Follow the nose: using whole-skull IHC to map olfactory involvement in Parkinson disease

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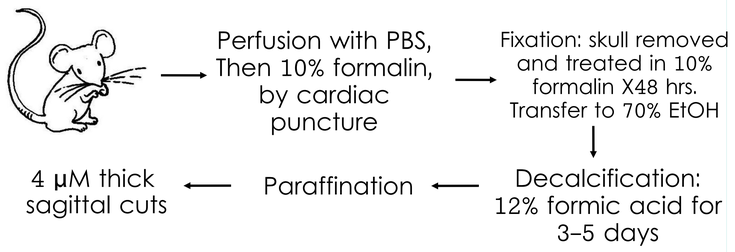
INTRODUCTION

Parkinson disease (PD) is a neurodegenerative disease characterized by the dysregulation and deposition of α-synuclein in the central nervous system, producing Lewy bodies in a stereotypic and progressive pattern. The average age of onset is 60 years, though it is known that olfactory impairment and gastrointestinal dysfunction may precede clinical parkinsonism by decades. In 2003, Braak and colleagues postulated the "dual-hit" hypothesis, whereby the PD process begins after an environmental toxin enters the CNS through either a nasal or a gastric route (or both), then spreads trans-synaptically into vulnerable brain regions.[1] This hypothesis is supported by a Lewy body deposition pattern that correlates with clinical disease progression.[2] Though research on the gastric pathway in mouse models has been productive, studies on the nasal pathway has been sparse due the difficulties in visualizing the nasal sinuses and cranial nerves in their natural, anatomic form. Our team has developed a novel whole-skull preparation technique utilizing formic acid to decalcify bone, conserving the integrity of the nasal cavity and permitting direct observation of neuroanatomy and pathology in the olfactory system of mice by routine immunohistochemistry (IHC). The useful applications of this technique are presented.

METHODOLOGY

Skull preparation and immunohistochemistry staining



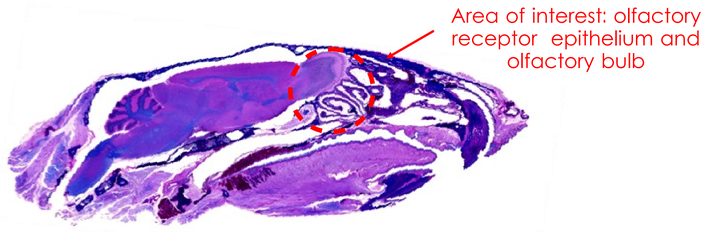
The skull slices were treated under a standard IHC protocol, using antibodies against neuronal pathology (α-synuclein, Tau, and reovirus). Immunoreactivity was revealed by DAB staining; hematoxylin was used as counterstain. Photographs of the individual compartments of the ethmoidal sinus were obtained using a Zeiss light microscope and a Zeiss AxioCam ICC3 digital camera.

Quantifying reovirus infection in the olfactory epithelium

Adult mice 6 weeks of age were inoculated with reovirus ($1.7X10^5$ pfu) via nasal cavity and sacrificed 2 days post-infection.[3] The skulls were prepared as above. Compartments of the ethmoidal sinus were numbered rostral to caudal. For each, the region of the neuroepithelium with the highest number of immunoreactive cells was captured by light microscopy and digital camera. Circular snapshots of these regions were sampled using ImageJ, prioritizing regions with the highest number of immunoreactive cells. The total number of IHC positive and negative cells were counted manually using the Cell counter plugin on Image J.

WHOLE-SKULL IHC AND ITS APPLICATIONS

Figure 1: Sagittal section (4 µM thick) of a whole-skull mount of mouse stained with Luxol Fast Blue.



1 α-synuclein and Tau are abundant in the olfactory receptor epithelium of mice

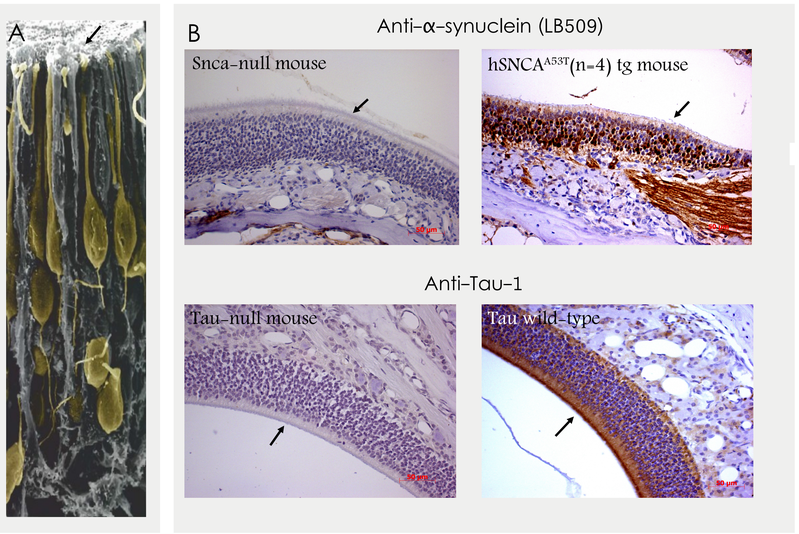


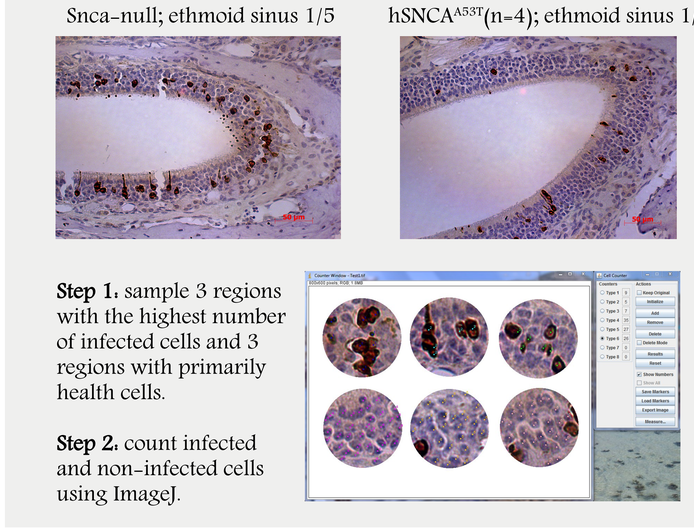
Figure 2-A: scanning electron micrograph of the rat olfactory epithelium observed from a perpendicular plane, showing the olfactory neurons (yellow) with a thick apical process (dendrite) extending to the surface and large cell body. Photo taken from Nomura et al., 2004.

Figure 2-B: light microscopy images of mouse olfactory epithelium (20X) stained by immunohistochemitry, using antibodies against oligomeric α-synuclein (LB509) and Tau (anti-Tau-1).

2 Reovirus invades the neurons of mouse olfactory receptor epithelium, with positive signals in CNI (arrow; CNV and CNS not shown)

3 From pictures to numbers: quantifying the severity of reovirus infection in the olfactory receptor epithelium

We devised a counting method to quantify and compare the reovirus infection in the olfactory receptor epithelium of (1) a snca-null mouse and (2) a humanized SNCA mouse, with 4 copies of the human SNCA locus carrying the Parkinson-linked A53T mutation.



Step 1: sample 3 regions with the highest number of infected cells and 3 regions with primarily health cells.

Step 2: count infected and non-infected cells using ImageJ.

Table 1: Quantification of reovirus infection in the olfactory epithelium of snca-null and hSNCA^{A53T} mouse

Mouse genotype	Reovirus infected cells per sinus	Healthy cells per sinus	Ratio infected/healthy
Snca knock-out mouse	8 ± 3	128 ± 11	0.06 ± 0.02
hSNCA^{A53T} (n=4) mouse	19 ± 16	121 ± 24	**0.16 ± 0.14**

The severity of infection in the olfactory epithelium is represented by the ratio between reovirus-infected cells and healthy cells; the higher the ratio, the greater the infection. These preliminary results seem to indicate a relative "vulnerability" of the hSNCA^{A53T} mouse to reovirus. This data needs to be validated using a larger sample size.

CONCLUSION

We have developed a novel immunohistochemical staining technique that allows direct visualization of the olfactory structures, in particular the olfactory receptor epithelium and cranial nerve-1 en route to the olfactory bulb.

Combining the most current knowledge and hypotheses on Parkinson disease pathogenesis, including Braak's "two-hit" hypothesis by nasal and gastric entry of an unknown environmental pathogen, our protocol allows for the exploration of the interactions between environmental factors (including microbial agents) and genetic susceptibilities to late-onset neurological disorders in mammals.

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ACKNOWLEDGEMENTS

- This project was graciously funded by **UROP** and **CIHR**..
- **Dr. John Woulfe** (Ottawa Hospital Research Institute) for valuable expertise in the recognition of mouse neuropathology.
- **Dr. Eric Robertson** (University of Alabama at Birmingham) for the kind donation of tau-null transgenic mice.
- **Dr. Omar El Agnaf** for kindly providing us with oligomeric SNCA-specific antibodies.
- **Ms. Dina Elleithy** for training and useful tips in IHC.
- **Ms. Jacqueline Tokarew** for her help with the review of this poster.

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