

Genetically modified rabies tracing of global circuit connections to corticotropin-releasing hormone neurons in the hypothalamic paraventricular nucleus

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Corticotropin-releasing hormone (CRH)-containing neurons in the paraventricular nucleus (PVN) of the hypothalamus play a key role in the hypothalamic-pituitary-adrenal (HPA) axis stress responses. The existing knowledge about PVN circuit connections comes from conventional anatomical studies which tend to have low spatial and cell type resolution. Many aspects of neural circuit organization of CRH⁺ PVN neurons are poorly understood. Thus we have mapped global synaptic connections to these CRH⁺ neurons in order to better understand how they integrate stress-relevant signals from brain-wide neural networks to regulate the neuroendocrine response to stress. A new Cre dependent monosynaptic rabies tracing system with the helper AAV expressing optimized glycoprotein (oG) were used to map direct circuit connections to CRH⁺ neurons in the PVN of CRH-ires-Cre mice. Reproducible results of synaptic input patterns were obtained from 6 high quality cases. We found that these CRH neurons received synaptic connections from more than one hundred brain regions. Hypothalamic regions account for the majority of inputs, as dorsomedial, ventromedial, lateral hypothalamus, lateral and medial preoptic nucleus, and the arcuate nucleus have on average, 11.5%, 10.9%, 8.1%, 17%, and 18% of total labeled presynaptic neurons, respectively. While there are no or little direct inputs from sensory and motor cortex, hippocampus or amygdalar nuclei, there are readily identifiable inputs from infralimbic cortex (0.4% of total labeled presynaptic neurons), the nucleus accumbens (2.8%), anteromedial and ventral thalamus (0.9%), lateral and medial septum (4%), dorsal and ventral subregions of the bed nucleus of the stria terminalis (3.3%), supraoptic nucleus (0.26%), zona incerta (0.7), periaqueductal gray area (0.9%), periventricular nucleus (0.6%), and mammillary nuclei (0.6%). Antibody immunostaining were used to further identify excitatory or inhibitory nature of input mapped neurons. All these regions including previously un-described input sources have been significantly implicated in emotion, reward and stress regulation. Our work aided by the new viral genetic mapping approach allows for a detailed analysis of global information flow directly impinged on CRH⁺ PVN neurons, and will shed new light on specific neural circuit mechanisms underlying direct control of HPA axis responses.