid of vibration insulation (vibration isolation is the process of isolating an object, such as a piece of equipment, from the source of vibrations)
Automated test system or keyboard for scoring behaviors

EPM apparatus setup

1. Set up the EPM in the test room at least seven days after the PSS exposure/sham exposure. Mount the video camera directly above the maze.

   The EPM apparatus consists of two open arms (sized for rats: 50 × 10–cm; for mice: 30 × 6–cm), and two enclosed arms (for rats: 50 × 10 × 40–cm; for mice: 30 × 6 × 30–cm), with an open roof, arranged such that the two open arms are opposite to each other. The maze is elevated from the floor (for rats: to a height of 50 cm and for mice: 30 cm) (Fig. 9.45.2).

2. Activate the video camera and record, and enter the rat's code into the video display.

EPM experiment initiation

IMPORTANT: test each animal once only!

3. Place each animal in the central platform, facing an open arm.
4. Observe behavior for 5 min.

5. Remove the animal from the maze into the transporting cage and return to the home cage until subsequent testing.
6. Remove any feces; wipe the maze with a damp cloth and wipe dry (avoid using strong smelling detergent).

EPM data analysis

7. Use a strict definition of an arm entry (all four paws must enter the arm) and arm exit (both forepaws must leave the arm).

   Data for any animal which falls off the maze are excluded.

8. Measure the following:

   a. Time spent (duration) in open arms
   b. Time spent in closed arms
   c. Time spent in the central platform
   d. Number of entries into open arms
   e. Number of entries into closed arms.

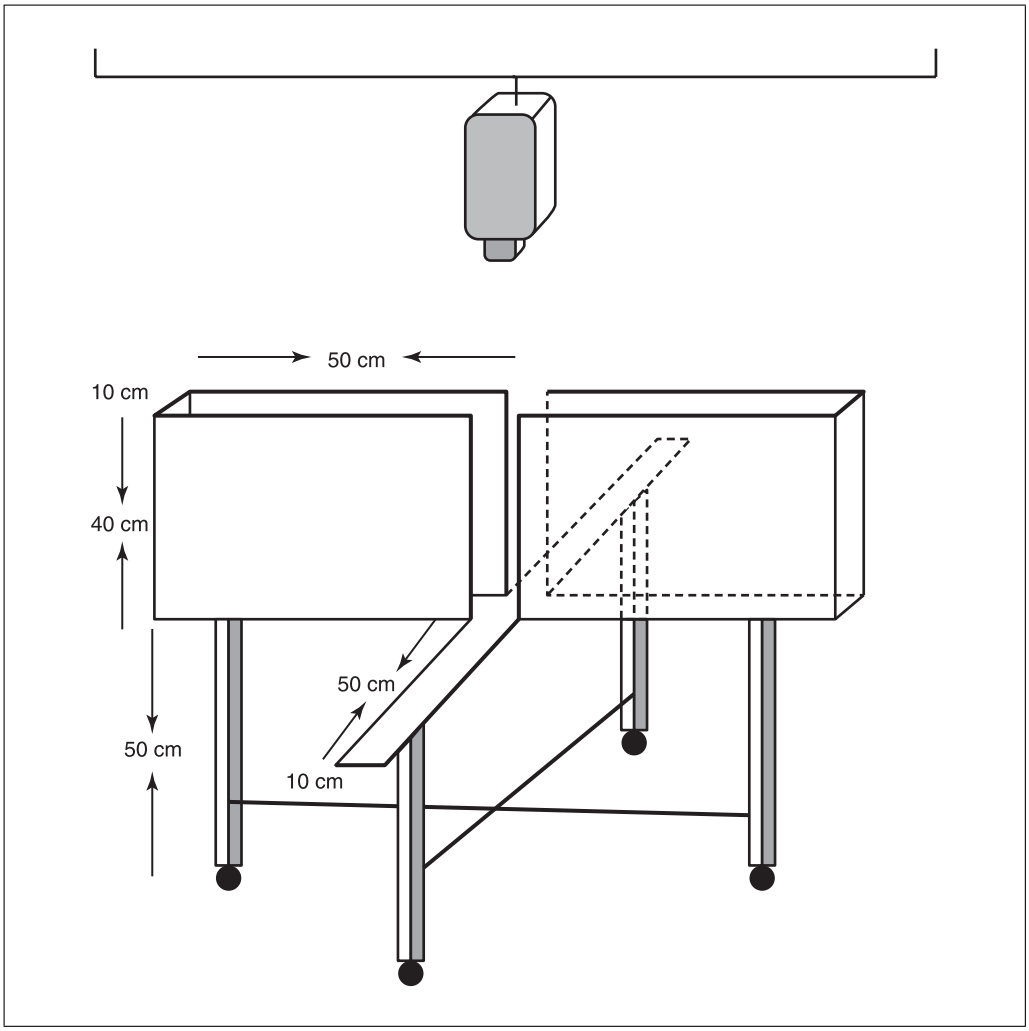

Figure 9.45.2 The elevated plus-maze apparatus. The maze is made of black Plexiglas, consists of four arms in form of a maze: two open arms (for rats: 50 × 10 cm; for mice: 30 × 6 cm) and two arms of the same size, that are enclosed by walls (for rats: 40 cm high; for mice: 30 cm). The open arms are opposite each other and converge into a central platform (for rats: 10 × 10 cm; for mice: 6 × 6 cm). A video camera mounted above the maze is used to observe the animal's behavior and record the trails for automatic computer analysis, for later additional scoring or both.

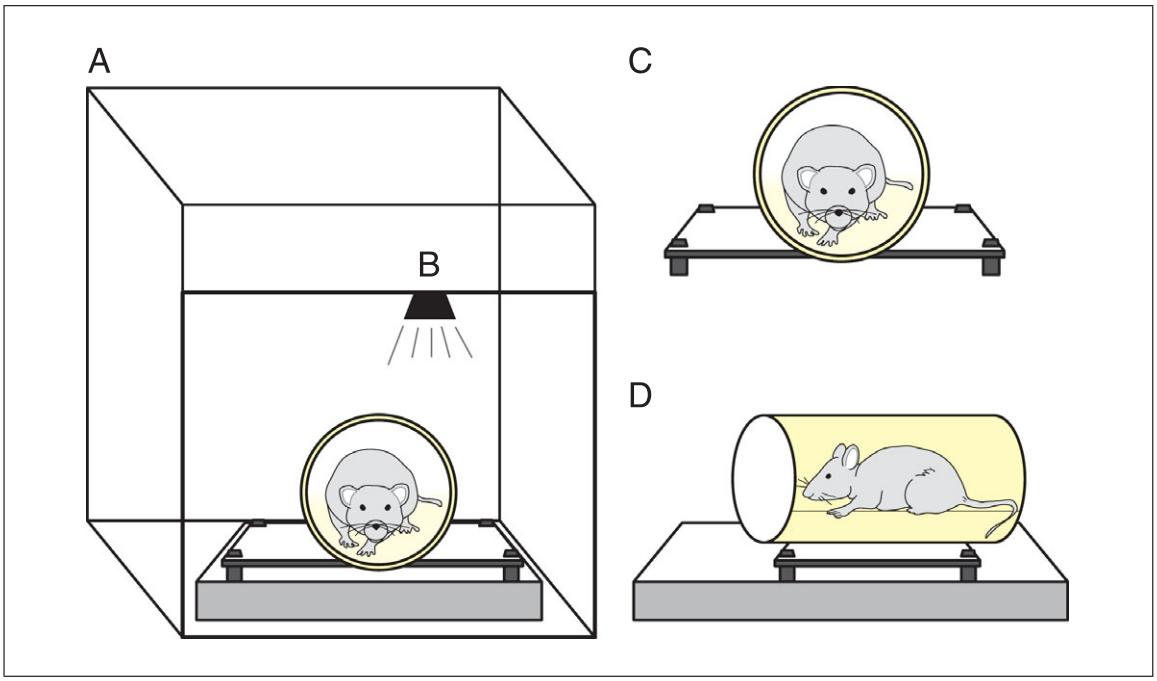

Figure 9.45.3 The acoustic startle response apparatus. (**A**) The cabinet contains a complete sound generation system for white noise production, separately adjustable background noise levels, and accessory connections for optional stimuli. The audio source module is used to provide acoustic stimuli. For tone stimuli (**B**), amplitude, frequency, and duration are computer controlled. Amplitude and duration of noise bursts are computer controlled. Specialized amplifier circuitry, contained in the cabinet, permits the use of a dynamic standardization system that emulates actual startle response movements. The dynamic response sensor design ignores static animal weights enabling the full range of the transducer capacity to be available for response recording. (**D**) Animal enclosures are designed to locate the subject without using restraint so the animals do not suffer from restraint stress and confound the results of the startle testing.

9. Calculate the following:

   a. Total exploration on the plus maze—the number of entries into any arm of the maze. This serves to distinguish between diminished overall exploration, exploration limited to closed arms (avoidance) and free exploration.
   b. "Anxiety index"—integrates all the EPM measurements as follows:

$$\text{Anxiety Index} = 1 - \left[\frac{\left(\frac{\text{time spent in the open arms}}{\text{total time on the maze}} \right) + \left(\frac{\text{number of entries to the open arms}}{\text{total activity on the maze}} \right)}{2} \right]$$

Anxiety index values range from 0 to 1, where an increase in the index expresses increased anxiety-like behavior. The anxiety index bring together the data for each of the individual parameters of exploratory behavior in the EPM and the accepted ratios used to date into a unified parameter, which reflects not only the absolute measures but also indicates a(n) (overall) tendency.

Set up the ASR apparatus

10. Place the startle box on a vibration isolation platform to minimize environmental vibration (Fig. 9.45.3).

    The system is computer-based and all stimulus parameters are specified by completing entries in software available from a single screen.

Do not stack the isolation boxes one on top of one another.

The acoustic startle apparatus is made of a Plexiglas cylinder (sized for rats: 180 × 85 × 90–mm; for mice: 100 × 60 × 70–mm) mounted on a Plexiglas platform and enclosed in a ventilated sound-attenuated cubicle equipped with high-frequency loudspeakers. A stabilimeter measures the whole-body flinch elicited by acoustic stimuli. Movements within the cylinder are detected and transduced by a piezoelectric accelerometer attached to the platform, digitized, and stored by the operating computer.

ASR commencement and timing

11. Commence the ASR test at least 1 hr after the EPM assessment. Both must be performed on the same day and within the same diurnal phase.
12. Take the animal to the test room, and enter the rat's code into the computer.
13. Place the startle cage and speakers that deliver the startle stimulus and the background noise into the sound-attenuating chamber.

    Set up startle equipment according to the manufacturer's instructions.

14. Select and define testing parameters: startle intensity, number of acoustic stimulus, background noise level, etc.

    In this animal model, each test session starts with a 5-min acclimatization period to background white noise of 68 dB, followed by 30 acoustic trial stimuli (40-msec burst of 120-dB pulse of background noise, at intervals averaging 15 sec (ranging from 12 to 30 sec) between stimuli).

Perform the ASR experiment

15. Clean and dry the animal chamber before testing each animal.
16. Calibrate the stimulus delivery and response recording system before each test.

    It is important to calibrate startle cages so that one cage is just as sensitive as another in measuring startle.

17. Place the animal in the test chambers.
18. Allow 5-min period of acclimation to the *darkened apparatus with a* background white noise level of 60 dB.
19. Begin the test session as part of a series of 30, startle-eliciting noise bursts using an average of 15-sec intertrail interval (ITI) (ranging from 12 to 30 sec).

    If there is no initial response to the startle stimuli: (a) assess hearing damage, (b) rule out general debilitation, and (c) assess general ability to exhibit a startle reflex using air puffs.

20. Remove the animal from the chamber into the transporting cage and return to the home cage until subsequent testing.
21. Remove any feces; wipe the chamber with a damp cloth and dry thoroughly (avoid using strong smelling detergent).

ASR data analysis

22. Calculate the following:

    a. Mean startle amplitude (averaged over all trials)
    b. Percent of startle habituation to repeated presentation of the acoustic pulse.

Percent habituation, the percent change between the response to the first block (average the responses across blocks of first six trains) of sound stimuli and the last—is calculated as follows:

$$\text{Percent habituation} = 100 \times \frac{\left[(\text{average startle amplitude in Block 1}) - (\text{average startle amplitude in Block 6})\right]}{(\text{average startle amplitude in Block 1})}$$

CLASSIFICATION ACCORDING TO CUT-OFF BEHAVIORAL CRITERIA (CBC)

BASIC PROTOCOL 3

Researchers who work with animals have long been aware that individual study subjects tend to display a varying range of responses to stimuli, especially when stress paradigms are concerned. This heterogeneity in responses was accepted for many years and regarded as unavoidable. Since humans clearly do not respond homogeneously to potentially traumatic experiences, any heterogeneity in animal responses might be regarded as confirming the validity of animal studies, rather than as a problem. It stands to reason that a model of diagnostic criteria for psychiatric disorders could be applied to animal responses to augment the validity of study data, as long as the criteria for classification are clearly defined, reliably reproducible and yield results that conform to findings in human subjects.

To maximize resolution and minimize false positives, the responses of animals on both the EPM and ASR tests serve as tools for classifying individuals as exhibiting either "extreme behavioral response" (EBR), "minimal behavioral response" (MBR), or "partial behavioral response" (PBR). The classification of individuals according to the degree to which their individual behavior is affected by a stressor is based on the premise that in the natural environment, such extremely compromised behavior in response to the priming trigger may compromise behaviors essential for survival, and is thus inadequate and maladaptive, representing a pathological degree of response.

Since clinical diagnostic criteria require a sufficient number of symptoms from three symptom clusters in order to achieve satisfactory diagnostic specificity, the CBC response classification process requires that a given animal fulfill all criteria on both tests, performed in series. The standard algorithm for the CBC classification model also requires that prior to classification, a significant overall effect be demonstrated (Fig. 9.45.4).

Materials

EPM data
ASR data

NOTE: Please refer closely to Figure 9.45.4 for the steps below.

Verify global effect

The data must demonstrate that the stressor had a significant effect on the overall behavior of exposed versus unexposed populations at the time of assessment. Step A is intended to test our null hypothesis, i.e., that exposure would have an overall effect on the rodents as a group compared to controls, and yet that there would be individual differences in behavioral effects. This heterogeneity would form the basis for the definition of cut-off criteria for two groups of animals. We hypothesized that it would be possible to identify one group of rats, which demonstrated significant behavioral change and other with almost none. These two groups would then provide the data by which behavioral criteria could be defined for the second step.

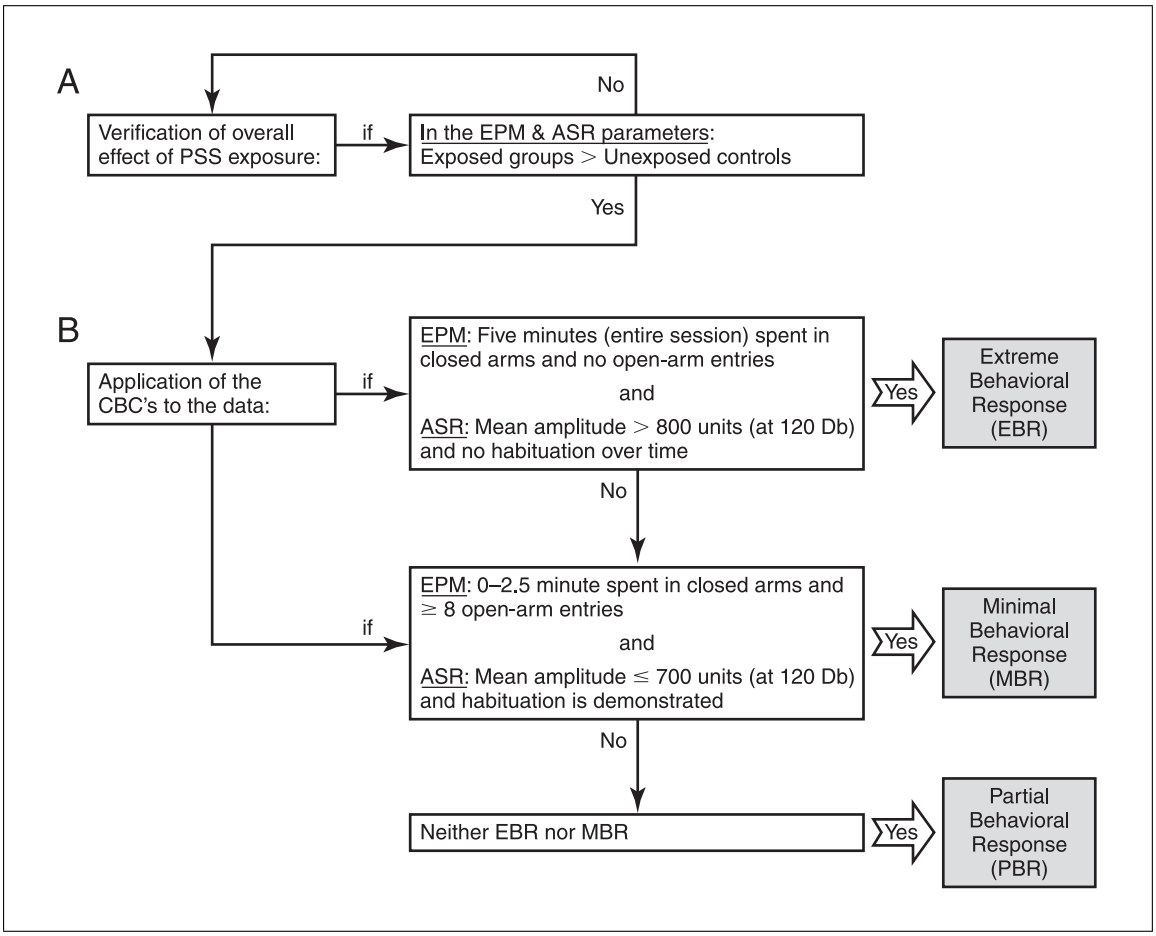

Figure 9.45.4 The cut-off behavioral criteria (CBC) algorithm. Behavioral models can be more closely matched with contemporary clinical conceptions of PTSD by using an approach that enables the classification of study animals into groups according to degree of response to the stressor, i.e., the degree to which individual behavior is altered or disrupted. This was achieved by defining both the behavioral criteria and the cut-off criteria that reflect response severity, a design that parallels the inclusion and exclusion criteria applied in clinical research. (**A**) The data must demonstrate that the stressor had a significant effect on the overall behavior of exposed versus non-exposed populations at the time of assessment. (**B**) To maximize the resolution and minimize false positives, extreme responses to both of these paradigms performed in sequence are required for "inclusion" in the EBR group, whereas a negligible degree of response to both is required for inclusion in the MBR group.

Apply CBCs to data

In order to maximize the resolution and minimize false positives, extreme responses to both EPM and ASR paradigms, performed in sequence, are required for "inclusion" into the EBR group, whereas a negligible degree of response to both is required for inclusion in the MBR group.

BASIC PROTOCOL 4

EXPOSURE TO TRAUMA CUES

A disproportionate psychophysiological response to trauma cues is integral to the clinical definition of PTSD. In order to model this component of PTSD, a stimulus that is not intrinsically threatening, yet acts as a clear-cut reminder of the traumatic stressor, is employed. The behavioral outcome measure which we found reliable and valid is freezing behavior (duration of immobility) of the rodent placed on the unused litter. As freezing behavior indicates a sense of immediate threat and intense fear, the fact that this behavior was engendered by a neutral reminder 8 days (or more) after stress exposure implies that a memory-related process and contextual association of the stimulus must have occurred.

Materials

Fresh, unused cat litter bedding
Experimental subject (rat or mouse) of appropriate strain, sex, and age
Quiet yard or test room
Trauma-cue apparatus: Plexiglas behavioral arena 40 × 40 × 40–cm—identical to sham-exposure
Video camera and monitor (optional: television/monitor screen connected to the video camera, located in an adjacent room)
Automated test system or keyboard for scoring behaviors

NOTE: Timing of trauma cue: assessments: Since the clinical symptom stands out because it occurs long after the actual event, the test must be timed accordingly. It is preferable to allow extended periods of time, certainly no less than 8 days. Many of our studies span a 30-day period and the trauma-cue exposure is thus performed on day 31, 24 hr after the EPM and ASR assessments and in a separate environment (free of traces of odors associated with anxiety).

1. Place the test apparatus for sham exposure in the test room and line it with a 5- to 8-cm depth of fresh, unused cat litter bedding.
2. Habituate the animals to the test room for >1 hr.
3. Place the animal in the fresh, unused litter.
4. Observe the animals for 15 min.
5. Remove feces from the bedding and smooth the surface.
6. Use a strict definition for freezing behavior: Freezing behavior is defined as the absence of all movements except for those related to respiration (Cohen et al., 2006a,b, 2008, 2010; Kozlovsky et al., 2009).

   Total cumulative freezing time (total seconds spent freezing during each assessment period) is measured and calculated as a percentage of total time.

COMMENTARY

Background Information

Post-traumatic stress disorder (PTSD) is a pathological response produced by (resulting from) exposure to a traumatic event, with behavioral, emotional, functional, and physiological components according to the diagnostic criteria of the Diagnostic and Statistic Manual (DSM). PTSD has been recognized as a diagnosis by the DSM since 1980. A diagnosis of PTSD is conditional on a number of required symptoms being present one month or more after exposure to a triggering event: (1) intrusive re-experiencing of the traumatic event in the form of nightmares and flashbacks, with an exaggerated response to trauma-related reminders/cues; (2) persistent avoidance of stimuli associated with the trauma and emotional numbing; and (3) persistent symptoms of exaggerated startle response, increased physiological arousal, and sustained preparedness for an instant alarm response (American Psychiatric Association, 1994).

In the first hours to days following the experience, the vast majority of individuals exposed to an extreme event will demonstrate, to a varying degree, symptoms such as intense fear, helplessness, or horror followed by anxiety, depression, agitation, shock, or dissociation, and may have trouble functioning in their usual manner for a while (Shalev, 2002; Bryant, 2006; Davidson, 2006). Retrospective and prospective epidemiological studies indicate that most individuals affected by a potential traumatic experience will adapt within a period of 1 to 4 weeks following exposure (Bryant, 2006; Foa et al., 2006). Epidemiological data from a range of events (from natural disasters to 9/11) indicate that only a proportion of persons exposed will develop long-term psychopathology (Bryant, 2006; Foa et al., 2006). In the United States, studies report that the rate of lifetime exposure to at least one "serious" traumatic event (excluding grief and mourning) is quite high; a conservative

estimate reported 61% among men and 51% among women (Breslau et al., 1998). Other studies have found similar rates (Helzer et al., 1987; Shore et al., 1989; Breslau et al., 1991; Resnick et al., 1995). The lifetime prevalence of PTSD in the general population reaches about 7% overall (Fairbank et al., 1995), suggesting that about 20% to 30% of individuals exposed to severe stressors will develop PTSD (Breslau et al., 1991). This figure varies depending on the type of trauma studied, where male rape victims suffer very high rates and populations exposed to natural disasters significantly less (Peri et al., 2000). The discrepancy between the proportion of the general population exposed to potentially traumatic experience and those who eventually fulfill criteria for the disorder suggests qualitative differences in vulnerability and/or resilience.

The fact that it is possible to distinguish between affected and unaffected individuals reflects the fact that animals display a range of responses to stimuli, as do the humans they are intended to model. The inclusion of all exposed animals in data analysis overlooks the individual variability in their behavioral response to the stressor, and represents a source for potential bias. Drawing a distinction between animals whom we choose to regard as affected to a sufficient degree by a stimulus in order to be included in a study from those whom we do not consider to have been sufficiently affected may be seen as being conceptually similar to the application of inclusion and exclusion criteria when diagnosing patients or deciding whether to include them in a clinical study. This approach exposes patterns of responses which are significantly different from those seen when it is not applied, and which resemble patterns seen in human studies regarding the more severe type of long-term sequelae of exposure to stressors, including PTSD. Thus, just as one would define the study population for a clinical PTSD study as "meeting DSM IV criteria," it seems sensible to consider redefining the study population in animal models of stress and PTSD as "those animals who were exposed to the paradigm and developed certain anxious/fearful behavioral changes."

This approach enables researchers to test interventions that might be impossible or difficult to do in a clinical setting without any proper preclinical basis. The animal model also enables the researcher to go one step further and correlate specific anatomic biomolecular and physiological parameters with the degree and pattern of individual behavioral response.

Anticipated Results

Classification according to cut-off behavioral criteria

Data from a large series of studies had previously shown that 7 days after a single 10-min predator scent exposure, the overall exposed population displayed significantly decreased time spent in the open arms and increased time in the closed arms of the EPM (which is translated to "avoidant" and "anxiety-like behavior"), higher mean startle responses, and lower startle habituation as compared to control animals (Fig. 9.45.5). It is important to note that the rodents' behavior was not uniformly disturbed, but rather demonstrated a broad range of variation in response severity (Fig. 9.45.6). Since the distribution of the individual variations in behavioral response was clearly nonnormal, tending toward discontinuous, it was justifiable to classify the animals according to extent of behavioral change (Fig. 9.45.7).

The behavioral measures for each of these groups on the EPM and ASR tests were employed to define the basic Cut-Off Behavioral Criteria (CBC). The classification of individuals according to the degree to which their individual behavior is affected by a stressor is based on the premise that in the natural environment, such extremely compromised behavior in response to the priming trigger may compromise behaviors essential for survival, and is thus inadequate and maladaptive, representing a pathological degree of response. Since clinical diagnostic criteria require a sufficient number of symptoms from three symptom clusters in order to achieve satisfactory diagnostic specificity, the CBC response classification process requires that a given animal fulfill all criteria on both tests, performed in series. The standard algorithm for the CBC classification model also requires that prior to classification, a significant overall effect be demonstrated.

The pooled behavioral data for entire PSS-exposed populations were re-examined according to the CBC, revealing that the overall prevalence rate for EBR animals was ~21.3%, as compared to 1.3% in unexposed control populations. The prevalence of MBR animals in the PSS-exposed groups was 6.7% as compared to 20.0% in the control groups (Fig. 9.45.8).

The implication of this initial finding was that all prior study analyses must have included

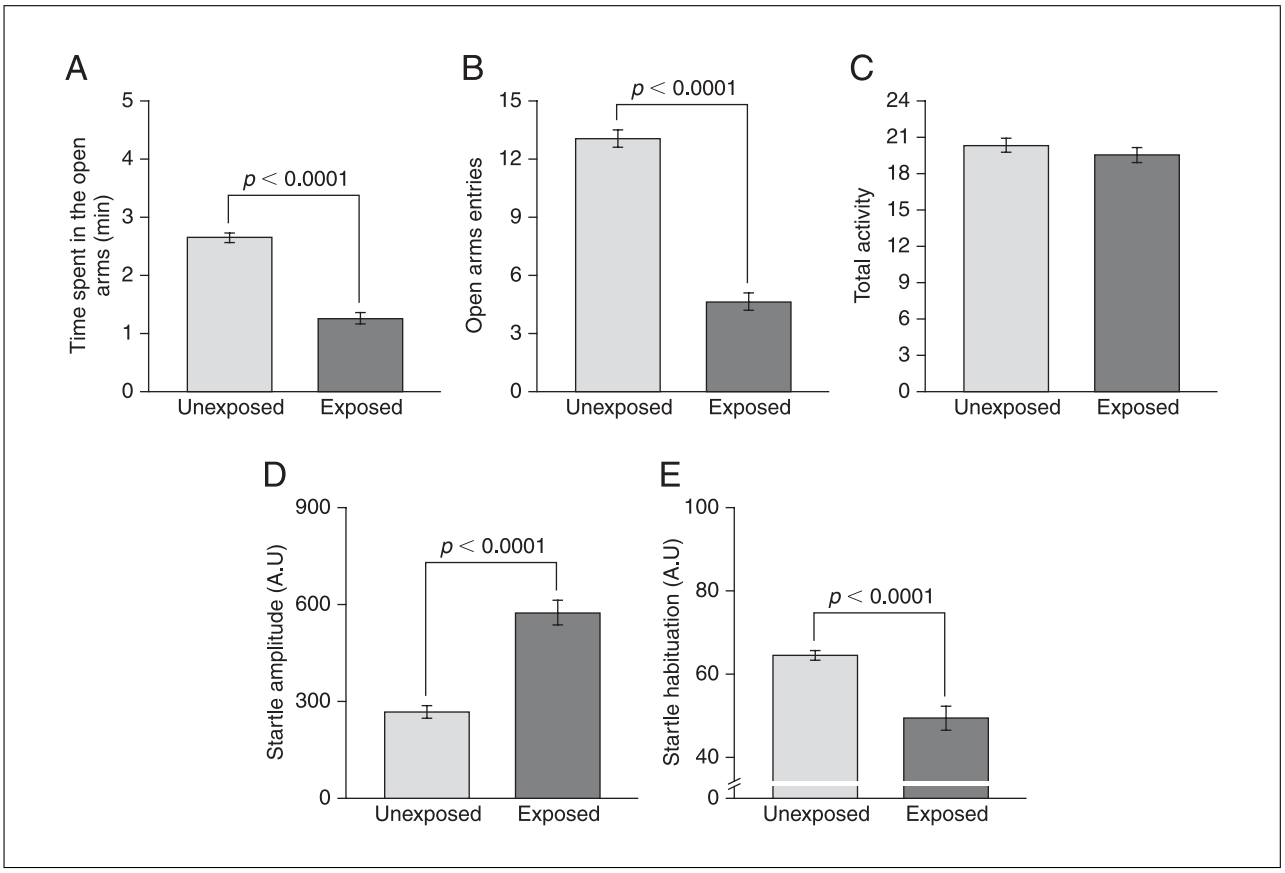

Figure 9.45.5 The effect of the predator scent stress (PSS) paradigm on overall anxiety-like behavior and acoustic startle response. A single ten-minute exposure to PSS significantly increased anxiety-like behavior/avoidance of open spaces as compared to unexposed controls. Time spent in the open arms (**A**) and entries into open arms (**B**) were significantly decreased after a single exposure to the stressor, as compared to control conditions [$F(1,298) = 126.4$, $p < 0.0001$ and $F(1,298) = 68.25$, $p < 0.0001$, respectively]. There were no differences in total exploration (**C**) of the maze between groups. This result suggests overall anxiety and avoidance of exploration in the open arms, as opposed to an impairment of locomotion/exploration. PSS exposure significantly increased the mean startle amplitude (**D**) and caused a significant deficit in the startle habituation of ASR (**E**) in exposed rats as compared to controls [$F(1,298) = 51.04$, $p < 0.0001$ and $F(1, 298) = 45.25$, $p < 0.0001$, respectively]. All data represent group mean ± S.E.M.

a significant proportion of animals whose behavior had not been affected by the stressor (MBR) and many animals whose response was of uncertain significance (PBR), alongside those whose response was unequivocally one of severely disrupted behavioral patterns (EBR). Hence, the method offered a feasible means for classifying animal response patterns to trauma, thereby increasing the conceptual accuracy of the data.

It is of interest to note that the proportion of the entire exposed population fulfilling criteria for extreme responses (EBR) was compatible with epidemiological data for PTSD amongst trauma-exposed human populations (Breslau et al., 1991), which report that between 15% to 35% fulfill criteria for PTSD and that ~20% to 30% display partial or sub-symptomatic clinical pictures (Breslau et al., 1998; Resnick et al., 1995). This compatibility further supports the concept of criterion-based classification in terms of face validity.

The animal model presented here, which is a combination of exposure to a predator and a focus on setting apart the affected based on behavioral cut-off criteria, has demonstrated high face validity. The cumulative results of our studies indicate that the contribution of animal models can be further enhanced by classifying individual animal study subjects according to their response patterns. This approach enables researchers to test interventions that might be impossible or difficult to do in a clinical setting without any proper preclinical basis. The animal model also enables the researcher to go one step further and correlate specific anatomic biomolecular and physiological parameters with the degree and pattern of individual behavioral response.

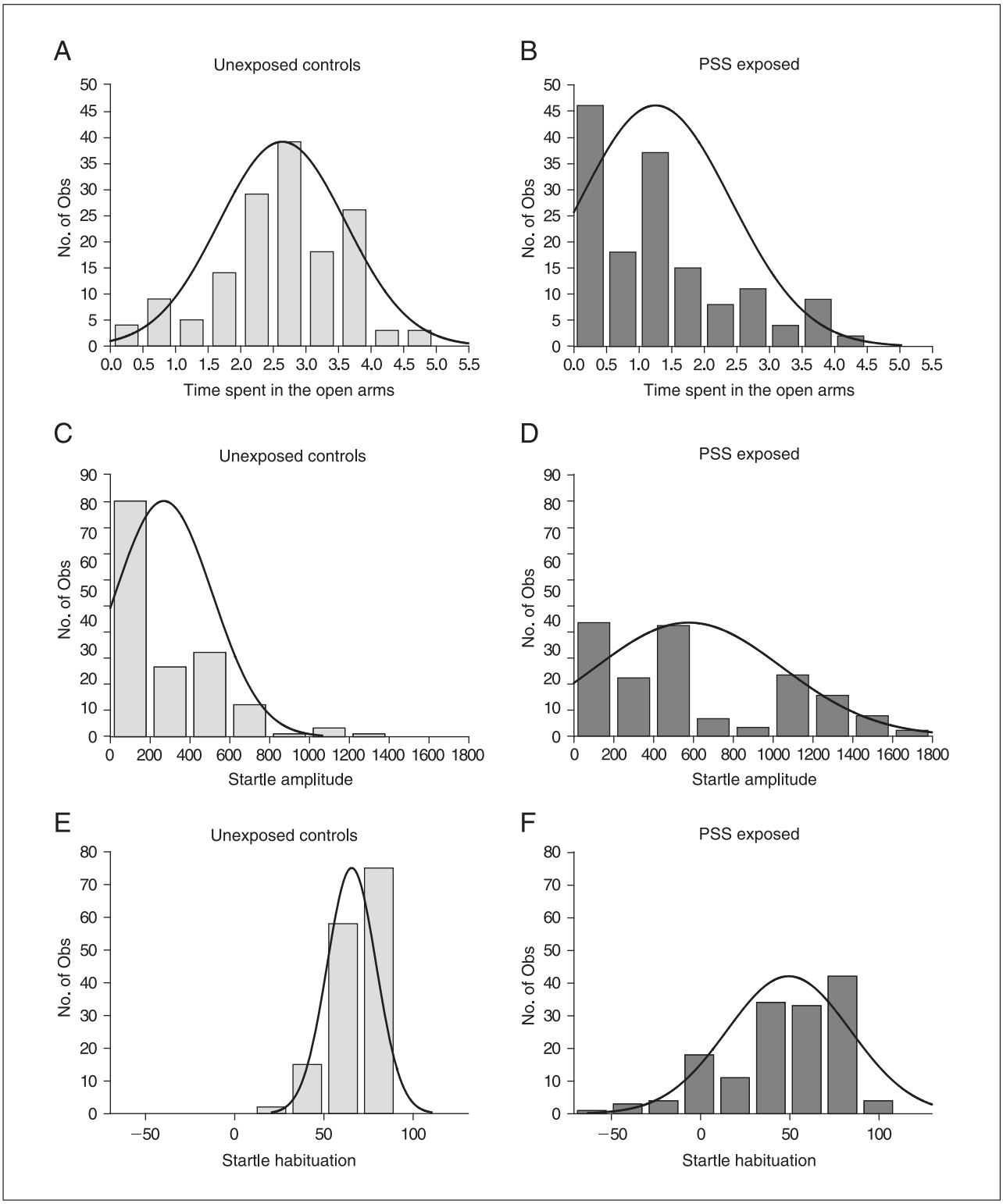

Figure 9.45.6 The distributions of the exposed groups and the control group. The distributions of the exposed groups and the control group (time spent in the closed arms, startle response and startle habituation) were compared by a Kolmogorov-Smirnov statistic. The results showed that there are differences in the general shapes of the distributions curve in the two populations ($p < 0.001$). The distribution curve for exposed animals has a distinct shifts (in the parameter of time spent in the open arms: shift to the left; startle response: a discontinuous nature, tending towards a bimodal distribution; Startle habituation: shift to the left).

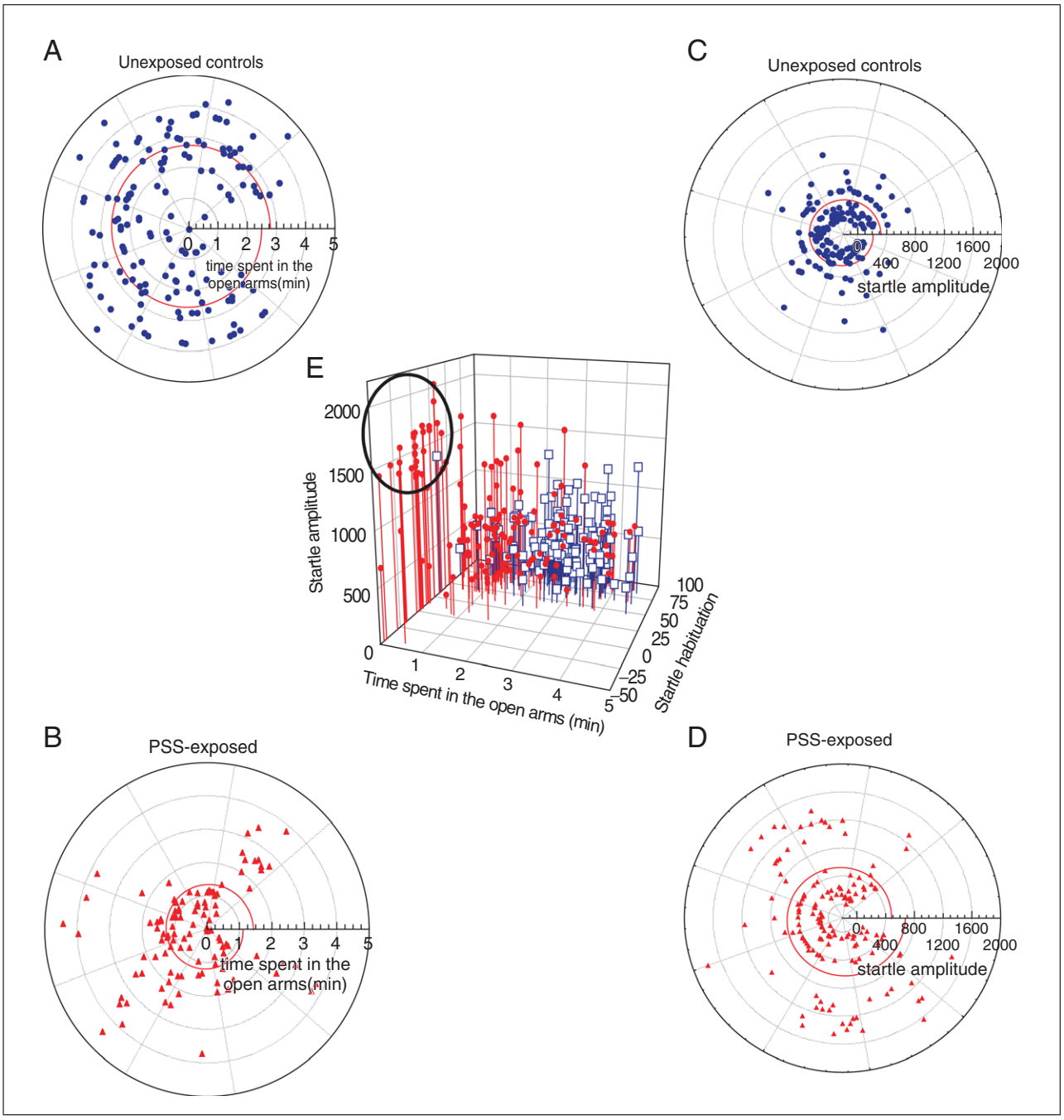

Figure 9.45.7 The effect of the predator scent stress (PSS) paradigm on anxiety-like behavior and acoustic startle response. The figures represent time spent in the open arms (**A**,**B**) of the elevated plus maze and in the mean acoustic startle amplitude (**C**,**D**) in rats exposed to a cat-scent, as compared to controls. Exposed rats spent less time in the open arms (center) and exhibited higher mean startle amplitude (circumference) as compared to controls. Overall, a wide distribution in results was observed within the exposed rats with a broad range of variation in behavioral response. We thus hypothesized that the group is not homogeneous, and we may be dealing with several subgroups in this population. Based on the results of this phase of the study the animals were subdivided into groups reflecting magnitude of response according to the CBC's, focusing selectively on EBR, PBR, and MBR. In **E**, representation of the data from both the elevated plus maze (EPM) and ASR paradigms reveals two distinct features. First, PSS exposure alters the response of the majority of individuals to at least some degree. Second, the cluster of individuals that forms in the upper left hand corner of the graph (i.e., more extreme responses to exposure) is distinct from the majority of individuals.

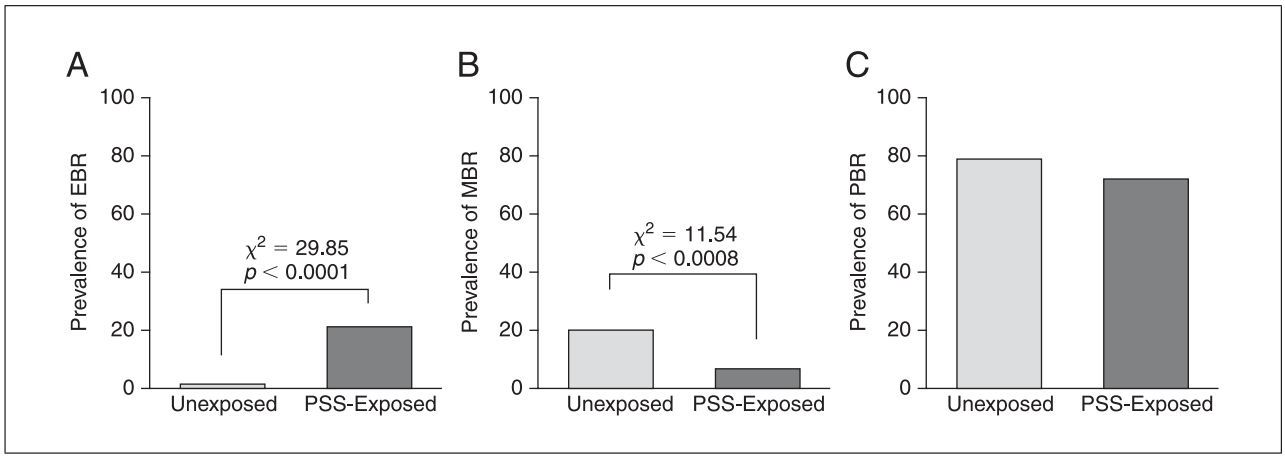

Figure 9.45.8 Relative prevalence rates according to CBCs. Re-analysis of data applying Cut-off Behavioral Criteria: (**A**) Prevalence of extreme behavioral response (EBR) rats. (**B**) Prevalence of minimal behavioral response (MBR) rats. (**C**) Prevalence of partial behavioral response (PBR) rats. There were significant differences in the prevalence rates of individuals displaying EBR among groups (Pearson $\chi^2 = 296.11$, df = 3, $p < 0.0001$). PSS exposure increased the prevalence of PTSD-like behavioral responses (EBR) ($x^2 = 29.85$, $p < 0.0001$) and concomitantly reduced prevalence rates of minimal behavioral response (MBR) ($x^2 = 11.54$, $p < 0.0008$), relative to unexposed controls.

Critical Parameters and Troubleshooting

In our studies, roughly 25% of exposed animals respond with EBR pattern. Thus, no less than 40 animals/groups must be exposed to the stressor in order to yield a sufficient population of EBR.

If the initial gross data show little or no effect for the PSS exposure in downstream tests, i.e., little or no difference between exposed and unexposed population during the EPM and/or ASR tests:

- Check whether the litter is scented to a degree which masks the urine scent; change litter and try again.
- Try changing the cat—there appear to be differences between individual cats of both genders.
- Fur can sometimes be helpful. Add some fur. Usually there are sufficient levels of fur in the soiled litter.

Colony maintenance and handling

- Acclimate the animals to the vivarium and light cycle for 2 weeks before testing. We have generally used a reversed cycle. If the animals are housed under reversed lighting, test them under dim light.
- House animals four to five per cage in standard cages.
- Correct handling and housing of the animals throughout all tests is crucial, but of particular importance when testing anxiety-like behaviors. It is critical that only the specific test conditions (and not other extraneous factors) determine the anxiety generated (File et al., 1993). Handling consists of picking the animals up with a gloved hand around the shoulder for 5 min/day.
- To monitor health, weigh each animal daily throughout training and testing.
- Randomly allocate animals to the various condition/drug groups and manipulate/inject them at the interval appropriate to the route of injection of a particular drug.

Time Considerations

The seven-day protocol takes at least 3 to 4 weeks to perform, in total, since acclimation to the vivarium and light/dark cycle conditions (14 days) must be taken into account.

PSS exposure requires 15 min per animal and ~10 min for preparation and cleanup between each animal.

The behavioral tests are performed seven days later, on the same day and within the same diurnal phase.

The time required for the EPM test is about 15 min per animal, including 5 min preparation, 5 min testing, and 5 min cleanup. The time required for the ASR test is significantly longer, about 35 min for the test session itself, including 5 min habituation and must start within 1 hr of the EPM. Therefore, the number of ASR chambers available will determine how many animals can be processed per batch.

Exposure to trauma cue occurs 24 hr later and requires ~25 min per animal (15 min reminder exposure and 10 min for preparation and cleanup).

For surgical manipulations, the time needed to recover from surgery will depend on the nature of the surgery and it could take about 7 to 10 days for fully recovery.

For pharmacological manipulations, animals given drugs or any other manipulation may require twice the time to complete the protocol.

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