Abstract of Doctoral Dissertation

Degree requested Doctor of Life Science Applicant’s name Miguel Sánchez Valpuesta

Title of Doctoral Dissertation

Study of the function of pallial to basal ganglia projecting neurons
in vocal learning and maintenance in songbirds
（鳴禽類の外套–基底核投射ニューロンの発声学習、及び維持における神経機能の研究）

This study investigated the basis of motor skill learning and maintenance at the neuronal level. The specific motor skill learning paradigm examined in this dissertation is vocal learning, for its ease of analysis and direct relevance to human behaviors such as speech. Learned vocalizations consist of a sequence of temporally precise vocal movements acquired by hearing the sound to be learned from a tutor and learning how to match the animals’ own vocalizations to the tutor sound through a period of repeated practice. It has been previously found that the learning of sequential motor actions depends on the neural function of the motor cortex and basal ganglia. A dense pathway directly links the motor cortical areas to the downstream basal ganglia but how these connections between cortical and basal ganglia components contribute to vocal motor skill learning still remains unknown. In experimentally tractable mammalian species such as rodents or marmoset monkeys motor cortices and basal ganglia areas serve multiple types of motor action at the neuronal level. Furthermore, at the behavioral level, these animals either produce innate vocalizations without clear development or at best exhibit a developmental process that is unaffected by learning from a tutor. For these reasons, mammalian models are unsuitable for this study. Therefore, the zebra finch, a songbird, was leveraged as an animal model to explore the function of motor cortical to basal ganglia projecting neurons in vocal learning and maintenance. Two main aspects make zebra finches attractive to answer this question. First, zebra finches learn their songs from a tutor birds during a restricted period in their juvenile stage through vocal learning, while they maintain their songs during the rest of their adulthood. Secondly, songbirds possess a series of neural pathways in their brain specialized in song learning and well characterized called the song system. Crucially for this study, in this song system there exists a specific neuron population projecting from the motor cortex (HVC) to the basal ganglia (Area X), the $HVC_{(X)}$ neuron population. $HVC_{(X)}$ neurons fire temporally precise bursts locked to specific time points in the song, and this capacity to convey “temporal context” information into the basal ganglia has been hypothesized by previous authors to be important for song learning through reinforcement learning. To assess the role of these $HVC_{(X)}$ projection neurons in song learning and maintenance, a three-pronged strategy was used. First, a viral vector system was developed in order to cell-specifically kill $HVC_{(X)}$ neurons. This development culminated in the construction of a system with two viruses that, injected each in HVC or Area X, activated cell death only in $HVC_{(X)}$ neurons ablating a large fraction (over 70%) of this $HVC_{(X)}$ neuron population. Second and in order to test the effects of $HVC_{(X)}$ neuron ablation in song learning, zebra finch juveniles were injected with the $HVC_{(X)}$-ablating viral mix before presentation of the tutor song and their whole learning process recorded. Ablation of $HVC_{(X)}$ neurons at the juvenile stage caused deficits in the learning of tutor song acoustics and in the development of species-specific sequence patterns. These results indicate that $HVC_{(X)}$ neurons are necessary for the correct learning of song at the acoustic and sequence levels. Thirdly, $HVC_{(X)}$ neurons were ablated in adult zebra finches in order to test for ablation effects on song maintenance. Two strategies were used to probe song maintenance, studying the effects of $HVC_{(X)}$-ablation on the fine song acoustics and sequence of hearing adult songbirds and to study the effects of $HVC_{(X)}$ neuron ablation on song degradation caused by the lack of auditory feedback through deafening. Both strategies provided the same results, $HVC_{(X)}$ ablation levels comparable to the ones that caused serious learning deficits in juveniles caused no deficits in adult birdsong maintenance. Summarizing the above results, by using cell-specific ablation of cortical to basal ganglia projection neurons in songbirds these neurons were found to be crucially necessary for vocal skill learning in the songbird but not necessary or largely dispensable for the maintenance of this same skill when already learned by the adult songbird. These results confirm several expected results of the reinforcement learning model for vocal learning and opens new avenues to understand cortical-basal ganglia function.