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RESEARCH HIGHLIGHT

Neuromedin U: a neuropeptide modulator of GABA transmission contributes to cocaine seeking

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The study of neuropeptide function in behavioral neuroscience has come a long way since the first neuropeptide, substance P, was isolated in 1931. Following this discovery, many neuropeptides have been discovered and they make up one of the largest classes of chemicals that alter neuronal function [1]. There are some common features among neuropeptide function. Neuropeptide receptors tend to be metabotropic G protein coupled receptors [1]. As a result, neuropeptides typically modulate neuronal activity on a longer time scale than other neurotransmitter classes that act as ligands at ionotropic receptors. Compared to other neurotransmitter types, the release of neuropeptides almost exclusively requires high frequency or burst firing activity [1]. This prerequisite of high signaling input supports the putative functionality of neuropeptides. Neuropeptides play a key role in homeostatic regulation – from food intake to stress response to alertness [1]. In summary, when the organism is challenged in ways of energy balance, stress, and pain, neuropeptide signaling is likely involved.

There has been increasing recognition of neuropeptides in another physiological challenge: repeated exposure to a drug reward. With accumulating evidence, it has become apparent that the same neuropeptide circuits involved in homeostatic regulation also govern the response to drugs of abuse. Neuromedins, a newer neuropeptide subclass isolated from porcine spinal cord in 1985, has been posited to have a role in drug motivated behavior. Initially, in the history of neuromedin U (NMU) specifically, its function was attributed to muscle contraction, anorexia, thermogenesis, and nociception response [2]. To add to the repertoire of NMU functionality, the report by Kasper et al. [3] in this issue of *Neuropsychopharmacology* highlights the role of NMU in regulating cocaine self-administration, and they investigate the function of NMU on multiple dimensions: behavior, circuit, and cell-type.

To characterize the function of NMU in cocaine self-administration, the authors used systemic pharmacology and viral knockdown of the NMU 2 receptor (NMUR2) in nucleus accumbens shell. Rats were trained to self-administer intravenous cocaine on a fixed ratio schedule before advancing to a progressive ratio (PR) schedule in which the response requirement (or effort) is increased after each drug infusion. One cohort of rats were administered different doses of NMU during the PR schedule. For a second cohort, a short hairpin RNA (shRNA) was used to virally knockdown NMUR2 from afferent projections of the nucleus accumbens shell. The higher doses of NMU decreased responding under the PR schedule. In addition, shRNA knockdown of NMUR2 had no effect on cocaine self-administration under the fixed ratio schedule but significantly increased responding under PR schedule (Fig. 1). Microdialysis sampling of GABA in the nucleus accumbens shell after concurrent delivery of NMU and cocaine revealed a cocaine-induced efflux of GABA that was decreased by NMU. These results suggest that NMU regulates the rat's effort for cocaine reward, and the mechanism of action is through inhibition of GABA release in the nucleus accumbens shell. The results corroborate with previous findings that NMU modulates inhibitory synaptic tone by decreasing GABAergic transmission, a mechanism that is observed broadly in some neuropeptides [1, 4].

After establishing the role of NMU in cocaine self-administration, the authors worked on determining the NMU-activated circuitry from the nucleus accumbens shell using c-Fos immunohistochemistry and a retrograde viral tracing approach. A retrograde viral vector expressing mCherry was injected in the ventral tegmental area (VTA) and a different GFP-expressing retrograde vector was injected in the ventral pallidum in order to label the targets of nucleus accumbens shell neurons. c-Fos expression was quantified after NMU administration in the nucleus accumbens shell for each pathway. Overall, NMU increased c-Fos expression in the nucleus accumbens shell and the NMU-induced increase in c-Fos expression was mostly attributed to the ventral pallidum projection pathway rather than the VTA projection pathway.

The remaining question was the identity of the neurons that project to the nucleus accumbens shell that then project to either the ventral pallidum or VTA. A retrograde viral vector containing TVA, rabies glycoprotein, and mCherry was injected into the ventral pallidum or VTA. Following injection of avian-enveloped rabies GFP into the nucleus accumbens shell, only neurons in nucleus accumbens shell that project to ventral pallidum or VTA with TVA expression were transfected, ensuring pathway specificity. The authors found distinct rabies labeling in the dorsal raphe nucleus upstream of both nucleus accumbens pathways to the ventral pallidum or VTA with no significant difference in labeling. Using immunohistochemistry, the dorsal raphe neurons were identified as mostly GABAergic rather than serotonergic for the nucleus accumbens shell to VTA pathway. However, for the ventral pallidum pathway, the dorsal raphe neurons were roughly equal proportions GABAergic and serotonergic.

Kasper et al. [3] note that because the percentage of dorsal raphe neurons that are GABAergic or serotonergic is greater than 100%, there may be co-labeling of dorsal raphe neurons projecting to the nucleus accumbens shell → ventral pallidum

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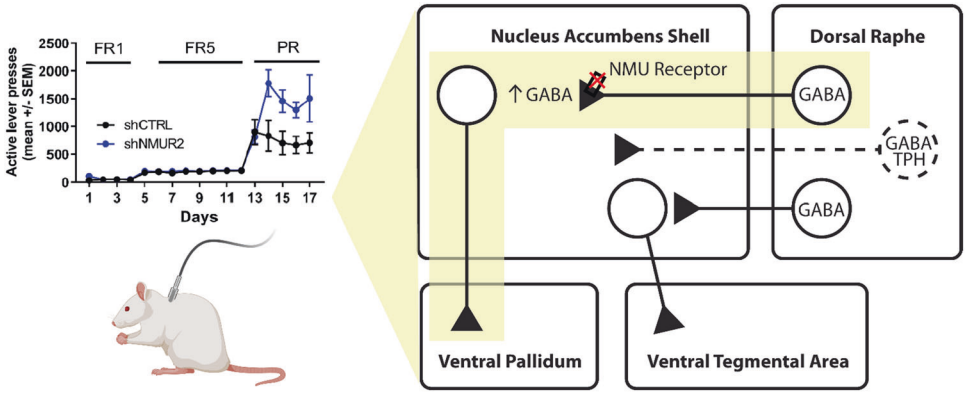

Fig. 1 Proposed GABAergic pathway regulated by neuromedin and play a role in cocaine self-administration. Rats were trained on fixed ratio (FR) schedules of cocaine self-administration before advancing to a progressive ratio schedule. Viral knockdown of the NMU receptor by short hairpin RNA (shNMUR2) from afferent projections of the nucleus accumbens shell increased lever presses for cocaine infusions under the progressive ratio (PR) reinforcement schedule.

pathway that are both GABAergic and serotonergic. Dual neurotransmitter expression by single neurons have been documented in discrete brain regions, including the dorsal raphe [5]. Further examination of raphe cell-types using mRNA-based techniques may be informative in further delineating pathway-specific cell-types. Nevertheless, because 1) NMU and the NMU2 receptor is located on terminals of dorsal raphe GABA neurons, and 2) NMU preferentially increased c-Fos expression on ventral pallidum-projecting shell neurons over VTA-projecting shell neurons, the results suggest that dorsal raphe GABA neurons control effort for cocaine self-administration via an NMU projection to the nucleus accumbens shell that then projects to the ventral pallidum.

The work from Kasper et al. [3] is a testament to the significance of neuropeptide transmission in drug motivated behavior and also to how little we still know about neuropeptide systems in regard to drug self-administration. While neuropeptides generally exist in small concentrations in the central nervous system, their mechanism of action is non-trivial. Future studies will become necessary to investigate the specificity of neuropeptides at different stages of drug-taking behavior, in response to different drugs of abuse, and their cell-type expression.

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AUTHOR CONTRIBUTIONS

AL wrote the initial draft of the manuscript and created the figure. DHR wrote and edited the final manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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