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NICOTINE-INDUCED ACUTE HYPERACTIVITY IS MEDIATED BY DOPAMINERGIC SYSTEM IN A SEXUALLY DIMORPHIC MANNER

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Abstract—Short-term exposure to nicotine induces positive effects in mice, monkeys and humans, including mild euphoria, hyperactivity, and enhanced cognition. However, the underlying neural basis and molecular mechanisms for these effects remain poorly understood. Here, using a video recording system, we find that acute nicotine administration induces locomotor hyperactivity in *Drosophila*, similar to observations made in higher model organisms. Suppressing dopaminergic neurons or down-regulating dopamine 1-like receptor (*DopR*) abolishes this acute nicotine response, but surprisingly, does so only in male flies. Using a GFP reconstitution across synaptic partners (GRASP) approach, we show that dopaminergic neurons possess potential synaptic connections with acetylcholinergic neurons in wide regions of the brain. Furthermore, dopaminergic neurons are widely activated upon nicotine perfusion in both sexes, while the response curve differs significantly between the sexes. Moreover, knockdown of the β1 nicotine acetylcholine receptor (nAChR) in dopaminergic neurons abolishes the acute nicotine response only in male flies, while panneural knock-down occurs in both sexes. Taken together, our results reveal that in fruit flies, dopaminergic neurons mediate nicotine-induced acute locomotor hyperactivity in a sexually dimorphic manner, and *Drosophila* β1 nAChR subunit plays a crucial role in this nicotine response. These findings provide important insights into the molecular and neural basis of acute nicotine effects, and the underlying mechanisms may play conserved roles across species. **© 2016 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: nicotine, locomotor hyperactivity, *Drosophila*, dopaminergic neurons, sexual dimorphism, β1 nAChR.

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Abbreviations: cAMP, cyclic adenosine monophosphate; CREB, cAMP-response element binding; *DopR*, D1-like DA receptor; EB, ellipsoid body; FB, fan-shaped body; GRASP, GFP reconstitution across synaptic partners; MB, mushroom body; nAChR, nicotinic acetylcholine receptor; RNAi, RNA interference.

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INTRODUCTION

Nicotine, a major compound found in tobacco, is the primary factor leading to addiction to smoking (Robinson and Pritchard, 1992; Benowitz, 2010). In mammals, repeated or long-term nicotine exposure results in a series of complicated changes at both molecular and behavioral levels, and may ultimately cause nicotine dependence (Markou, 2008; Ray et al., 2009; Subramaniyan and Dani, 2015). Among various nicotine effects, changes in locomotor activity have been frequently observed, thus it has been used as a main parameter to evaluate nicotine effects (Philpot et al., 2012). For example, both acute and chronic nicotine exposure induces locomotor hyperactivity in rats (Dwoskin et al., 1999).

Locomotor activity has recently been found to change upon nicotine exposure of fruit flies. In the startle-induced negative geotaxis assay using vertical columns, flies showed reduced climbing activity in response to nicotine treatment, and the acute and long-lasting effects were found to be mediated by dopaminergic system and cyclic adenosine monophosphate (cAMP)/cAMP-response element binding (CREB) pathway, respectively (Bainton et al., 2000; Hou et al., 2004). Using a video recording system where flies are tested in horizontal tubes (Zimmerman et al., 2008), our previous study revealed that chronic nicotine treatment induces locomotor hyperactivity in flies, and a protein in the decapping complex, decapping protein 2, was found to be a key factor in mediating nicotine-induced locomotor hyperactivity (Ren et al., 2012). Early onset of hyperactivity upon nicotine exposure has been observed in flies, though the mechanistic details behind this observation have so far not been studied (Bainton et al., 2000). To access the acute hyperactivity of the acute nicotine response in flies, we adopted the same video recording system to analyze locomotion activity accurately.

Nicotinic acetylcholine receptors (nAChRs), are transmembrane oligomers consisting of five subunits (Cooper et al., 1991). In mammals, neuronal nAChRs are constituted by α- and β- subunits (α2–α10 and β2–β4) (Millar, 2003) in various combinations. Widely distributed in the peripheral and central nervous system (Wu and Lukas, 2011), nAChRs play fundamental roles in the regulation of neural activity (Dani and Bertrand, 2007; Albuquerque et al., 2009). In particular, different types of nAChR subunits have been found to regulate certain effects of nicotine (Dupuis et al., 2012), e.g., the α5

nAChR subunit is involved in nicotine short-term effects in mice, and the β2 nAChR subunit participates in the modulation of protective responses to nicotine stress in mice (Cohen et al., 2002; Salas et al., 2003). In *Drosophila*, ten nAChR subunits have been identified and designated as either α-type (α1–α7) or β-type (β1–β3) (Sattelle et al., 2005), both of which are exclusively located in the central nervous system (Schuster et al., 1993). These receptors allow fast excitatory synaptic transmission (Campusano et al., 2007), and were shown to regulate sensory and cognitive processes (Dupuis et al., 2012).

In mammals, dopamine represents a reward signal and is crucial for the reinforcement that promotes the self-administration of nicotine (Nestler, 2005). Similarly, the nicotine response was also blocked in flies treated with dopamine inhibitor, suggesting a conserved role of dopaminergic system in mediating nicotine effects (Bainton et al., 2000). Here, we characterized the acute nicotine response of locomoter hyperactivity in flies. In addition, we found that dopaminergic neurons and the β1 nAChR subunit play crucial roles in mediating this nicotine response.

EXPERIMENTAL PROCEDURES

Fly strains

Fruit fly strains were courtesy of the following groups/researchers: Dumb^{-} by Dr. F. W. Wolf (Ernest Gallo Clinic and Research Center); c5-Gal4, c205-Gal4, c232-Gal4, and c819-Gal4 by Dr. L. Liu (Institute of Biophysics); UAS-GCaMP3 by Dr. L. L. Looger (Howard Hughes Medical Institute, Janelia Farm Research Campus); 247-Gal4 by Dr. M. Heisenberg (University of Würzburg); Cha-LexA by Dr. M. Landgraf (Cambridge United Kingdom); TH-Gal4 by Dr. J. Hirsh (University of Virginia); Trh-Gal4 and UAS-*shibire*^{ts} by Dr. Y. Rao (Peking University), LexAop-CD4::GPF11; UAS-CD4::GFP1–10 by K. Scott (University of California); and PL00420/TM3Sb, *elav*-Gal4 and OK107-Gal4 by the Bloomington *Drosophila* Stock Center. UAS-*DopR*-RNAi (RNA interference) (107058) was provided by the Vienna *Drosophila* RNAi Center; RNAi fly strains for nAChRα1, nAChRα2, nAChRα3, nAChRα4, nAChRα5, nAChRα6, nAChRα7, nAChRβ1, nAChRβ2, and nAChRβ3 were obtained from the Tsinghua fly center. Flies were raised on Bloomington *Drosophila* Stock Center standard medium at 25 °C and 60% relative humidity under 12 h/12 h light/dark (LD) conditions. Flies were collected at eclosion and aged for 3–4 days before experiments.

Behavior assay

Individual flies were placed into 65 mm × 5 mm monitor tubes with 2% agar food at ZT 12, and 2-μl liquid food containing 5% yeast and 5% sucrose as the normal food was delivered to the tube at ZT 2. For experimental groups, nicotine (Sigma, N3876) was added to the normal food at given concentrations. Locomotor activity was monitored in the LD cycle using the video recording system (Zimmerman et al., 2008). Web cameras with a 640 × 480 resolution were pretreated to allow reception of infrared light. Images were acquired every 1 s and further processed by Pysolo24 (Gilestro and Cirelli, 2009) to determine the location of the flies. The total walking distance was calculated to represent locomotor activity.

Food-intake assay

To examine the food intake in our setup, flies were entrained in the recording tubes with the same paradigm above. Brilliant Blue (Care, Chemodist Industries) was added to the food to a final concentration of 1% before fed to flies for 15 min. According to a standard method (Edgecomb et al., 1994), flies were frozen on dry ice immediately and homogenized in PBS and centrifuged (12,000 rpm) for 30 min. Supernatants were transferred to a new tube, and its absorbance was measured in the cuvettes at 625 nm to reflect the amount of food consumption.

GFP reconstitution across synaptic partners (GRASP) experiments

GRASP experiments were performed according to a standard method (Feinberg et al., 2008). Briefly, the two parts of the split-GFP were separately expressed in two types of neurons, using the UAS/Gal4 and the LexA/LexAop dual expression systems. We constructed the flies of LexAop-CD4::GPF11/+;TH-Gal4,UAS-CD4::GFP1–10/Cha-LexA, in which CD4::spGFP11 was expressed under the control of Cha-LexA (labeling cholinergic neurons), and CD4::spGFP1-10 was expressed under the control of TH-Gal4 (labeling dopaminergic neurons), respectively.

Immunohistochemistry and imaging

Brains of adult fruit flies were processed according to a standard immunohistochemistry protocol (Wolff et al., 2014). Briefly, we dissected the brains in cold phosphate-buffered saline (PBS, containing 1.86 mM NaH_2PO_4, 8.41 mM NaH_2PO_4, and 175 mM NaCl) and fixed them in 4% paraformaldehyde in PBST (0.3% Triton in PBS) at room temperature for 40 min. After three 20-min washes in PBST brain samples were blocked in 5% NGS for 1 h at 25 °C, then incubated in mouse antibody against FasII (1:200, DSHB) (Fushima and Tsujimura, 2007) overnight at 4 °C. After three 20-min washes, a goat anti-mouse Alexa 555 secondary antibody (1:500, Invitrogen, Calrsbad, California, America) was applied overnight at 4 °C. Brain samples were imaged using a Leica SP5 II confocal microscope and Z stack images were scanned at 1-μm section intervals with a resolution of 1024 × 1024 pixels. Confocal stacks were analyzed with Image J (National Institutes of Health).

Calcium imaging with nicotine perfusion

Flies of TH-Gal4; GCaMP3.0 were anesthetized on ice, and fly brains were dissected gently in saline (Wilson et al., 2004). Brain samples were pre-incubated in 1 μM TTX (KangTe, 120516) in saline for 10 min, followed by

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