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Dopamine Modulation Of Honey Bees

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Abstract:

Primary olfactory centers [antennal lobes (ALs)] of the honey bee brain are invaded by dopamine (DA)-immunoreactive neurons early in development (pupal stage 3), immediately before a period of rapid growth and compartmentalization of the AL neuropil. Here we examine the modulatory actions of DA on honey bee AL neurons during this period. The honeybees play an important role in the pollination and are considered as friends to farmers. Results suggest that the delayed rectifier-like current (IKV) also remains intact in the presence of DA. Taken together, our data indicate that Ca2+-dependent K+ currents are targets of DA modulation in honey bee AL neurons. This study lends support to the hypothesis that DA plays a role in the developing brain of the bee.

Key words: Dopamine, Modulation, Honey, Bees.

1. Introduction

India is a tropical country bestowed with highly diversified ecosystems inhabited by a variety of bee species. The varied ecological conditions with diversified flora have provided favorable habitat for various honeybee species in India. Several honeybee species viz., giant honeybee (A. dorsata F.), Indian bee (Apis cerana F.), dwarf bee (A. florea F.), and stingless bees (Trigona sp. and Melipona sp.), were widely distributed across the plains, hills and foothills of both urban and rural areas of Karnataka (Anonymous, 2005, 2007; Basavarajappa, 1998, 2004; Reddy and Reddy, 1989). These species pollinate various plants (Bright et al., 1998), and produce hive products such as honey, wax, pollen, etc. which are useful to mankind (Shukla and Upadhyay, 2007). While A. dorsata and A. florea (Hymenoptera: Apidae : Apini) show the strong tendency of migration from place to place (Basavarajappa, 2004; Neupane, 2004; Oldroyd et al., 2000; Paar et al., 2000), Trigona iridipennis (Hymenoptera : Apidae : Meliponini), a perennial species, rarely undertakes migration (Basavarajappa, 2006; Inoue et al., 1984; Solomon Raju et al., 2009). During emigration and immigration, these bees stay at various human built structures (Basavarajappa, 2008; Manjunath and Basavarajappa, 2008), and in the ground, hollow tree trunks, and branches (Solomon Raju et al., 2009). They share common foraging niche in human inhabited ecosystem, agricultural lands, and scrubby vegetation. During their foraging/ nesting at various ecosystems, wild honeybees encounter severe threat by manmade activities (Basavarajappa, 1998) which is not understood properly. The present

investigation, therefore, was undertaken to record the wild honeybee colony decline as influenced by man-made activities.

During metamorphosis, the CNS of the honey bee, Apis mellifera , undergoes dramatic growth and reorganization. Nowhere are the changes more striking than in the primary olfactory centers [antennal lobes (ALs)] of the brain. Around pupal stage 2 of the nine stages of metamorphic adult development, antennal sensory afferent neurons enter the ALs.

Their arrival triggers the formation of prominent subunits of synaptic neuropil called glomeruli which are the functional subunits of the AL neuropil. Each glomerulus contains the terminal arbors of antennal sensory afferent neurons, processes of local interneurons, dendrites of projection (output) neurons, and ramifications of centrifugal neurons that project to the ALs from other sites in the brain.

Behaviors in social insects are plastic and change responding to the environment. The behavioral changes are controlled by neural factors. The nervous system in social insects is divided into two highly structured, intertwined systems. The first is the visceral (also called stomatogastric or sympathetic) system that controls alimentary canal movements and is closely concerned with the process of neurosecretion. The second is the central nervous system, which coordinates the peripheral sense organs and muscles. Brain is a large group of neurons that lies above the esophagus. For that reason it is sometimes called the supraesophageal ganglion. Three parts are generally recognized. The most anterior section, called the protocerebrum, is the most complex part of the insect brain. It directs neural traffic at the crossroads between sensory input and motor output. At each side, optic lobes extend to the compound eyes; at its center, a pair of large mushroom bodies process olfactory information and control tasks that require visual coordination of locomotor activity and spatial orientation. The mushroom bodies and associated cells provide a structure for elaborate interconnections that allow learning and memory to occur. The second brain section, called the deutocerebrum, connects to the antennae. Its neurons are of two types—one type processes chemosensory information; the other, mechanosensory. The third and smallest part of the brain, the tritocerebrum, connects the central nervous system to the ventral nerve cord through the circumesophageal connectives. It also innervates the labrum (upper lip), pharynx (region between mouth and digestive system), and the rest of the visceral nervous system. The nervous system in social insects with fewer numbers of neurons compared to vertebrates regulates the behavioral changes adequately. The studies of neurophysiological mechanisms controlling the changes of behaviors are important to understand the functions of nervous system in social insects.

2. Material and methods

Apis Mellifera:

Frames of honey bee brood collected from hives at the Department of Zoology, University of Otago, were kept for periods of ≤1 wk in a humidified incubator at 35°C. Metamorphic adult development in the honey bee occurs over an 8- to 9-day period. Pupal honey bees at stages 4–6 (P4–P6) of the nine stages of metamorphic adult development were collected from the brood frames.

The stage of development was ascertained by using a method based on external cues, such as eye color and head pigmentation. The experiments described in this work comply with the laws of New Zealand regulating scientific research.

Whole Cell Recording:

Patch-clamp recordings in whole cell configuration were used to examine DA modulation of ionic currents in honey bee AL neurons in vitro. The voltage-gated and Ca2+-dependent currents examined in this study have been described in detail elsewhere. These currents include a transient A-type K+ current (I A), a sustained, delayed rectifier-like current (I KV), Ca2+-activated K+ currents (I KCa), a Ca2+ current (I Ca), and a rapidly activating transient TTX-sensitive current carried by Na+ (I Na).

Cells were viewed under an IMT-2 microscope (Olympus) using phase-contrast optics. All experiments were conducted at room temperature. Recording electrodes (2–3 MΩ) were prepared from borosilicate glass (100-µl micropipettes, 1.71 mm OD, 1.32 mm ID; VWR Scientific, West Chester, CA) using a Flaming-Brown micropipette puller (P-87, Sutter Instruments) and backfilled with a solution containing (in mM) 100 K-aspartate, 40 KF, 20 KCl, 2.5 MgCl2, 1 EGTA, 160 sucrose, and 10 HEPES (pH 7.2).

Isolation of Currents:

Whole cell current profiles were examined initially in the absence of any blocking agents. Components of the whole cell current profile were isolated using routine pharmacological techniques described elsewhere.

Na+ currents were blocked with TTX (10−7 M), whereas Ca2+ currents (and Ca2+-activated K+ currents) were blocked with 5 × 10−5 M CdCl2. Rapidly activating, transient (A-type) current (I A) was blocked with 4-aminopyridine (4-AP; 5 × 10−3 M), quinidine (5 × 10−5 M) was used to block the delayed-rectifier-like current, I KV, and K+ currents collectively were blocked by substituting K+ in the electrode solution with Cs+.

Statistical Analysis:

Repeated-measures ANOVA was performed to determine the overall effect of DA application. This accounted for any serial correlation between data recorded from the same cell over time. In groups of cells recorded in the absence of blockers, and in cell groups recorded in the presence of TTX and 4-AP, small numbers of missing values at the +8-min time-point were estimated using multiple regression techniques, where the values of control or DA-treated groups recorded over the initial 6-min recording period were used to predict missing 8-min data points.

Where significant overall, or group, effects were detected by ANOVA, Student's t -test for independent samples were performed post hoc to determine at which specific time-points the current amplitudes of DA-treated and untreated cell groups were significantly different. Student's t -test were also used to assess statistical differences in time-to-peak data.

Significance was accepted at $P = 0.05$ except when multiple comparisons were made, where the level of significance was reduced according to Bonferroni's correction. Mean values are expressed as mean ± SE. All analyses were performed using SPSS 11.0 (SPSS, Chicago, IL).

3. Results and Discussion

Current Profiles in Apis AL Neurons:

Based on the composition of outward currents, AL neurons examined in this study (n = 51) could be grouped into two main categories. In 71% of cells (n = 36), the outward current profile exhibited a rapidly activating transient component followed by a sustained component that showed little or no inactivation during the voltage step. With voltage steps above approximately −30 mV, the amplitude of the sustained outward currents in these cells increased linearly with each voltage step.

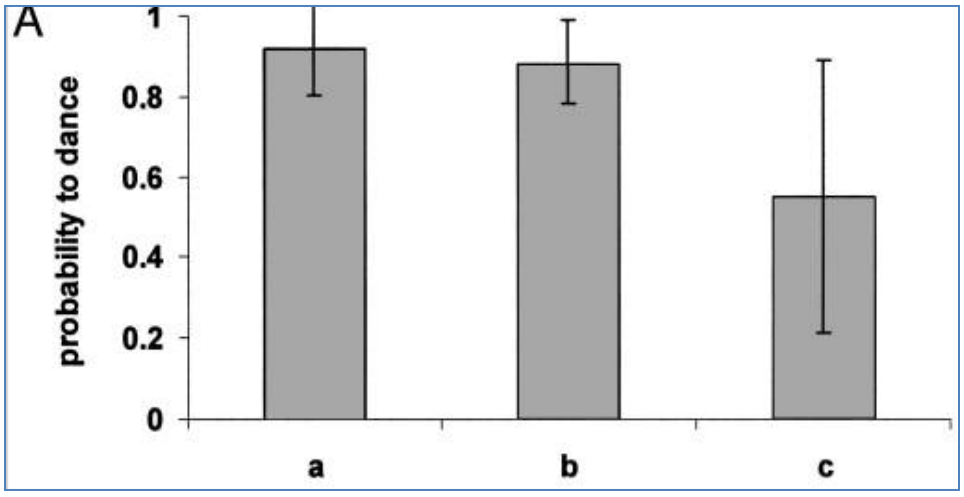
We assume therefore that type 1 current profiles originate from cells belonging to the second major category of AL neurons, namely local AL interneurons, but this has yet to be confirmed. Here, attention is focused predominantly on cells exhibiting type 1 current profiles, although small numbers of type 2 cells were found to be responsive to DA **.**

Effects of DA on Outward Current Profiles:

Effects of DA on ionic currents were examined initially in the absence of any channel blockers. Changes in the amplitude of outward currents in cells exposed to DA (DA-treated; n = 13) were compared with those observed in cells receiving no DA treatment (untreated, n = 18).

Emergence Probability and In-Hive Behavior:

We found no significant differences in the emergence probability among the three temperature groups (32°C, 100%; 34.5°C, 99%; 36°C, 98%). On emergence, none of the young adult bees exhibited obvious morphological defects or slow, uncoordinated movement of the type that have been described in bees raised at more extreme temperatures (2, 12–14). All of the bees raised at 32°C or 36°C introduced into the foster colony in the observation hive behaved apparently normally and started foraging after ≈2 weeks, just as their untreated control nest mates did. However, although the number of 36°C-treated bees did not decrease noticeably, fewer and fewer of those raised at 32°C were seen in the hive as time passed. Individuals treated at 32°C were seen to exit the hive on their normal orientation flights but then disappear. Their bodies were not found within the hive nor at the entrance, suggesting that they had suffered some subtle motor deficiency that prevented them from flying or feeding.



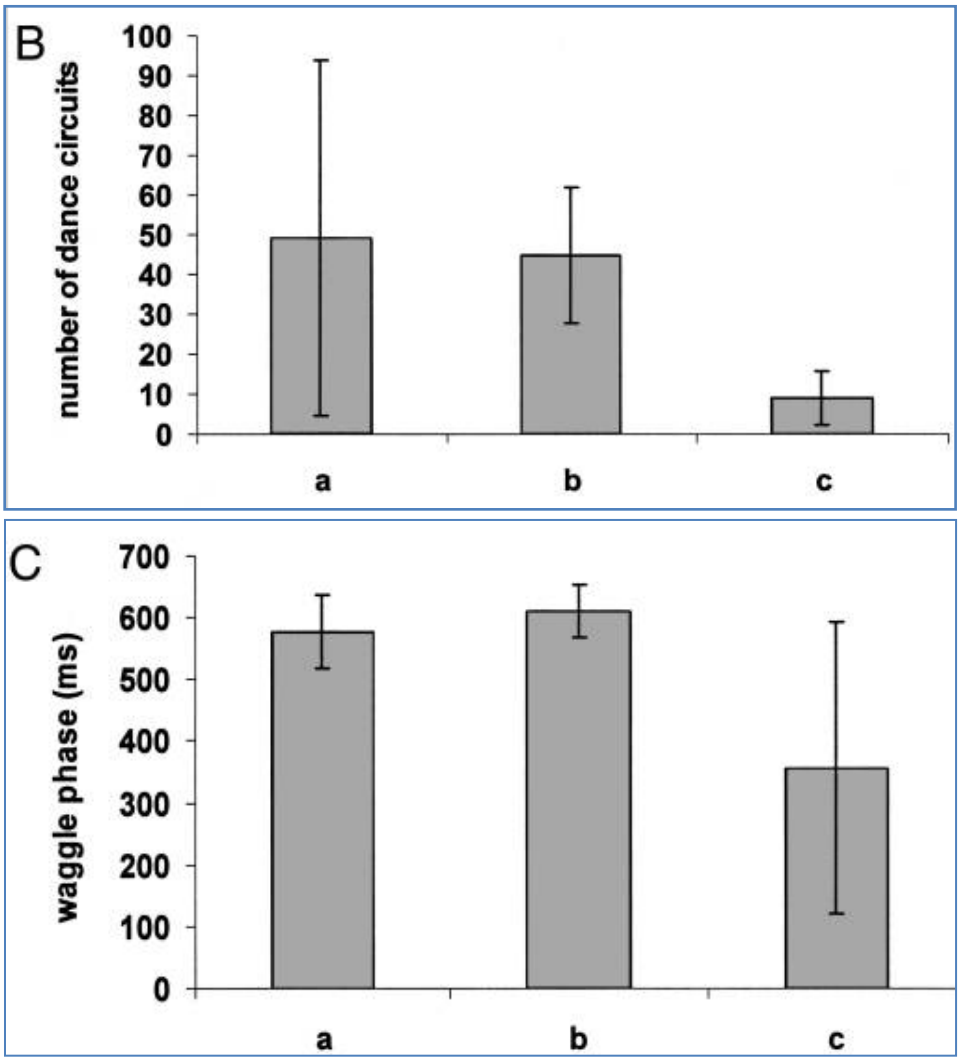


Figure 1: (A) Probability (mean ± SD) that bees would dance after a visit to the feeder. Column a, probability for bees raised naturally in a standard hive (25 visits from five foragers); column b, probability for bees that were treated as pupae at 36°C (25 visits from five foragers); column c, those treated at 32°C (20 visits from four foragers). There is no significant difference among the three groups. (B) Number of dance circuits (mean ± SD) performed by the bees after each visit to the feeder. Columns represent the same three groups as described in A. Column a, 23 dances from five foragers; b, 22 dances from five foragers; c, 11 dances from four foragers. There is a significant difference between the bees raised at 32°C (c) and those raised at 36°C (b). (C) Duration of the waggle phase (mean ± SD, ms) in the dances performed by the bees. Columns represent the same three groups as described in A. Column a, 1,127 dance circuits from five foragers; b, 1,224 dance circuits from five foragers; c, 176 dance circuits from four foragers.

Waggle Dance Performance:

Three groups of bees were compared. The first group consisted of five individuals from the foster colony in the observation hive that had been raised under natural conditions. These constituted the control

group. The second group consisted of five individuals of the 36°C-treated bees. The third group consisted of individuals of the 32°C-treated bees.

After the bees had made five visits to the feeder, we assumed that they had sufficient experience in terms of the orientation of the feeder and to the hive to convey information through the dance to dance followers.

The three criteria of the dance that were analyzed from the video recordings were the probability to perform waggle dances after a return from the feeder (Fig. 1A), the number of dance circuits (Fig. 1B), and the duration of the waggle phase (Fig. 1C). The three columns in each of the figures depict the performances of the five control bees that were raised under normal conditions (column a), the five bees raised at 36°C (column b), and the four bees that were raised at 32°C (column c), respectively. We could not discern any obvious differences in the behavior of the bees of the three groups in terms of moving in the hive, leaving the hive, or arriving and feeding at the feeders.

Learning and Memory Consolidation:

Because testing the learning and memory consolidation is far less time consuming than measuring the dancing behavior, we were able to include bees from all three temperature-treated groups, namely those raised at 32°C, 34.5°C, and 36°C. The learning behavior of bees raised under natural conditions has been studied extensively (19), so it was not considered necessary to repeat the experiments here with a group of control animals.

Each bee from each of the three temperature regimes was given one conditioning trial and then was tested for the conditioned response either 1 or 10 min after the conditioning trial. Both time intervals test for nonassociative (sensitization) or for associative learning and for the ability to consolidate memory.

The results of the learning and memory consolidation tests are shown in Fig. 2, and the results of the statistical analysis of these data are given in Table 1. We found that bees raised at 36°C performed significantly better at both test intervals than bees raised at either 32°C or 34.5°C (Fig. 2). The differences in response level between the 36°C group and the two other groups are similar to those found for what have been termed “good” and “bad” learners (ranges given in ref. 20: “bad” learners, 55–70% for 1 min and 50–68% for 10-min interval; “good” learners, 50–70% for 1 min and 72–80% for 10-min intervals). In the 1-min test, bees raised at 36°C showed a response level of 70%, and in the 10-min test, the response level increased to 85%, a performance that is close to the maximum expected in the conditioning of the proboscis extension. There were no significant differences in the response level between the bees reared at 32°C or 34.5°C at both time intervals tested.

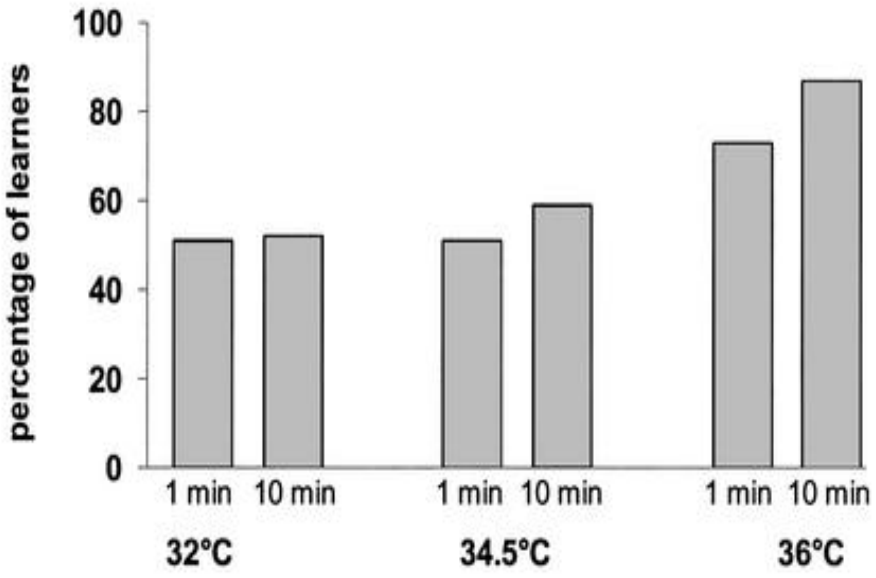


Figure 2: Proboscis-extension reflex response 1 or 10 min after one-trial odor conditioning in bees, the pupae of which were reared at 32°C, 34.5°C, and 36°C. Columns represent the percentage of responders after 1 min (55 bees raised at 32°C, 43 raised at 34.5°C, and 40 raised at 36°C) or 10 min (58 bees raised at 32°C, 54 raised at 34.5°C, and 23 raised at 36°C).

Table 1:

Statistical χ^2 test on the data presented in Fig. 2

Temperature, °C	1 min	10 min	1 min vs. 10 min
32			$P = 0.93$
32 vs. 34.5	$P = 0.98$	$P = 0.42$	
34.5			$P = 0.42$
34.5 vs. 36	$P = 0.04$	$P = 0.01$	
36			$P = 0.18$
32 vs. 36	$P = 0.04$	$P = 0.014$	

$P < 0.05$, significant

4. Conclusion

Reports describing the outcome of I KCa modulation in other invertebrate systems provide clues as to likely effects of DA modulation of Ca2+-activated K+ currents on the excitability of Apis AL neurons. In molluscan central pattern generator neurons, serotonin-induced attenuation of spike afterhyperpolarization, attributed to a reduction in I KCa amplitude, promotes repetitive spiking in these

neurons. Applying DA to isolated pyloric dilator neurons in the lobster stomatogastric ganglion, on the other hand, increases the amplitude of total I KCa contributing to an increase in interspike interval and a reduction in action potential frequency in these neurons. These results, together with reports in other systems suggest that DA-induced attenuation of I KCa will increase the excitability of honey bee AL neurons.

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