Biological Psychiatry: Celebrating 50 Years

From Structure to Behavior: Circuit Specificity of Stress-Induced Synaptic Plasticity in the Basolateral Amygdala Projection Neurons

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In this issue of *Biological Psychiatry*, Zhang *et al.* (1) aim to bridge the gap between brain structure and complex behavior by dissecting the impact of chronic stress on circuit-specific remodeling of amygdala output projection neurons (PNs) and, ultimately, on stress-induced behavioral pathology.

The amygdala, anatomically located in the medial temporal lobe of the brain, is currently one of the most extensively studied brain regions. It plays essential roles in normal brain functioning and roles in different psychiatric diseases. The basolateral amygdala (BLA), including the lateral amygdala and the basal amygdala, receives dense sensory inputs and is necessary for the acquisition of fear conditioning, thus playing an important role in the regulation of fear and anxiety (2).

Neuroimaging studies have revealed abnormal amygdala activity in patients with major depressive disorder (3), and these studies are supported by preclinical studies wherein animals exposed to stress paradigms, which induce some of the features of mood and anxiety disorders, showed altered amygdala function and morphology. Particularly, chronic stress altered the morphology of PNs within the BLA, causing hypertrophy (4), while acute stress caused dendritic retraction (5).

The amygdala, which contains anatomically distinguishable nuclei, is highly interconnected with various other brain regions, and a better understanding of the heterogeneity of the amygdala output is needed to analyze, in detail, the regulation of amygdala PNs during pathophysiological conditions (e.g., in the stress response). The challenge of current and future studies is to identify the specific dysregulations that occur within distinct amygdala circuits and neuron subpopulations with a long-term goal of identifying potential targets for specific therapeutic intervention.

In a first step, Zhang *et al.* (1) confirmed that chronic restrained stress (CRS), a well-known model of chronic stress in rodents, caused a generalized dendritic hypertrophy in the BLA PNs. To tackle the question of circuit specificity, they took advantage of available techniques to retrogradely label and trace neurons projecting from the BLA to specific brain areas. This allowed them to identify BLA PNs targeting the dorsomedial prefrontal cortex (dmPFC) and to distinguish them from PNs targeting brain regions other than the dmPFC. Consistent with their hypothesis, the authors confirmed that CRS affected dendritic spine density in a circuit-specific manner, via a specific increase in the spine head diameter restricted to PNs projecting from the BLA to regions other than the dmPFC.

Neuronal circuits are modified by activity-dependent mechanisms and environmental cues (6). Among the neuronal subcompartments involved in structural plasticity, dendritic spines have a remarkable ability to undergo changes. Dendritic spines can be classified according to their morphology. In their structural analyses, Zhang *et al.* (1) considered three types of spines: mushroom spines, which are mature spines with an enlarged spine head; stubby spines, which are short spines without a well-defined spine neck; and thin spines, which are filopodia-like protrusions. The latter two types of spines are immature spines. CRS specifically increased the density of mushroom spines in the PNs not targeting the dmPFC.

Alterations in spine density are associated with changes in synaptic transmission. Indeed, dendritic spines are the postsynaptic components of most excitatory synapses. An increased density of mushroom spines could reflect changes in glutamatergic neurotransmission. A classical method to measure changes in glutamatergic transmission is to record miniature excitatory postsynaptic currents (mEPSCs) at the PNs in the presence of tetrodotoxin, an action potential blocker, and picrotoxin, a gamma-aminobutyric acidergic transmission blocker. Zhang *et al.* (1) showed that CRS increased the frequency of mEPSCs in the PNs projecting from the amygdala to areas other than the dmPFC without altering the amplitude. An increase in mEPSC frequency is observed as a result of an increase in synaptic spine density as well as an increase in presynaptic glutamate release. To address this question in more detail, Zhang *et al.* (1) recorded EPSCs evoked by two consecutive electrical stimuli and calculated the paired pulse ratio as an index of neurotransmitter release probability. Interestingly, no difference was observed between the two groups. To specifically target glutamate release, the authors examined the decay in the evoked *N*-methyl-D-aspartate receptor–mediated currents in the presence of its noncompetitive antagonist MK-801 and did not observe any differences. Taken together, these results showed that the increase in mEPSC frequency in BLA PNs not targeting the dmPFC is a direct consequence of the increase in mushroom mature spine density in CRS animals, thereby linking circuit-specific morphological alterations to functional changes (increase of mEPSC frequency).

One of the key behavioral domains classically involving amygdala circuitries is anxiety. To finally bridge the gap from structure to behavior, Zhang *et al.* (1) investigated whether the changes observed in glutamatergic neurotransmission and

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increase in mushroom spine density were also translated into pathological behavior, i.e., differences in anxiety-related behavioral tasks. To this end, the animals were tested in the elevated plus maze and open field test, two tests commonly used to study anxiety-like behavior in rodents. Interestingly, CRS mice spent less time in the open arm of the elevated plus maze and less time in the center of the open field, supporting an increased anxiety-like behavior phenotype. The time spent by the mice in the open arm, the number of entries into the open arm of the elevated plus maze, and the time spent in the center of the open field were inversely correlated with the mEPSC frequency in PNs not projecting from the BLA to the dmPFC.

Still, the target area(s) of BLA PNs that did not target the dmPFC remained to be elucidated. Zhang *et al.* (1) hypothesized an involvement of two neuroanatomical structures known to play a crucial role in mediating stress-related phenotypes and anxiety-related behavior, namely the ventral hippocampus (vHPC) and the nucleus accumbens. Using an approach similar to that outlined above, Zhang *et al.* (1) found that CRS specifically increased the spine density in the PNs projecting to the vHPC and the spine head diameter as well. Surprisingly—and unlike the results obtained in the aforementioned study—an analysis of the dendritic spines on PNs targeting the vHPC revealed that the density of all three spine types (i.e., mushroom, thin, and stubby) was altered. Those data revealed that CRS increased the density not only of functionally mature spines but also of immature, thin, and stubby spines. This increase in small spines might be associated with the generation of silent synapses after stress, as previously reported (7). Furthermore, Zhang *et al.* (1) complemented their morphological analyses with electrophysiological recordings and identified an increased mEPSC frequency in PNs targeting the vHPC, and this was inversely correlated with the time spent by the mice in the open arm of the elevated plus maze and in the center of the open field as measures of anxiety-related behavior.

Taken together, these data strongly support that the vHPC is a key brain region that is targeted by output PNs from the BLA. Even more importantly, these data highlight the importance of this circuit and its specific activation in mediating stress-related behavioral pathologies.

This study clearly revealed the importance of dissecting brain circuits to better understand their individual contribution to pathological phenotypes. The data highlight that the balance between activation and inhibition of specific brain circuits finally shapes the behavioral alterations, thus enabling an anatomical and functional characterization of the pathways altered in stress-related disorders. As previously shown by Felix-Ortiz *et al.* (8), optogenetic activation of the BLA–vHPC synapses increases anxiety-like behavior, while inhibition of the BLA–vHPC synapses decreases anxiety-like behavior. Zhang *et al.* (1) expanded this concept into disease-relevant conditions (i.e., CRS) and showed that chronic stress specifically targets this specific brain circuit (from BLA to vHPC) by increasing spine density and glutamatergic neurotransmission. How can stress affect specific brain circuits and what are the molecular mechanisms that translate stressful environmental stimuli into stress-induced changes of synaptic plasticity? The answer to this question will help pave the way for targeted manipulations of specific circuits that could promote resilience to specific stressors in individuals who are at risk.

We are currently facing exciting times for translational research into neuropsychiatric disorders. To overcome the current limitations in drug development in neuropsychiatry, increasing attention has been given to circuit-based approaches. In line with the concept of a transdiagnostic approach [e.g., as conceptualized in the Research Domain Criteria initiative (9)], a circuit-based taxonomy of psychiatric symptoms and phenotypes would be suited to specifying symptoms in terms of underlying neural dysfunction at the individual level. The recent technical advances in closed-loop brain state–dependent stimulation allow researchers to define the brain states within certain regions or networks by electrophysiological recordings and neuroimaging. Those data are then used to individually tailor the stimulation parameters, enabling brain state–dependent brain stimulation (10).

Ultimately, the possible combination of personalized therapy with targeted brain stimulation of specific neuronal circuits could support or restore synaptic plasticity in key brain circuits that are responsible for diseases in the future.

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