Original article

Dietary dopamine causes ovary activation in queenless *Apis mellifera* workers

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Abstract – Groups of young honey bee workers were fed a diet containing dopamine while confined in small cages at 34 °C and 80% RH in absence of a queen for 8 to 13 days. The bees in eight pairs of cages, each pair containing an equal number of workers, received a pollen-rich diet supplemented with dopamine (10 µg/g of diet) (DOP groups), or not supplemented (controls). The rate of consumption of the diet was monitored continuously during the confinement period, after which the workers were dissected to assess follicle development in the ovaries. The results showed a significantly higher proportion ($P = 0.004$) of workers with activated ovaries in the DOP groups than in control groups. The number of bees surviving confinement was significantly higher in the control groups than in the DOP groups ($P \leq 0.01$), possibly reflecting a deleterious effect of dopamine. The surviving bees from both groups consumed equivalent amounts of diet ($P = 0.687$), showing that ovary activation was not due to differential diet consumption. The results suggest a role of dopamine in the chain of events mediating changes in the reproductive status of orphan honey bee workers.

Apis mellifera / honeybees / dopamine / ovary activation

1. INTRODUCTION

In *Apis mellifera* L., reproduction is an attribute of the queen, whereas workers perform tasks related to the organization and maintenance of the hive, such as construction of brood cells, provisioning, thermoregulation, defense, and caring for the developing larvae. The inactive ovaries of workers impair egg production; however, under specific circumstances, their ovaries can be activated and oocytes begin to grow, resulting in the production of haploid eggs that will give rise to drones. Pheromones from egg-laying queens (Butler et al., 1961; Slessor et al., 1988, 1990; Winston et al., 1989, 1990), and also signals from larvae (Kropáková and Haslbachová, 1971; Le Conte et al., 1990), suppress follicle development in worker ovaries (Velthuis et al., 1990). Aging or death of the queen, and the consequent decrease in the amount of developing brood, elicit physiological changes leading to ovary activation in workers. Free from queen pheromone and brood signal, a number of orphaned workers in a colony will then start to lay haploid eggs.

The complete chain of events mediating the change in the reproductive status of orphaned worker bees is still unknown. Biogenic amines in the central nervous system, especially dopamine, octopamine and serotonin, are candidates to respond to variations in queen

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pheromone. By functioning as neurotransmitters, neuromodulators and neurohormones (Bicker and Menzel, 1989; Roeder, 1994; Osborne, 1996) the biogenic amines modulate a diversity of physiological and behavioral functions in honey bees, such as learning and olfactory memory (Bicker and Menzel, 1989; Erber et al., 1993; Menzel and Müller, 1996; Blenau et al., 1998), recruitment behavior (Bozic and Woodring, 1998), age-related division of labor (Taylor et al., 1992; Schulz and Robinson, 1999, 2001; Wagener-Hulme et al., 1999), nestmate recognition (Robinson et al., 1999), foraging behavior (Barron et al., 2002), and changes in morphological development (Taylor et al., 1992). Also, in honey bees, as well as in the bumble bee *Bombus terrestris* L., high levels of dopamine in the brain correlate with physiologically active ovaries and fully developed oocytes (Harris and Woodring, 1995; Bloch et al., 2000; Sasaki and Nagao, 2001). These results, however, were not attributed to a putative effect of dopamine on ovary activation. Instead, it was hypothesized that the elevated dopamine levels were triggered by processes associated with the onset of oogenesis (Harris and Woodring, 1995). In a recent study, however, Sasaki and Nagao (2001) suggested that high levels of dopamine in the brain of queenless workers could cause physiological changes in reproduction.

The current study was conducted to test whether increased levels of dopamine could lead to ovary activation in orphaned honey bee workers. For this purpose, a confinement strategy using small cages was designed to monitor the ingestion of a diet containing dopamine. The results showed a significant increase in the number of orphaned workers with activated ovaries in the dopamine-fed group as compared to control workers.

2. MATERIALS AND METHODS

Honey bees used for the experiments

Africanized *Apis mellifera* worker pupae were collected from queenright colonies maintained in the Apiary of the Department of Genetics at the Faculty of Medicine in Ribeirão Preto, São Paulo University, Brazil.

### 2.1. Confinement for dopamine treatment

Combs containing worker pupae ready to emerge were transported from the apiary to incubators where they were maintained at 34 °C and 80% relative humidity for 4 days. Sufficient honey and pollen reserves were available in the comb cells to feed the workers emerging during this period of time. Water was given ad libitum. On the fourth day, groups of workers that emerged from the combs were transferred to small wooden cages measuring $8 \times 11 \times 13$ cm, equipped with a sliding glass front and a screened bottom. Pairs of cages, each containing equal number of worker bees taken from the same hive, were prepared for each experiment. Eight repetitions were performed using bees from different hives. In most of the 8 experimental series, we used 50 bees per cage, with exception of Experiment 2, in which 47 bees per cage were used, and in Experiments 6 and 7, in which 106 bees per cage were used. In each set up, the worker bees from the test cage received a diet supplemented with dopamine, while bees in the control cage were fed on the same diet but without dopamine. All cages were maintained in incubators at 34 °C and 80% relative humidity. Water and diet were supplied ad libitum to all groups. The confinement lasted 8 to 13 days, depending on the experiment.

2.2. Diet administered to groups of caged worker bees

The paste-like diet was prepared with 30 g pollen (beebread) freshly collected from colonies in our apiary, 70 g powdered sucrose and 2 mL honey. Dopamine was added to the diet of the experimental groups (10 µg dopamine/g of diet), but not to the diet offered to the control groups. Each day, a new batch of food was supplied to the cages. The leftover food from the day before was weighed, and the number of surviving bees was counted daily. Food consumption, expressed as mg/bee/day, was calculated by $(N - NC)/n$, where N was the weight of administered diet, NC was the weight of the non-consumed diet (left in the feeding dishes), and n was the number of surviving bees. The mean daily consumption per bee (in mg of diet), over the entire experimental period, was calculated and used to compare the amount of diet ingested by dopamine-treated and control bees.

2.3. Ovary examination

After confinement, the worker bees were dissected. Their ovaries were examined and classified as activated if they showed developing
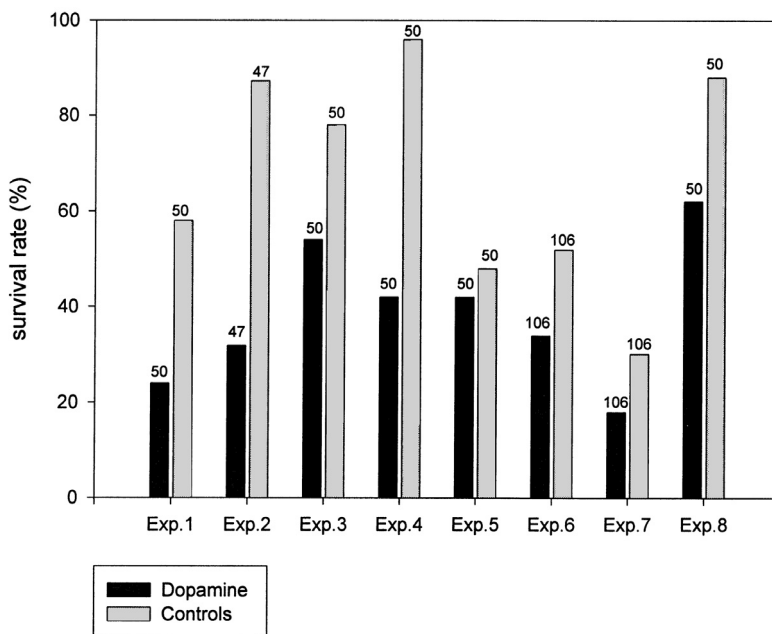
Figure 1. Survival of groups of workers fed dopamine or not fed dopamine (controls) in the confinement experiments (Exp. 1 to Exp. 8). The number of confined bees is indicated above each bar.

follicles, or as inactive if the ovarioles did not contain developing follicles.

2.4. Statistical analysis

The differences in ovarian development between the dopamine-fed and control groups was evaluated using z-test for comparing proportions, set at confidence level of $P < 0.05$. Analysis of variance (one-way ANOVA) was also performed to analyze the amount of diet consumed between treated and control groups of bees.

3. RESULTS

The confinement strategy used for experimental series had to be favorable for ovary activation. Several variations in the confinement conditions were tested (data not shown) to develop an experimental strategy that optimized bee survival and ovary activation. Figure 1 shows the survival rates of worker bees fed and not fed dopamine. In the control groups, the percentage of surviving bees varied from 30.2 to 96%, whereas in dopamine-fed groups the percentage of surviving bees

ranged from 17.9 to 62%. In each experimental series, the percentage of bees surviving confinement was higher in the control groups than in the respective dopamine-treated groups (z-test, $P \leq 0.01$). These results showed that dopamine feeding increased bee mortality. However, it was also possible that mortality was influenced by the number of bees confined together. In Experiments 6 and 7, we confined 106 bees per cage, instead of 47 or 50 bees used in the majority of the experiments. A significant difference was observed (z-test, $P \leq 0.001$) between the number of bees surviving in the control cages filled with 47 or 50 bees, and in the cages containing 106 bees, showing that a crowded confinement condition (106 bees) negatively affected survival. The same result was observed when survival was compared between dopamine-treated groups composed of 47–50 bees or of 106 bees (z-test, $P \leq 0.001$). Although the cages apparently were large enough to house many more than 100 bees, survival was significantly higher when cages were filled with only 47–50 bees. Survival was significantly lower (z-test, $P \leq 0.001$) in dopamine groups than in
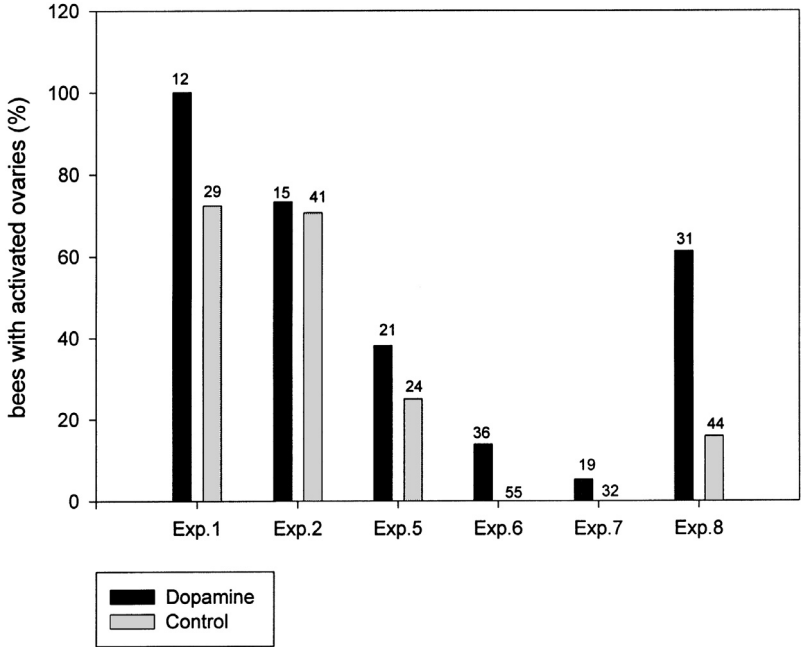
Figure 2. Percentages of worker bees with activated ovaries in confined groups fed dopamine or not fed dopamine (controls) in Experiments (Exp.) 1, 2, 5, 6, 7 and 8. Experiments 3 and 4 were not represented in this figure because none of the bees had activated ovaries. The number of bees that survived confinement and were measured for ovary activation are indicated above the bars.

control groups, even when the data obtained from experiments 6 and 7 (106 bees per cage) were removed from the statistical analysis. In conclusion, the data showed that feeding dopamine decreased survival of the bees, and that this effect was intensified in a crowded confinement condition.

The confinement conditions permitted ovary activation in worker bees in six of the eight experimental series (workers from experiments 3 and 4, fed or not fed dopamine-diet did not exhibit activated ovaries). The percentage of workers with active ovaries was significantly higher (z-test, $P = 0.004$) in the dopamine-fed groups than in the respective control groups (Fig. 2). The ovary activation was also affected by the size of the caged bee population. A smaller proportion of bees with activated ovaries was observed in dopamine-treated as well as in control groups when 106 bees were confined together (Experiments 6 and 7) than when the confined groups were composed of 47–50 bees (z-test, $P \leq 0.001$).

The higher proportion of bees with activated ovaries in the dopamine-fed groups was not due to differences in the amount of diet ingested. Bees from the experimental and control groups consumed equivalent amounts of diet (one way ANOVA, $p = 0.687$), showing that dopamine did not affect the quantity of food ingested (Fig. 3).

Taken together, the results showed a positive effect of dietary dopamine on ovary activation in queenless workers. The fact that experimental and control bees ingested equivalent amounts of diet, excluded a possible effect of differential food consumption rate on ovary activation.

4. DISCUSSION

The present study showed that a diet supplemented with dopamine promoted follicle development in queenless groups of honey bee workers. The experimental design to test the effect of dopamine on ovary growth had to
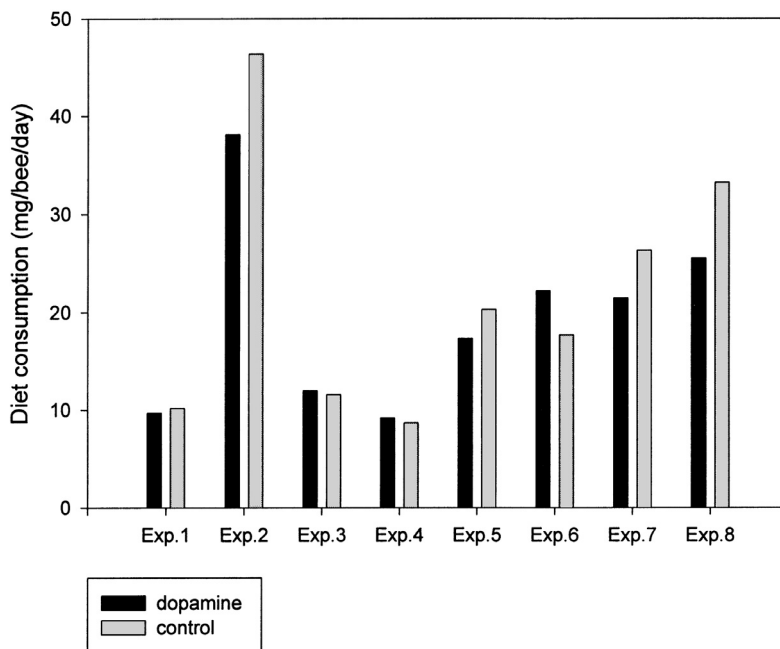
Figure 3. Amount of diet ingested (mean daily consumption per bee) by confined worker bees from the dopamine and control groups, for all experiments (Exp. 1 to Exp. 8).

circumvent the difficulty of keeping the worker bees alive in confinement for sufficient time to initiate ovary activation. This situation was attained by placing the caged bees in incubators held at the same temperature and humidity as a colony in the field. Also, the caged bees were fed a pollen-rich diet, which served as a protein source for the secretion of normal levels of vitellogenin – the egg protein precursor (Bitondi and Simões, 1996). However, even under these conditions, bees did not survive for a long time in confinement, and the experiments could not be conducted for more than 13 days.

Confinement in a queenless condition is not a guarantee that a high proportion of worker bees will show activated ovaries. Even in the hive, only a proportion of queenless workers will have active ovaries. There is evidence indicating that the changes in the physiological status of individual queenless workers leading to ovary growth are genetically determined. Worker bees derived from different hives, or even from the same hive but from different patrilines, can differ in the probability that their ovaries will be activated in the

absence of the queen (Page and Erickson, 1988; Robinson et al., 1990; Makert and Hartfelder, 2001). The percentage of bee workers that had activated ovaries in our experiments varied between 70–100% (Experiments 1 and 2) and 0% (Experiments 3 and 4), which could be a reflection of whether certain genotypes were capable of activating their ovaries.

Studies on the action of dopamine on oocyte growth have been conducted in *Blattella germanica* (Pastor et al., 1991), and *Drosophila melanogaster* (Neckameyer, 1996). In the former species, dopamine stimulated oocyte growth when injected during the beginning of the first ovarian cycle, just before vitellogenesis, although an inverse effect was observed in females injected at the end of vitellogenesis. In *D. melanogaster*, the inhibition of the activity of tyrosine hydroxylase, an essential enzyme in the biosynthesis of catecholamines, resulted in depletion of dopamine levels and in abnormally developed ovaries. To our knowledge, the present study is the first to causally demonstrate a dopamine effect on honey bee ovary growth. Previous studies

on honey bees demonstrated a positive correlation between high endogenous dopamine level and ovariole width (Harris and Woodring, 1995) or ovary development (Sasaki and Nagao, 2001). In the bumblebee, *Bombus terrestris*, high dopamine levels were also associated with the last stages of oocyte development (Bloch et al., 2000).

Sasaki and Nagao (2001) showed significantly higher brain dopamine levels in 17-day-old queenless workers living naturally in colonies (non-caged), than in their 0- or 10-day-old nestmates. Caged 18- and 12-day-old queenless workers also showed higher dopamine levels than 6-day-old workers living under the same conditions (Harris and Woodring, 1995). Thus, it seems clear that newly emerged and very young workers have characteristically lower dopamine levels than older workers, and aging correlates with a rise in brain dopamine levels, at least in the queenless situations studied. In queenright workers (caged or uncaged), an age-dependent increase in brain dopamine levels was also observed (Taylor et al., 1992; Harris and Woodring, 1995). However, this result contrasts with that obtained by Sasaki and Nagao (2001) where increased dopamine levels were only detected in queenless workers, whereas 17-day-old queenright workers showed the same dopamine levels as newly emerged or 10-day-old ones. In our experiments, dopamine administered to young caged workers supposedly caused an increase in the levels of endogenous dopamine that ultimately resulted in activation of the ovarioles.

The action of dopamine in modulating juvenile hormone biosynthesis in *corpora allata* incubated in vitro was demonstrated in *B. germanica* (Pastor et al., 1991). These glands, isolated from *A. mellifera* late larvae, were not affected by dopamine, even though other biogenic amines, such as octopamine and serotonin, had a positive effect on juvenile hormone release in vitro (Rachinsky, 1994). To our knowledge, in adult honey bees, only octopamine was demonstrated to play a role in the control of juvenile hormone biosynthesis (Kaatz et al., 1994). These results seem to exclude a putative function of dopamine on honey bee ovary growth, via activation of juvenile hormone biosynthesis in *corpora allata*. Moreover, reproduction in worker honey bees is associated with low juvenile hormone titers (Hartfelder and Engels, 1998). Although in honey bee pupae, this hormone has an inductive effect on the initiation of the synthesis of vitellogenin – the major egg protein (Barchuk et al., 2002), it clearly does not increase vitellogenin synthesis in adult bees. On the contrary, when a juvenile hormone analogue, pyriproxyfen, was applied on adult workers, or when their fat bodies were incubated with this analogue, a significant decrease in vitellogenin synthesis was observed (Pinto et al., 2000).

If dopamine does not activate honey bee ovaries by stimulating the *corpora allata*, it could be that dopamine has a direct effect on the fat body – the site of vitellogenin synthesis – or on the ovary proper, via circulating dopamine molecules. In insects, dopamine as well as other biogenic amines, exert their effects by binding to specific membrane proteins belonging to the superfamily of G protein-coupled receptors. Studies on these receptors and their related intracellular signaling have been reviewed by Blenau and Baumann (2001). Dopamine receptors were characterized in the *corpora allata* of *Manduca sexta* (Granger et al., 2000), in the antennal lobe neurons (Kirchhof and Mercer, 1997) and in the brain (Kokay and Mercer, 1996) of the honey bee, and in the mushroom bodies of locust (Degen et al., 2000). Characterization of dopamine receptors in ovaries or fat body could clarify the observed effect of dopamine on honey bee worker ovary growth. Being the site of vitellogenin synthesis, the fat body is the first candidate for studies searching for dopamine receptors and intracellular signaling mediating synthesis of this protein. In *Blaberus discoidalis*, octopamine, but not dopamine or serotonin, had a stimulating effect on fat body glycogen phosphorylase activity and trehalose synthesis. The octopaminergic receptor for phosphorylase activation in *B. discoidalis* fat body appears to belong to the octopamine I class that uses Ca^{2+} as a second messenger (Park and Keeley, 1998). Besides being the major storage organ in insects, the fat body is also the site of vitellogenin synthesis. The search for a dopamine receptor in honey bee fat body cells, as well as for the signal transduction mechanisms elicited by the receptor, might be useful in testing

the direct effect of dopamine on vitellogenin synthesis and ultimately on ovariole growth.

ACKNOWLEDGMENTS

Résumé – La dopamine contenue dans la nourriture active les ovaires des ouvrières des colonies d'*Apis mellifera* orphelines. L'influence de la dopamine sur l'activation ovarienne a été étudiée sur des groupes de jeunes ouvrières orphelines confinées dans des cagettes (8 × 11 × 13 cm) à 34 °C et 80 % HR durant 8 à 13 jours. Pour chaque expérience (8 répétitions) un même nombre d'abeilles âgées de 4 j a été confiné dans des paires de cages où elles étaient nourries avec un régime riche en pollen supplémenté ou non en dopamine (10 µg/g de régime). La nourriture sous forme de pâte était préparée avec 30 g de pollen fraîchement récolté, 7 g de sucre en poudre et 2 mL de miel. L'eau et la nourriture étaient fournies ad libitum aux deux groupes. La nourriture était renouvelée chaque jour et la consommation (mg de régime/abeille/j) était enregistrée durant toute la période de confinement. A la fin on a recherché la présence de follicules en développement dans les ovaires des ouvrières survivantes. Dans les groupes témoins le pourcentage d'abeilles en vie à la fin du confinement a varié entre 30,2 et 96 %. Chez les groupes supplémentés en dopamine (groupes DOP) les taux de survie étaient moindres (17,9 à 62 %). Pour chaque série d'expériences, le pourcentage d'abeilles survivant au confinement était significativement plus élevé chez les témoins que dans les groupes DOP (test z, $P \leq 0,01$). Ces résultats montrent que le nourrissement à la dopamine, et non le confinement lui-même, était la principale cause de mortalité. Les conditions de confinement utilisées ont permis aux ouvrières de survivre suffisamment longtemps pour développer leurs ovaires et montré que la dopamine avait une influence positive sur ce processus. On a trouvé une plus grande proportion d'abeilles avec des ovaires développés dans les groupes DOP que chez les témoins (test-z, $P = 0,004$). Des ouvrières présentant une croissance des follicules ont été observées dans six des huit séries expérimentales et, dans ces six séries, le pourcentage d'ouvrières ayant des ovaires développés était plus grand dans les groupes DOP que chez les témoins. Les abeilles des groupes témoins et des groupes DOP ont consommé les mêmes quantités de nourriture (ANOVA à un facteur, $P = 0,687$), ce qui montre que la dopamine n'a pas stimulé la prise de nourriture. Au total ces résultats montrent un effet positif de la dopamine sur l'activation ovarienne des ouvrières orphelines

et suggère fortement une fonction de cette amine biogène dans le changement du statut reproducteur des ouvrières orphelines.

Apis mellifera / dopamine / activation ovarienne

Zusammenfassung – Dopaminhaltige Nahrung aktiviert die Ovarien von *Apis mellifera* Arbeiterinnen in weisellosen Völkern. Wir untersuchten über einen Zeitraum von 8 bis 13 Tagen den Einfluß von Dopamin auf die Aktivierung der Ovarien in Gruppen junger weiselloser Bienenarbeiterinnen in kleinen Käfigen (8 × 11 × 13 cm) bei 34 °C und 80 % rel. Feuchte. In jedem Experiment (8 Wiederholungen) wurde eine gleiche Anzahl vier Tage alter Arbeiterinnen in jeweils zwei Käfigen eingebracht und mit pollenreicher Nahrung gefüttert, die entweder mit Dopamin versetzt war oder nicht (10 µg/g Nahrung)). Die pastöse Nahrung wurde aus 30 g frischgesammelten Pollen, 70 g Puderzucker und 2 mL Honig zubereitet. Wasser und Nahrung wurde beiden Bienengruppen ad libitum gegeben. Das Futter wurde täglich gewechselt und die Futterabnahme (mg Futter pro Tag und Biene) über den gesamten Versuchszeitraum protokolliert. Zum Ende der Käfighaft wurden die Ovarien der überlebenden Arbeiterinnen auf sich entwickelnde Follikel untersucht.
Durch die Käfighaft konnte den Bienen eine bestimmte Nahrung zu verabreicht werden, musste allerdings ebenfalls Bedingungen bieten, in denen die Bienen lange genug überlebten, um ihre Ovarien zu entwickeln. Der Anteil überlebender Bienen in den Kontrollgruppen schwankte zwischen 30,2 und 96 %. In den mit Dopamin gefütterten Gruppen war die Überlebensrate geringer (17,9 to 62 %). In jeder der Experimentserien war der Prozentsatz überlebender Bienen in der Kontrollgruppe signifikant höher als in der entsprechenden mit Dopamin behandelten Gruppe (z-test, $P \leq 0,01$). Die Ergebnisse zeigen, dass die Fütterung mit Dopamin und nicht die Haltungsbedingungen der Hauptgrund der Bienensterblichkeit war.
Die in dem Experiment verwendeten Käfighaftbedingungen ermöglichten den Bienen ihre Ovarien zu entwickeln. Die Ergebnisse zeigten, dass Dopamin einen fördernden Einfluss auf diesen Prozess hatte. In den Dopamingruppen wurde ein höherer Anteil an Bienen mit entwickelten Ovarien festgestellt als in den Kontrollgruppen (z-test, $P = 0,004$). Arbeiterinnen mit Follikelwachstum traten in 6 der 8 Versuchsserien auf. In all diesen 6 Experimenten war der Prozentsatz von Arbeiterinnen mit aktivierten Ovarien in der mit Dopamin gefütterten Gruppe höher als in der Kontrollgruppe.
Bienen aus Test – und Kontrollgruppen nahmen die gleiche Menge des Futters auf (ONE-WAY ANOVA, $P = 0,687$). Dies zeigt, dass Dopamin die Nahrungsaufnahme nicht angeregt hatte. Insgesamt zeigen die Ergebnisse einen positiven Effekt von Dopamin auf die Aktivierung der Ovarien und

weisen deutlich auf eine Funktion dieses biogenen Amins innerhalb der den Wechsel des reproduktiven Status weiselloser Arbeiterinnen herbeiführenden Ereignisse hin.

***Apis mellifera* / Honigbienen / Dopamin / Ovarienaktivierung**

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