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Research Plan:

a) Rationale:

Obsessive Compulsive Disorder, more commonly known as OCD, affects 2.3% of the population, or about 1 in 40 people. Although not detrimental to a person's health, OCD severely inhibits a person's quality of life. However, there are limited treatments due to a lack of knowledge of the disease's pathophysiology. It is known that the cortico-striato-thalamo-cortical circuit, or action selection feedforward loop, is implicated in the disease, but the specifics are unknown. Previous studies suggest that altered cholinergic tone results in OCD like symptoms; however, these studies directly altered cholinergic interneuron (ChI) function (releasing striatal ACh), ergo there is no direct evidence of ChIs directly participating in OCD's circuitry. Preliminary data from our lab suggests that direct spiny projection neurons (dSPNs) exhibit an increase in excitability and indirect spiny projection neurons exhibit a decrease in excitability in SAPAP3 deficient OCD mouse models when compared to WT mice. These neurons respectively promote and inhibit action selection within the basal ganglia's main input nucleus, the striatum. SPN excitability can be modulated through a change in acetylcholine binding. Consequently, we will seek to investigate if ChIs are implicated in the OCD circuit by directly measuring them, while also indirectly observing if acetylcholine fluctuations are responsible for SPN excitability changes.

b) Hypothesis:

Previous studies indicate that a change in cholinergic tone results in OCD-like symptoms within mice; however, cholinergic interneurons have never been directly observed when studying the circuitry of OCD . Acetylcholine modulates dSPN and iSPN excitation: a decrease promotes dSPN excitation while inhibiting iSPN excitation - and preliminary data finds a decrease in iSPN and an increase in dSPN excitation. For these aforementioned reasons, we hypothesize that there will be a decrease in cholinergic interneurons in OCD mouse models when compared to wild type mice.

c) Procedure, Risk and Safety, Data Analysis

i) Procedure:

Immunohistochemistry

Mouse brain slices of 200 microns will be mounted 8 slices per well. Each slice will be rinsed with PBS solution for 10 minutes at room temperature. After the rinse, each slice will be incubated for 2 hours at room temperature with 1500µL/well of blocking buffer containing 3% normal donkey serum in diluent solution (.1% Triton X-100 in PBS). The blocking buffer will then be discarded, and the slices will be incubated overnight at 4 °C in a diluent solution containing .5% normal donkey serum, .1% Triton X-100, and primary antibodies of 1:500 goat anti-ChAT and 1:2000 rabbit anti-µOR. Subsequently, the slices will be washed for a duration of 10 minutes 3 times at room temperature in a diluent solution of 5% normal donkey serum and .1% Triton X-100. The slices will be counterstained in diluent solution of 5% normal donkey serum and .1% Triton X-100 with 1:2000 donkey anti-goat 594 and 1:2000 donkey anti-rabbit 488 for 2-3 hours at room temperature. They'll then be washed again 3 times, rinsed in PBS for 3 minutes, and then mounted onto a glass slide using Fluromount-G for microscopic analyses.

ii) Risk and Safety

1) Human Subjects: N/A

2) Vertebrate Animals: N/A

3) Potentially Hazardous Biological Agents (PHBA):

All mice will be treated with proper care. Only properly trained personnel will directly handle/euthanize the mice. All brain tissue will be stored in a fridge of 4 °C when not in use, and when in use there will be proper supervision. Appropriate safety equipment such as closed-toed shoes, long pants, and gloves will be worn at all times. Mouse brain tissue of SAPAP3 KO and WT mice will be BSL-1. After use, the tissue will be discarded in the proper hazardous bin, and the equipment will be washed with alconox cleaning solution.

4) Hazardous Chemical/Activities/Devices:

Phosphate Buffer Solution (PBS)

It may cause irritation to the eyes and skin through contact. If ingested, PBS may cause irritation to the digestive tract. If inhaled, it may cause irritation to the respiratory tract. If one's eyes comes into contact with PBS, one can use water to flush out the chemical.

In the case of skin irritation, the chemical can be washed off with water. In the case of ingestion, one may rinse the mouth with water.

Triton X-100

If ingested orally, it may cause category 4 acute toxicity. If it comes into contact with one's eyes, it may cause category 1 serious eye damage. If swallowed, call a poison center/doctor if you feel unwell. Rinse mouth. If in your eyes, rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a poison center/doctor. Contents will be disposed of in an approved waste disposal plant.

Optimal Cooling Temperature Compound(OCT)

If inhaled, move to fresh air. Call a physician if symptoms develop or persist. If skin contact, wash off with soap and water. Get medical attention if irritation develops and persists. If it comes into contact with the eyes, rinse with water. Get medical attention if irritation develops and persists. If ingested, rinse mouth. Get medical attention if symptoms occur. Most importantly, direct contact with eyes may cause temporary irritation.

iii) Data Analysis:

ImageJ Analysis

Microscope images will be uploaded into ImageJ to outline/measure the area of the striatum and calculate the number of cholinergic interneurons within the striatum. Each slice will be uploaded with a z-stack of 31 images, which will then be converted to a composite image of the 15 clearest stacks. The composite images will then be pseudo colored red for cholinergic interneurons. The primary measurement for analysis will be cholinergic interneuron striatal density.

d) Bibliography:

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Striosomes and Matrix by an Amphetamine-Sensitive Mechanism." *Frontiers in Neuroanatomy*, Frontiers Media S.A., 21 Mar. 2017, www.ncbi.nlm.nih.gov/pubmed/28377698.

Lim, Sean Austin O., et al. "Striatal Cholinergic Interneuron Regulation and Circuit Effects." *Frontiers in Synaptic Neuroscience*, vol. 6, 2014, doi:10.3389/fnsyn.2014.00022.

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- NO ADDENDUMS EXIST -