EVALUATION OF ANIMAL MODELS AND PREVIOUS STUDIES ON PTSD

By Nhi Doan

Introduction

Anxiety has long been a socioeconomic and public health burden because of its persisting impact on patients and costly government investment along with a high chance of placebo, ineffective treatment, and adverse side effects. With the outbreak of COVID-19, the mental health of the general population has worsened. A combined study conducted in China, Spain, Italy, Iran, the US, Turkey, Nepal, and Denmark shows a relatively high rate of mental health problems: people report experiencing anxiety, varying from 6.33% to 50.9%, stress from 8.1% to 81.9%, and psychological distress 34.43% to 38% (Xiong et al., 2020). Furthermore, anxiety is usually comorbid with other medical complications, such as hypertension, migraine, asthma, and cardiovascular diseases (Cryan & Sweeney, 2011). The surge in anxiety disorders calls for better, more effective drugs more than ever.

The need for animal models

The problem of finding clinically effective drugs has never been an easy task. The development of new anxiolytic drugs depends on both diagnostic improvement and behavioral, genetic, and neurobiological understanding of humans. While our knowledge about psychiatric disorders is still in its infancy, the urge to treat a growing population of patients and the huge cost of phase II and III clinical trials force pharmaceutical companies to find a model that can exhibit similar phenotypes and is relevant to the disorder in question. The well-known and well-established observation of Darwin notes a considerable similarity of behavioral and pharmacological responses across species. Therefore, it is beneficial for our understanding of human anatomy and physiology to study the neural mechanisms and behavioral responses of a
different species (Cryan & Sweeney, 2011). Animal models are also able to incorporate other ethically salient factors (such as the impact of early development, a low level of maternal care, and the stressful estrangement from parent animals) to see the impact of stress on behavioral and biological reactivity, which can never be found if we rely only on human observations. Animal models also allow researchers to look at the biochemistry in different brain areas and test out the most promising pharmacological agents to understand the pharmacokinetics of these compounds on the brain (Cohen et al., 2011). Thus, animal models have become a useful tool to enhance our understanding of the pathophysiology of psychiatric diseases and assess drug efficacy.

**Evaluation of animal models validity**

It must be noted that there are several incompatible elements between animal models and the human body. For example, animal models cannot address some symptoms in humans such as thoughts, dreams, or images, or the feeling of shortcoming. Nor is their perception of an event (a life-threatening event for instance) the same as that of humans.

The choice of animals is also a problem. Amongst different species, the rat (*Rattus norvegicus*) and the mouse (*Mus musculus*) are the most prevalent choices of researchers due to their size, superior cognitive ability, and performance in memory tasks and operant conditioned tasks (Cryan & Sweeney, 2011). However, their size makes it harder to collect blood or analyze biomarkers. The diversity of rodent strains (with each of them having their own biological and behavioral responses to the same stimulus) also poses a question for researchers to consider which one is the most compatible with the human body. The ambiguity in using and classifying animal models into appropriate diseases is a challenge to researchers and continues to widen the disparity between drug efficacy and the target (Neumann et al., 2011).
To assess the similarity between the model and the human body, researchers employ certain standardized criteria, such as construct, face, and predictive validity. Construct validity pertains to the comparable underlying biological mechanisms of the models and human physiology. Face validity refers to the similarity in the model and the condition the model tries to imitate (even though in some cases it assumes the behavioral similarity between animals and symptoms of humans). Predictive validity suggests the performance in the test could accurately predict the disorder in question and is more reliable because any factors that influence both models and human conditions increases the validity of this test (Joel et al., 2006). To assess face validity, researchers rely on behavioral tests, which are a combination of anxiety, arousal, and fear, such as open-field, elevated plus maze, or startle response. To demonstrate the predictive and construct validity, post-stress measurements assess drug and hormone response of anxiolytic or antidepressant, behavioral and endocrine stress responses of immune reactivity or cardiovascular system activity, and neurobiological processes, like histochemistry or electrophysiology (Daskalakis et al., 2013).

**PTSD as a start**

Amongst a cluster of anxiety disorders, post-traumatic stress disorder (PTSD) turns out to be a prevalent starting point. PTSD is a subset of anxiety disorder. PTSD is caused by a dramatic event that can lead to a series of changes in behaviors and reactions to even the cue of the memory (Daskalakis et al., 2013). From the first time to weeks after experiencing a traumatized event, people will have intense fear, helplessness, accompanied by fear, anxiety, depression, and dissociation. Persistent and pervasive thoughts about past stressful events, hypervigilance, sleep disorder, or personality change are coping methods to adapt to the new distressing event. These
symptoms directly interfere with the patient's normal life, leaving them dysfunctional and thus unfit in a striving world (Neumann et al., 2011)

When compared with other psychiatric syndromes, PTSD has a clear triggering etiology, thus giving hope for a better understanding of this disorder. The intensity, as well as the quality of the stressors of this disorder, also correspond to previously studied stimuli (Neumann et al., 2011). Using animal models, PTSD can be studied in a timely and uniform manner, from which researchers can incorporate other risk factors (such as low parental care, substance abuse, adversities at early stages of life, bad habits during adulthood, etc.) into the model and study their contributions and impacts on PTSD.

The more bewildering part of researching for effective drugs for any psychiatric disorders (PTSD is one example) is that there are more confusing, overlapping complications of the condition more than the advantages. PTSD is not a well-defined disorder as most of the symptoms in DSM-5 are self-reported (such as intrusive thoughts, dreams, and images -- symptoms that are unique to humans). Intrusive and unwanted flashbacks cannot be measured in animal models, thus lacking objective and standardized parameters (Neumann et al., 2011). PTSD also shares major symptoms with other disorders, such as significant dysfunctional behaviors or negative alterations in moods. A drug for a psychiatric disorder is regarded as effective as they respond to the disease in question but not others. The overlapping symptoms amongst different disorders make it harder for researchers to distinguish if the drugs are indeed inefficacy or it is efficacy only for a subgroup of the disorder in question (Joel, 2006).

Another of the challenging aspects of PTSD is that its basis is not yet clear. The lack of understanding of what induces a life-long impact on some people why leaving others intact is the core issue of PTSD. Many patients do not experience complete remission even though their
symptoms improve with drugs. Around 75-80% of the population experience stressful or traumatizing events in their lifetime, but only 10% of them cannot function normally and suffer from the event for the rest of their life (Richter-Levin et al., 2018). Because of the complexity and uncertainty of the disorder, the overlapping symptoms with other syndromes, there is currently no effective treatment for PTSD. First-line treatment includes cognitive-behavioral therapy, exposure therapy, or cognitive therapy; some of these can have unsuccessful rates as high as 50% (Richter-Levin et al., 2018).

**Translatability of animal models to PTSD**

Even though there is an incompatibility between animal models and PTSD patients, researchers have been carefully tackling small details to create the closest possible model to approach this complex matter. Starting with a trauma/stress-based model, the goal of animal model tests is to mimic an originating, triggering event that has long-lasting impacts and engraves into the memory of the survivor. There are currently some tests to model this type of event, even though the duration and the repeated exposure varies from test to test. The more natural setting (predator-scented for instance) is preferred to other artificial stressors, such as electrical-shocking or restraint.

A range of mazes, open field, and acoustic startle response is used to measure the behavioral alteration or adaptations after exposure to trauma. A variety of memory and learning is also incorporated into these tests to assess the performance of animals and the impact of trauma on cognitive ability. In these tests, the elevated plus maze test (EPM) and acoustic startle response (ASR) are two commonly employed tests to evaluate behaviors impacted by the trauma. While EPM is employed as a platform to interpret overall anxious and exploratory behaviors, ASR gives a measurable parameter of hyper-vigilance/ hyper-alertness (Cohen et al., 2012).
The timing of behavioral measurement is also vital. In assessing the translatable validity of animal models, Richter-Levin and colleagues question whether or not a duration of animal tasks is comparable or translatable to symptoms evolving in a significant amount of time in human experience. Cohen and her colleagues observed that behavioral changes of rodents are unlikely to change for the 30 days after day 7 from the exposure. In addition, the average lifespan of rodents falls between 2.5 and 3 years, so suggesting their behavioral responses at day 7 and afterward is reliable to represent symptoms of PTSD seems reasonable (Cohen et al., 2012). This threshold of timing is translatable to a month of distressing experience in humans, which fits with the description of PTSD from DSM-5 (APA, 2013).

In addition, a good animal model will be able to demonstrate variability between individuals, but the variability leads to a biased mean number and consequently invalid conclusion. In real life, of all people who experience shocking, disturbing events, only a portion of them eventually develop PTSD as a way to cope with intrusive cues or reminders, whereas other resilient individuals adapt within 1 to 4 weeks of the events. About 7% of the general population has PTSD at some points in their life, suggesting around 20-30% of stressor-exposing individuals develop PTSD (Cohen et al., 2012). Cohen and her group proposed cut-off points to classify these behaviors. Overall, the exposed group in the animal model usually demonstrates a shrinking time in open-arms of the EPM, a longer period spent in the closed-arms, a higher startle response, and a lower habituation period. Cohen’s group organized animals into behavioral response patterns (extreme behavioral response, minimal behavioral response, and partial behavioral response), using time spent on the closed arm of EPM and mean startle response as a criterion (Cohen et al., 2012). This method excludes any animals whose behaviors
do not demonstrate a significant alteration by the stressors and thereby increases the accuracy of data interpretation.

In addition to the general guidelines of Cohen’s group, Richter-Levin and colleagues proposed to assess animal models differently to better understand the underlying causes and symptoms of PTSD. They suggest that instead of immediately assessing the impact of the trauma on the models, researchers should wait for a period of time. In real life, the majority of people (around 80%) experience trauma, but these memories fade away as time passes. The ability to accept reality and move past trauma is a valuable skill, and the lack of it puts people at a greater risk for developing other psychiatric disorders, such as PTSD. Understanding the early symptoms of PTSD is important, but solely using this knowledge as a guideline for PTSD might not be accurate.

Richter-Levin also pointed out some drawbacks of animal models. First of all, the triggering event in experimental design is also very challenging to manipulate. The test is created based on an assumption that any exposure to sufficient traumatized events can lead to long-lasting impacts. The fear-conditioning based-model may not be a good test as they are not clear if the PTSD patients have an abnormally stronger response to fear. Another research group, Daskalakis and colleagues, agrees that the notion of the fear mechanism as an underlying cause for PTSD might be simplistic. There are many other factors, such as habituation or sensitization that can interfere with the experimental model. It is also possible that PTSD patients produce a normal fear response in the return from severe trauma.

Secondly, the trauma alone will not make animal models an effective method for researchers. Only a small number of people who are exposed to traumatic events develop PTSD. The exposure may be the foundation to understand PTSD, but it alone is not sufficient to produce
any valid conclusion. Some risk factors (early childhood activities, prenatal health, bad habits including a high level of alcohol assumption, drug abuse) and resilience factors (such as personality or genetics) can predispose people to these disorders. This high level of variability amongst people makes it difficult to explain the underlying factors that help some get past their stressful experiences while holding onto others.

There are currently some common tests trying to understand the mechanism and reaction of animals at different stress levels. Even though these tests focus on inducing exposure to severe stress or a single-prolonged trauma, they employed different methods and thus a variety of outcomes. That is the third obstacle in trying to solve the PTSD puzzle. In addition, researchers are subjected to the ethical code of conduct, so the tests themselves might not go beyond the handling ability of animals, which can misinterpret the outcome of the experiment.

Sex is another factor that strongly influences the responses of individuals to stress. An epidemic study shows that women are twice as likely to have PTSD as men are, and female reproductive cycle and emotional memory processing mainly contribute to this gap (Daskalakis et al., 2013). Estradiol has been proved to correlate to the rate of memory extinction, while ovarian hormones interact with hormones of the hypothalamus-pituitary-adrenal axis (HPA) to modify memory consolidation (Daskalakis et al., 2013). In real life, the experience of a combat soldier witnessing his friend’s disembowelment is surely very different from that of a sexually assaulted woman. Their responses to stress are also expected differently by society, which leads to different emotional responses (such as a sense of guilt or moral crises) that animals cannot model. Genetics, gender, and social expectation make it even more complicated for the understanding of PTSD.
These factors do not mean to discourage the use of animal models, but rather, they encourage the development of a more reliable and comprehensive one. This paper will present and evaluate two previous studies. One establishes face validity, and the other assesses construct and predictive validity using animal models. The advantage of having different assessments to understand a complicated and individualistic disorder like PTSD is that it integrates different factors and approaches into a collective understanding and provides an insight into the matter under the lenses of biologists, behaviorists, epidemiologists, and geneticists. Each field has its own strengths and each contributes a valuable perspective on the matter.

**Previous studies of PTSD using animal models**

1. **Face validity of animal models for PTSD**

   Faraday and her group have been systematically exploring whether there are physiological and behavioral differences between individuals within a species and the role of gender and gene in predisposing individuals to a certain type of stress (Faraday, 2002). Understanding this diversity helps explain the disparity amongst individuals (i.e., some people develop PTSD and others resilient adapt normally), advance the understanding of stress vulnerability, and better treat or prevent stress-related disorders in susceptible organisms.

   Faraday’s work is compelling because it addresses some limitations that previous studies neglected. Before, studies had focused on the extreme stress expressions by deliberately breeding for extreme sensitivity and insensitivity. The detailed mechanism of this genetic extreme is partly understood, but it coincidently leaves the medium between two phenotypes unexplored. Her study also includes female organisms, a large number of dependent variables, and repeated assessment points. A diversity of variables evaluates if one triggering variable has an impact on
the subjects while others leave it intact and if together these triggering variables might be predictive of certain types of stress. To be more certain, her group routinely measures body weight, food intake, open-field activities, acoustic startle response with and without prepulse inhibition, in order to consider if the rats are in a process of habituation, a vital concept in PTSD patients who are unable to cope with novel situations. Her group carefully chooses food intake and body weight as dependent variables due to their central roles in other health complications, such as cardiovascular diseases, eating disorders, or obesity. Open-field activities, acoustic startle response (ASR), and prepulse inhibition (PPI) will be used to assess the innate reflective and defense mechanisms, establishing the face validity from animals to humans, and provide insight into gender and genotype sensitivity to stress.

Faraday recruited 66 Sprague-Dawley and 64 Long-Evan subjects with equal numbers of males and females, gave them access to food and water, and measured their activities during the night. For the first ten days of the experiment, subjects were acclimated to open-field conditions and startle testing to minimize any stress that might occur. After this period, animals were assigned according to sex and strain to stressed and no stressed groups. Stressed groups were immobilized by tightening the fingers (without inflicting any pain) for the next 21 days. Bodyweight and feeding were measured every other day while open-field activities were assessed at 15-minute and 2-hour periods on days 1, 6, 9, and 19. ASR and PPI were evaluated one day after the measurement of open-field activities.

**Result**

a. **Bodyweight and food intake**
Fig. 1

(a) Body weights of nonstressed and stressed Sprague–Dawley and Long–Evans males (mean in g±S.E.M.) at Baseline and during the Stress Phase; (b) Body weights of nonstressed and stressed Sprague–Dawley and Long–Evans females (mean in g±S.E.M.) at Baseline and during the Stress Phase; asterisks (*) indicate significant difference ($P<.05$) between nonstressed and stressed same-sex Long–Evans on that measurement day; number sign (#) indicates significant difference ($P<.05$) between nonstressed and stressed same-sex Sprague–Dawley rats on that measurement day.

Fig. 2

(a) Food consumption of nonstressed and stressed Sprague–Dawley and Long–Evans males (mean in g±S.E.M.) at Baseline and during the Stress Phase; (b) Food consumption of nonstressed and stressed Sprague–Dawley and Long–Evans females (mean in g±S.E.M.) at Baseline and during the Stress Phase; asterisks (*) indicate significant difference ($P<.05$, unless otherwise noted) between nonstressed and stressed same-sex Long–Evans on that measurement day; number sign (#) indicates significant difference ($P<.05$) between nonstressed and stressed same-sex Sprague–Dawleys on that measurement day. Inset graphs depict cumulative food consumption during the Stress Phase.

b. Open-field activities, ASR and PPI

Fig. 3

(a) Open-field activity over 15 min (mean beam breaks±S.E.M.) of nonstressed and stressed Sprague–Dawley and Long–Evans males at Baseline and on Stress Days 1, 6, 9,
and 19; (b) Open-field activity over 15 min (mean beam breaks±S.E.M.) of nonstressed and stressed Sprague–Dawley and Long–Evans females at Baseline and on Stress Days 1, 6, 9, and 19; asterisks (*) indicate a significant difference between nonstressed and stressed Sprague–Dawley females on that measurement day.

**Fig. 4**
(a) Startle responses to the 120-dB stimulus (mean in arbitrary units±S.E.M.) of nonstressed and stressed Sprague–Dawley and Long–Evans males at Baseline and on Stress Days 2, 7, 10, and 20; (b) Startle responses to the 120-dB stimulus (mean in arbitrary units±S.E.M.) of nonstressed and stressed Sprague–Dawley and Long–Evans females at Baseline and on Stress Days 2, 7, 10, and 20; asterisks (*) indicate significant difference ($P<.05$, unless otherwise noted) between nonstressed and stressed same-sex Sprague–Dawleys on that measurement day.

**Fig. 5**
(a) Percent PPI responses (±S.E.M.) to 120 dB stimulus with 82 dB prepulse of Sprague–Dawley and Long–Evans males at Baseline and on Stress Days 2, 7, 10, and 20; (b) Percent PPI responses (±S.E.M.) to 120 dB stimulus with 82 dB prepulse of Sprague–Dawley and Long–Evans females at Baseline and on Stress Days 2, 7, 10, and 20; asterisks (*) indicate statistically significant ($P<.05$, unless otherwise noted) differences between nonstressed and stressed same-sex Long–Evans on that measurement day.
Discussion

Figures 1 and 2 show that even though there are some significant differences between rat strains, these differences are observed in only male groups. In particular, nonstressed and stressed Long-Evans male groups differ dramatically starting experimental day 11 while the two groups of Sprague-Dawley male strain see a difference starting day 19. These findings indicate that males are more susceptible to a decrease in food intake and consequently body weight. Conversely, stressed female rats react differently than the rest of the subjects in open-field activities and prepulse inhibition. More specifically, stressed female Sprague-Dawley rats show a significantly lower number of beam breaks in open-field activities, compared to stressed female Long-Evan and the rest. Stressed female Long-Evan in prepulse inhibition measurement achieves a much lower percentage compared to nonstressed ones, those of Sprague-Dawley and male group. The difference in variables, as Faraday hypothesized, varies, and so do the outcomes. Figure 4 indicates a clear disparity between two strains when Sprague-Dawley strain, both males and females of the stressed group, show a more sensitive reactivity toward acoustic startle while there is no clear gap observed in Long-Evans strain.

The study shows that a certain strain and sex can have different responses to the same stressor and different dependent variables can indicate distinctive features of the independent variables. A lot of negligible details, such as the female population, various variables, activity measurement at night, and repeated assessment points, help contribute to the overall validity of
the experiment. Even when the experiment does not focus on PTSD, the model becomes a good foundation for future studies to study the impact of prolonged stress on the well-being of the exposed. The result also leaves room for interactions between different variables (strain and sex for instance) and reminds future researchers to take these interactions into account when designing new experiments.

2. Construct validity of animal models for PTSD

As mentioned above, the trauma alone will not be a sufficient factor to determine if a person will develop PTSD. Other risk factors, such as personal habits, genetics, and early childhood experience, play important roles in predicting if people are more prone to develop PTSD. If Faraday’s experiment zooms in on behavioral elements that appear after a prolonged period of stress, Zoladz and Diamond group, another group studying PTSD, explored the complexity of PTSD syndrome by incorporating risk factors of PTSD to create the closest animal model possible.

Previous research has shown that predator scent can provoke stress through activating hypothalamus-pituitary-adrenal (HPA), impairing spatial memory and synaptic plasticity in the hippocampus, and reinforcing synaptic plasticity in the amygdala (Zoladz & Diamond, 2016). The method, therefore, is relevant to the goal of mimicking PTSD syndrome, in which animal models can generate a learned fear response and create abnormally behavioral and physiological reactions. Amongst various methods, the PTSD model of Cohen and colleagues has attracted much attention due to its resemblance to survival threats that happen often in nature. In the experiment design, they exposed rats to a cat urine scent for 10 minutes and measured the physiological and behavioral responses 7 days after. Zoladz and Diamond explored this model deeper and studied why only a portion of people develop PTSD after experiencing a serious,
life-threatening event. They established construct validity by analyzing the impact of PTSD at cognitive, cardiovascular, and hormonal levels. They incorporated more factors (such as loss of control, a sense of reliving the events, and lack of social support) into the model with the target of inducing PTSD-like effects in more subjects instead of just a subgroup.

For Zoladz and Diamond’s experiment, the researchers divided the subjects into three groups: no stress (home cage only), stressx2 (exposure to a cat at the first and second testing time and stay in the cage the third testing time), and stressx3 (exposure to the cat at the first, second, and third testing time). They exposed rats to a cat while immobilizing them to mimic the sense of uncontrollability in a life-threatening occasion even though there is no real physical contact between the cat and rats. They then re-exposed the rats after 10 days to activate the intensive repeated flashbacks. The third exposure happened 21 days after the second one. Between the exposures, the rats were constantly moved from house to house to imitate the social instability that PTSD patients face. After 4 months, the subjects were tested again on the same chamber in which they encountered but did not contact the cat.

Previous studies often reported an elevation in cortisol level, which can be explained by the activity of fight or flight response (Zaladz & Diamond, 2012). When PTSD patients are treated with dexamethasone, a synthetic glucocorticoid, they experience depression in cortisol level. At the hormonal level, the researchers used the same paradigm as described (but they use only the nonstressed and stressx2 groups) to test dexamethasone. They picked 10 rats from both stress and nonstressed groups. Each rat received an injection of dexamethasone at 10 μg/kg, 25 μg/kg, 50 μg/kg, or 1 ml/kg to or vehicle solution, which contains sodium sulfite (1 mg/ml) and sodium citrate (19.4 mg/ml).

*Result*
a. Immobility observed in three stressed groups

Immobility during the three stress sessions and the context and cue tests. Blue represents the nonstressed group. Red represents the group that has the 2nd exposure to a stress source (cat) after 10 day. Burgundy represents the 3rd exposure to stress source 21 days after the second exposure. Data are means ± SEM *p < 0.05 vs. the no stress group; **p < 0.05 vs. all other group (Zoladz et al., 2015)

b. Level of corticosterone levels and diastolic blood pressure

Effects of psychosocial stress on diastolic blood pressure (left) and corticosterone levels (right) when combining psychosocially stressed animals given 2 or 3 cat exposures. Data are means ± SEM. β p = 0.068 vs. the no stressed group; *p < 0.05 vs. the

c. Effect of dexamethasone on stressed and nonstressed groups

Injection of dexamethasone or vehicle solution to stressed and nonstressed groups. Blood
sampling occurred three weeks following the second cat/home cage exposure between 1700 h and 2000 h. Data are presented as mean ± SEM. The dark bar from 0 min to 20 min indicates the time when rats were immobilized. *p < 0.05 relative to the psychosocial stress group (Zoladz et al., 2012)

Discussion

Figure 6 indicates there are no significant differences during the first two sessions when rats in Stressx2 and Stressx3 groups are exposed to the cat twice. By the third session, both Stressx2 and Stressx3 groups demonstrate approximately equal inactivity. The context memory test, conducted 4 months after all the exposures, observes a disparity between the three groups. In particular, the Stressx2 and Stressx3 groups both exhibit immobility but the Stress×3 group shows greater inactivity than the other. This finding suggests that frequent exposure to stress sources can leave long-lasting impacts on patients, and more frequent exposure might engrave into the mind deeper.

The psychosocial stressed group (Stress × 2 + Stress × 3) in figure 7 displays a hefty elevation of diastolic BP and a significant depression level of corticosterone relative to the no-stress group. This finding is reiterated in figure 8 when the psychosocial stressed group exhibits significantly curtailed corticosterone levels than the nonstressed group at the baseline. Vehicle injection groups experience an elevation in corticosterone level after 80 minutes. As for dexamethasone-injected groups, after the injection of 10 μg/kg dexamethasone, the stressed
group does experience a surge in corticosterone momentarily before falling to a threshold of 3 μg/kg. The same pattern is observed in the group that is administered the dose of 25μg/kg. There is no difference in the administration of 50 μg/kg of dexamethasone. It is worthy to note that the low level of cortisol is only observed in uninjected groups, while both vehicle and dexamethasone injected groups do not see the difference in cortisol levels.

In sum, this model has produced a wide range of physiological and behavioral changes like those in PTSD, such as heightened anxiety, impaired learning ability, elevated cardiovascular and hormonal reactivity and startle response to a stressor (Zoladz & Diamond, 2016). However, Zoladz and Diamond’s model produces a low baseline level of cortisol in stressed animals, which was not a prevalent result observed in previous studies. After administering dexamethasone, the animals experience a surge in corticosterone instead of depression, which is usually observed in the empirical data of dexamethasone-treated patients with PTSD. Interestingly, animal models that employed prolonged stress or reliving the stressful event paradigm also note the low baseline level of cortisol in the stressed group. The researchers explain that this abnormality can be due to blood sampling. In particular, they collect the subjects’ blood during the earlier hours of the evening, the time in which the level of corticosterone is rising. Additionally, even though the experiment has approached PTSD syndrome from multiple angles, their subjects are male Sprague-Dawley only. From the experiment of Faraday, it is clear that different stains react differently to different variables due to genetic diversity. Moreover, the empirical data are collected based on a large population including both males and females, and their experience with trauma can be very different from another (for instance, men usually experience combat trauma because they are soldiers, and women experience sexual assault since they are physically more vulnerable). It should be noted that the researchers experiment with the light portion of the
day, which is not an active time for the subjects. Future research should include more strains, sexes, and different doses of dexamethasone. The data are better collected during the dark portion of the circadian cycle. To understand the hormone at the molecular level, researchers suggest employing more biomarkers to understand the physiological and pathological processes of PTSD.

3. Predictive validity of animal models for PTSD

Zoladz and Diamond’s research also establishes predictive validity by evaluating the impact of proven pharmacotherapeutic treatment and clinically based treatment strategy. As for pharmacotherapies, the group chooses amitriptyline, clonidine, and tianeptine as testing variables. Amitriptyline, an antidepressant, is known to mitigate a subset of symptoms of PTSD, while clonidine, a noradrenergic receptor, alleviates any impacts on anxiety and hyperarousal. Tianeptine is well-established as an effective antidepressant as its main function is to stabilize glutamatergic neurotransmission and enhance synaptic plasticity. They employed the same paradigm: Sprague-Dawley males in the stressed group are exposed to cat scent for 1 hour on two different occasions, each is separated by 10 days. Those in the stressed group was also changed their cage mate every day to mimic social instability. 24 hours after the first exposure, the rats in both stressed and nonstressed groups were administered with amitriptyline, clonidine, tianeptine, or vehicle solution every day for the rest of the testing period (Zoladz et al., 2016).

Besides, in their previous work, they find that inevitable, life-threatening periods of predator exposure in combination with daily social instability causes animals to have conditions like those observed in PTSD patients (Zoladz et al., 2008). In another work, Seetharaman and colleagues develop a new non-pharmacological strategy to hamper the development of PTSD in animal models. On the hypothesis that daily social interaction would be able to alleviate any
development of PTSD aftermath, they employed the same animal paradigm as described. In the
two stressed and nonstressed groups, they divided the group into 2 smaller groups: social group
and nonsocial group (so in total, they have 4 groups: stressed and social, stressed and nonsocial,
nonstressed and social, nonstressed and nonsocial). The animals in the social group were placed
in an enriched environment for 2 hours everyday (social interaction). The animals in the stressed
group are changed the cohort pair combination randomly (social instability).

**Result**

**a. Effects of different antidepressants on growth rate, adrenal gland and thymus gland weight**

**Fig. 9**

Growth rate and organ weights for all groups. The data are presented as the mean ± SEM. * $p < 0.05$ relative to the vehicle-treated no psychosocial stressed group; $\beta = p < 0.05$ relative to the vehicle-treated psychosocial stressed group; $\tau = p < 0.05$ relative to the respective drug-treated no psychosocial stressed group; $\# = p < 0.05$ relative to all other groups (Zoladz et al., 2016)

**b. Effects of different antidepressants on startle responses**

**Fig. 10**

Startle responses exhibited by rats following presentation of 90 dB (top left), 100 dB (top right) and 110 dB (bottom) auditory stimuli. The data are presented as the mean ± SEM. * $p < 0.05$ relative to the
vehicle-treated no psychosocial stressed group; $\beta = p < 0.05$ relative to the vehicle-treated psychosocial stressed group; $\tau = p < 0.05$ relative to the respective drug-treated no psychosocial stressed group; $\omega = p = 0.056$ relative to the vehicle-treated no psychosocial stressed group (Zoladz et al., 2016)

c. Effects of different antidepressants on cardiovascular activities

![Fig. 11](image)

Heart rate, systolic blood pressure, and diastolic blood pressure of rats on the final day of testing. The data are presented as the mean ± SEM. * $p < 0.05$ relative to the vehicle-treated no psychosocial stressed group; $\beta = p < 0.05$ relative to the vehicle-treated psychosocial stressed group; $\tau = p < 0.05$ relative to the respective drug-treated no psychosocial stressed groups (Zoladz et al., 2016)

d. Effects of social interaction on activities on elevated plus maze model

![Fig. 12](image)

Activity in the elevated plus maze (EPM). * $P < 0.05$ relative to No Social/No Stress. # $P < 0.05$ relative to Stressed/No Social. Data are
presented as mean ± SEM (Seetharaman et al., 2016).

e. Effects of social interaction on startle responses

![Acoustic Startle Response](image)

Fig. 13

Startle response when presented with an auditory stimuli. Data are presented as the mean startle response (Newton) to the 90, 100, and 110 dB acoustic stimuli ± SEM. *P < 0.05 relative to all other groups at 110 dB stimulus intensity (Seetharaman et al., 2016)

f. Effects of social interaction on growth rates and organ weight.

![Growth Rate and Thymus Gland](image)

Fig. 14

Influence of psychosocial stressed on growth rate and organ weights. Organ weights are shown as mean mg/100 g body weight. *P < 0.05 relative to No
Social/No Stressed. \#P < 0.05 relative to Stressed/No Social. (Seetharaman et al., 2016)

Discussion

The injection of amitriptyline, clonidine, and tianeptine shows different responses on the measured variables. Figure 9 indicates that the tianeptine produces the most similar effect in the psychological stressed group, compared to the nonstressed group. This effect is consistent with the growth rate, adrenal gland weight, and thymus gland weight. Meanwhile, tianeptine and higher doses of both amitriptyline and clonidine can repress the startle responses in rats at all levels of auditory stimuli (figure 10). In figure 11, tianeptine and higher dose clonidine are the most effective in controlling heart rate, systolic, and diastolic blood pressure. In general, when tested on the model of Zoladz and Diamond, tianeptine is the only drug that delivers the most consistent results across different variables -- whether it is to hamper the effect of stress on startle response or control cardiovascular responses and organ weight.

As for the testing of social support on animal models of PTSD, Zoladz and Diamond prove that sufficient social interaction can alleviate any stress that a subject might have to face. In figure 12, social support groups are far more active and exploratory on both the close and far open arms of the elevated plus-maze. The stressed/social group even has more body movements and head dips than the control (no social and no stressed) group. In figure 13, even though there are no significant differences at 90 or 100dB across the groups, no social/stressed group reacts more intensively than the other three groups, indicating heightened anxiety. Meanwhile, the social/stressed group exhibits similar responses to the no social/no stressed group. They are also
two groups that exhibit the least startle responses to the stimuli and the highest growth rate in figure 14. It is worth noting that the three groups -- no social/stressed, social/stressed, and social/no stressed -- experience increases in weight of the thymus gland, heart, and adrenal glands. The social/no stressed group is expected to share similar physiological characteristics with no social/no stressed group, but in the experiment, it shares features with the stressed group. The researchers attribute this characteristic to the increase in physical activity, which produces organ hypertrophy (Seetharaman et al., 2016). Overall, Zoladz and Diamond find that even daily 2-hour social interaction can block the development of fear-conditioned responses, anxiety-like behaviors, and startle reactions.

In sum, the result from clinically based strategy proves that sufficient and timely social support can help alleviate the impact of trauma. The pharmacological treatment indicates the efficacy of three testing drugs on different variables and tianeptine is shown to have a consistent result on cardiovascular activities, organ weights, and auditory stimuli.

**Conclusion**

The research described here has proved that animal models are an effective tool for researchers to gain insight into our bodies. Faraday’s research looks at the behavioral responses of animals after being exposed to prolonged stress periods. Her research shows that the diversity of sex and strain does account for differences in outcome. The researcher has diligently collected the data over a long period of time, carefully paid attention to the active time of the subjects, and included a more diverse population of rats. These details overall help to establish a thorough and reliable study.

Research conducted by Zoladz and Diamond demonstrates that animals can model the biological and pharmacological aspects of the human body. Incorporating different risk factors
(social instability, prolonged stress, and social support) into their research has shown the results to be comparable with the symptoms observed in PTSD patients, like immobility, heightened anxiety, cognitive ability, hormonal adjustment, and reactions to therapeutic solutions.

Each study offers a unique vantage point onto PTSD: one looks at the behavioral responses while the other assesses the biological and pharmacological aspects. Each has its own advantages and limits, but they have been successful in designing the models based on their specialty and opening new ways of thinking for future research. Subsequent studies might focus on incorporating risk factors, including a more diverse population of subjects, using a natural trauma-causing factor, and combining the strengths of each type of research to produce a more comprehensive study.
Bibliography


