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Commentary

Defeating the fear: New insights into the neurobiology of stress susceptibility

Vaishnav Krishnan[1]

Department of Neurology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215, USA

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ABSTRACT

The psychopathological impact of emotional stress on a specific individual varies markedly: while most escape the development of post-traumatic stress disorder and/or major depression, a select group of individuals demonstrate a vulnerability to succumb to these conditions. The past decade has witnessed an explosion in animal research into the underlying neurobiological mechanisms that govern both vulnerability and resilience to such stressors. In the May 2014 issue, Chou and colleagues employ the mouse social defeat model of chronic stress to demonstrate that defeated susceptible mice display an exaggerated conditioned fear response associated with more pronounced autonomic changes. These physiological alterations were found to be mediated via local increases in the levels of brain derived neurotrophic factor (BDNF) within the basolateral amygdala and could be inhibited by the systemic administration of a beta adrenergic antagonist. This mini-review critically examines this manuscript's new mechanistic insights in light of previous results employing similar approaches. The strengths and limitations of the social defeat model, as well as the relevance of these findings to neurologic illness are discussed briefly.

Introduction

There has been a recent paradigm shift in preclinical studies designed to explore the neurobiological basis of stress-related disorders such as depression and anxiety disorders. Historically, rats or mice were divided into two groups, "stressed" and "control", and any appreciated changes in neurochemistry and neuronal morphology in the "stressed" group would be attributed to the effects of stress. Combining a variety of acute and chronic stressors with molecular and cellular anatomical techniques, this approach has been crucial to putting forward several theories pertaining to the detrimental effects of stress on a variety of limbic and cortical nodes of emotional processing. One such theory born out of this methodology is the neurotrophic hypothesis of depression, wherein stressful exposures result in neuritic atrophy, reduce neurogenesis, and decrease neurotrophin levels and downstream signaling in the hippocampus. These effects are reversed by exercise and antidepressant therapy.

Human beings display a wide variability in their response to a traumatic stressor (Russo et al., 2012; Rutter, 2006). Santiago and colleagues recently conducted a systematic review of the prevalence of post-traumatic stress disorder (PTSD) in trauma-exposed individuals, and classified trauma as either intentional (e.g., physical assault, combat) or unintentional (e.g., motor vehicle accident, earthquake). At one year following the traumatic exposure, the prevalence of PTSD in either category was less than 25% (Santiago et al., 2013). This is a compelling result, as it demonstrates that *most* individuals exposed to a traumatic stressor *do not* develop PTSD, and questions the relevance of animal studies that infer a "stressed state" in animals that have received a stressor. Just as human beings display widely varied responses to emotional stress, there can be an impressive degree of variability in behavior within a group of laboratory animals exposed to the same stressor, but appreciating this variability requires that a stressor is combined with some test of coping or exploration. For instance, the application of daily restraint stress to mice or rats results in decreased levels of brain-derived neurotrophic factor (BDNF) mRNA in the hippocampus (Smith et al., 1995), but it is not obvious whether this neuroplastic change is i) a compensatory response to promote better coping, ii) a maladaptive change that mediates depressive behavior, or iii) an epiphenomenon that is unrelated to any behavior that the animal displays following a stressful experience. Coupling the stressful exposure with some test of emotional behavior allows one to begin to explore these various possibilities.

The mouse social defeat paradigm

It is for this reason that there has been a resurgence of interest in the mouse social defeat model of depression (Kudryavtseva et al., 1991). In this model, an intruder mouse is physically subordinated by an aggressive resident mouse for 5 to 10 min daily over ten days. In between these defeat episodes, the mouse is housed opposite the aggressor, providing a second non-physical social stressor throughout the day through

E-mail address: vkrish@bidmc.harvard.edu.
[1] Fax: +1 617 667 2987.

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sensory contact (Golden et al., 2011). Following this chronic stress, a significant proportion of defeated mice display a persistent decrement in social interaction that is reversed by chronic but not acute administration of antidepressant drugs (Berton et al., 2006). Scores on a social interaction task can be used to distinguish between susceptible and resilient mice, and on average, resilient mice display social interaction scores that are equivalent to nondefeated controls. When compared with resilient mice, susceptible mice also have reduced sucrose preference, enhanced cocaine place preference, display a greater weight loss over the 10 day period and also demonstrate a significant hyperthermic response to social stress, thereby exhibiting a syndrome of emotional phenotypes ethologically similar to various depression and anxiety-related syndromes in humans (Krishnan et al., 2007). This adaptation of the social defeat model has been utilized for both correlative (i.e., demonstrating relationships between vulnerable/resilient behavior and changes in neurochemistry and neuroanatomy within limbic brain regions) and causative purposes (i.e., by studying how the effects of social defeat may vary with local or systemic genetic manipulation) (Krishnan and Nestler, 2011). Individual differences have also been studied using the chronic mild stress model of depression (Strekalova et al., 2004).

By manipulating the duration and number of defeat episodes, the quantitative hostility of aggressor mice (such as by measuring attack latency), and the genetic background of intruder mice, the intensity of the social stress can be easily modulated. The model's foremost advantage lies in its ethological relevance, as the application of this type of stress utilizes an innate behavior, rather than relying on other mechanical stressors such as footshock or restraint. This feature can at times serve as a critical limitation of this paradigm, as relatively minor variations in the experimenter's technique and other physical factors (e.g., bedding, cage size) can lead to *both* heightened aggression and excessive physical injury to poor attack rates, ultimately resulting in significant variability across experimenters and animal colonies (Golden et al., 2011; Krishnan and Nestler, 2011). Several portions of the paradigm also require single housing, which can dramatically increase the space required and costs of animal housing. Finally, while human depression is more common in women, mouse social defeat is unable to employ female animals given the low levels of aggression between female mice. As a step towards overcoming this limitation, Trainor and colleagues have established a social defeat model in the California mouse (*Peromyscus californicus*), since both males and females of this family display a high incidence of territorial aggression (Greenberg et al., 2014).

The precise interpretation of social avoidance following repeated bouts of social subordination stress is complicated. First and foremost, one could posit that social avoidance is in fact *not* pathological, i.e., avoiding a physically dominant conspecific animal is an adaptive and logical response to prolonged bouts of social stress (Russo et al., 2012). Several key pieces of data argue against this perspective. First, the avoidance seen in susceptible mice is quite long-lasting (up to several months) and extends not only to unfamiliar aggressors, but also to other unfamiliar members of the same strain. Second, chronic antidepressant treatments reverse this avoidance behavior and have no effect on the social behavior of control or resilient mice (Berton et al., 2006). Third, scores on social interaction testing correlate reasonably well with other measures of anhedonia (a state of reduced sensitivity to naturally rewarding stimuli), including sucrose preference and weight loss (Krishnan et al., 2007). Finally, a number of neuroplastic changes observed in a variety of brain regions in susceptible mice are mirrored in brains from depressed humans, together suggesting that overall, defeated mice that display social avoidance do in fact display a type of murine stress syndrome analogous to depression.

While some groups believe that avoidance represents a state of diminished *motivation* to socially interact (measuring anhedonia), others may interpret this avoidance as a state of heightened social anxiety. Interestingly, both susceptible and resilient mice had reduced exploratory behavior on standard laboratory "anxiety" assays and showed exaggerated corticosterone levels following a swim stressor, suggesting that the interaction score used to segregate out susceptible mice measures a phenotype that is distinct from (and likely orthogonal to) pure anxiety-related behavior. Finally, a third interpretation of defeat-induced avoidance may be that it is an expression of learned fear, i.e., susceptible mice more robustly learn the negative association between an otherwise neutral social cue and the possibility of severe social trauma. This is an important theme within current working models of the pathophysiology of post-traumatic stress disorder (PTSD), whereby in vulnerable individuals, a traumatic event induces an appropriate learned fear that is *inappropriately* overgeneralized leading to a profound sympathoadrenal flight/fight response to a safe and otherwise neutral stimulus (Mahan and Ressler, 2012). The vulnerability to develop social avoidance following social defeat is likely to map across *multiple* behavioral domains, and understanding how susceptible and resilient mice differ in measures of fear learning and memory will provide a better understanding of the "social defeat syndrome" and its ethological validity to a range of stress-related disorders.

The role of BDNF in the basolateral amygdala

In the May 2014 edition of *Experimental Neurology*, Chou et al. (2014) wanted to understand the relationship between social defeat, social avoidance and learned fear. Following ten days of social defeat, defeated *c57bl6* mice were classified as susceptible (~60%) or resilient (~40%) and 7 days later were exposed to a tone-shock pairing fear conditioning paradigm. In this task of classical conditioning, a previously innocuous tone (CS, tone) is repeatedly paired with an aversive unconditioned stimulus (US, footshock) so as to ultimately produce a fear response to the tone alone. On the following day, when mice were placed in a different context and received the tone stimulus, susceptible mice displayed a significant increase in freezing behavior, while resilient mice were similar to controls. This discrepancy between groups was even present 7 days later. Resilient and susceptible mice were not different on measures of extinction (i.e., rates at which freezing behavior diminished over time when presented with just the tone). Similarly, this difference was not related to changes in pain sensitivity and did not extend to other simple measures of memory for object novelty, object placement or familiar odors.

Given the critical role of BDNF signaling within the amygdala complex in mediating the acquisition, consolidation and extinction of learned fear (Mahan and Ressler, 2012), the authors measured how this classical fear conditioning paradigm altered BDNF levels within the basolateral amygdala (BLA). In susceptible mice, the application of footshocks paired with an auditory conditioning stimulus led to a transient two-fold increase in BDNF levels within the BLA that returned to baseline levels in 24 h. Resilient mice displayed a much weaker increase in BDNF levels that returned to baseline within 2 h. BLA neurons of susceptible mice also displayed a relatively greater induction of cFos 90 min following the application of the CS–US pairing paradigm. The authors employed two complementary approaches to test whether this "spike" in BDNF levels was necessary for the expression of this fear response. First, a broad forebrain deletion of BDNF was obtained by crossing mice expressing *Cre* recombinase under the control of the calmodulin kinase II promoter (CamK-Cre) with floxed BDNF mice ($BDNF^{los/lox}$). Second, *Cre* recombinase expressed in a lentiviral vector was stereotaxically injected into the BLA of $BDNF^{los/lox}$ mice to achieve a local deletion of this gene. In both conditions, the loss of BDNF not only impaired this type of conditioned freezing following social defeat, but also produced mice that were resilient to the avoidance promoting effects of social defeat. These findings are broadly in line with results that have been obtained previously using a Syrian hamster model: when two unfamiliar hamsters are paired together, their physical interactions can be examined closely to identify a *winner* and a *loser*. In this paradigm, *winners* were found to have lower BDNF mRNA levels in the