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EFFECTS OF STRESS, AGE, SEASON, AND SOURCE COLONY ON LEVELS OF OCTOPAMINE, DOPAMINE AND SEROTONIN IN THE HONEY BEE (*APIS MELLIFERA* L.) BRAIN

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Abstract—The effects of environmental and genetic factors on levels of octopamine, dopamine and serotonin in brains from worker honey bees (*Apis mellifera* L.) were measured using high-performance liquid chromatography with electrochemical detection. Exiting foragers were stressed by clamping their legs, which resulted in peak elevation of octopamine and serotonin after 10 min. Significant seasonal differences in levels of all three biogenic amines were found for bees sampled from an observation colony during the spring, summer and autumn of 1990. Levels of the amines were highest during June–September, corresponding to high levels of colony foraging activity. These differences may reflect seasonal changes in colony nutrition, population size, or brood-rearing activity. The levels of all three amines were significantly lower in the brains of newly emerged bees than in brains from randomly aged worker bees from the same colony. Since the total protein content in bee brains was not different for bees from these two groups, age-related differences were not related to brain growth. Significantly different levels of octopamine, dopamine and serotonin were detected among workers in colonies that contained unrelated queens.

Key Word Index: Octopamine; dopamine; serotonin; *Apis mellifera*

INTRODUCTION

Insect nervous systems contain high levels of octopamine, dopamine and serotonin or 5-hydroxytryptamine (Evans, 1980, 1986). Octopamine has been the most widely studied because of its relationship to the insect "fight or flight" response. It regulates lipid and carbohydrate mobilization (Orchard *et al.*, 1981, 1982, 1983; Downer *et al.*, 1984; Pannabecker and Orchard, 1986). It serves as a neuromodulator of neuromuscular transmission and muscle contraction in insect skeletal muscle (Evans and O'Shea, 1978; Evans, 1981) and controls insect visceral muscles in the gut and ovaries (Orchard and Lange, 1987). Octopamine also regulates flight muscle metabolism (Candy, 1978). It initiates light emission from the firefly light organ (Nathanson, 1979), and mediates some insect behaviours (Brookhart *et al.*, 1988; Mercer and Menzel, 1982).

Levels of octopamine in the insect central nervous system also change in response to metamorphic development (Fuzeau-Braesch *et al.*, 1979; Bodnaryk, 1980; Woodring *et al.*, 1988), starvation (Davenport and Evans, 1984a), flight (Goosey and Candy, 1980; Bailey *et al.*, 1984) and handling stress (Downer, 1979; Davenport and Evans, 1984b; Woodring *et al.*, 1988, 1989).

In honey bees (*Apis mellifera* L.), large amounts of octopamine, dopamine, serotonin and other neuroactive compounds (acetylcholine, γ-amino butyric acid, glutamate, tryptophan and kynurenine) are found in the central nervous system (Mercer *et al.*, 1983; Mercer, 1987; Fuchs *et al.*, 1989). Octopamine affects the behaviour and responses of bees to odours (Mercer and Menzel, 1982; Mercer, 1982). Tryptophan and serotonin depress neural (central and peripheral systems) and behavioural activity while kynurenine stimulates central nervous system activity in bees (Lopatina and Dolotovskaya, 1984; Lopatina *et al.*, 1985).

The present study represents a preliminary evaluation of environmental and genetic factors that affect levels of biogenic amines in the brains of honey bees. Our immediate goal is to demonstrate that handling stress, season, worker age and queen mother influence the levels of biogenic amines

found in the cerebral cortex of honey bee workers. Consequently, such factors must be considered when designing experiments examining behavioural relationships to these neuroactive compounds.

MATERIALS AND METHODS

Biogenic amine detection and quantitation

Octopamine, dopamine and serotonin were separated through a C_{18} reverse phase column (4.6 × 100 mm Alltech column with Adsorbosphere packing) and detected simultaneously using two coulometric detectors (ESA Model 5000) in series; the first set to a potential of 300 mV (for dopamine and serotonin) and the second to 700 mV (for octopamine). A running buffer consisting of 3.35 g chloroacetic acid, 6.0 g monobasic sodium phosphate, and 0.5 g sodium dodecyl sulphate dissolved in 720 ml water, 200 ml acetonitrile, and 80 ml methanol (pH 3.0–3.1) was run isocratically (1 ml/min).

Biogenic amines were identified and quantified by comparison of peak areas with known standards. We used 3,4-dihydroxy-benzylamine (DHBA) as an internal standard for each sample. Spiking samples with standards and altering the running buffer were methods employed to confirm sample peak identities.

Brains (cerebral ganglia) were removed from bee heads in cold bee saline (0.2 g KCl, 0.2 g $CaCl_2$, 4.0 g saccharose and 9.0 g NaCl per litre). Only cleanly dissected brains with intact connective membranes were used to avoid loss of biogenic amines from damaged tissue. Only 3–5 min was needed to remove a brain from a bee. Brains were placed (3 brains/sample) into 50 μl of 0.4 N perchloric acid in 1.5 ml Eppendorf tubes, sonicated for 15–20 s, centrifuged at 10,000 *g* for 15–20 min, and filtered through Amicon YMT membrane filters (at 2500 *g* for 15 min) to remove large proteins. The samples were then frozen (−20°C) until needed, and tests indicated no loss of amine content in frozen samples over a 3–4 week period. Just prior to analysis, samples were thawed and the supernatant injected directly onto the high-performance liquid chromatography column.

Workers of different ages

To reduce variation in worker bees that might be related to different drone fathers, a heterozygous cordovan honey bee queen was instrumentally inseminated with a single, unrelated cordovan drone. One expects 50% of the resulting brood to be phenotypically wild type and 50% to be cordovan. Only the cordovan workers were sampled to avoid sampling wild-type workers that may have drifted into the colony from other colonies. No other cordovan source colonies were in close proximity to the colony. Brain samples were collected from newly emerged cordovan bees (<24 h old) that had been collected from brood combs in the laboratory and from randomly aged cordovan bees that were picked with forceps from the top bars of the colony brood nest on the same day that the young bees had emerged. Bees were cold immobilized prior to removing their brains. The total protein content of brains from newly emerged and randomly aged bees was compared using the Biorad protein assay (Bradford, 1976).

Seasonal sampling

Four sampling periods were used: 26 April–7 May, 25 June–9 July, 28 August–10 September, and 27 October–4 November. During each period, 15–20 samples (3 brains/sample) of non-agitated (see below) bee brains were collected from an observation colony maintained in the laboratory. All samples were collected during the middle of the day (10:00 a.m. to 3:00 p.m.).

Establishment of different colonies

All colonies were established from 1.5 lb packages of a homogeneous mixture of bees that had been shaken from several source colonies. Each new colony was given a queen that had been inseminated with a single, unrelated drone. Bees were not sampled from the colonies until after the queens had laid several complete brood cycles to ensure that all bees from a single colony were genetically uniform and that none of the unrelated bees that had been used to establish the colonies were included in the samples.

Collection of stressed and non-stressed bees

An observation colony containing two standard depth Langstroth wax combs, between 3500–5000 worker bees and a young, naturally mated queen was maintained in the laboratory. The colony was supplemented with honey during long periods of dearth to avoid starving the bees. The colony was connected to the outside by two adjacent pieces of Tygon tubing (13 mm i.d.) through holes cut through a window.

Non-stressed bees (0 min) were collected by disconnecting a length of the exit tubing as bees were following their footprint pheromone path to the outside exit. The tube could be disconnected, and if handled gently, the bees were not visibly agitated before being anaesthetized with carbon dioxide (≤1 min exposure).

Stressed bees were collected from the exit tubes by grabbing their legs with forceps, and stress was maintained by pinching and holding their legs with an alligator clamp. Bees were stressed for 0, 3, 10 or 20 min. We did not stress them beyond

20 min because they showed a reduction in stressed behaviour (biting, twisting and sting extrusion).

Statistical treatment of the results

Multiple comparisons were made using the least significant difference mean separation test (SAS) after significant differences in biogenic amine levels for the stress, season and colony data were indicated by analysis of variance (SAS). The Student's t-test was used to compare biogenic amine levels and total protein content of brains between newly emerged and randomly aged bees (SAS).

RESULTS

Age-related changes in biogenic amine levels

Brain levels of octopamine ($P = 0.0224$), dopamine ($P = 0.0017$) and serotonin ($P = 0.0037$) were significantly lower in newly emerged worker bees ($\leqslant 24$ h) when compared to their randomly aged sisters (Fig. 1). Since the total protein content in brains from workers of both age groups were not significantly different ($P = 0.8280$), the increased biogenic amine levels in older bees must be attributed to something other than growth of the brain.

Seasonal variation in biogenic amine levels

Significant seasonal differences for levels of octopamine ($P \leqslant 0.0001$), dopamine ($P = 0.0003$) and serotonin ($P \leqslant 0.0001$) were found in worker bee brains (Fig. 2). Amine levels were lowest during the spring (April–May) and autumn (October–November) and highest during the summer (June–July and August–September). March–June corresponds to high levels of colony activity in regard to population growth and nectar foraging in Louisiana (Harbo, 1986). Winter bees (December–February) were not sampled.

Source colony variation in biogenic amine levels

Levels of octopamine ($P = 0.0001$), dopamine ($P = 0.0020$) and serotonin ($P = 0.0334$) varied significantly between the five colonies (Fig. 3). Levels of octopamine were directly related to levels of serotonin. For example, the highest octopamine and serotonin levels were seen in colonies E and B, and the lowest levels for both amines were seen in colonies C and D (Fig. 3). Levels of dopamine seemed to be unrelated to octopamine or serotonin levels.

Stress-induced changes in biogenic amine levels

Handling stress elevated levels of all three biogenic amines in worker honey bee brains (Fig. 4). Octopamine levels showed the greatest increase; the levels doubled ($P = 0.0131$) after 10 min of stress and started to decrease after 20 min. Serotonin levels increased to a lesser, but statistically significant amount ($P = 0.0004$) after 10 min. The apparent elevation in dopamine was not statistically significant ($P = 0.0961$).

DISCUSSION

The results clearly indicate that the levels of biogenic amines in the brains of honey bees are influenced by at least four critical factors. These are the

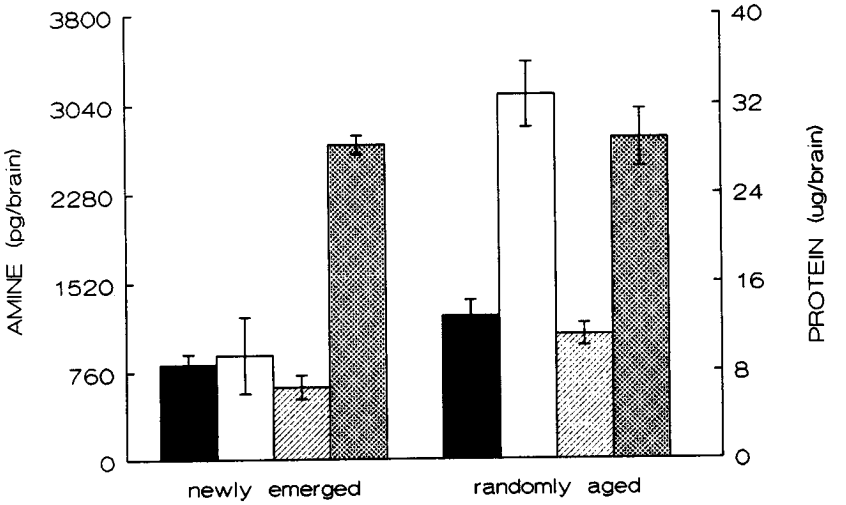

Fig. 1. Effects of age on biogenic amine levels in the brain of *Apis mellifera* L. (left axis). The solid column is octopamine; the open column is dopamine, and the striped column is serotonin. Each amine column represents the average (mean ± SE) of 12–15 samples (3 brains/sample). All three amines were significantly lower in newly emerged bees ($P < 0.05$). Protein content of the brain (right axis) did not change with increased age (dotted column). Each protein column represents the average (mean ± SE) for 10 individuals.

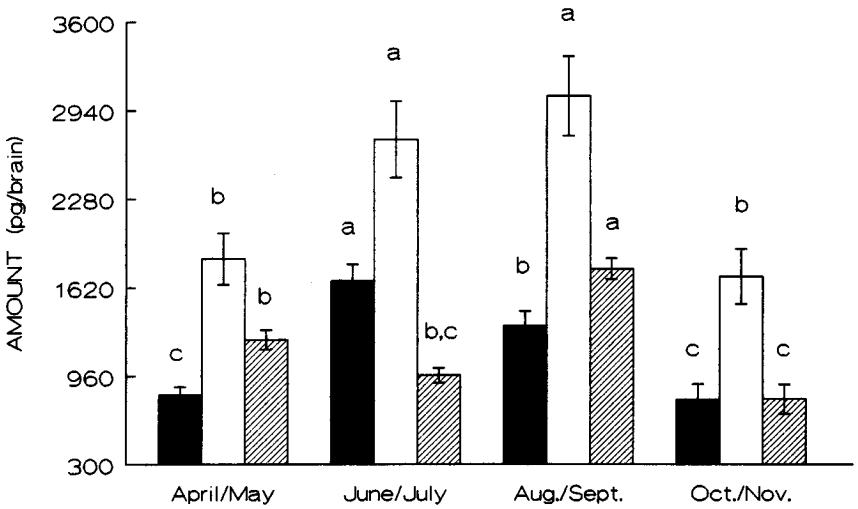

Fig. 2. Effects of season on biogenic amine levels in the brain of *Apis mellifera* L. The solid column is octopamine; the open column is dopamine, and the striped column is serotonin. Each column represents the average (mean ± SE) for 14–20 samples (3 brains/sample). For each amine, columns with the same letter are not significantly different ($\alpha = 0.05$).

source colony, the season of the year, the age of the bee, and the extent to which the bee is stressed. This baseline information indicates that these factors must be considered in any study of biogenic amines, particularly in studying the role of biogenic amines in insect behaviour.

Fuchs *et al.* (1989) found that the levels of various neuroactive compounds were different in brains from adult worker honey bees of different ages. In their study, glutamate and GABA levels were found to correlate with worker age, while serotonin and dopamine did not. The current study differs in that levels of dopamine and serotonin were found to be different between very young bees and older bees.

An increase in the optic lobe octopamine content of recently emerged *Manduca* and the pharate noctuid *Mamestra configurata* is thought related to an increased sensory input and behavioural repertoire of the adult moth (Bodnaryk, 1980; Klassen and Kammer, 1985; Davenport and Wright, 1986). The increased levels of all three biogenic amines in the brains of honey bees during periods of high colony activity might also be related to increased behavioural repertoire associated with foraging activities. Insufficient ages of worker bees were

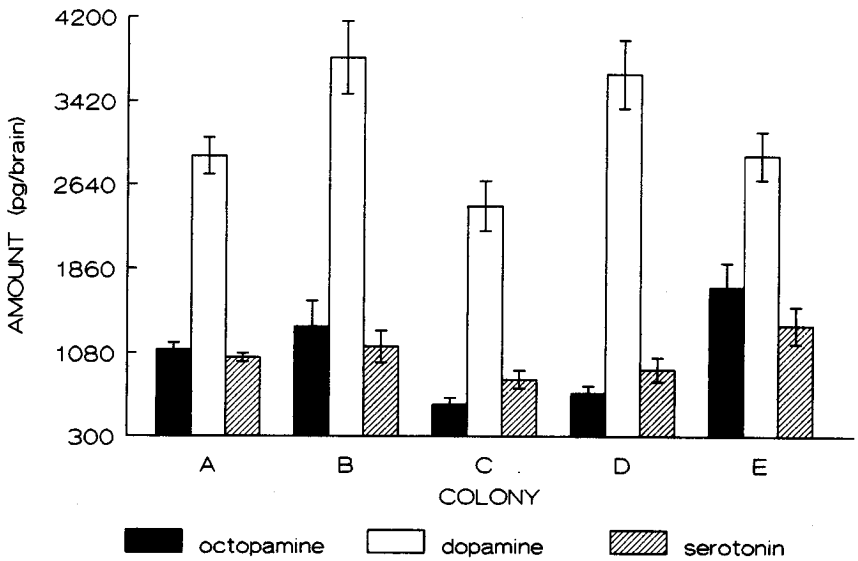

Fig. 3. Effects of source colony on biogenic amine levels in the brain of *Apis mellifera* L. Five colonies were sampled (A–E). The solid column is octopamine; the open column is dopamine, and the striped column is serotonin. Each column represents the average (mean ± SE) for 6–12 samples (3 brains/sample). For each amine, columns with the same letter are not significantly different ($\alpha = 0.05$).

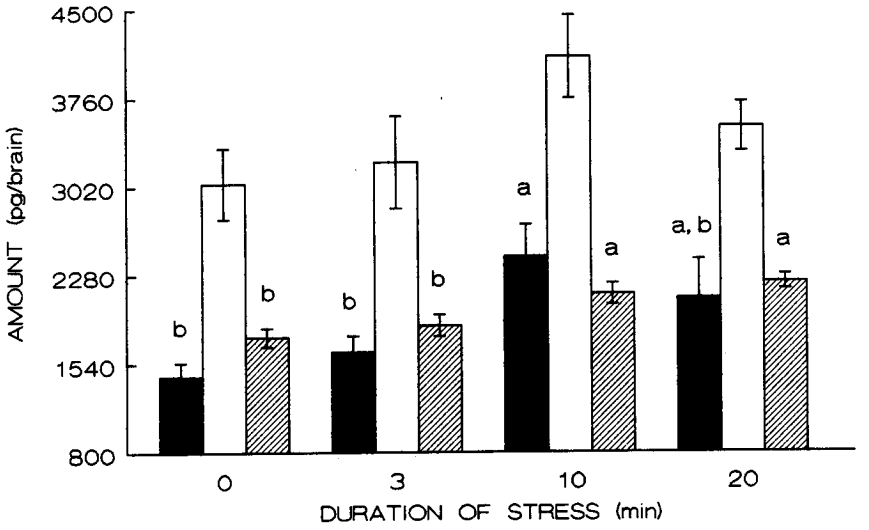

Fig. 4. Effects of stress on biogenic amine levels in the brain of *Apis mellifera* L. The solid column is octopamine; the open column is dopamine, and the striped column is serotonin. Each column represents the average (mean ± SE) of 13–15 samples (3 brains/sample). For octopamine and serotonin, columns with the same letter are not significantly different ($\alpha = 0.05$). Dopamine levels did not change significantly with stress ($\alpha = 0.05$).

checked to determine a possible relationship between biogenic amines and age-related tasks that are mediated by juvenile hormone (Robinson and Ratnieks, 1987; Robinson *et al.*, 1989), but more experiments are in progress.

The changes in biogenic amine levels of bees at different times of the year might be related to the colony's nutritional state, population size or levels of stress related to a variety of factors. Many other physiological parameters are affected by season. Seasonal changes in fat-body stores and blood sugar content have been noted for both workers and queens (Shehata *et al.* 1981). Worker survival is related to colony size and brood-rearing rate (Harbo, 1986), and brood rearing is directly related to seasonal changes in abundance of nectar and pollen (Harbo, 1986; Newton and Michl, 1974). Juvenile hormone III, thought to control age-related behavioural and physiological changes in bees (Fluri *et al.*, 1982; Robinson *et al.*, 1989), continuously increases in the blood of ageing summer bees but remains low in winter bees (Fluri *et al.*, 1982).

Both the increased level of biogenic amines in older bees and the higher levels in bees during the season of high foraging activity support the hypothesis relating biogenic amines levels to behaviour. There are several studies that have examined the effects of various amines on honey bee behaviour. Mercer and Menzel (1982) found that dopamine and serotonin injected into the brain reduced the percentage of bees responding to a conditioned olfactory stimulus, while octopamine enhanced the degree of responsiveness. The electric potentials caused by stimulation of the antennae with air or scents are reduced by dopamine, while octopamine increased potentials formed in the α-lobes of the mushroom bodies in response to light (Mercer, 1982).

The roles of serotonin, tryptophan and kynurenine in controlling honey bee behaviour were examined in bees expressing different chemotypes of the snow group of eye-colour mutations of the tryptophan–kynurenine–ommochrome pathway) (Lopatina and Dolotovskaya, 1984; Lopatina *et al.*, 1985; for a review of the honey bee eye mutants see Tucker, 1986). These studies showed that serotonin and tryptophan depress neural (central and peripheral systems) and behavioural activity while kynurenine stimulates central nervous system activity in bees.

The variation found in colonies with different genetic backgrounds is potentially the most interesting result of this study. It has been clearly established that the Africanized honey bee is genetically (Page, 1989) and behaviourally (Collins *et al.*, 1982) different from the European honey bee races. Our results present some evidence that at the effector level, the genes responsible for augmented defensive behaviour may be mediated via biogenic amines in the brain. The increased octopamine levels in the bee brain in response to stressing the bees lends further support to the idea that "fight or flight" responses in insects are mediated by octopamine (Matthews and Downer, 1974). In several species the octopamine titres in both the blood and brain increase in response to various kinds of stress (Downer, 1979; Davenport and Evans, 1984a; Woodring *et al.*, 1988, 1989), and it is

possible that the central nervous system of Africanized bees has higher levels of various biogenic amines or responds to them differently than less defensive races of bees.

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