Notice of the Final Oral Examination
for the Degree of Master of Science

of

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BSc (University of Victoria, 2018)

“Differential Distribution of Functional Co-transmitted Cholinergic and GABAergic Synaptic Inputs onto Substantia Nigra Dopaminergic Neurons”

Division of Medical Sciences

Tuesday, April 13, 2021
10:00 A.M.
Conducted Virtually

Supervisory Committee:
Dr. Raad Nashmi, Division of Medical Sciences, University of Victoria (Supervisor)
Dr. Craig Brown, Division of Medical Sciences, UVic (Member)
Dr. Kerry Delaney, Division of Medical Sciences, UVic (Member)

External Examiner:
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Chair of Oral Examination:
Dr. Lorelei Newton, School of Nursing, UVic

Dr. Stephen Evans, Acting Dean, Faculty of Graduate Studies
Abstract

Neuronal communication in the mammalian brain relies on the presynaptic release of neurotransmitters which bind to ligand-gated ion channels found on postsynaptic neurons to modulate neuronal excitability. One such neurotransmitter is acetylcholine (ACh), a small molecule that is the signalling messenger of the cholinergic system. The cholinergic system is involved in a variety of behavioural functions including motor activity, sensory function, and higher executive commands. Dopaminergic neurons in the substantia nigra pars compacta (SNc) and the basal ganglia in general have long been implicated in initiation and completion of voluntary movement. Following an increased focus on the investigation of neural circuits responsible for motor control, studies have shown that cholinergic neurons from two brainstem nuclei, the laterodorsal tegmental nucleus and the pedunculopontine nucleus, project onto substantia nigra dopaminergic (DA) neurons in the midbrain and can release ACh, GABA or both to modulate motor behaviours. However, with prior research primarily focused on demonstrating the phenomenon of co-transmission itself, the subcellular distribution and dynamics of ACh and GABA release onto SN DA neurons receiving co-transmitted inputs largely remains to be investigated.

The present study investigated the spatial and physiological nature of ACh/GABA cotransmission from brainstem cholinergic axons synapsing onto medial SN DA neurons to understand its role in tuning the neuron’s excitatory-inhibitory balance. To that end, we developed a channelrhodopsin (ChR2)-based functional input mapping technique with high spatial resolution to probe the dendritic distribution of ACh and GABA synaptic inputs onto DA neurons in *Chatcre::ChR2* mice. Using this technique, we discovered three different types of monosynaptic inputs from cholinergic axons onto DA cells: co-transmitted ACh/GABA, GABA only, and Ach only. Furthermore, we revealed a somatodendritic patterning of cholinergic input distribution onto DA cells with a predominant GABA conductance along the lateral dendrites and a soma-centered ACh/GABA transmission. Physiological findings were corroborated using immunolabeling against VGAT and VACHT, which showed many closely spatially clustered ACh and GABA-specific cholinergic terminals and few truly colocalized VACHT and VGAT terminals. This result revealed that true co-transmission represents a minority of the presynaptic mode of release from cholinergic axons onto medial SN DA neurons, and that the majority actually share closely spatially clustered ACh and GABA-specific cholinergic terminals.
To investigate the dynamic properties of soma-centered ACh/GABA transmission, we restricted our stimulation field to the cell body in order to measure the contribution of nAChR and GABAR-mediated conductances without recruiting the lateralized population of primary GABA inputs. We then employed a deconvolution method to understand the relative plasticity of contributions of nAChRs and GABARs to ACh/GABA transmission onto DA cells. We confirmed an initial dominant GABAergic component of ACh/GABA transmission that was previously reported. However, we found that the weight of the GABAergic contribution had a greater decay compared to the ACh component with repeated stimulations, with the initial inhibition followed by a subsequent equalization of excitatory and inhibitory conductances. Finally, we performed similar experiments to compare the short-term plasticity of the isolated GABA conductance during 15 Hz stimulation between the populations of mix ACh/GABA inputs proximally and the population of primary GABA inputs found on the lateral dendrites 160 μm away from the cell body. Interestingly, the lateral GABA component was more sustained across repeated stimulations compared to the proximal GABA conductance, suggesting a differential contribution to excitation/inhibition balance by spatially distributed populations of ACh and GABA inputs from cholinergic axons onto the dendrites of medial SN DA neurons. To our knowledge, this is the first study to examine the distribution and dynamics of ACh/GABA transmission onto midbrain DA system using fine-scale ChR2-assisted subcellular input mapping and conductance deconvolution.