Lynx2 on Nicotine-Induced Anxiety and Locomotion

Introduction

Nicotine addiction is a leading cause of preventable death in the United States, with nearly one in five deaths being related to cigarette smoking. In addition, about 1,600 minors smoke their first cigarette every day (Centers for Disease Control and Prevention, 2020). Nicotine is the main addictive constituent in tobacco and its use is associated with an increased risk of cardiovascular, respiratory, gastrointestinal, and immunological disorders (Mishra et al., 2015). To understand how nicotine addiction develops, neurobiological mechanisms underlying nicotine use needs to be studied.

When nicotine is consumed through smoking, it is absorbed into the pulmonary veins and diffuses rapidly to the brain. It binds to nicotinic acetylcholine receptors (nAChRs), which are ligand-gated ion channels and induces the release of dopamine (Benowitz, 2009). Lynx2 is a prototoxin gene that encodes proteins involved in nAChR modulation. It is primarily expressed in the prefrontal cortex and amygdala, as shown in Figure 1, which are involved in anxiety control (Tekinay et al., 2009). Previous studies have tested the role of lynx2 in fear and anxiety, but its effects on nicotine-induced behaviors has not been determined yet (Tekinay et al., 2009). This project aims to analyze the role of lynx2 in locomotion and anxiety-related behaviors in the presence of nicotine.

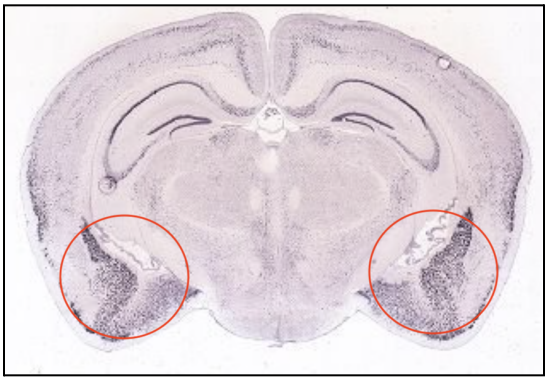

Figure 1. The expression of lynx2 mRNA in the mouse amygdala, retrieved from the Allen Brain Atlas

Background

Nicotine produces a psychoactive effect through its interaction with nAChRs in the brain. It is an exogenous agonist that binds to nAChRs and opens the channels, allowing for the flow of cations. When injected subcutaneously, it quickly enters the bloodstream and achieves peak levels after around 15 minutes (Barik & Wonnacott, 2009). In the amygdala, nAChR activity

plays a significant role in stress responses. Specifically, the *α5, α2, α3*, and *β4* subunits are involved in nicotine withdrawal, anxiety, and depression (Mineur et al., 2016). *α7* and *β2** nAChR signaling is significantly involved in stress-related behaviors (Mineur et al., 2016). Through *α7* nAChR activity, nicotine enables glutamatergic signaling in the amygdala (Mineur et al., 2016).

Lynx2 is a membrane-bound GPI anchored prototoxin. It binds to *α7* and *α4β2* nAChRs and suppresses their activity. The presence of lynx2 decreases the expression of *α4β2*, which lessens agonist responsiveness. Previous studies suggest that lynx2 and nicotine compete to bind at the ligand binding site (Miwa et al., 2019). In prior experiments with lynx2 *in vivo*, lynx 2 knockout mice experienced no differences in motor and sensory behaviors compared to wildtype mice. However, they had increased anxiety, elevated fear levels, and decreased social behavior. Lynx2 gene deletion also resulted in enhanced glutamatergic activity in the medial prefrontal cortex in response to nicotine (Tekinay et al., 2009).

The elevated plus maze (EPM) is an apparatus consisting of a square platform with four arms. Two of the arms are open with no walls and two of them are closed with walls. More time in the open arms represent decreased anxiety while more time in the closed arms represent increased anxiety. Nicotine's effects on anxiety-related behaviors is dependent on age and sex. In experiments with EPM on rats, nicotine had varying effects on adolescent males and females. While adolescent females spent less time in the open arms, adolescent males spent more time. This indicates that while nicotine relieved anxiety for young males, it increased anxiety in young females (Elliot et al., 2004).

Sex also plays a role in nicotine's effects on locomotion. Specifically, the amount of nicotine administered affects the responses in males and females differently. At a high dose of nicotine (200μg/ml), both male and female mice experience increased locomotion, but at moderate dose (100μg/ml), only male mice have increased locomotion (Calderone et al., 2008). Previous studies suggest that males are more sensitive to the stimulative effects of nicotine, explaining their increased movement.

Materials and Methods

Adult male and female lynx2 knockout and wildtype mice from the breeding colony in the lab will be used. They are housed in a humidity and temperature-controlled environment and approved by the Institutional Animal Care and Use Committee for use at the University of California, Irvine.

Initially, subjects will be habituated to the room and experimenter daily for three days prior to the behavioral assessment. During the day of the assessment, animals will be habituated in the testing room for 1 hour. Following habituation to the room and the experimenter, subjects will be injected subcutaneously with 0.5 mg/kg nicotine and after 30 minutes, tested in the Elevated Plus Maze. The mice will then be given adequate time to prevent confounds for the locomotor behavioral assessment. On the next testing day, mice will undergo a similar procedure of a subcutaneous nicotine injection and a locomotion assessment 30 minutes afterwards. During these assessments, behavior will be recorded and scored using the Anymaze software available on the lab computers.

Student Responsibilities

Under the guidance of a graduate student in the lab and my principle investigator, I will be responsible for habituating mice, injecting them subcutaneously with

nicotine, administering EPM and locomotion tests, scoring behavioral data using Anymaze software, and analyzing the results.

Timeline

	Fall 2022	Winter 2023	Spring 2023
Injections			
EPM/Locomotion			
Behavioral Analysis			
Writing			

Itemized Budget

	Cost
Animals	
7 cages of mice (35 animals)	$690
Drug Treatment	
Nicotine Tartrate	$200
Saline Solution, sterile, USP	$65
Syringes	$45
Total Cost	$1,000

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