

Physiological Effects of Electroshock Stimulation on Western Honey Bee

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Abstract

Bee venom contains various bioactive substances, and numerous studies have confirmed its potential for medical applications. Consequently, the production and purification of bee venom have gradually developed into a commercial model. Electroshock is one of the methods used to stimulate honey bees to produce a larger amount of clean crude bee venom. This study investigates whether electroshock affects bee behavior and internal physiology. Commercial bee venom extractors were used applying electric shocks the intensities of 58, 61, 64, 67, and 70 for 3 seconds with 3 second intervals, continuously for 10 minutes. The average temperature increase at the center of the honeycomb was 1.9 °C, 1.8°C, 1.6°C, 2.1°C, and 1.8°C, respectively. The temperature increases at the top of the beehive, where the electric shock plate was placed, was 6.8 °C, 5.8°C, 5.6°C, 5.7°C, and 5.2°C, respectively compared to before the treatment. The top temperature peaked 2.2 to 9.8 minutes after the end of the electric shocks and then gradually returned to a stable temperature within approximately 90 minutes. RT-qPCR (Reverse transcription quantitative PCR) was used to measure the RNA expression levels of *abaeicin*, *hymenoptaeicin*, and heat shock protein 70 genes in worker bees after electroshock treatment. The results showed higher expression levels of *abaeicin* 1.3 to 3.3 times, *hymenoptaeicin* 3.5 to 4.6 times, and *heat shock protein 70* 1.2 to 4.8 times respectively, from treatments compared to before treatment, indicating increased expression of antibacterial peptides and heat shock protein due to electroshock. Additionally, the lifespan of newly emerged worker bees subjected to electroshock was observed in the laboratory, revealing that the average lifespan of electroshock-treated worker bees (23.5 days) was longer than the control group's (21.8 days). In conclusion, the results suggest that electroshock treatment may positively impact bee lifespan and antibacterial peptide expression.

Keywords: electroshock, colony temperature, lifespan, antibacterial peptide

Research aim:

Honey bees (*Apis mellifera*) exhibit a remarkable defensive behavior known as "heat balling" to neutralize predators like hornets. This involves a coordinated aggregation of bees to generate a localized increase in temperature, effectively overheating the intruder. Our prior research on electroshock stimulation for bee venom collection serendipitously revealed a similar aggregation response in honey bees. This intriguing observation prompts us to investigate whether electroshock stimulation, beyond its venom-inducing properties, also elicits a measurable temperature increase within bee colonies, potentially mimicking aspects of their natural heat-balling defense mechanism. Furthermore, we aim to systematically quantify the immediate and delayed thermal responses, as well as the broader biological impacts, of varying electroshock parameters on honey bee colonies.

Experiment 1: Electroshock Stimulation Induces Temperature Increase in Bee Colonies

We studied how electric shocks affect bee colony temperature. Four experimental and four control colonies (each 8 frames of bees) were set up as shown in Figure 1 (A), with temperature loggers in the brood nest. Temperatures were recorded minute-by-minute from 8:00 AM to 7:00 PM. At 11:00 AM, experimental colonies received an electric shock (intensity 67, 20 minutes, 3-sec on/3-sec off pulses), and temperature changes were monitored.

Result 1: The temperature peaked at a 2.9°C increase 22 minutes after the shock began (2 minutes after it ended). By 30 minutes (10 minutes after it ended), the temperature had dropped by 0.5°C from its peak (Figure 1 (B)).

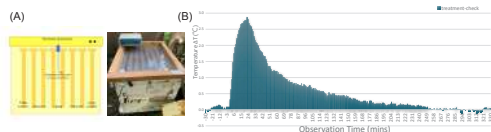


Figure 1: Brood Frame Distribution and Temperature Logger Placement in Exp.1(A). Brood Nest Temperature Changes in Electrically Stimulated Bee Colonies (B) shows how electric shock treatment affects bee colony brood nest temperature. The x-axis indicates time relative to the shock (0 = start), and the y-axis shows the temperature difference (ΔT) between experimental and control groups.

Experiment 2: Localization of Temperature Increase Response to Electroshock in Bee Colonies

Four experimental and four control colonies (8 frames each) were placed temperature loggers in the central brood nest and at the top of the frames, recorded data minute-by-minute from 10:00 AM to 4:00 PM. At 11:00 AM, experimental colonies received a 120-minute shock (intensity 67, 3-sec pulse/30-sec pause). We then recorded temperature changes in both locations.

Results 2: The central brood nest temperature rose by a maximum of 2.7°C, peaking at 67 minutes after the treatment began. It then dropped to within 0.5°C of its peak by 133 minutes (13 minutes after the treatment concluded) (Figure 2). The temperature at the top of the frames showed a larger increase, peaking at 6.4°C after 51 minutes. This temperature declined to within 0.5°C of its peak by 109 minutes (11 minutes before the treatment stopped).

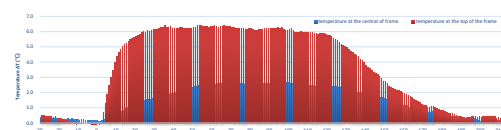


Figure 2: Brood Nest Center and Frame Top Temperature Changes in Electrically Stimulated Bee Colonies. This figure shows the temperature changes (ΔT) in bee colony brood nests and frame tops after electric shock. The x-axis represents time relative to the shock (0 = start), and ΔT is the experimental group's average temperature minus the control group's average.

Experiment 3: Effect of Different Electric Intensity on Bee Colony Temperature Increase

This experiment used four experimental and four control bee colonies, each with eight frames. Electric shock treatments were applied weekly to one experimental colony at a time, cycling through intensities of 58, 61, 64, 67, and 70 over five weeks. Each shock lasted 10 minutes with 3-second pulses and pauses. Before and after each treatment, we measured temperatures in the central brood nest and at the top of the frames, using the same logger setup as Experiment 2.

Results 3: The average temperature increase at the center of the honeycomb was 1.9 °C, 1.8°C, 1.6°C, 2.1°C, and 1.8°C, respectively (Table 1). The temperature increases at the top of the beehive, where the electric shock plate was placed, was 6.8 °C, 5.8°C, 5.6°C, 5.7°C, and 5.2°C, respectively compared to before the treatment (Table 2). The top temperature peaked 2.2 to 9.8 minutes after the end of the electric shocks and then gradually returned to a stable temperature within approximately 90 minutes.

Table 1: Effects of Different Electric Shock Intensities on Brood Nest Center Temperature Increase

Intensity	Time to High Temp (min)	Temperature before treatment (°C)	Highest Temp after treatment (°C)	ΔTemp (°C)	Time to Highest Temp decrease 0.5 degree after treatment (min)
58	26.6±5.9	35.8±0.3	37.7±0.6	1.9±0.4	53.2±8.0
61	19.8±5.1	35.8±0.3	37.5±0.4	1.8±0.4	55.2±15.3
64	21.8±5.8	35.8±0.2	37.4±0.2	1.6±0.2	36.9±0.2
67	20.4±6.4	35.7±0.4	37.8±0.5	2.1±0.6	45.8±8.9
70	19.8±9.0	35.6±0.3	37.5±0.2	1.8±0.2	47.6±11.2

Mean ± SD, n=5; Analysis of variance (ANOVA) showed $P > F > 0.05$, indicating no significant difference in brood nest center temperature increase among different intensities.

Table 2: Effects of Different Electric Shock Intensities on Frame Top Temperature Increase

Intensity	Time to High Temp (min)	Temperature before treatment (°C)	Highest Temp after treatment (°C)	ΔTemp (°C)	Time to Highest Temp decrease 0.5 degree after treatment (min)
58	12.2±0.4	33.6±0.8	40.4±0.7	6.8±0.6	24±4.4
61	19.8±5.1	34.0±0.8	39.8±0.8	5.8±0.7	27.2±7.2
64	13.2±3.8	34.3±0.9	40.0±0.5	5.6±1.1	25.6±5.9
67	13.0±2.9	34.1±0.7	39.9±0.7	5.7±1.2	26.6±5.0
70	11.2±1.9	34.6±0.6	39.8±0.6	5.2±0.9	23.4±8.5

Mean ± SD, n=5; Analysis of variance (ANOVA) showed $P > F > 0.05$, indicating no significant difference in frame top temperature increase among different intensities.

Experiment 4: Effect of Electric Voltage Shock on Bee Longevity

We collected 800 newly emerged bees, splitting them into experimental (400) and control (400) groups. On day 5, experimental bees were chilled (-18°C, 15 min) and then received two electric shocks (intensity 67, 3-sec on/off). Control bees were only chilled. After recovery, both groups were housed in rearing cages (100 bees/cage, with pollen and sugar water) in a 33°C, 70% humidity incubator. We daily recorded bee mortality until all bees died.

Results 4: The lifespan of newly emerged worker bees subjected to electroshock was observed in the laboratory, revealing that the average lifespan of electroshock-treated worker bees (23.5 days) was longer than the control group's (21.8 days) (Figure 3 (B)). There was no significant difference in longevity between shocked (n=379) and control (n=381) bees (Welch's t-test, $p < 0.05$).

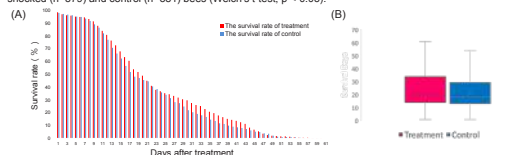
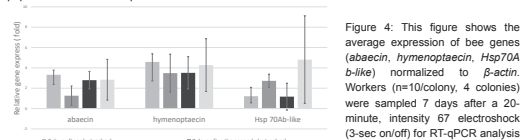


Figure 3: Longevity of Worker Bees After Electric Shock Treatment (A) shows the observed longevity of worker bees following electric shock treatment and control. Average Longevity of Worker Bees After Electric Shock Treatment (B) shows the average lifespan of worker bees after electric shock and control. There was no significant difference in longevity between shocked (n=379) and control (n=381) bees (Welch's t-test, $p < 0.05$).

Experiment 5: Effects of Electrical Shock on Honeybee Gene Expression

We studied how electric shocks affect honeybee gene expression. Four experimental and four control colonies (each with eight frames) were set up, and the comb center temperatures were recorded from 8:00 AM to 7:00 PM. Experimental colonies received a 20-minute electric shock (intensity 67, 3-sec on/off) at 11 AM, with temperature changes monitored. Shocks were repeated weekly. Before each shock, 10 worker bees were collected and frozen. Total RNA was extracted from bee abdomens, then reverse-transcribed and analyzed via RT-PCR to measure *abaeicin*, *hymenoptaeicin*, and *hsp70Ab-like* gene expression, using *β-actin* as a control, and the sample collected before the first shock as the gene expression baseline.

Results 5: RT-qPCR (Reverse transcription quantitative PCR) was used to measure the RNA expression levels of *abaeicin*, *hymenoptaeicin*, and *heat shock protein 70* genes in worker bees after electroshock treatment. The results showed higher expression levels of *abaeicin* 1.3 to 3.3 times, *hymenoptaeicin* 3.5 to 4.6 times, and *heat shock protein 70* 1.2 to 4.8 times respectively, from treatments compared to before treatment (Figure 4), indicating increased expression of antibacterial peptides and heat shock protein due to electroshock.



Conclusions:

- Electric shock stimulation induces an increase in bee colony temperature. The central brood nest temperature increased after electric shock treatment, for example, by a maximum of 2.9°C at intensity 67. After the shock stopped, the temperature gradually returned to stable levels. Temperatures at the top of the frames, closer to the shock plate, showed a larger increase, peaking at 6.4°C.
- There was no significant difference in bee colony central temperature increase across different electric shock intensities. Analysis of variance (ANOVA) indicated no significant difference in the increase of brood nest center temperature among various intensities (58, 61, 64, 67, 70). Similarly, no significant difference was observed in the frame top temperature increase across these intensities.
- Electric shock significantly increased the gene expression of antimicrobial peptides and heat shock protein in honeybees. Compared to pre-treatment levels, *abaeicin* gene expression increased 1.3 to 3.3 times, *hymenoptaeicin* 3.5 to 4.6 times, and *heat shock protein 70* (Hsp70Ab-like) 1.2 to 4.8 times after electric shock treatment. This indicates increased expression of antibacterial peptides and heat shock protein due to electroshock.
- Electric shock treatment did not significantly negatively impact the average longevity of worker bees. The average lifespan of electroshock-treated worker bees (23.5 days) was observed to be longer than that of the control group (21.8 days). However, a Welch's t-test revealed no significant statistical difference between the treated group (n=379) and the control group (n=381) ($p < 0.05$).
- Electric shock treatment may positively influence bee lifespan and antibacterial peptide expression. The study's findings suggest that electroshock treatment, in addition to its use for bee venom collection, could have positive effects on bee longevity and the expression of antibacterial peptides.

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