Journal of Apicultural Research 35(2): 69–78 (1996)

Effects of carbon dioxide on levels of biogenic amines in the brains of queenless worker and virgin queen honey bees (*Apis mellifera*)

JEFFREY W HARRIS[1]; JOSEPH WOODRING[1]; JOHN R HARBO[2]

[1]Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803, USA

[2]USDA-ARS Honey Bee Breeding, Genetics and Physiology Laboratory, 1157 Ben Hur Road, Baton Rouge, LA 70820, USA

(Received 15 September 1994, accepted subject to revision 9 November 1994, accepted for publication 20 September 1996)

Keywords: queen honey bees, worker honey bees, *Apis mellifera*, brain, feeding pollen, CO_2 narcosis, amines, dopamine, octopamine, serotonin, tryptophan, tyrosine, ovarian development

SUMMARY

Several experiments were conducted to examine the effects of exposure to CO_2 or CO_2 containing 20% O_2 on the amounts of biogenic amines in the brain, and on development of ovaries in worker and queen honey bees. When workers were fed pollen and exposed to CO_2, their ovaries were less developed and brain dopamine (DA), tryptophan (TRP) and tyrosine (TYR) levels were significantly reduced *versus* untreated controls that were also fed pollen. If workers were not fed pollen and exposed to CO_2, the differences in brain amine content between untreated controls and CO_2-treated workers were not significant; however, a significant reduction in brain TRP and TYR levels through time was found for both groups of workers. Treatment with CO_2 stimulated free-running queens to lay eggs sooner than untreated free-running queens, but CO_2 narcosis had no significant affect on queen brain chemistry. However, the brain chemistry of caged queens (many queens caged within a single colony) responded differently from free-running queens. Treatment with CO_2 caused a 45% reduction in dopamine levels in the brains of free-running queens, whereas dopamine levels of caged queens were not affected by treatment with CO_2. This study shows that the brain dopamine levels of workers and queens do not change to the same degree in response to CO_2 treatment. For workers, this study suggests a positive relationship between brain dopamine and the extent of ovarian development.

INTRODUCTION

Although CO_2 has been shown to greatly influence insect behaviour and physiology, insects are frequently immobilized with CO_2 during routine studies by biologists. Treatment with CO_2 has been shown to affect insect reproduction (Engels *et al.*, 1976), development (Woodring *et al.*, 1978), feeding (Birkenmeyer & Dame, 1970; Woodring *et al.*, 1978) and other behaviour (Ralph, 1959; Whisenant & Brady, 1965; Mardan & Rinderer, 1980; Schneider & Gary, 1984). Woodring *et al.* (1978) showed that in crickets (*Acheta domesticus*) the effects of short-term exposures to CO_2 are associated with a true anaesthetic effect on nervous tissue, while longer exposures cause asphyxiation that leads to anoxia of tissues. The anaesthetic effect results from the direct diffusion of CO_2 gas to nerve cells followed by a reduction in neuroplasmic pH resulting from the conversion of CO_2 to carbonic acid (Edwards & Patton, 1965) and not by the drop in haemolymph pH associated with exposure to CO_2 (Woodring *et al.*, 1978).

In honey bees (*Apis mellifera*), exposure to CO_2 is frequently used during the instrumental insemination of queens (Mackensen, 1947). Exposure to CO_2 increases vitellogenin titres in the haemolymph and accelerates ovarian development and eventual oviposition in queens (Engels *et al.*, 1976; Engels & Ramamurty, 1976). In unmated queens aged 5–20 days old, the rate of vitellogenin synthesis rarely exceeds 30% of total protein synthesis; however, within 24 hours of two exposures to CO_2 and introduction into a colony, the vitellogenin synthesis rate increases to 60% of all protein synthesis (Engels *et al.*, 1976). Virgin queens and instrumentally inseminated queens not given CO_2 begin egg laying when 18–67 days old. Queens given two exposures to CO_2 and introduced into colonies begin egg laying when 11–23 days old (Mackensen, 1947).

In worker honey bees, exposure to CO_2 inhibits ovarian development. Fyg (1950) found that 67% of the worker bees in queenless cages developed ovaries after four weeks, but if they were given a single exposure or a series of three exposures to CO_2, only 5% of the workers had developed ovaries. Biedermann (1964) and Kropacova *et al.* (1968) also found that exposure to CO_2 reduces ovary development in workers, and that workers receiving CO_2 eat less pollen than untreated workers (see also Harris & Harbo, 1990). Kropacova *et al.* (1968) found that groups of queenless bees allowed to eat pollen for a 3-day period prior to exposure to CO_2 will begin developing ovaries, but after narcosis, ovary development slows or stops. In contrast, Harris and Harbo (1990) found that a single 15 min exposure to CO_2 was effective at inhibiting ovary development only if given when the worker bee was less than 3 days old. If workers are not given pollen until after narcosis, ovarian development remains low.

Although the effects of CO_2 on the reproductive systems of queen and worker honey bees are well known, the mechanism leading to these effects is unknown. These effects on reproduction are, at least initially, most probably mediated by changes in the nervous system triggered by exposure to CO_2. As an example of neuroendocrine functions affected by narcosis, exposure to CO_2 increases the volume of the corpora allata and the titre of haemolymph juvenile hormone (JH) in worker honey bees (Bühler *et al.*, 1983). Unlike many orthopteran insects in which JH directly stimulates vitellogenesis and oogenesis (Hentschel, 1981), JH has an atypical, indirect role in reproduction in worker and queen honey bees (Robinson, *et al.*, 1991) but has a pronounced role in the control of age-related behavioural patterns in worker bees (Robinson *et al.*, 1989).

Other mechanisms to explain the effects of CO_2 on honey bee behaviour and reproduction might initially involve changes in biogenic amine neurotransmitters and neuromodulaters in the brain. Harris and Woodring (1995) showed that brain dopamine (DA) levels are higher in bees from queenless colonies than in bees from queenright colonies and that brain DA levels were correlated with ovariole width in queenless worker bees.

The purpose of the current study is to test the effects of exposure to CO_2 on brain levels of octopamine (OA), dopamine (DA) and serotonin (5HT) and some of their precursors (tryptophan (TRP) and tyrosine (TYR)) in worker and queen bees. Because brain DA levels have been related to ovarian development in workers, CO_2 narcosis could alter levels of DA and/or other neuroactive compounds.

MATERIALS AND METHODS

Biogenic amine detection and quantification (HPLC analysis)

OA, DA, 5HT, TRP and TYR were separated as previously described (Harris & Woodring, 1992) through a C_{18} reverse phase column (4.6 × 100 mm Alltech column with Adsorbosphere matrix) and detected simultaneously using two coulombmetric detectors (ESA Model 5000) in series; the first set to a potential of 300 mV (for DA and 5HT) and the second to 700 mV (for OA, TRP and TYR). A running buffer consisting of 3.35 g monochloroacetic 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 with a constant flow rate (1 ml/min).

Biogenic amines were identified and quantified by comparison of peak areas with known standards. We used 3,4-dihyroxy-benzylamine (DHBA) as an internal standard for each sample. Spiking samples with standards and altering the running buffer were methods employed to confirm peak identities. Analog output from our ESA detector was connected to a computer through an analog-digital converter, and peak areas were quantified using the Shimadzu EZChrom chromatography data system software.

Brains (cerebral ganglia minus the optic lobes) were dry dissected from honey bees that had previously been flash-frozen in liquid nitrogen during sampling. Single brains were placed into 100 µl of running buffer in 1.5 ml Eppendorf tubes, sonicated for 15–20 s, centrifuged at 10 000 g for 15–20 min, and the supernatant was stored at –70°C until high performance liquid chromatography (HPLC) analysis.

CO_2 treatment of queenless workers given pollen

Fifty newly-emerged bees (≤ 15 h old) were placed into each of 15 wooden cages (7.5 × 11 × 12.5 cm^3) similar to those used by Kulinčevic and Rothenbuhler (1973) during early August 1993. The bees used were the progeny of a naturally-mated queen. Each cage was supplied with 50% sucrose solution and water in gravity feed vials. Pollen was available *ad libitum* in small plastic trays on the floor of each cage. The pollen fed to the bees had been collected two months prior to this experiment (and frozen until needed) from several field colonies. Five cages were randomly assigned to each of three treatments: (1), controls received no narcosis; (2), cages exposed for 10 min to CO_2 narcosis; and (3), cages exposed for 10 min to a mixture of CO_2 containing 20% O_2. All cages were kept in an incubator held at 31° ± 0.5°C and 65% relative humidity (RH) throughout the experiment.

All narcoses were applied on the third day of the experiment. Each test gas (CO_2 or CO_2/O_2) was administered at room temperature (*c.* 23°C) by placing the cages in a clear plastic bag and quickly filling the bag with test gas. Timing of the 10-min exposure began when the first bees fell to the bottom of their cages. After the bag was fully expanded, a flow rate of 50 ml/min was maintained to keep positive pressure within the bag during the exposure.

Brains were removed for HPLC analysis of biogenic amine content from a subsample of three bees from each cage on the eighth day of the experiment. A hierarchial analysis of variance for a completely randomized design was used to compare amine levels between the three treatment groups. We tested for potential effects of individual cages on variation in amine content within the main treatment effects. Following the rules for pooling variances given by Bancroft and Han (1983), cage effects were pooled with the random error term to estimate a common variance when appropriate, and the main treatment effects were retested with more power (SAS). The Tukey's HSD was used for mean separations after significant differences ($\alpha = 0.05$) in amounts of an amine were found between treatments (SAS).

CO_2 treatment of queenless workers kept without pollen

Each of twenty-four wooden cages (same as in the previous experiment) were given 50 newly-emerged (≤ 15 h old) bees and supplied water and 50% sucrose *ad libitum* during the middle of September 1993. The bees used in this experiment were from the same colony as used in the previous experiment. The cages were given no pollen throughout the experiment. Twelve cages were treated to a single 10-min exposure to CO_2 on the third day, while the remaining 12 cages were not treated. Cages were held in an incubator for 4, 6 or 8 days. On each sampling day, the brains from three bees were sampled for HPLC analysis from each of four control cages and four cages that had been treated with CO_2. Levels of OA, DA, 5HT, TYR and TRP were compared between the two different treatments and the three sampling days using an ANOVA that included a main treatment term (TRT), a sampling day term (DAY) and an interaction term (TRT*DAY) (SAS).

CO_2 treatment of queens that were free-running or caged

During June 1994, virgin queens of the same age were obtained by grafting larvae from two different lines or stocks of bees. A single queenless colony was used to rear the queen cells from both stocks, and the capped cells were removed to individual vials stored in an incubator. A 2 × 2 × 2 factorial arrangement of treatments was used in a completely randomized designed to test the effects of: (1), CO_2-treatment *versus* untreated controls; (2), differences between the two stocks; and (3), the effects of caging queens *versus* allowing them to run freely within small colonies on brain biogenic amine content. We included the third factor because the practice of caging queens for extended periods of time reduces their acceptability to workers (Woyke, 1988, 1989) and may affect reproductive potential. The statistical analysis included consideration of all possible main factors and interaction terms (SAS).

A total of 23 queens (11 from one stock (A) and 12 from the other stock (B)) were treated with a 15 minute CO_2 narcosis on the 11th and 12th days after emergence as adults. Eleven of the treated queens were each placed in a small cage (four from stock A and seven from stock B) and stored in a single, queenless colony. Each of the remaining 12 treated queens had been introduced into a small colony as a pupa in a queen cell. Each queen was captured to be marked and treated with CO_2 and subsequently returned to their colonies. (Colonies were started from a homogeneous mixture of bees shaken from several field colonies on 3 June 1994. Each small colony was provided two frames of capped honey, one frame of capped brood and about 6000 bees. Queen excluders were secured across the colony entrances to prevent mating flights by the queens.) An additional 21 queens (10 queens from stock A and 11 from stock B) were not treated with CO_2. Of these, 12 queens (four from stock A and eight from stock B) were caged and placed in the same colony used for the CO_2-treated and caged queens. The remaining nine queens (six from stock A and three from stock B) were introduced into small colonies as queen cells, and they were

captured and marked in the same manner as the CO_2-treated, free-running queens. The colonies used were from the same mixture of bees used for the CO_2-treated queens. All colonies were monitored for the appearance of eggs through a total of 23 days when the brains from all queens were removed for HPLC analysis.

CO_2 treatment of workers kept with and without queens

During late April–early May 1995, an experiment was conducted to test the effects of CO_2 narcosis on the brain biogenic amine content and ovarian development of workers kept with or without naturally-mated queens in incubator cages. Fifty newly-emerged bees (≤ 15 h) from a single source colony containing a naturally-mated queen were placed into each of 12 incubator cages. Each cage was provided with a small piece of wax foundation fastened to the wall of the cage, and 50% sucrose and water were provided by gravity feed vials. Six cages were given young naturally-mated queens (all queens were sisters) and the remaining six cages were kept queenless. Within each of these sets, three cages were treated with a 15-min exposure to CO_2 (as in the first experiment) on the first day, while the remaining three cages were not treated. The queens were not treated with CO_2 and were introduced after the workers began to recover from the narcosis. Cages were held in an incubator (at 31° ± 0.5°C and 65% RH) for 10 days.

Ten days after narcosis four workers were sampled from each cage for measurements of brain biogenic amine content and the extent of ovarian development. Brains from the workers were prepared for HPLC analysis before the ovaries were removed. Both the left and right ovaries were classified using a grading scale previously described by Harris and Harbo (1990). Level 1 ovaries were those with the most undeveloped ovarioles; level 2 were those with rounded to bean-shaped eggs; level 3 ovaries were those with more elongated, sausage-shaped eggs. The sum of the grading levels for the right and left ovaries of each bee was used in the statistical analysis of effects of CO_2 and the presence or absence of a queen on worker ovary development. Ovarian development and biogenic amine levels were then compared between treatment groups using an analysis of variance that incorportated the 2×2 factorial arrangement of treatment levels with the effects of individual cages nested in the main treatments (SAS).

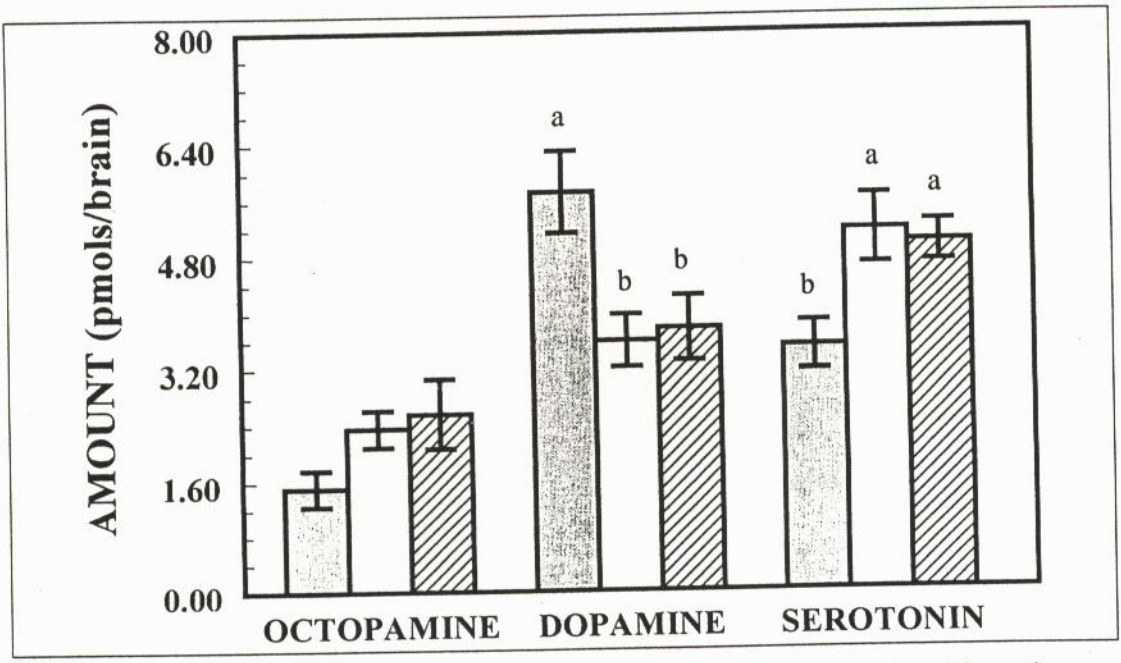

FIG. 1. Effects of CO_2 narcosis on brain biogenic amine levels in workers fed pollen. Fifty newly-emerged bees were placed into each of 15 incubator cages, and each cage was given either (1) no CO_2 (gray bars), (2) a 10-min exposure to CO_2 (open bars) or (3) a 10-min exposure to a mixture of CO_2 and 20% O_2 (striped bars) (5 cages per treatment). The CO_2 treatments were given on the third day of the experiment, and brains were sampled on the eigth day of the experiment. Three bees were sampled from each cage for amine analysis. Octopamine levels were not significantly different between treatments ($\alpha = 0.05$). For serotonin and dopamine levels, treatment mean (± s.e.) bars having the same letter do not differ as determined by the Tukey's mean separations test.

RESULTS

Effects of CO_2 narcosis on workers given pollen

Exposure to CO_2 significantly affected brain levels of two of the three amines measured (fig. 3). OA levels were not significantly different among the three treatments ($F = 1.93$; d.f. = 2,12; $P > 0.18$), but 5HT levels ($F = 10.81$; d.f. = 2,12; $P < 0.01$) were significantly higher and DA levels ($F = 5.87$; d.f. = 2,12; $P < 0.02$) were significantly lower in either CO_2 or CO_2/O_2 treated bees *versus* untreated bees (fig. 3). Levels of both amine precursors, TRP ($F = 4.58$; d.f. = 2,12; $P < 0.04$) and TYR ($F = 23.37$; d.f. = 2,12; $P < 0.001$), were significantly lower in either CO_2 or CO_2/O_2 treated bees versus untreated bees (fig. 3). Although TRP levels were found to be significantly lower by ANOVA, the Tukey's mean separations test could not differentiate the three treatment means at an $\alpha = 0.05$ confidence level.

Nested cage effects were not significant for OA ($F = 1.61$; d.f. = 12,30; $P > 0.14$) or TYR ($F = 1.38$; d.f. = 12,30; $P > 0.2$), but according to Bancroft and Han (1983), this nested factor could not be pooled with the random error term for testing main treatment effects. Nested cage effects were not significant for DA ($F = 1.08$; d.f. = 12,30; $P > 0.40$), TRP ($F = 0.64$; d.f. = 12,30; $P > 0.7$) or 5HT ($F = 0.41$; d.f. = 12,30; $P > 0.9$) levels; however, because cages were a significant source of variation for OA and TYR, the nested experimental error term was not pooled with the random error term to test the main effects.

Effects of CO_2 narcosis on workers kept without pollen

There were no significant differences in levels of OA ($F = 0.83$; d.f. = 1,18; $P > 0.3$), 5HT ($F = 0.61$; d.f. = 1,18; $P > 0.4$), DA ($F = 2.80$; d.f. = 1,18; $P > 0.10$) (fig. 3) or TRP ($F < 0.01$; d.f. = 1,18; $P > 0.95$) (fig. 3) in the brains of CO_2-treated *versus* untreated worker bees. However, TYR levels ($F = 7.18$; d.f. = 1,18; $P < 0.02$) were significantly reduced by narcosis on day 4 (fig. 3).

Worker age (DAY term) significantly influenced levels of some of the compounds that were measured. OA levels ($F = 12.15$; d.f. = 2,18; $P < 0.001$) and 5HT levels ($F = 4.66$; d.f. = 2,18; $P < 0.03$) were significantly elevated from day 6 to day 8 (fig. 3). TRP ($F = 76.18$; d.f. = 2,18; $P < 0.001$) levels and TYR ($F = 11.36$; d.f. = 2,18; $P < 0.001$) levels were significantly reduced through time (fig. 3). The TRT*DAY interaction terms were not significant causes of variation for levels of OA ($F = 2.87$; d.f. = 2,18; $P > 0.08$) and 5HT ($F = 0.59$; d.f. = 2,18; $P > 0.5$) or TRP ($F = 0.17$; d.f. = 2,18; $P > 0.8$) and TYR ($F = 0.93$; d.f. = 2,18; $P > 0.4$) levels.

Worker age did not affect brain levels of DA ($F = 0.85$; d.f. = 2,18; $P > 0.4$) (fig. 3). As with the previous compounds, the TRT*DAY interaction term was not a

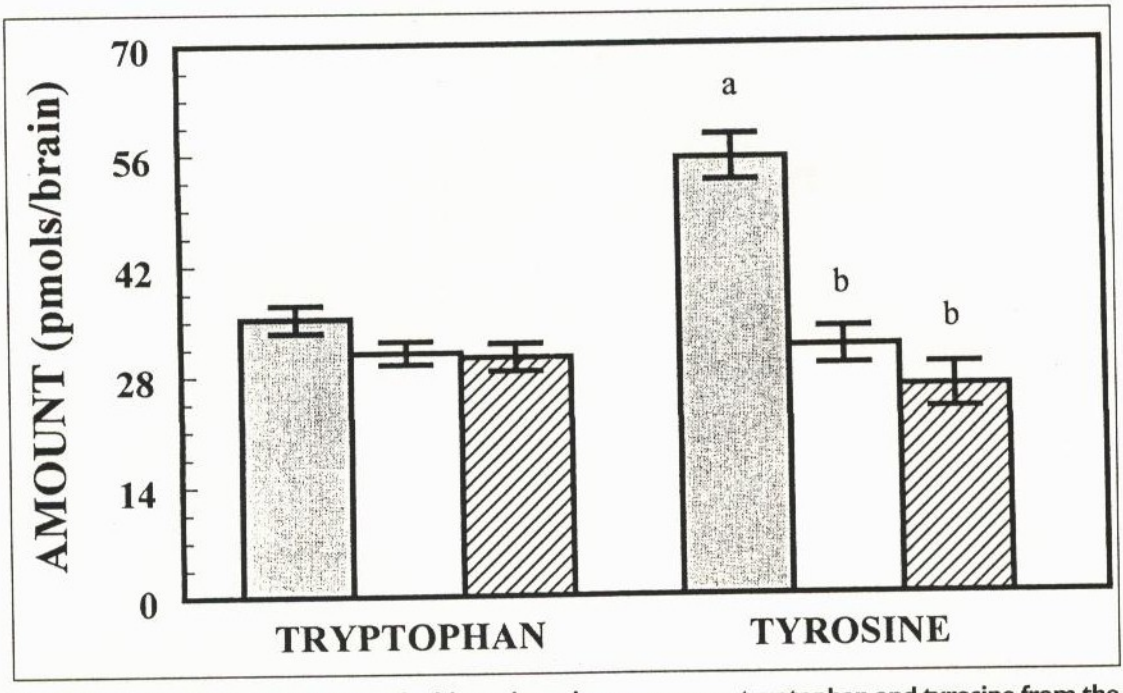

FIG. 2. Effects of CO_2 narcosis on the biogenic amine precursors tryptophan and tyrosine from the brains of bees that were fed pollen. Controls = gray bars; CO_2-treated bees = open bars and CO_2/O_2-treated bees = striped bars (see fig. 3 caption for experimental details). Mean tryptophan levels for the different treatments could not be separated with Tukey's mean separations test. For tyrosine, treatment mean (± s.e.) bars with the same letter were not significantly different ($\alpha = 0.05$).

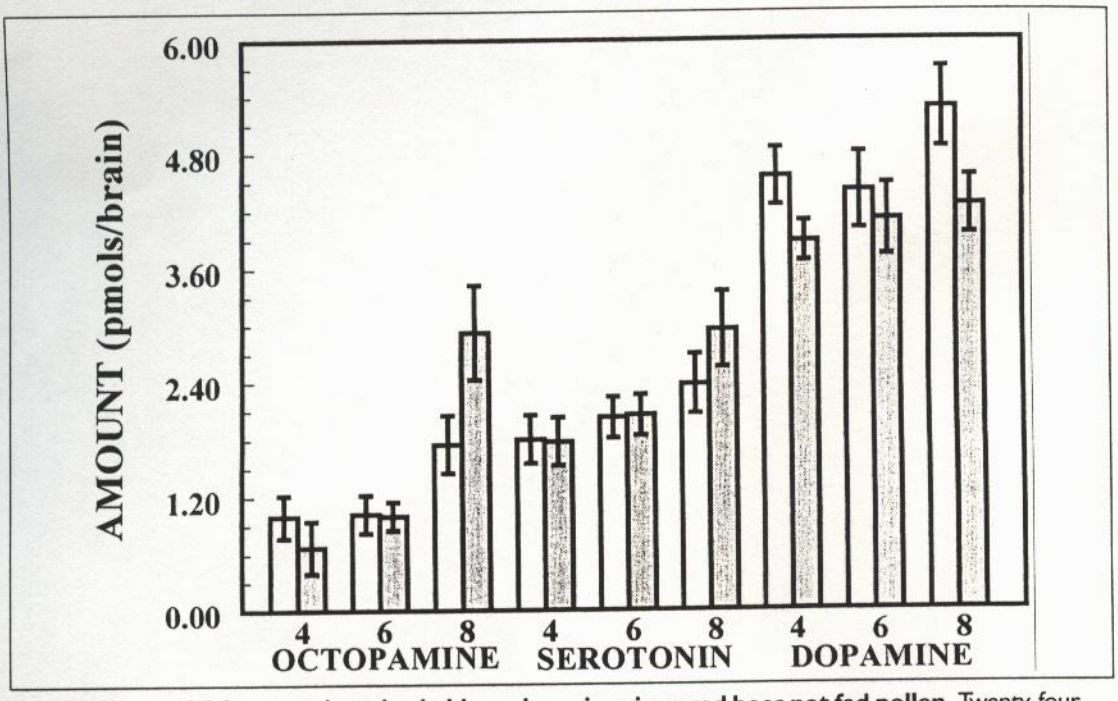

FIG. 3. Effects of CO_2 narcosis on brain biogenic amines in caged bees not fed pollen. Twenty-four cages were given 50 newly-emerged bees, and on the third day of the experiment 12 cages were exposed for 10 min to CO_2 (gray bars) and the remaining 12 cages were untreated (open bars). Cages were held in the incubator for an additional 1, 3 or 5 days after treatment (for a total of 4, 6 and 8 days respectively). Three bees were sampled for biogenic amine analysis from 4 control cages and 4 CO_2-treated cages on each sampling day. Cages were sampled only once. There were no significant differences between treatment groups for any of the three compounds measured. Octopamine levels were significantly elevated between day 6 and day 8.

significant source of variation for levels of DA ($F = 0.27$; d.f. = 2,18; $P > 0.7$).

Effects of CO_2 treatment on caged and free-running virgin queens from two stocks

All CO_2-treated queens that were kept in small colonies produced eggs between days 19 and 23 of the experiment ($n = 12$). None of the untreated queens that were kept in small colonies produced eggs during this period ($n = 9$). Egg production was not determined for caged queens.

Treatment with CO_2 did not have a significant effect on levels of OA ($F = 0.03$; d.f. = 1,36; $P > 0.85$), 5HT ($F = 2.36$; d.f. = 1,39; $P > 0.13$) or DA ($F = 3.10$; d.f. = 1,39; $P > 0.09$) (fig. 3). The effects of caging queens (*versus* free-running conditions) were not significant for OA ($F = 1.99$; d.f. = 1,36; $P > 0.15$) and DA ($F = 0.04$; d.f. = 1,39; $P > 0.8$), but 5HT ($F = 9.56$; d.f. = 1,39; $P < 0.004$) levels were significantly elevated in caged queens (fig. 3). The differences between the two stocks were not significant sources of variations for OA ($F = 0.12$; d.f. = 1,36; $P > 0.7$), DA ($F = 1.56$; d.f. = 1,39; $P > 0.2$) or 5HT ($F = 2.79$; d.f. = 1,39; $P > 0.10$). None of the two-factor interaction terms (treatment by stock; treatment by caging condition; and stock by caging condition) and the three-factor interaction term (treatment by stock by caging condition) were significant causes of variation for OA and 5HT. For DA, the treatment by caging condition term was the only significant source of variation ($F = 4.47$; d.f. = 1,39; $P < 0.04$). This result indicates that the freerunning and caged queens were affected by CO_2 treatment to different extents (fig. 3): for free-running queens, treatment with CO_2 reduced DA levels by 45% while treatment with CO_2 did not reduce DA levels of caged queens (fig. 3).

Effects of CO_2 on amines and ovarian development in workers caged with and without queens

Eggs were found on the ninth day of the experiment in all three cages of bees that were not given CO_2 and contained no queens. None of the cages containing bees that were given a CO_2 treatment and contained no queens had eggs. All six cages that contained queens had eggs throughout the experiment.

Significant differences in extent of ovarian development were found for both main factors: (1), exposure or no exposure to CO_2 ($F = 75.00$; d.f. = 1,8; $P < 0.001$) and (2), presence or absence of a queen ($F = 75.00$; d.f. = 1,8;

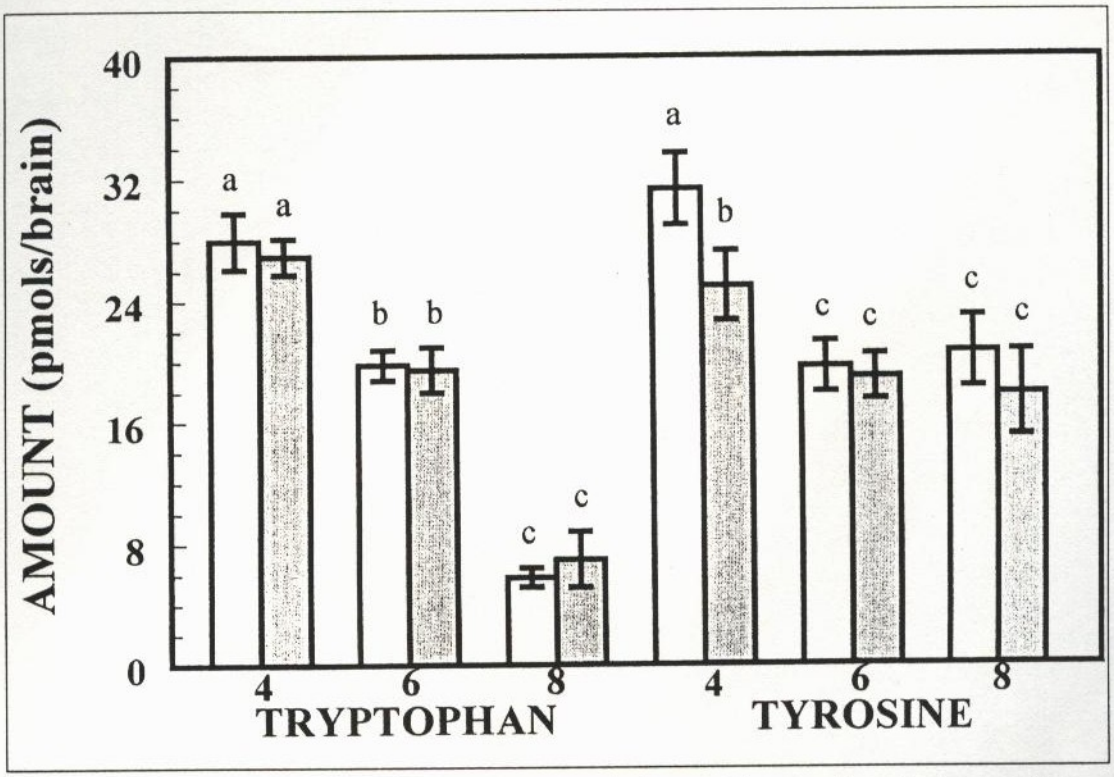

FIG. 4. Effects of CO_2 narcosis on the precursors tryptophan and tyrosine from the brains of bees not fed pollen (see fig. 3 caption for experimental details). Absence of pollen led to significant reductions in levels of tryptophan and tyrosine in both control (open bars) and CO_2-treated bees (gray bars). Only tyrosine levels were significantly different between the two treatments on day 4. Treatment means (± s.e.) having the same letter do not differ ($\alpha = 0.05$) as determined by Tukey's mean separations test.

$P < 0.001$). The ovaries of worker bees remained undeveloped after 10 days in all cages containing queens or in cages that had been treated with CO_2. Only workers from untreated cages without queens had developed ovaries. The average (mean ± s.e.) sum of the right and left ovary scores for these bees was 3.25 ± 0.328 ($n = 12$). A worker with both ovaries undeveloped would have the sum of 2.00.

Of the three compounds measured from the worker brains, OA ($F = 0.81$; d.f. = 1,8; $P > 0.30$) and 5HT ($F = 0.00$; d.f. = 1,8; $P > 0.95$) levels were not significantly affected by CO_2 treatment. Only DA levels were significantly affected by CO_2 treatment ($F = 20.48$; d.f. = 1,8; $P < 0.002$) (fig. 3). The presence or absence of a queen did not significantly affect OA ($F = 0.21$; d.f. = 1,8; $P > 0.65$), 5HT ($F = 0.77$; d.f. = 1,8; $P > 0.40$) or DA ($F = 4.97$; d.f. = 1,8; $P > 0.05$) levels. The CO_2 treatment by presence or absence of queen interaction term was not a significant source of variation for OA ($F = 1.16$; d.f. = 1,8; $P > 0.31$) and DA ($F = 0.43$; d.f. = 1,8; $P > 0.50$) levels. This interaction term was a significant source of variation for serotonin levels ($F = 9.28$; d.f. = 1,8; $P < 0.02$).

DISCUSSION

A 10–15-min exposure to CO_2 or CO_2 containing 20% O_2 led to significant reductions in levels of TRP, TYR and DA in worker honey bees given pollen, but had no effect in workers kept without pollen. The present work showed an age-related reduction in TRP and TYR in workers that were not fed pollen. Untreated and CO_2-treated bees with no available pollen had only 5–7 pmoles TRP and 18–20 pmoles TYR in their brains after eight days, considerably lower than the 25–30 pmoles TRP and the 27–30 pmoles TYR found on the fourth day. In bees that were continuously fed pollen, levels of TRP and TYR (although significantly reduced by CO_2) remained high after eight days (30 pmoles).

The reduction in brain TRP and TYR levels in pollen deprived bees is not surprising since many essential amino acids and vitamins are supplied to bees in their pollen (Groot, 1953; Haydak & Dietz, 1972; Herbert & Shimanuki, 1977). However, one might expect a concomitant decrease in 5HT and the catecholamine (OA and DA) levels because TRP and TYR are the dietary precursors to these neurotransmitters. The levels of OA, DA and 5HT levels did not significantly decrease in the absence of pollen. The ability of the nervous system

to maintain levels of these neuroactive substances without dietary intake of their precursors seems remarkable, but it is clear that treatment with CO_2 can cause dramatic changes in levels of important neuroactive substances in the brains of worker honey bees.

Unlike workers, treatment with CO_2 had no statistically significant effect on brain DA levels in queens (TRP and TYR were not examined in the queens). However, CO_2 treatment reduced DA levels more in free-running queens than in queens that were caged and stored together in a single colony. This trend in free-running queens was similar to the effects of CO_2 on brain DA levels in workers. Why dopamine levels were not reduced in queens that were caged and stored together is not clear, but these results do indicate that the nervous systems of workers and queens respond to CO_2 treatment differently. Also, the brains of queens had 3–5 times higher levels of DA than those of workers. Similar results were previously reported (Brandes *et al.*, 1990).

If dopamine in the brain is related to the control of reproduction in workers, this study suggests that regulation of oogenesis in queens and workers may be different. Although 5HT levels were statistically higher in caged queens than in queens that were free-running in small colonies, the differences were not great.

The current study also showed that brain DA levels in worker honey bees were reduced by treatment with CO_2, whether the bees were caged with or without queens. Ovarian development in workers was reduced either by treatment with CO_2 or by the presence of a queen. The presence of a queen did not significantly reduce brain DA levels in workers. At best these results are only correlative, but they suggest a positive relationship between brain DA and reproductive processes in worker honey bees. How changes in DA levels are related to reproduction remains unclear. However, a similar study by us found that brain DA levels in workers were elevated with increased ovarian development, and the

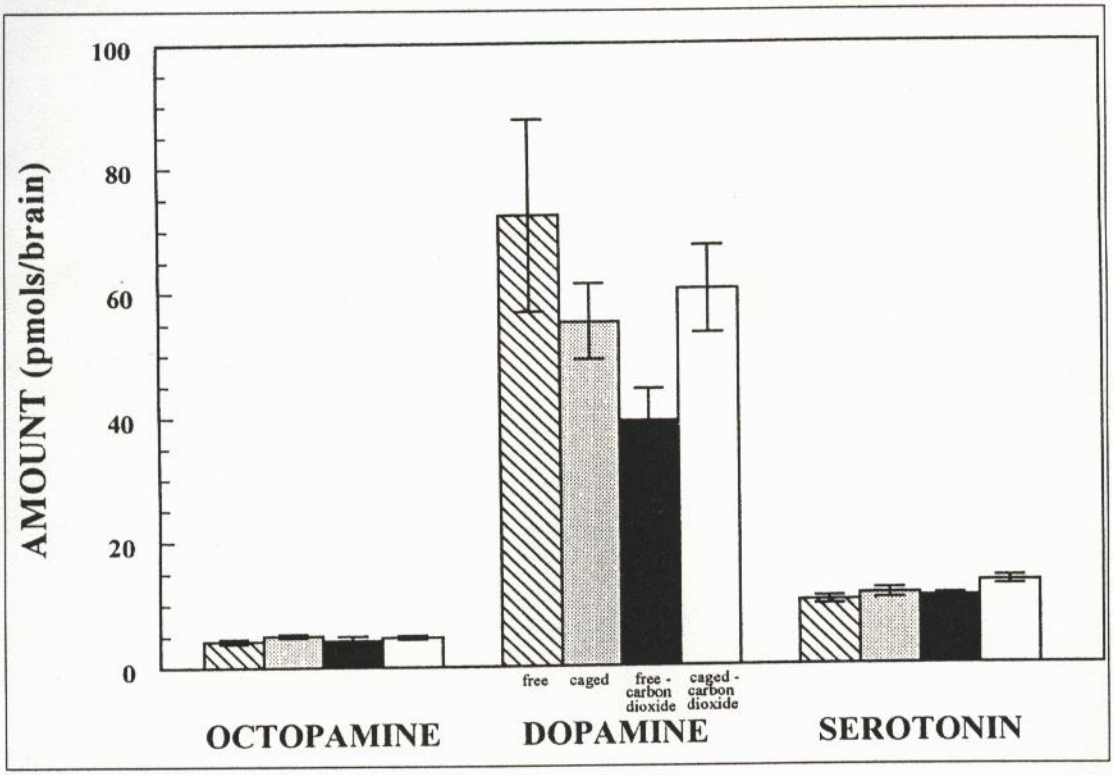

FIG. 5. Effects of CO_2 narcosis on virgin queens that were caged or kept free-running in small colonies. Treated queens (23 queens from two stocks) were given a 10-minute CO_2 narcosis when queens were 11 and 12 days old, while control queens (21 queens from the same two stocks) were not given CO_2. Queens from both groups were then either caged (in a single large colony) or placed individually into small colonies fitted with queen excluders at the entrance to prevent mating flights (12 control queens and 11 treated queens were banked; 9 control queens and 12 treated queens were maintained in colonies). The brains from all queens were removed for biogenic amine analysis on day 23 of the experiment. Octopamine levels were not affected by CO_2 treatment or caging. Serotonin levels were significantly elevated by caging. Dopamine levels were not significantly affected by CO_2 or caging, but reductions in dopamine levels were more pronounced in free-running queens than in banked queens. Striped bars = free-running, untreated queens; gray bars = caged, untreated queens; dark bars = free-running queens given CO_2; and the open bars = caged queens given CO_2.

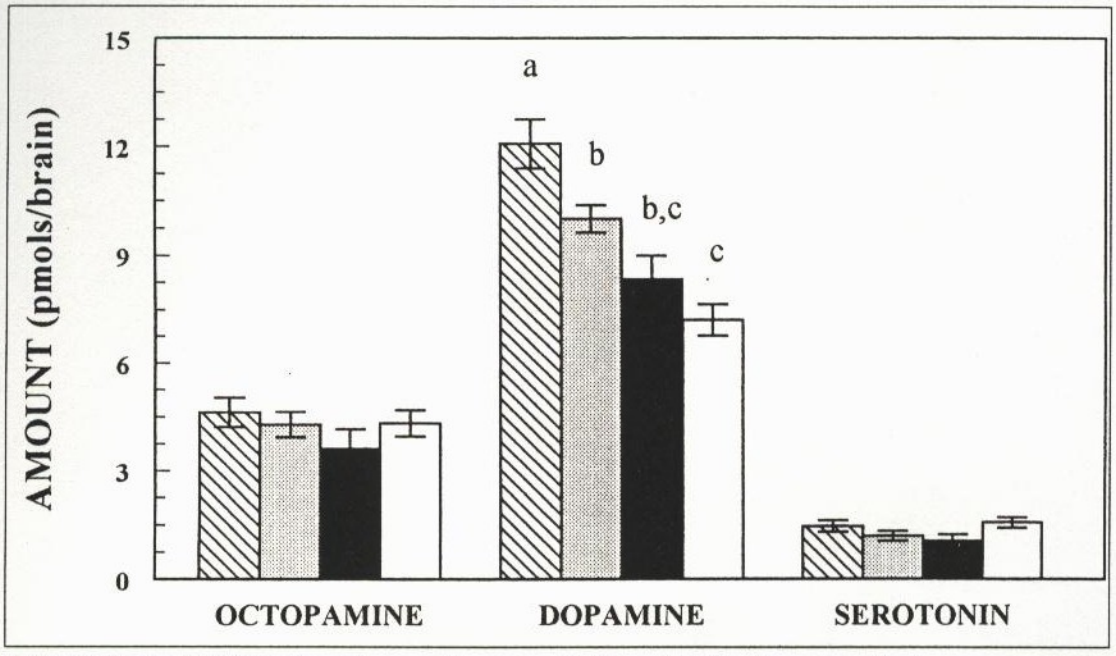

FIG. 6. Effects of CO_2 treatment on workers caged with and without queens. Twelve cages were kept in an incubator through 10 days when the brains from 4 bees per cage were sampled for amine content (6 cages each with a queen; 6 cages without a queen). Within both groups of bees, three cages were given a single 15 minute CO_2 narcosis on the first day while the remaining three cages were not treated. Only dopamine was significantly affected by CO_2 treatment. Each bar represents the mean (± s.e.) for 9–12 bees. Striped bars = bees with no queen and no CO_2; dark bars = bees with a queen and no CO_2; open bars = bees without a queen but given CO_2; gray bars = bees with a queen and given CO_2.

elevated DA levels were correlated to ovariole width (Harris & Woodring, 1995). If brain levels of DA are involved in the stimulation of worker honey bee reproduction, reductions in DA levels caused by exposure to CO_2 may partially explain reductions in ovary development associated with narcosis (Harris & Harbo, 1990).

Exposure to CO_2 had no effect on brain OA levels in any of the experiments with workers or queens. Effects of CO_2 treatment on brain 5HT levels were mixed. In one experiment with workers 5HT levels were elevated by CO_2 treatment, but in all other experiments levels of 5HT were unaffected. Levels of 5HT in queen brains were also unaffected by CO_2 treatment.

The reduction in TRP and elevation of 5HT might retard worker ovarian development independent of changes in DA levels. Because 5HT has been shown to decrease or inhibit many behaviours and metabolic processes in honey bees (Erber *et al.*, 1993), an increase in serotonergic activity within the central nervous system might decrease neuroendocrine activity related to reproduction. Changes in TRP levels may only indicate an increased biosynthetic conversion to 5HT; however, TRP has been shown to exhibit effects on neural activity in honey bees that is independent of the actions of 5HT (Lopatina & Dolotovskaya, 1984; Lopatina *et al.*, 1985). Changes in TYR levels could potentially affect many parts of the nervous system because it is the precursor to the neuroactive catecholamines (DOPA, DA, norepinephrine and epinephrine) and phenolamines (tyramine, OA, synephrine).

Changes in levels of TRP, TYR, 5HT and DA may only be symptomatic of disruptive changes in the central nervous system resulting from narcosis or anoxia suffered during long exposures to CO_2 (Woodring *et al.*, 1978) and may not be related to worker reproduction. Because the effects of treatment with CO_2 were similar to effects from treatment with a mixture of CO_2 containing 20% O_2, the current study indicates that the effects of a 10-min exposure to CO_2 in bee brains are probably the result of narcosis and not anoxia. In contrast, Woodring *et al.* (1978) indicated that a 10-min exposure marked the beginning of anoxia in the house cricket, *Acheta domesticus*, and that the major long-term effects of longer exposures to CO_2 are probably caused by anoxia rather than narcosis.

REFERENCES

The numbers given at the end of references denote entries in *Apicultural Abstracts*.

BANCROFT, T A; HAN, C (1983) A note on pooling variances. *Journal of the American Statistical Association* 78: 981–983.

BIEDERMANN, M Z (1964) Neurosekretion bei Arbeiterinnen und Koniginnen von *Apis mellifica* L. unter naturlichen und experimentellen Bedingungen. *Zeitschrift für Wissenschaftliche Zoologie* 170: 256–308.

BIRKENMEYER, D K; DAME, D A (1970) Effects of carbon dioxide and nitrogen in *Glossina morsitans orientalis*. *Annals of Tropical Medicine and Parasitology* 64: 269–275.

BÜHLER, A; LANZREIN, B; WILLE, H (1983) Influence of temperature and carbon dioxide concentration on juvenile hormone titre and dependent parameters of adult worker honey bees (*Apis mellifera* L.). *Journal of Insect Physiology* 29(12): 885–893. 155/86

BRANDES, C; SUGAUA, M; MENZEL, R (1990) High-performance liquid chromatography measurement of catecholamines in single brains reveals caste-specific differences between worker bees and queens in *Apis mellifera*. *Comparative Biochemistry and Physiology, C* 97: 53–57.

EDWARDS, L J; PATTON, R L (1965) Effects of CO_2 anesthesia on the house cricket, *Acheta domesticus*. *Annals of the Entomological Society of America* 58: 828–832.

ENGELS, W; RAMAMURTY, R S (1976) Initiation of oogenesis in allectomized virgin honey bee queens by carbon dioxide treatment. *Journal of Insect Physiology* 22: 1427–1432. 890/77

ENGELS, W; GONCALVES, L S; ENGELS, E (1976) Effects of carbon dioxide on vitellogenin metabolism in unmated queen honeybees. *Journal of Apicultural Research* 15(1): 3–10. 774/76

ERBER, J; KLOPPENBURG, P; SCHEIDLER, A (1993) Neuromodulation by serotonin and octopamine in the honeybee: behaviour, neuroanatomy and electrophysiology. *Experientia* 49: 1073–1083.

FYG, W (1950) Beobachtungen über die Wirkungen der Kohlensäure-Narkose auf Arbeitsbienen. *Schweizerische Bienenzeitung* 73: 174–184.

GROOT, A P de (1953) Protein and amino acid requirements of the honeybee (*Apis mellifica* L.). Thesis; University of Utrecht, Netherlands; 90 pp.

HARRIS, J W; HARBO, J R (1990) Suppression of ovary development of worker honey bees by association with workers treated with carbon dioxide. *Journal of Apicultural Research* 29(4): 187–193. 161/92

HARRIS, J W; WOODRING, J P (1992) Effects of stress, age, season, and source colony on levels of octopamine, dopamine and serotonin in the honey bee (*Apis mellifera* L.) brain. *Journal of Insect Physiology* 38(1): 29–35. 161/94

HARRIS, J W; WOODRING, J P (1995) Elevated brain dopamine levels associated with ovary development in queenless worker honey bees (*Apis mellifera* L.). *Comparative Biochemistry and Physiology, C* 111: 271–279.

HAYDAK, M H; DIETZ, A (1972) Cholesterol, pantothenic acid, pyridoxine and thiamine requirements of honeybees for brood rearing. *Journal of Apicultural Research* 11(2): 105–109. 358/73

HENTSCHEL, E (1981) Investigations on catecholamine integrated influences on reproduction in *Periplaneta americana* (L.). *Advances in Physiological Science* 22: 205–234.

HERBERT, E W Jr; SHIMANUKI, H (1977) Brood-rearing capability of caged honeybees fed synthetic diets. *Journal of Apicultural Research* 16(3): 150–153. 885/78

KROPACOVA, S; HASLBACHOVA, H; NOVAK, V (1968) Development of honeybee ovaries as affected by narcosis and injection of certain substances. *Sbornik Vysoke Zemedelske v Brne* 16: 537–543.

KULINČEVIC, J M; ROTHENBUHLER, W C (1973) Laboratory and field measurements of hoarding behaviour in the honeybee. *Journal of Apicultural Research* 12(3): 179–182. 145/74

LOPATINA, N G; DOLOTOVSKAYA, L Z (1984) Role of serotonin in the behavioral and neurological effects of snow and snow-laranja mutations of the bee, *Apis mellifera*. *Zhurnal Evolyutsionnoi Biokhimii i Fiziologii* 20(4): 370–373. 1182/87

LOPATINA, N G; CHESNOKOVA, E G; PONOMARENKO, V V (1985) Tryptophan and its metabolites in the function of the nervous system of the honey bee, *Apis mellifera*. *Zhurnal Evolyutsionnoi Biokhimii i Fiziologii* 21: 25–32. 1183/87

MACKENSEN, O (1947) Effect of carbon dioxide on initial oviposition of artificially inseminated and virgin queen bees. *Journal of Economic Entomology* 40: 344–349.

MARDAN, M; RINDERER, T E (1980) Effects of carbon dioxide and cold anaethesia on the hoarding behaviour of the honeybee. *Journal of Apicultural Research* 19(3): 149–153. 558/81

RALPH, C L (1959) Modification of activity rhythms of *Periplaneta americana* induced by carbon dioxide and nitrogen. *Physiological Zoology* 32: 57–62.

ROBINSON, G E; PAGE, R E Jr; STRAMBI, C; STRAMBI, A (1989) Hormonal and genetic control of behavioral integration in honey bee colonies. *Science, USA* 246: 109–112. 503/93

ROBINSON, G E; STRAMBI, C; STRAMBI, A; FELDLAUFER, M F (1991) Comparison of juvenile hormone and ecdysteroid haemolymph titres in adult worker and queen honey bees (*Apis mellifera*). *Journal of Insect Physiology* 37(12): 929–935. 876/94

SCHNEIDER, S S; GARY, N E (1984) 'Quacking': a sound produced by worker honeybees after exposure to carbon dioxide. *Journal of Apicultural Research* 23(1): 25–30. 1207/84

WHISENANT, B R; BRADY, U E (1965) Effects of anethesia on the subsequent mating behavior of *Plodia interpunctella* males. *Journal of the Georgia Entomological Society* 2: 27–30.

WOODRING, J P; CLIFFORD, C W; ROE, R M; BECKMAN, B R (1978) Effects of CO_2 and anoxia on feeding, growth, metabolism, water balance, and blood composition in larval female house crickets, *Acheta domesticus*. *Journal of Insect Physiology* 24: 499–509.

WOYKE, J (1988) Problems with queen banks. *American Bee Journal* 128(4): 276–278. 246/89

WOYKE, J (1989) Correct queen maintenance before and after instrumental insemination, tested in Egypt. *Journal of Apicultural Research* 28(4): 187–190. 243/91