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Short communication

# Nicotine injected into the antennal lobes induces a rapid modulation of sucrose threshold and improves short-term memory in the honeybee *Apis mellifera*

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**Abstract**

In honeybee, although it is known that the food perception and the olfactory memory can be modulated by many environmental and biochemical factors, nothing is known on the effects of nicotine on these processes. Using microinjections of nicotine in the antennal lobes, we show that nicotine at $10^{-3}$ M and $10^{-4}$ M but not at $10^{-5}$ M induced an increase of sucrose sensitivity and that post-training injection of $10^{-5}$ M nicotine improved retention of olfactory learning. These results demonstrate that potentiation of the cholinergic system in the honeybee enhances sucrose perception and facilitates olfactory memory.


*Theme:* Neural basis of behaviour
*Topic:* Learning and memory: pharmacology

*Keywords:* Honeybee; Proboscis extension; Antennal lobes; Sucrose threshold; Olfactory learning; Nicotine

The proboscis extension reflex of the honeybee (*Apis mellifera*) is an appetitive component of feeding behavior. When the antennae are touched with a droplet of sucrose solution, the honeybee reflexively extends its proboscis, a behavior that is referred to as proboscis extension response (PER). The PER to sucrose depends on different parameters such as the sucrose concentration, the repletion level or the individual sucrose sensitivity [12,13] and can be modulated by pharmacological agents, like biogenic amines such as serotonin, dopamine, octopamine, or tyramine [14]. Further use of the PER experimental approach has shown that one paired presentation of an odor associated to a sucrose solution is generally sufficient to induce later on a conditioned response when the odor is presented alone. This procedure is known as the olfactory conditioning of the PER [1,7]. Using olfactory or tactile learning, we have shown that brain injections of nicotinic antagonists such as mecamylamine or α-bungarotoxine differently impair learning and memory processes in the honeybee [2,5]. In vertebrates, the same nicotinic antagonists target different nicotinic receptors [4,17], suggesting that a broad panel of nicotinic receptors is involved in the memory processes [5]. Moreover, we have identified four nicotinic acetylcholine receptor α-subunits in the honeybee brain [15,16] which are expressed in different brain regions such as the antennal lobes (ALs). The ALs are the first relay of olfactory information coming from the antennal olfactory receptors and going to higher-order brain centers [11], and indeed the gustatory fibers arising from the antennal chemo-tactile receptors go through the ALs before reaching the gustatory centers.

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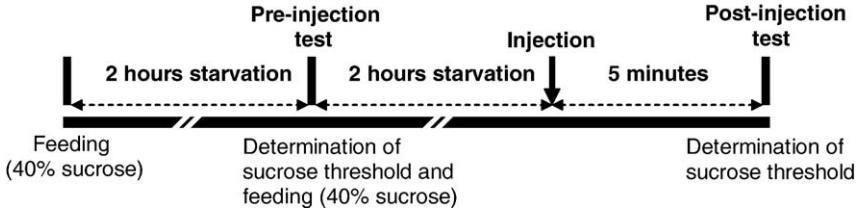


Fig. 1. Paradigm used to test nicotine effect on the sucrose threshold.

Because none of the former studies have analyzed the role of the cholinergic modulation in these processes, we have designed studies to analyze the nicotinic modulation of sucrose sensitivity and of the olfactory memory in the honeybee using the PER to sucrose experimental approach.

In the first study, restrained individual honeybees were fed to repletion with a 40% (W/V) sucrose solution the evening preceding the experiments. The next morning, honeybees were fed with one droplet of 40% sucrose solution, then starved for 2 h before the first sucrose threshold determination. Afterwards, they were fed again with one droplet of 40% sucrose solution and starved for 2 h (Fig. 1). During this period, animals were operated to give access to ALs: the head was immobilized with an adhesive tape and a small window in the cuticle was cut behind the basis of the antennae. Then, they received a bilateral saline or nicotine injection into the ALs and 5 min later they were submitted to the second sucrose threshold determination. Pressure injections (100 ms) of nicotine or saline solutions were made by the means of a custom-made microinjection system, using a glass micropipette drawn out by a micro-electrode puller (Campden). 0.5 nl of the following concentrations of nicotine ($10^{-5}$ M, $10^{-4}$ M and $10^{-3}$ M) were bilaterally injected into the ALs. Nicotine was prepared in a bee saline solution [3] containing (in mmol/l): 2.6 KCl, 1.8 $CaCl_2$, 150 NaCl and 11 sucrose (pH: 7.2, 320 mOsm/l). Sucrose threshold were measured according to Lambin et al. [9] 5 min after saline or nicotine injection: antennae were stimulated with 1-min inter-trial interval with increasing concentrations of sucrose solutions following a geometric progression from M/1024 to 2M and numbered from 1 (M/1024) to 12 (2M). The range of increasing sucrose solutions was presented twice with a 5-min interval. The lowest concentration of sugar solution that elicits the PER was defined as the sucrose perception threshold. Honeybees that did not respond to any sucrose solution were discarded. For statistical analysis, we used a modulation index (MI) inspired by Scheiner et al. [14]. Two numbers corresponding to sucrose threshold before injection ($ST_0$) and after injection (ST) were attributed to each honeybee. The MI calculated as $(ST - ST_0) / (ST + ST_0)$ can range between −1 and +1. A positive index marks an increase in sucrose threshold, a negative index reflects a decrease in sucrose threshold, and an index of 0 indicates that sucrose threshold did not change after treatment. Data from the control and nicotine treated groups were analyzed using the one-tailed non-parametric Mann–Whitney $U$ test. Results show that injections of nicotine at $10^{-4}$ M and $10^{-3}$ M into the ALs significantly affect the sucrose threshold (Fig. 2) as the MI of bees injected at these concentrations were negative and significantly different from those of control groups ($10^{-4}$ M: $U = 52$, $P \leq 0.05$; $10^{-3}$ M: $U = 88.5$, $P \leq 0.05$). On the other hand, the $10^{-5}$ M concentration did not induce significative change in the MI. This suggests that nicotine can increase the sensitivity to sucrose in bees. In the same time, bees were tested for PER to 40% sucrose solution to check for the functionality

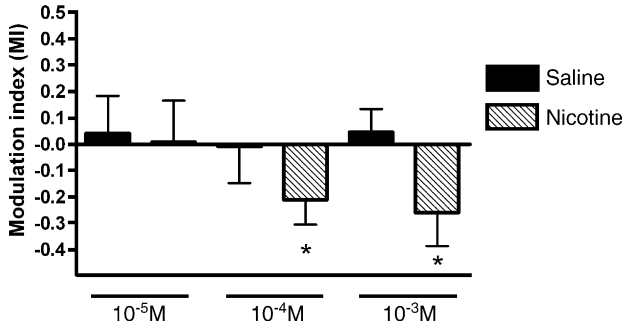


Fig. 2. Modulation index of sucrose thresholds by nicotine injection into the ALs. Black and hatched blocks represent saline and nicotine groups, respectively. Median modulation indices and quartiles (error bars refer to quartiles) are shown. Median modulation indexes are significantly different between the treated and the controls groups (30 bees in each group) for nicotine concentrations of $10^{-4}$ M ($U = 52$, $P \leq 0.05$) and $10^{-3}$ M ($U = 88.5$, $P \leq 0.05$). Asterisks denote significance in the one tailed Mann–Whitney $U$ test for $P \leq 0.05$.

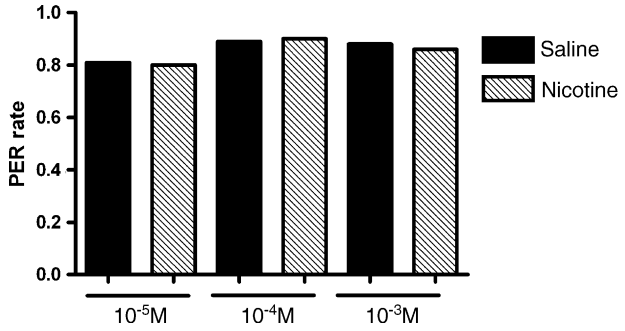


Fig. 3. Effects of different nicotine concentrations ($10^{-3}$ M, $10^{-4}$ M, and $10^{-5}$ M) comparatively to saline injections on the PER, 5 min after injection (30 bees in each group). The PER rate is the proportion of honeybees releasing a PER during presentation of 40% sucrose. No significant difference was found between control and nicotine groups.