

Casting a (Perineuronal) Net: Connecting Early Life Stress to Neuropathological Changes and Enhanced Anxiety in Adults

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Genetics and environment, especially early life environment, contribute to the genesis of mood and anxiety disorders in older children and adults. While there have been considerable advances in experiments with rodents in linking circuit activity manipulations to anxiety-related behaviors, determining how early stressors change the brain to predispose it to later elevations of anxiety has been elusive. Murthy *et al.* (1) have contributed to this endeavor by linking early stress to histochemical changes within a subclass of inhibitory interneurons in the dentate gyrus (DG) of the ventral hippocampus. This subclass has previously been implicated in the generation of theta band oscillations, as well as theta–gamma coupling, that are also associated with early stress in rodents and humans. Although the mechanisms whereby early stress changes interneuron neurochemistry have not been established, nor has what these neurochemical changes mean for circuit activity in adults, the results of this study are an important step in the critical process of discovering the neuropathological changes and circuitry dysfunctions in the adult brain that result from early life stress.

To study how early life stressors might promote anxiety responses in adulthood, several rodent models have been developed, including prenatal stress, maternal deprivation, and adolescent social isolation models. The behavioral phenotypes produced by these environments endure throughout the lifespan and indicate that the rodent brain in prenatal and early postnatal life is highly vulnerable. Multiple lines of evidence suggest that human neural development is similarly vulnerable in early life (2).

In the model used by Murthy *et al.* (1), mouse pups are exposed to 4 hours daily of separation from their mother from postnatal days (PDs) 2 to 5, then 8 hours of daily separation from PDs 6 to 16, and then are weaned at PD 17. Control mice receive no separation and are weaned at PD 21. Adult male mice exposed to this maternal separation with early weaning (MSEW) regimen display fewer entries into the open arm of an elevated plus maze and locomotor hyperactivity, findings that are interpreted as anxiety-like and attention-deficit/hyperactivity disorder-like phenotypes. Female MSEW mice are resilient to this behavioral phenotype, which is an initially counterintuitive finding because women are more likely than men to be diagnosed with anxiety disorders. However, this resilience is consistent with studies showing increased locomotor hyperactivity in male mice exposed in utero to maternal stressors, an effect thought to be mediated through placental inflammation in male pups (3). An important issue to resolve,

therefore, is whether approaches to measure anxiety in rodents are inadequate in females or whether female rodents are indeed more resilient to stressors during late prenatal and early postnatal life.

Murthy *et al.* (1) describe several phenotypes in adult MSEW mice, including an alteration in hippocampal theta–gamma coupling and altered neurochemical and gene expression in ventral hippocampal DG interneurons. Taking the interneurons first, they find reduced expression of parvalbumin (PV), a calcium-binding protein associated with “fast-spiking” responses to excitatory inputs. PV expression at least partially covaries with activity (4), and MSEW probably affects their intrinsic spiking capacity, enhances their inhibition, or reduces their excitation. In these same cells there is an increased detectability of perineuronal nets (PNNs) along with messenger RNA for orthodenticle homeobox protein 2 (Otx2), a transcription factor expressed by the choroid plexus and other cells. Otx2 uptake into PV interneurons is facilitated by PNNs and during development is generally associated with enhanced PV interneuron activity and higher PV levels (5). PNNs are mainly present on PV-expressing subgroups of hippocampal and neocortical interneurons, where they are thought to influence the stabilization of excitatory inputs associated with the end of regionally and functionally diverse sensitive periods in cortical plasticity.

Citing previous literature of increased excitatory hippocampal output in anxiety models (6), Murthy *et al.* (1) suggest that MSEW may result in enhanced rhythmic inhibition of PV interneurons in the DG, a result that could be consistent with enhanced theta–gamma coupling. The source of this enhanced inhibition is not clear but could be intrinsic to the PV interneuron network within the DG because PV interneurons can be electrically as well as synaptically coupled. Among alternative possibilities, there is a growing appreciation that there are long-range inhibitory inputs to hippocampal interneurons from the entorhinal cortex (7) and that these inputs may regulate anxiety-related responses. Either way, an important next step will be to determine whether the intrinsic firing properties of DG PV interneurons are affected by MSEW, and if not, whether the functional effects of their inputs are altered and from where. Of note, Murthy *et al.* (1) also found fewer DG interneurons expressing somatostatin, a neuropeptide related to anxiety responses that is not known to be activity dependent. Therefore, this is an additional curious finding, like the disjunction between PV expression and of the PNNs/Otx2, that may be important in future studies.

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The holy grail of any study examining the effects of an environmental or genetic manipulation on behavioral outcomes and their underlying neuronal architecture is to determine whether the neuronal response to the manipulation correlates with the specific behavioral abnormalities observed. In studies of brain and behavior, even in tightly controlled rodent models, there is considerable intersubject variability in both the observed behavior and the neuronal phenotype. If the neuronal changes observed occur within a principal neuronal substrate of a behavioral response, this should be reflected in a correlation between the cellular phenotype and the behavior across individuals. For example, a positive correlation across individual rats has been reported between their degree of prepulse inhibition and their expression of the *N*-methyl-D-aspartate receptor GluN1 subunit in dendrites of the nucleus accumbens (8). While correlation cannot establish causation, the lack of a correlation might imply that another brain region or cellular substrate may have a more significant contribution to a specific behavioral manifestation. In the case of Murthy *et al.* (1), it would be useful to know whether the level of theta–gamma coupling, reduced entry into the open arm on elevated plus maze, and the levels of PV expression in the ventral hippocampal DG (or PNN/Otx2) correlate across individual MSEW mice.

That said, a strength of the Murthy *et al.* (1) study is connecting a stress paradigm in early postnatal development both with an alteration of potentially relevant neurons and with electrophysiological measures that have been associated with enhanced anxiety in humans. While it is fair to point out that stress during the first postnatal week could influence processes in cortical development that mostly occur prenatally in humans, such as neuronal migration and initial synaptogenesis, the results of this study open the door for additional cross-species studies. With both the theta–gamma coupling and elevated plus maze to work from, this could be a highly useful paradigm for the development of preventative measures in the postweaning prepubertal age range in mice (PDs 21–28) that could be evaluated by similar measures in humans. In addition, it has recently been demonstrated that optogenetic activation of axon terminals in the lateral hypothalamic area that arise from “anxiety cells” of ventral hippocampal CA1 enhance anxiety-related behaviors in mice (9). If the MSEW paradigm (perhaps via DG disinhibition) also enhances the activity or lateral hypothalamic area connectivity of these cells, this may be an opportunity to connect early stress to later pathology at both the cellular level and the circuitry level. Maltreatment during early childhood correlates with altered functional magnetic resonance imaging measures of tractography in both resilient and vulnerable adults, but only the resilient adults showed reduced, presumably compensatory changes in nodal efficiency involving anxiety-related forebrain regions (10). If the MSEW paradigm can be shown to functionally alter anxiety-related circuits in a manner that predicts

an individual animal’s response to that stress, that would further enhance the validity of this paradigm for the study of gene–environment interactions and for the development of preventative or corrective interventions.

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Article Information

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