Glutamatergic Neurons from the Ventral Tegmental Area Establish Multiple Synapses on Single Parvalbumin-GABAergic Interneurons in the Nucleus Accumbens

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The ventral tegmental area (VTA) is thought to play a role in goal directed behavior and in the reward processing of natural rewards and on several drugs of abuse [1, 2]. Two populations of VTA neurons (dopaminergic neurons and GABAergic neurons) have been extensively characterized. However, recent findings indicate that the VTA has neurons that express the vesicular glutamate transporter 2 (VGluT2). VGluT2 is integrated into membranes of synaptic vesicles for the accumulation of glutamate. The VTA-VGluT2 neurons are diverse in their biochemical composition and connectivity [3]. We recently found that nucleus accumbens (nAcc) optical stimulation of fibers from VTA-VGluT2 neurons evokes conditioned place aversion mediated by local release of GABA, which is accompanied by expression of c-Fos in parvalbumin GABAergic interneurons [4]. To determine the synaptic connectivity established by VTA-VGluT2 neurons in the nAcc, we used a combination of cell specific viral vector tracing and immunoelectron microscopy. In these studies, axons from VTA glutamatergic neurons were tagged by the in vivo expression of channelrhodopsin-2 [(ChR2) tethered to mCherry] in the VTA-VGluT2 neurons of VGluT2::Cre mice. By double immunolabeling and electron microscopy, we detected in the nAcc medial shell axon terminals containing immunoreactivity (IR) for both VGluT2 and mCherry, indicating that these VGluT2-IR/mCherry-IR-terminals were originated from VTA glutamatergic neurons. Further analysis of these VGluT2-IR/mCherry-IR-terminals showed that they made asymmetric (excitatory type) synapses on dendrites or cell bodies. Next, by triple immunoelectron microscopy, we found that the VTA VGluT2 neurons establish multiple synapses on a single parvalbumin-GABAergic dendrite or with a single parvalbumin-GABAergic cell body. The mesoaccumbens glutamatergic pathway shown here to form multiple asymmetric synapses on parvalbumin-GABAergic interneuron is the first anatomically identified glutamatergic pathway to nAcc GABAergic interneurons.

Key words: VGluT2, parvalbumin, GABAergic interneurons, ventral tegmental area, nucleus accumbens, aversion, VTA
References:

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**Figure** A single nAcc mShell PV interneuron forms asymmetric synapses with several axon terminals from VTA VGluT2 neurons.