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Inhibition of gustatory plasticity due to acute nicotine exposure in the nematode *Caenorhabditis elegans*

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ABSTRACT

The present study investigated the effect of nicotine exposure on gustatory plasticity in the nematode *Caenorhabditis elegans*. The chemotactic response of wild-type N2 nematodes pre-exposed to 100 mM NaCl with 3.0 mM nicotine was almost the same as that of mock-conditioned nematodes unexposed to NaCl; however, the response of N2 nematodes pre-exposed to NaCl without nicotine was significantly lower than that of mock-conditioned nematodes. These results indicate that gustatory plasticity is inhibited by acute nicotine exposure. Inhibition of gustatory plasticity was observed when *cat-2* mutants with a defect in dopamine biosynthesis were pre-exposed to NaCl with 3.0 mM nicotine. Acute nicotine exposure did not cause inhibition of gustatory plasticity in *bas-1* mutants, which had defects in both serotonin and dopamine secretion, and *tph-1* mutants, which had a defect in serotonin secretion only. However, inhibition of gustatory plasticity was observed when *bas-1* and *tph-1* mutants were maintained on a growth plate that included serotonin. These results suggest that serotonin signaling plays an essential role in the modulation of the acute effects of nicotine.

1. Introduction

Cigarette smoking is the most prevalent drug addiction in humans, and nicotine, the main psychoactive ingredient in cigarettes, is responsible for the psychopharmacological effects such as mild euphoria, increased energy, heightened arousal, and reduced stress and anxiety (Herman and Sofuoglu, 2010). In animal experiments, acute administration of nicotine evokes changes in locomotory activities, grooming, and feeding (Scheufele et al., 2000; Levin et al., 2006) and can improve attentional flexibility (Allison and Shoaib, 2013). Although the roles of nicotine in directly modulating the function of the nicotinic acetylcholine receptor (nAChR) are well investigated, the genetic mechanisms leading to behavioral and neuronal plasticity remain poorly understood.

The nematode *Caenorhabditis elegans* is frequently used as a model organism to study behavioral plasticity and to evaluate the effects of drugs because it has a simple neuronal network comprising 302 neurons and a known genomic sequence (The *C. elegans* Sequencing Consortium, 1998; White et al., 1986). *Caenorhabditis elegans* lives in soil and at air–water interfaces where it feeds on soil bacteria (Andrew and Nicholas, 1976). It shows a chemotactic response to various compounds including water-soluble substrates (e.g., NaCl and lysine), volatile odorants (e.g., alcohol and diacetyl), and amino acids that are produced by food bacteria and act as natural chemotactic attractants for the nematode (Bargmann and Horvitz, 1991; Bargmann et al., 1993). These chemicals are sensed by chemosensory neurons in the head sensory organ called the amphid and in the tail organ called the phasmid (Bargmann and Horvitz, 1991; Hilliard et al., 2002). Information about these chemicals is transmitted to several interneurons and plays a significant role in mediating behavioral response.

Caenorhabditis elegans expresses at least 27 nAChR subunits (Jones and Sattelle, 2004), and a subset of these contribute to the nicotine modulating response, such as egg-laying and body-wall muscle activity (Waggoner et al., 2000; Gottschalk et al., 2005). Nicotine is known to cause several types of behavioral responses, including a continuous higher locomotion speed (acute response), tolerance, withdrawal, and sensitization via nAChR (Feng et al., 2006). Dual effects of nicotine on locomotion speed, which are dependent on differences in its dosage and treatment duration, have also been revealed (Sobkowiak et al., 2011). Although some studies have investigated the effects of nicotine on the motor activity in nematodes, the mechanisms underlying the effects of nicotine on behavioral plasticity, such as associative learning, remain unclear.

As mentioned above, *C. elegans* is an excellent model organism for studying behavioral plasticity. Nematodes are capable of associating NaCl with the absence or presence of food, as

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demonstrated in salt chemotaxis learning paradigms (Tomioka et al., 2006). Recently it was shown that administration of ethanol interferes with salt chemotaxis learning in *C. elegans* and serotonin signaling plays an essential role in this effect (Wang et al., 2011). In the present study, we demonstrated the effect of nicotine on gustatory plasticity, i.e., salt chemotaxis learning, and found that nicotine impaired gustatory plasticity. We also revealed that serotonin signaling has an essential role in modulating the acute effects of nicotine. Our findings should lead to a better understanding of the effects of drugs on neuronal plasticity that underlies the learning process.

2. Materials and methods

2.1. Animals

The wild-type strain of *C. elegans* (Bristol N2) and *lev-1(e211)*, *lev-1 (ok3201)*, *unc-29(e1072)*, *bas-1(tm351)*, *bas-1(ad446)*, *cat-2(e1112)*, and *tph-1(mg280)* mutants were obtained from the *Caenorhabditis* Genetics Center of the University of Minnesota. The nematodes were grown and maintained on nematode growth medium (NGM) agar plates [3 g/l NaCl, 2.5 g/l polypeptone, 5 mg/l cholesterol, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM KH_2PO_4 (pH 6.0), and 17 g/l agar] in an incubator (Fukushima FMU-133I, Osaka, Japan) at 20 °C with *Escherichia coli* strain OP50 as food.

Synchronously staged young adult (YA) hermaphrodites were used in all experiments (Matsuura et al., 2007, 2010). L4 (last larvae) stage nematodes have a crescent structure around the vulva; this structure disappears in the YA stage and thus was absent in all experimental animals. To obtain synchronous hermaphrodites, 50 gravid nematodes were transferred to fresh NGM plates and incubated for 3 h at 25 °C to lay eggs. Subsequently, these gravid nematodes were removed and the plates with the eggs were maintained in an incubator at 20 °C.

2.2. Preference assay

For the nicotine preference assays, tissue culture dishes (9 cm in diameter) containing 1 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM KH_2PO_4 (pH 6.0), and 17 g/l agar with nicotine (0.1 mM, 0.3 mM, 1.0 mM, 3.0 mM, and 5.0 mM) (Wako 104-01211, Osaka, Japan) were used. Ten minutes after the agar gel was allowed to set, a central circular plug (6 mm in radius) and both opposite quarters of the agar plate were removed from the assay plate, and replaced with the same amount of agar solution [1 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM KH_2PO_4 (pH 6.0), and 17 g/l agar] (control area: Fig. 1A).

N2 nematodes in the NGM plates were collected using wash buffer and washed thrice with the wash buffer [1 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM KH_2PO_4 (pH 6.0), and 0.05% Tween 20] by centrifugation for 20 s at approximately 900 × *g*. Approximately 30 nematodes were placed at the original location (center of the preference assay plate: Fig. 1A) to avoid the confounding effect of population density on chemotactic behavior (Matsuura et al., 2005). The nematodes at the original locations were dried, separated by gently touching them with a Kimwipe, and then allowed to move freely on the assay plate for 90 min. Every 30 min, the number of nematodes at the nicotine and control areas was determined using a microscope (Olympus SZ40, Tokyo, Japan). A preference index (Lee et al., 2009) was calculated as follows: preference index = (number of nematodes at the nicotine area − number of nematodes at the control area)/(total number of nematodes on the plate). The assays were performed during the daytime in an experimental room maintained strictly at 20 °C, and each measurement was repeated more than four times.

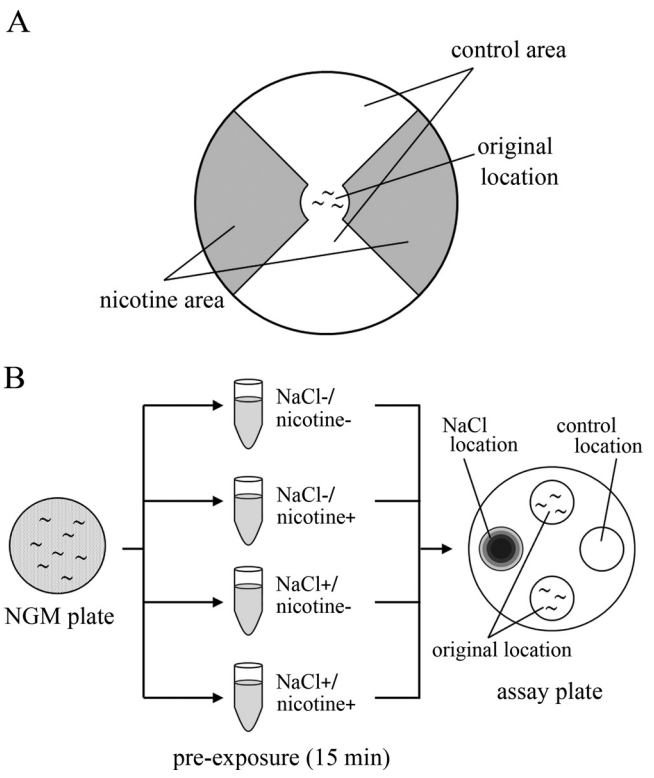

Fig. 1. (A) Configuration of the plate for the preference assay. A central circular plug (6 mm in radius) and opposite quarters of agar plates with nicotine (0.1 mM, 0.3 mM, 1.0 mM, 3.0 mM, and 5.0 mM) were removed from the assay plate, and replaced with the same amount of agar solution (control area). Approximately 30 nematodes were placed at the original location (center of the assay plate) and allowed to move freely on the plate for 90 min. (B) Experimental procedure for gustatory plasticity. Wild-type nematodes and mutants were transferred to a pre-exposure solution: (a) wash buffer without and with nicotine (referred to as NaCl−/nicotine− and NaCl−/nicotine+ pre-exposed nematodes, respectively) or (b) wash buffer containing NaCl without and with nicotine (referred to as NaCl+/nicotine− and NaCl+/nicotine+ pre-exposed nematodes, respectively). The nematodes were maintained at 20 °C for 15 min in their respective pre-exposure solutions. Approximately 30 nematodes were then placed at their original locations on the assay plate and allowed to move freely for 90 min.

2.3. Gustatory plasticity assays

In the gustatory plasticity assays (chemotaxis assays for NaCl), tissue culture dishes (9 cm in diameter) containing 1 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM KH_2PO_4 (pH 6.0), and 17 g/l agar (NaCl was omitted) were used. To obtain a concentration gradient of NaCl, 100 mM NaCl (7 μl) was spotted on the surface of an assay plate (NaCl location: Fig. 1B) at 18 h and 3 h before the start of the assay (Matsuura et al., 2004, 2005, 2007). The assay plate was stored in an incubator at 20 °C. A 1-μl aliquot of 0.5 M sodium azide was spotted on the same location as that of NaCl shortly before the chemotaxis assay to anesthetize the nematodes. As a control, 1 μl of 0.5 M sodium azide was also spotted on a control location.

To investigate the effects of nicotine on gustatory plasticity, nematodes in the NGM plates were collected and washed thrice with a pre-exposure solution: (a) wash buffer without and with nicotine (mock-conditioned nematodes; referred to as NaCl−/nicotine− and NaCl−/nicotine+ pre-exposed nematodes, respectively) or (b) wash buffer containing 100 mM NaCl without and with nicotine (NaCl-conditioned nematodes; referred to as NaCl+/nicotine− and NaCl+/nicotine+ pre-exposed nematodes, respectively). The nematodes were maintained at 20 °C for 15 min in their respective pre-exposure solutions (Fig. 1B). After the pre-exposure period, they were washed once with the wash buffer,

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