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Pipeline for whole-brain analysis of METH-induced changes in neurotransmitter phenotype

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Methamphetamine (METH) is widely misused as a stimulant. Understanding the extent to which METH induces maladaptive neuroplasticity is essential to addressing the consequences of its misuse. However, METH's ability to cause neurons to change their transmitter phenotype has received scarce attention.

We previously showed that repeated exposure to METH induces an activity-dependent gain of GABA in prelimbic cortex glutamatergic neurons that is linked to cognitive deficits. Since METH causes a widespread increase in neuronal activity, we hypothesized that it induces neurons in multiple brain regions to change their transmitter phenotype. To test this hypothesis, since many neurons express either glutamate or GABA, we developed a pipeline for whole-brain analysis of neurotransmitter changes from glutamate to GABA or vice versa. We generated a $VGAT^{FLP}$::$VGLUT2^{CRE}$::$TdTomato^{cON/fON}$ reporter mouse line, in which neurons that express or have expressed both VGAT and VGLUT2 are permanently labeled with TdTomato. After a ten-day treatment with either METH or saline as a control, we acquired coronal sections of the entire brain with multiphoton tomography, performed image registration into the Allen Brain Atlas, and automatically quantified TdTomato+ neurons. METH treatment increased the number of TdTomato+ neurons in more than 10 brain regions. Fluorescent *in situ* hybridization against VGLUT2 and VGAT validates changes in transmitter phenotype within TdTomato+ neurons.

These ongoing studies are allowing an unprecedented understanding of the extent to which drug use can affect the neurotransmitters that neurons express, and will provide the basis for future investigation into the behavioral effects of METH-induced changes in neuron transmitter phenotype.