


# Transcriptional Profiling of Amygdala Neurons Implicates PKC $\delta$ in Primate Anxious Temperament

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fear, anxiety, stress, central nucleus of the amygdala, bed nucleus of the stria terminalis, microcircuitry, protein kinase C type delta, PKC $\delta$ , somatostatin, retrograde tracing

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## Comment on

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## Commentary

Even during infancy, parents recognize aspects of their child's temperament. Although children interact with and are affected by their environment, their temperament is, in part, genetically determined and relatively stable across time. Importantly, variations in early-life temperament can predict the emergence of later-life psychopathology. For example, children with an extreme anxious temperament (AT) are at an increased risk for developing later-life depression and anxiety.<sup>1</sup> Deciphering the neural circuits, cell types, and molecular mechanisms underlying AT is critical for understanding how neuropsychiatric disorders arise and will aid in the development of early-life treatment strategies.

Because of their recent evolutionary divergence from humans, non-human primates are ideally suited to study the conserved neural, cellular, and molecular components of threat processing. This is relevant for understanding human stress-related neuropsychiatric disorders which are characterized by alterations in behavioral, emotional and physiological responses to potential threats. Our laboratory developed and characterized a young rhesus monkey model of AT that shows remarkable similarities to childhood AT.<sup>2</sup> Monkeys with extreme AT freeze for

long durations in response to a potential threat,<sup>1</sup> similar to the behavioral inhibition exhibited by shy children when meeting a stranger. Using multimodal brain imaging, we identified an AT circuit that includes the central nucleus of the amygdala (Ce) and bed nucleus of the stria terminalis (BST)<sup>2</sup> and we demonstrated that Ce neurotoxic lesions decrease AT.<sup>3</sup> The Ce can be divided into at least two subnuclei, the lateral Ce (CeL) and the medial Ce (CeM). The CeM projects out of the amygdala. The CeL coordinates threat responses by integrating threat-relevant information and modulating the CeM's output through a GABAergic microcircuit comprised of neuronal subpopulations e.g., protein kinase C type delta (gene: *PRKCD*, protein: PKC $\delta$ ) and somatostatin (gene: *SST*, protein: SST) neurons.<sup>4</sup>

In our recently published study,<sup>5</sup> we describe our efforts to understand Ce gene expression in relation to individual differences in AT. Among our top hits was *PRKCD*, a marker of a CeL neuronal subpopulation that is critical for threat responses.<sup>4</sup> Mouse studies have focused on the function of *PRKCD* neurons in threat responses and CeL circuit function.<sup>4</sup> However, our finding demonstrates that *PRKCD* is not only a neuronal marker, but that its expression levels are positively associated with AT, a clinically-relevant phenotype. Together, our finding and the known role of CeL

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*PRKCD* neurons make *PRKCD* and its downstream pathway a potential treatment target. Future studies knocking out *PRKCD* expression are required to understand the role of CeL *PRKCD* in primate AT, threat processing, and CeL function.

In mice, PKC $\delta$  neurons receive input from SST neurons and this microcircuit modulates CeM and BST function, and threat responses.<sup>4</sup> While this circuit is well characterized in mice, little work has been done in primates. Following up on our *PRKCD* finding, we performed a stereological analysis of PKC $\delta$  and SST neurons in mouse and monkey and identified several potentially important species differences. Specifically, we found that monkey PKC $\delta$  neurons are evenly distributed across the CeL's anterior-posterior extent whereas in mice, they are concentrated in the posterior CeL. We also found that monkey CeL SST neurons are less abundant than in mice. Although descriptive, these findings suggest species differences in circuitry organization with implications for the complex responses of primates to threats in their environment. The characterization of species differences in cell types and microcircuitry organization will enable us to better understand the extent to which rodent findings translate to humans.

The BST, like the Ce, is involved in responding to aversive stimuli. The CeL sends robust projections to the laterodorsal BST (BSTLd) and this pathway is important in threat responses. In our study,<sup>5</sup> we found that about half of PKC $\delta$  neurons project to the BSTLd, whereas very few SST neurons project to the BSTLd. We also noted dense SST varicosities surrounding BSTLd-projecting PKC $\delta$  neurons, suggesting that SST may modulate this pathway. The synaptic connection between CeL SST neurons and BSTLd-projecting PKC $\delta$  neurons may provide an additional point of intervention for modulating threat responses through this circuit.

Our transcriptome-wide study in CeL neurons provides evidence supporting a primate CeL to BSTLd microcircuit relevant for understanding AT and points to specific molecules within this circuit that could serve as potential treatment targets for human anxiety disorders. Building on this work, we are actively pursuing gene expression studies in other components of the AT circuit. These studies will allow us to identify transcriptomic changes that are shared across, or unique to, AT-related brain regions. It will be interesting to investigate whether these genes are associated with specific cell populations, which may provide insights into the relevant cellular components within the AT circuit. These studies in primates linking molecules, cell types, and circuits have the potential to inform the pathophysiology

underlying human stress-related disorders and ultimately guide the development of novel treatments.

### Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: NHK has received honoraria from CME Outfitters, Elsevier, and the Pritzker Consortium; has served on scientific advisory boards for Actify Neurotherapies and Neuronetics; currently serves as an advisor to the Pritzker Neuroscience Consortium and consultant to Corcept Therapeutics; has served as co-editor of Psychoneuroendocrinology and currently serves as Editor-in-Chief of The American Journal of Psychiatry; and has patents on promoter sequences for corticotropin-releasing factor CRF2 $\alpha$  and a method of identifying agents that alter the activity of the promoter sequences (70,71,323; 75,31,356), promoter sequences for urocortin II and the use thereof (70,87,385), and promoter sequences for corticotropin-releasing factor binding protein and the use thereof (71,22,650). All other authors report no biomedical financial interests or potential conflicts of interest.

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