

PIECING TOGETHER THE BUILDING BLOCKS OF THE VOCAL LEARNING BAT BRAIN

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1. Background

Vocal learning is the ability to learn to produce novel vocalizations and is an important component of the human capacity to acquire speech and spoken language (Janik & Slater, 2000). Our close relatives, non-human primates, do not show evidence of learning to produce novel vocalizations. However, a small group of more evolutionarily distant animals, such as bats, display evidence for vocal learning. This raises the question of how and when this skill evolved and how it is encoded in our brains and genomes. *Phyllostomus discolor* is a highly social, vocal learning bat that can be studied in a laboratory setting (Vermes, 2017). Little is currently known about the brain areas and networks involved in vocal learning in *P. discolor*. Acquiring a better understanding of the neurobiology of this skill in bats will bring us closer to understanding how this skill evolved, and subsequently shed light on the evolution of human speech and spoken language.

2. Approach

In the current study, we used two complementary approaches, neuroimaging and neurogenetic mapping, to start to map the connectivity of the *P. discolor* brain and to highlight potential brain areas of interest for the study of vocal learning in *P. discolor*.

We performed high quality structural and multi-shell diffusion tensor imaging of post-mortem formalin-fixed *P. discolor* brains on an 11.7 T Bruker BioSpin magnetic resonance imaging (MRI) scanner. Previous research has shown increased and altered connectivity of brain areas involved in vocal communication in vocal learners compared to vocal non-learners (e.g., Kumar et al., 2016). As no research thus far has been done on investigating the macro-scale brain networks of *P. discolor*, we set out to exploratively map the brain-wide connectivity of *P. discolor*.

In parallel, we performed immunohistochemistry, immunofluorescence, and *in situ* hybridization on juvenile and adult *P. discolor* brains. We investigated a set of genes of which previous research has shown that they have a distinct pattern of expression in voluntary vocal motor (planning) regions in the brains of humans and vocal learning songbirds (Wang et al., 2015; Pfenning et al., 2014). We hypothesized that these genes might show the same pattern of expression in potential homologous bat vocal motor (planning) cortical regions, and could hence point us to potential brain regions of interest.

3. Preliminary Results and Future Perspectives

We successfully acquired high-quality DTI data of post-mortem *P. discolor* brains and are currently in the process of reconstructing the major white matter tracts in the *P. discolor* brain and performing probabilistic tractography from two cortical regions involved in social communication in *P. discolor*: the auditory and frontal cortex. Via neurogenetic mapping, we show that expression of our set of marker genes is not dispersed uniformly across the *P. discolor* cortex, but is enriched or reduced in specific layers and cortical areas. This suggests that these expression patterns can be used to mark out distinct brain areas in *P. discolor*.

Exploring our dataset further in the future, we hope to define key areas and circuits involved in vocal learning in the *P. discolor* bat brain and that this teaches us about how this skill evolved in bats and humans.

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