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An Alpha-synuclein Overexpression Model of Vocal Symptoms in Parkinson's Disease

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Mutations in the gene, alpha-synuclein (α-syn), are common to inherited and sporadic cases of Parkinson's disease (PD). Abnormal accumulation of α-syn at synapses in the brain affects dopamine producing neurons [1]. The loss of dopamine to the basal ganglia circuits is one contributor to PD, resulting in limb motor deficits, such as resting tremors, and vocal deficits associated with PD such as soft and monotonous voice. Rodent models have recapitulated the parkinsonian-like vocal symptoms, but these models rely on the interpretation of vocalizations whose underlying neural circuitry are not well characterized [2, 3]. In contrast, the zebra finch songbird has well identified neural circuitry for vocalizations (Fig. 1) and considerable homology to human neural substrates, providing a better model for characterization of molecular/cellular pathways underlying early vocal deficits in PD [4-6]. We hypothesized that α-syn is important for modulation of vocalizations in adult male finches based on previous literature [2, 3, 7]. To investigate this, we use an adeno-associated virus (AAV) to increase expression of human α-syn in song-dedicated Area X of the basal ganglia compared to a control virus. Consequently, the song resembles the soft, monotonous voice detected in PD (Medina, Munger, Badwal et al. manuscript in prep.). The validation of our finch PD model requires doing fluorescent immunohistochemistry to identify neuronal cell types which co-express the control and α-syn viruses [8]. The *hypothesis* is that virally driven α-syn overexpression occurs in the synaptic terminals of medium spiny neurons (MSNs), supported by preliminary data (Fig. 2).

Adult male finches are injected with control or α-syn virus bilaterally into Area X using standard aseptic surgical techniques. Song is recorded pre and post virus injection. At experimental endpoints for song collection, birds are humanely sacrificed with an overdose of isoflurane anesthesia then perfused with 4% paraformaldehyde in Phosphate Buffer Saline (PBS). Brains are incubated in a 20% sucrose solution overnight prior to cryosectioning in the coronal plane at 14μm and collected.

Sections are hydrated in 0.1 M Tris Buffered Saline (TBS), blocked for non-specific binding for one hour at room temperature in 0.1 M TBS + 0.3% Triton-X + 5% goat serum, and washed in 1xTBS + 0.3% Triton-X + 1% goat serum. Slides are incubated overnight at 4˚C with cell type markers, either primary mouse antibodies against DARPP-32 for MSNs (1:1000, gift of Dr. Hugh Hemmings) or parvalbumin for interneurons (1:250, Sigma P3088), or a rabbit polyclonal antibody against the pallidal neuron marker LANT-6 (1:250, provided by A. Reiner and R. Carraway) [8-10]. The LANT-6 primary antibody requires the use of a Tyramide Signal Amplification (TSA) kit to amplify the primary antibody staining which I am still optimizing (TSA Fluorescein Plus Evaluation Kit, Perkin Elmer). Slides will be double labeled for primary antibody for α-syn (1:250 Proteintech #10842-1-AP), which labels synaptic processes. Slides are incubated with secondary antibody (1:2000 Alexaflour 488 nm, 1:1000 Alexaflour 568 nm, Life Technologies, #A11029, A11036) for three hours at room temperature. A negative control section, where the primary antibody is omitted, is included on each slide to confirm a lack of non-specific secondary antibody staining. Alternatively, we verify a lack of primary antibody staining in brain regions outside of the basal ganglia as a second negative control. Results are imaged using a Zeiss 880 inverted confocal

microscope. The confocal microscope z-stack feature is used to take optical sections of the finch brain and determine if there is co-association of α-syn nerve terminals with specific cell type markers in Area X [11].

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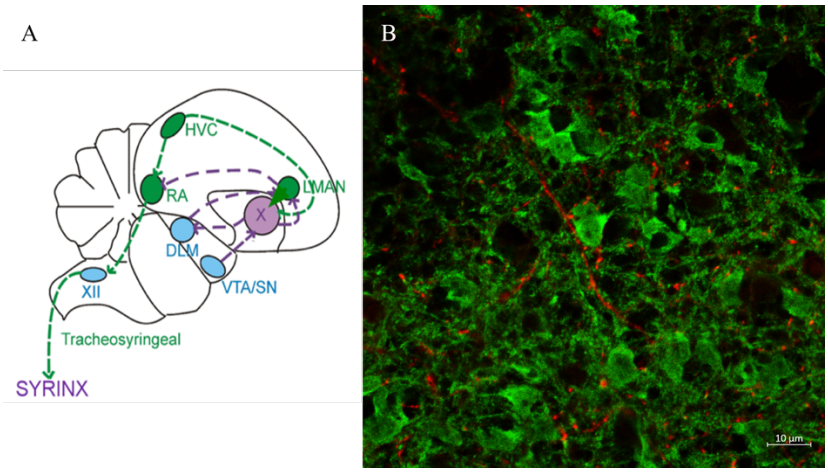

Figure 1. Zebra finch song circuitry, DARPP-32 labeled cell body and α-syn labeled processes.
A) The song control circuitry of the zebra finch consists of two interconnected loops; one for vocal production and the other for song learning and maintenance with nuclei abbreviated as proper names [6].
B) The cell bodies of MSNs in Area X (green signal), are labeled with the marker DARPP-32 with their neuronal processes expressing the α-syn label (red signal) driven by AAV5-human α-syn. Scale bar=10 µm. Images obtained from a Zeiss 880 inverted confocal microscope.

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