Phencyclidine-Induced Neurotransmitter Switching and its Impact on Behavior

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The proper function of brain circuits relies on the correct specification and modulation of neurotransmission. Environmental stimuli producing sustained alteration in neuronal firing can induce neurons in the adult brain to lose the transmitter they were expressing and gain a new one, a process called neurotransmitter switching (NTS). It typically involves re-specification from an excitatory to an inhibitory transmitter or vice versa, and has the potential to significantly impact the functioning of the neuronal circuits in which the neurons are involved.

Several types of psychoactive substances affect neuronal activity in the prelimbic cortex (PrL), a brain region that is crucial for cognitive processes and regulation of reward seeking and extinction. In particular, the dissociative anesthetic phencyclidine (PCP), a non-competitive antagonist of the NMDA receptor, profoundly affects PrL activity and function.

Methods: To determine if PCP induces changes in neurotransmitters expressed by glutamatergic neurons, we stably labeled them by combining a vGluT1::Cre mouse line with a Cre-dependent H2B-mCherry reporter. Mice were treated subchronically with either PCP (10 mg/kg s.c. once a day for 10 days) or saline. Two days after the end of the treatment, mice were sacrificed and PrL sections were collected. The number of mCherry-positive neurons co-expressing GABA, as well as the GABA synthetic enzyme GAD67, was detected by immunohistochemistry and quantified by cell counting. We evaluated PCP-induced behavioral sensitization by quantifying locomotor activity immediately after PCP injection on the first and last day of treatment. The presence of cognitive deficits was assessed 5 and 8 days after the end of the treatment with the novel object recognition (NORT) test and spontaneous alternation task (SAT) respectively.

Results: PCP induces a significant increase in the number of glutamatergic neurons that express GABA and GAD67 compared to control mice, suggesting that NTS is occurring. Furthermore, PCP induces both behavioral sensitization, and cognitive deficits in the NORT and SAT. To determine whether PCP-induced gain of GABA in glutamatergic neurons is causally linked to these behavioral alterations we are now stereotaxically injecting the PrL of vGluT1::Cre mice with a Cre-dependent AAV-GFP-GAD1-ShRNA viral vector to override the PCP-induced gain of GABA and test whether normal behaviors are recovered. PCP exposure also induces a decrease in the number of GABAergic neurons in the ventral tegmental area, suggesting that the drug induces NTS in more than one region of the brain. To test this hypothesis, we are now optimizing a strategy to perform faster, whole-brain screening of glutamate-to-GABA neurotransmitter switching that combines the use genetic labeling of switching neurons, whole brain clarification, light-sheet microscopy and automated cell counting.

Discussion: Subchronic PCP induces a gain of GABA and GAD67 in PrL glutamatergic neurons that is associated with drug-induced sensitization and cognitive deficits. The relationship between neurotransmitter switching and drugs of abuse has not been investigated previously. It therefore seems useful to study the extent to which drug-induced NTS causes behavioral alteration that can lead to the development of addiction.

References: Dulcis, D., Jamshidi, P., Leutgeb, S. and Spitzer, N.C. (2013) Neurotransmitter switching in the adult brain regulates behavior. *Science 340:* 449-453; Spitzer, N.C. (2017) Neurotransmitter switching in the developing and adult brain. *Ann. Rev. Neurosci*. *40*: 1-19.

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