Comparative Physiology – Lab A03
Independent Project Proposal
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A Drosophila Model for Developmental Nicotine Exposure

Hypothesis

If Drosophila melanogaster consume a diet containing nicotine throughout their lives, they will show a preference for food with nicotine over a control, compared to fruit flies that have never ingested nicotine.

Background

Nicotine is primary addictive chemical in tabaco products such as cigarettes or vaporizers and is a massive public health crisis. As of 2010, 45 million Americans smoke tabaco of some form and that number is rapidly increasing again after a long period of decline with the popular introduction of vaporizers notably in children and young adults. Nicotinic acetylcholine receptors (nAChRs) are ligand gated ion channels that are involved in processing nicotine within the body, these receptors function and respond similarly in both human and drosophila models. nAChRs are involved in a variety of physiological processes, including neurotransmission, nicotine reward, and addiction. They are also linked to several diseases, including Alzheimer's, Parkinson's, schizophrenia, depression, and epilepsy. Nicotinic receptor signaling is also important for normal development of the nervous system including roles in synapse formation, neuronal growth, neuronal differentiation, and the regulation of the GABA switch from an excitatory role early in development to its mature role as inhibitory neurotransmitter. Exposure to nicotine in developmental years in both rodents and insects in prior studies resulted in low birth weight, increased morality. A known outcome of prenatal and early developmental nicotine exposure in mammals is an increase in the number of nicotinic binding sites resulting in increased hyperactivity and increased nicotine self-administration. In *Drosophila*, there are ten genes encoding nAChR subunits, with *Dα7* being one of the most relevant to this study. *Dα7*encodes a subunit with high homology to vertebrate nAChRs and has been shown to mediate nicotine-induced behaviors, such as escape responses and hyperactivity. Given its involvement in both nicotine sensitivity and its expression in brain regions associated with reward pathways, *Dα7* is a strong candidate for mediating the effects of developmental nicotine exposure in *Drosophila*. This gene's role will be crucial in exploring whether nicotine exposure during development alters behavioral responses in adulthood, such as food preferences and nicotine self-administration.

Controls

To ensure that any observed preferences are due to nicotine exposure and not other factors, quinine will be used as a control. Quinine is known for its bitter taste, similar to nicotine, but does not possess the same addictive properties. By comparing the flies' responses to nicotine and quinine, we can control for the bitter taste factor and isolate the effect of nicotine addiction.

Variables

- **Independent Variable:** Nicotine consumption during development.
- **Dependent Variable:** Food preference in adulthood.

Possible Confounding Factors

Potential confounding factors include genetic variations, environmental conditions, and the nutritional content of the food media. To minimize these, the following steps will be taken:

- Use genetically similar fly strains.
- Maintain consistent environmental conditions (ex. temperature, humidity).
- Ensure that both nicotine-containing and control foods have comparable nutritional content and flavor profiles.

Methods

1. **Materials:**
    - *Drosophila melanogaster***
        - ***Would like to discuss what genetic lines are available. Wildtype is acceptable but preference to a genetic line that either lack bitter taste receptors or intentional choice of wildtype and Dα7 deletion line.*
    - Standard *Drosophila* food media
    - Nicotine
    - Quinine
    - Agarose solution
    - Dyes: Amaranth (red) and FD&C #1 (blue)
    - Sucrose
    - Hot plate/sand bath
    - Dissecting microscope
    - Incubator
    - Pipettes and tips
    - Fruit fly vials and stoppers
    - Cotton balls
2. **Procedure:**
    - **Developmental Exposure:**
        - Raise the treatment group on food media containing a high concentration of nicotine from the larval stage through to adulthood.
        - Maintain a control group on nicotine-free food media.
    - **Food Preference Assay:**
        - Prepare two types of food:
            - Nicotine-containing food dyed with Amaranth (red).
            - Quinine-containing food dyed with FD&C #1 (blue).
        - Add a small amount of sucrose to both foods to encourage feeding.
        - Place mature flies from both the treatment and control groups into separate vials containing both types of food.
        - Allow the flies to feed for a predetermined period (e.g., 24 hours).
        - After the feeding period, assess the flies' food preference by examining the color of their abdomens under a dissecting microscope.

        - Record the number of flies preferring nicotine-containing food versus quinine-containing food in both groups.
3. **Replication:**
    - Conduct the experiment with multiple cohorts to ensure statistical validity. For example, perform three independent trials with separate batches of flies.
4. **Statistical Analysis:**
    - Use a chi-squared test to compare the observed food preferences between the treatment and control groups.
    - Determine if there is a significant difference in the preference for nicotine-containing food between flies exposed to nicotine during development and those not exposed.

5. **References:**

Velazquez-Ulloa, N. A. (2017). A Drosophila model for developmental nicotine exposure. *PloS One*, *12*(5), e0177710–e0177710. https://doi.org/10.1371/journal.pone.0177710

Rimal, S., & Lee, Y. (2019). Molecular sensor of nicotine in taste of Drosophila melanogaster. *Insect Biochemistry and Molecular Biology*, *111*, 103178–103178. https://doi.org/10.1016/j.ibmb.2019.103178

Sanchez-Diaz, I., Rosales-Bravo, F., Luis Reyes-Taboada, J., Covarrubias, A. A., Narvaez-Padilla, V., & Reynaud, E. (2015). The Esg Gene Is Involved in Nicotine Sensitivity in Drosophila melanogaster. *PloS One*, *10*(7), e0133956-. https://doi.org/10.1371/journal.pone.0133956

Salas, R., Orr-Urtreger, A., Broide, R., Beaudet, A., Paylor, R., & De Biasi, M. (2003). The nicotinic acetylcholine receptor subunit alpha 5 mediates short-term effects of nicotine in vivo. *Molecular Pharmacology*, *63*(5), 1059–1066. https://doi.org/10.1124/mol.63.5.1059
Benowitz, N. L. (2009). Pharmacology of Nicotine: Addiction, Smoking-Induced Disease, and Therapeutics. *Annual Review of Pharmacology and Toxicology*, *49*(1), 57–71. https://doi.org/10.1146/annurev.pharmtox.48.113006.094742

Benowitz, N. L. (2010). Nicotine Addiction. *The New England Journal of Medicine*, *362*(24), 2295–2303. https://doi.org/10.1056/NEJMra0809890

Ping, Y., & Tsunoda, S. (2012). Inactivity-induced increase in nAChRs upregulates Shal K+ channels to stabilize synaptic potentials. *Nature Neuroscience*, *15*(1), 90–97. https://doi.org/10.1038/nn.2969

Mannett, B. T., Capt, B. C., Pearman, K., Buhlman, L. M., VandenBrooks, J. M., & Call, G. B. (2022). Nicotine Has a Therapeutic Window of Effectiveness in a Drosophila melanogaster Model of Parkinson's Disease. *Parkinson's Disease*, *2022*, 1–11. https://doi.org/10.1155/2022/9291077

Sellier, M.-J., Reeb, P., & Marion-Poll, F. (2011). Consumption of Bitter Alkaloids in Drosophila melanogaster in Multiple-Choice Test Conditions. *Chemical Senses*, *36*(4), 323–334. https://doi.org/10.1093/chemse/bjq133

Jones, A.K., Sattelle, D.B. (2010). Diversity of Insect Nicotinic Acetylcholine Receptor Subunits. In: Thany, S.H. (eds) Insect Nicotinic Acetylcholine Receptors. Advances in Experimental Medicine and Biology, vol 683. Springer, New York, NY. https://doi.org/10.1007/978-1-4419-6445-8_3