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RESEARCH ARTICLE

IMPACT OF TOBACCO ON CHEMOSENSORY RESPONSE IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Fruit flies react to taste molecules in a way which is quite similar to humans and within the detection range of mammals. They are attracted to sugars, avoid bitter and toxic molecules, and adapt their consumption of acids and salts to their internal needs. In *Drosophila* adults, contact chemoreception is mediated through hair-like structures, called sensilla, located on the mouthparts, the legs, the wings margin, and the ovipositor. The behavior paradigms are relatively complicated, it is necessary to understand how the fundamental behavior is organized at neural level, before a full understanding of the complex behavior. In the present study *Drosophila melanogaster* has shows biased preference when facing sensory stimulations towards varied concentrations of tobacco.

INTRODUCTION

Drosophila melanogaster adapt their food consumption to their internal needs and avoid ingesting noxious molecules. Defects in the genes involved in these decisions induce behavioral alterations that are usually screened by monitoring flies feeding in two-choice or in no-choice situations (Kuhar *et al.*, 2017). Although psychostimulants, opiates and ethanol all have different primary effects and modes of action in the central nervous system (CNS), current theories suggest that their positive reinforcing, or rewarding, properties are mediated in part by an elevation of extracellular dopamine in the nucleus accumbens (Di Chiara, 1995). Nicotine, the major addictive component of tobacco, affects mammalian behavior by activating nicotinic acetylcholine receptors (Nestler 2005). When exposed to volatilized nicotine, flies exhibit locomotor hyperactivity and spasmodic movements leading to grooming at low doses and hypokinesia and akinesia at higher doses (Bainton *et al.* 2000). Similar to cocaine, nicotine exposure dose-dependently impairs negative geotaxis in flies (De Gubareff and Sletator, 2011.). In mammals, the addictive properties of nicotine are thought to be mediated by both direct and indirect activation of dopaminergic neurons (Nestler 2005). The locomotor effects of nicotine in flies are similarly dependent on dopamine, as pharmacological depletion of dopamine reduces nicotine sensitivity (Andretic *et al.*, 2008).

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Aside from dopamine, little is known about the molecular mechanisms mediating nicotine sensitivity in flies. However, several genes known to mediate cocaine sensitivity in flies have also been shown to regulate nicotine sensitivity: moody mutant flies are sensitive to the effects of both drugs, whereas RhoGAP18B and tao mutants are resistant (Gerber and Stocker 2007; Gong 2012; Gordesky *et al.*, 2008). These genes suggest that certain shared mechanisms may regulate multiple types of drug addiction in flies. Understanding the relationship between mechanisms mediating acute and long-term responses to drugs is key to understanding the addictive properties of the drug.

MATERIALS AND METHODOLOGY

Fly Stock

The fly stocks were routinely cultured in standard wheat cream agar medium in uncrowded condition at 22± 1°C (rearing temperature), 12:12 h light and dark periods and relative humidity of 70%. The test flies were cultured in wheat cream agar medium along with different concentrations of the tobacco (20 mg/1000ml, 40 mg/1000ml and 60 mg/1000ml).

Larval Gustatory Preference

On the day of experiment the Petri dishes were prepared (1mm×100mm) for control a Petri dish was divided into 2 halves, both of which were filled with 1% agarose in distil

water and allowed to cool for 10 minutes. For the experiment a Petri dish was divided into 2 halves, 1 half was filled with 1% agarose in distilled water and allowed to cool for 10 minutes. The other half was filled with 1% agarose along with experimental concentration (i.e., 40 mg, 60 mg, 80 mg caffeine and nicotine) and allowed to cool. Ten larvae were introduced in the center of each petri dish and allowed to choose. The number of larvae on each half of the petri dish was counted and the gustatory preference index (GPI) was calculated, every 2 minutes for 20 minutes. GPI values range from -1 to +1 with negative values representing preference for pure and positive values, representing preference for nicotine or caffeine.

$$\text{GPI} = \frac{\text{number of experimental} - \text{control}}{\text{Total number larvae} - \text{upside}}$$

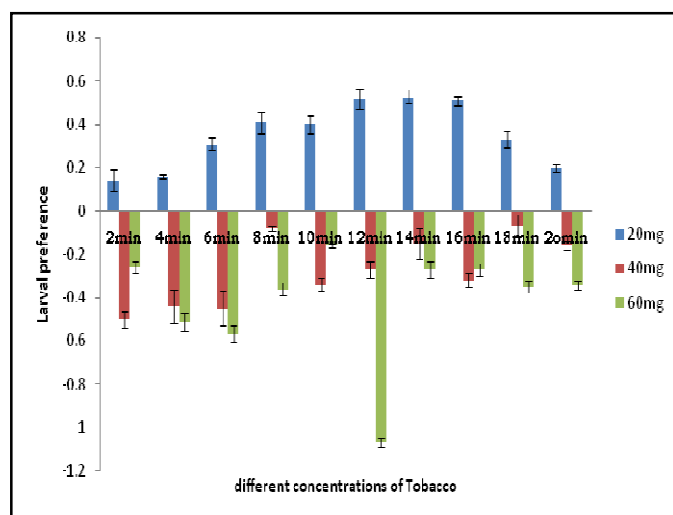
Larval Olfactory Choice preference

10 larvae were placed in the cap of the vial that is the start point and the movement of the larvae was observed. This was done simultaneously for all the experimental concentrations (i.e., 40mg, 60mg, and 80mg nicotine and caffeine) and control. The number of larvae and the distance travelled by it was tabulated for 50 minutes at 5 minute intervals. The odour choice index (OCI – equation 2) was calculated for the readings taken.

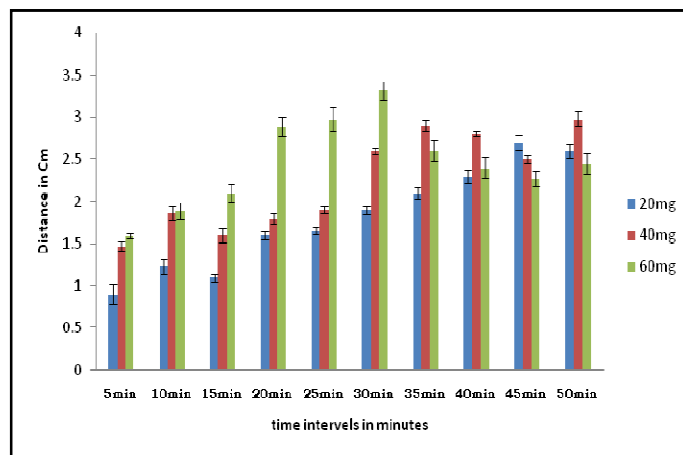
$$\text{OCI} = \frac{\text{number of flies in experiment-control}}{\text{Total number of flies}}$$

RESULTS

Graph1, shows the larvae fed with different concentrations of tobacco. The larvae fed with lower concentration of tobacco tend to prefer the same media when compared to other concentrations. The larvae exposed to mid and high concentrations preferred to move from treated media to control. The larvae treated with high concentrations have shown increased larval olfactory choice preference, after 30 min the same larvae gradually decreased the larval olfactory choice preference. Larvae fed with lower concentration has shown increased larval olfactory choice preference Graph 2.



Graph 1. Mean (\pm SE) larval gustatory preference of *Drosophila melanogaster* an exposure to different concentrations of tobacco



Graph 1. Mean (\pm SE) larval olfactory preference of *Drosophila melanogaster* an exposure to different concentrations of tobacco

DISCUSSION

The next decade should witness the discovery of many novel mechanisms underlying addiction-related behaviors in flies as the number of tools available to study molecular and neural processes is expanding at a rapid rate (Jones *et al.*, 2007). Based on what we have learned in from *Drosophila* addiction research, we expect that these novel mechanisms will be relevant to mammalian models and provide novel targets for the development of pharmacotherapies for drug addiction (Kuhar *et al.*, 2014). Upon exposure to volatilized free-base nicotine and caffeine, adult *Drosophila* exhibited dose-dependent behavioral responses. Low doses induced primarily grooming and hyperactivity. Moderate doses led to hypokinesia and stereotypic locomotion often manifested as circling (Millar and Denholm 2007). High doses induced spasmodic activity, tremor, and finally, complete loss of movement (akinesia). These behaviors are qualitatively very similar to those described by McClung and Hirsh (1998). The study emphasizes innate preference behaviors cannot be concluded that the presence of common mechanisms underlying different types of preferences, since the accumulated data is limited to the level of primary sensation (Pendleton *et al.*, 2000; Gargano *et al.*, 2005). Actually, there are signs that similarities between larval navigational strategy in chemotaxis and odor taxis can be found are diverse (Sokolowski 2001). It will be fascinating to look for the common basis across different preference behaviors of various modalities, but probably only after the full molecular and neural underlying mechanism is disclosed (Stocker 2004). It is evident that the molecular and neural basis for these preference behaviors is quite diverse. No common molecules or neurons are found to be involved in different types of preference behaviors (Todd and Staveley, 2004). Our results highlight the important role that nutrition plays in determining the phenotypic expression of starvation in *Drosophila* and provide broad implications for understanding tobacco responses to larval gustatory and larval olfactory choice preference with respect to tobacco. This study introduces insights into the evolution of tobacco responses to variable drug abuse.

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