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Profiling early growth response (Egr-1) gene expression in the honeybee brain during foraging

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Abstract

Immediate early genes (IEGs) are the first expressed genes in the brain upon induction by any stimulus. IEGs unique properties of immediate expression provide us ample opportunities to investigate the regulatory genes propelled by behavioral changes. Different types of IEGs have been found to be activated by different behavioral characteristics. Foraging of honeybees is one of the most well characterized behaviors among social insects. However, only a little knowledge has been accumulated about the molecular mechanisms that regulates the foraging behavior, due to availability of few studies. Finding which IEGs are involved in monitoring or inducing a behavior is likely the first promising adventure to open the lid for uncovering the underlying complex regulatory biology. *Egr-1* is one of most widely studied IEGs that was found to have been induced by different behaviors including learning and memory. Like the most IEGs, *Egr-1* expression is also transient, indicating activation of its downstream genes' role in monitoring the behavior. Honeybee foraging is a complex behavior in which learning and memory of food location is a primary goal of foraging. In our recent studies, involvement of *Egr-1* in honeybee foraging and associative learning had been demonstrated. In this study we aim to examine the expression dynamics of *Egr-1* during consecutive trips of foraging. It may be noted that, during foraging, a forager bee repeatedly flies back and forth multiple times from the hive to the feeder, communicate each other and motivate other foragers in the hive and accomplished the food collection. Examining at which flight trip, *Egr-1* reaches highest expression level, then drops, could help in finding the downstream regulatory pathway genes that monitors the complex foraging behavior.

Key words: Honeybee; Foraging; Immediate Early Genes; *Egr-1*

1. Introduction

Honeybees are one of most admirable eusocial insects. Even though they are tiny in size, they possess highly efficient well-structured social life culture that remains to be mysterious and captivated to humans. They share and distribute responsibilities or labour among different castes in the colony including the queen, the worker, and the drone; thus maintain and sustain their hive/colony (Singh & Chanu, 2021, 2022; Wojciechowski et al., 2018). Infect one of the most widely studied behaviours among social insects is the foraging of honeybees. While a vast number of studies are facing difficulties to uncover the mysterious behaviours of honeybees, knowledge on the underlying biology is still far underexplored and limited to a few studies. Moreover, the challenges are also greater to formulate experimental designs to study the biology of foraging. Because they live as colony and their survival and reproduction requires entire efforts of the caste and labour distribution ("The Colony and Its Organization, Mid-Atlantic Apiculture Research Extension Consortium, canr.udel.edu," ; Pan et al., 2024). Therefore, to conduct behavioural experiments on honeybees always

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preferred either semi or fully opened natural environment, a stringent condition cannot be applied as it would disturb their normal/ natural behaviour.

During foraging honeybees carry nectar and pollen from flowers or other available sources and store it to the hive for their colony. Most foragers repeatedly visit the same food source for many days and thereby a repetitive behaviour. Foraging is accomplished by appropriate coordination of many distinct complex behaviours including long-distance navigation, learning and memory of flower cues, evaluation of the reward value of the food source, and communication (Frisch, 1965). Notably, all the higher order sensory and cognitive abilities of honeybees have been identified through studying the foraging behaviour and training the honeybees to forage on artificial feeders (Chittka & Geiger, 1995; Hunt & Chittka, 2015). While the honeybee behaviours have been extensively studied, their regulatory mechanisms at molecular and cellular level have been far too little studied. In recent years, IEGs such as *kakusei*, *c-Jun* (*Jra*), *Hr-38* and *Egr-1*, have been used as markers for neural activity and to identify brain regions involved in innate and learned behaviours (Kiya et al., 2012; Singh & Chanu, 2022; Singh et al., 2018; Singh & Takhellambam, 2021; Singh et al., 2020; Sommerlandt et al., 2019). And the results have shown to be highly promising. Meanwhile, studies in vertebrates also reported that expression changes of IEGs is induced by neural activities and subsequently regulate the expression of downstream genes that are involved in neural homeostasis and synaptic plasticity (Clayton, 2000; Loebrich & Nedivi, 2009; Perez-Cadahia et al., 2011).

Our recent publications have shown that IEGs *kakusei*, *Hr-38* and *Egr-1* were upregulated during the entire time of foraging while the level was gradually increased, then decreased after reaching a peak level at about 30 min (Singh & Chanu, 2022; Singh & Chanu, 2024; Singh et al., 2018; Singh & Takhellambam, 2021). It indicates a clear involvement of the three IEGs in foraging activity and a possible co-operative performance during foraging. Additionally, sustain upregulation during the entire foraging period also indicates a strong indication of continuous foraging to hold the increased IEG expression associating to neural activity and behaviour. Among the three IEGs *Egr-1* has been the most extensively investigated and well characterised in the studies using vertebrate models. Notably, *Egr-1* expression changes have been implicated to be involved in learning processes during orientation flights as well as in priming the brain for navigational learning in honeybees (Lutz & Robinson, 2013; Naeger & Robinson, 2016). Subsequently, *Egr-1* expression changes have also be linked with associative learning during foraging (Singh et al., 2018).

As the *Egr-1* is immediately over-expressed and its expression level varied during the entire duration of foraging, in this study we are interested to examine whether there is difference between the flight trips during foraging. Our results showed that differential expression and upregulation of *Egr-1* during the early food reward foraging.

2. Materials and methods

2.1. Feeding training and adaptation

Honeybee house was located inside the campus of National Centre for Biological Sciences (NCBS), Tata Institute of Fundamental Research (TIFR), Bangalore, India. It was built as outdoor flight cage which makes the environment close to the nature, allowing the bees to forage freely from the hive to the feeder with minimal/negligible disturbance. Such designed bee house provided us the opportunity to conduct the behavioral training, tests and observe in a semi natural environment. The honeybees (*Apis Melifera*) were fed with pollen and 1 M sucrose solution every day from 14:00 hr. to 15:00 hr. in different feeder plates. This was continued everyday about two weeks before the sample collection began. So that before the experiment began the bees could learned and get adapted properly with feeing time, food location, food quality and the color of the feeding plates, green color for sucrose and red color of pollen. The entire procedures are similar with our previous reports (Singh et al., 2018; Singh & Takhellambam, 2021; Singh et al., 2020).

2.2. Sample collection during, before and after foraging

Sample collection started after the bees had learned and adapted about bee house environment, hive location, food quality, feeding time, feeder place and feeding plate etc. The bees were gently pen marked at the thorax using Uni POSCA Paint Markers (Uni Mitsubishi Pencil, UK), at their first arrival to the feeder and collection started from the consecutive arrivals. We collected 1-2 bees at each time point in each day and continued until the numbers were filled up to 5/7 bees at each group. We collected samples at 1st arrival at the feeder which was immediately after presenting the sucrose solution and labelled as 1st-arrival/trip group (14:00 hr.). The subsequent time points or groups are the 5th-trip (14:15 hr.), 8th-trip (14:20 hr.), 11th-trip (14:28 hr.) 15th-trip (14:35 hr.), 20th-trip (14:60 hr.) etc. We used 50 mL falcon tubes with multiple holes in it for the bee collection and immediately flash frozen after collection then stored at -80°C for the further processing.

To collect before foraging group, the honeybees were pen marked on the day before collection and the marked bees were collected on the next day morning at 9:00 hr. in the hive, before the bees flew out from the hive to forage. Whereas, for the after foraging group, the bees which were pen-marked during foraging were collected in the evening at 18:00 hr. in the hive, on the same day. The collected bees were immediately flash frozen and stored at -80°C. We collected 5/7 bees in both the groups. In our experimental settings, the bees stop foraging after 17:00 hr. (Singh et al., 2018; Singh & Takhellambam, 2021; Singh et al., 2020) (Singh et al., 2018; Singh & Takhellambam, 2021; Singh et al., 2020).

2.3. Brain dissection

The frozen bees from the -80˚C were proceed to lyophilization for 20 min at -50˚C and vacuum condition at 0.420 mBar in a lyophilizer (Freeze Zone1 PlusTM 4.5-liter cascade Freeze Dry System, Labconco Corporation, Kanas City). The lyophilized brains were dissected in a glass chamber containing 100% ethanol placed on dry ice. The dissected whole brains were immediately placed in 1.5 mL Eppendorf tube containing 500 µL Trizol (Trizol Reagent, ambion RNA, life technology) and placed on dry ice.

2.4. RNA isolation and cDNA synthesis

The frozen brain was thawed on ice and homogenized using electronic homogenizer (Micro-Grinder Pestle Mixer, RPI Research Products International) with pestle (Micro-Tube Sample Pestles, Research Products International). This was followed by centrifugation at 10000g for 5min at 4^0C. The upper portion containing RNA was removed without disturbing the lower DNA fraction, cell tissue debris, or protein fraction, and transferred into another tube in ice. The total RNA was quantified, and purity check done using nanodrop spectrophotometer. The samples with absorbance ratio 260/280 greater than 1.8 were considered having negligible contamination and proceed to cDNA synthesis. cDNA synthesis was carried out taking equal amount of total RNA from each sample, using cDNA kit manufacturer's protocol of SuperScript™III First-Strand Synthesis System supplied by Invitrogen (Thermo Fisher Scientific).

2.5. Quantitative real time PCR (qPCR).

The complementary cDNA was used for amplifying target IEG *Egr-1* by qPCR using 7900HT Fast Real Time PCR System (Applied Biosystem, Singapore). *Rp-49* gene was used as endogenous or housekeeping gene. Same amount of total cDNA was taken from each sample, and the final reaction volume was made up to 10µl, including specific oligonucleotide primers (Sigma Aldrich) and SYBR Green (KAPA Syber1 FAST PCR Master Mix (2X) ABI Prism1). The oligomeric sequences for the primers are *Egr-1*(mRNA): Forward Primer-GCTCTGAGGGTGATTTCTCG & Reverse Primer-GAGAAACCGTTCTGCTGTGA; *Egr-1*(pre-mRNA): Forward Primer-ATCCCTTGCGTACACACCTC and Reverse Primer-AATCGCACCAGATTCCACTC) *Rp-49*: Forward Primer-CAGTTGGCAACATATGACGAG & Reverse Primer-AAAGAGAAACTGGCGTAAACC. Each qPCR plate also had five standard reactions with five different serial dilutions (1/10, 1/100, 1/1000, 1/10000, 1/100000) for preparing standard curve. The reaction mixture and qPCR condition settings followed SYBR Green manufacturer's protocol and we have used the same procedures in our previous multiple publications as well (Singh et al., 2018; Singh & Takhellambam, 2021; Singh et al., 2020).

2.6. Statistics for relative gene expression analysis at different time points

A standard curve was plotted for each qPCR run and quantify the unknown gene expression levels for each gene *Egr-1* and *Rp-49*. The unknown quantities were measured by CT values based on relative standard curve method generated by SDS 2.4 software supplied with the 7900HT Fast Real system (Applied Biosystem, Singapore) and normalized with *Rp-49* CT values. To examine the expression level difference among the time points, fold change using the formula $2^{-\Delta\Delta CT}$ were calculated. Moreover, standard deviation (SD) was also calculated following the protocol provided by 7900HT Fast Real system (Applied Biosystem, Singapore). Following this, normality tests were performed. After each group passed normal/Gaussian distribution, One-Way-Anova test (with corrected for multiple comparison analyses) was carried out for checking statistical difference. P-values less than 0.05 were considered significantly different, whereas the difference of the means with P-values greater than 0.05 were considered to have happened by chance. The normal distribution and One-Way-Anova test was performed with the help of GraphPad Prism Version 10.4.1 (Motulsky, 2016) (http://www.graphpad.com).

3. Results

We initially planned to collect every trip until the last trip of foraging; however, the plan did not work due to multiple marked bees arriving at the same time, and we happened to miss the target. Nonetheless, it will be possible if marked only 1 or 2 bees per day of foraging and collected them only on the same day. Since we are interested to examine the upregulation of the gene during foraging, sample collection at every trip was not mandatory. Therefore, we collected the bees at different trips with uneven intervals and counted the time at which the bees were collected. When we

checked the number of trips and time it took, we observed that honeybee made about 20 trips during 1 hour of foraging in our experimental settings. The number of trips and the time at which we collected the samples are 1st-trip (14:00 hr.), 5th-trip (14:15 hr.), 8th-trip (14:20 hr.), 11th-trip (14:28 hr.), 15th-trip (14:35 hr.), 20th-trip (14:60 hr.) etc. We also collected before (9:00 hr.) and after foraging (18:00 hr.) samples.

Our results showed that the expression level of *Egr-1* in all other foraging trips and after-foraging is higher than the before-foraging as shown in Figure 1. However, trip-1 and after-foraging levels are not statistically significant. We also observed gradual increase until the trip-11, while the level of after-foraging is highly significantly lower than the 8th-trip, 11th-trip, 15th-trip and 20th-trip (Figure 1 and Table1). We also examined the level of *Egr-1* pre-mRNA level. Similarly, the pre-mRNA level of all the trips and after foraging is greater than the level at before-foraging and statistically significant including the after-foraging level; the results are summarized in Figure 2 and Table 2. The pre-mRNA levels were gradually increased until 8th-trip and then decreased. In conclusion, our results showed that both *Egr-1* and *Egr-1* pre-mRNA can be used as neural markers for investigating the biology of behavior.

4. Discussion

Dissecting behavioral phenotypes of animals and humans into molecular and cellular level is highly complex and until now there is no appropriate study model available for unwinding this complexity. As rodents are phylogenetically close to humans, they have been widely used to provide information on behavioral abnormalities of human but rarely on insects. With time it has been gradually realized that less complex social insects such as honeybees could be a better choice model for finding some basic answers to fundamental questions on the complex regulatory mechanism of behavior. Notably, even though small, honeybees demonstrate distinctive behaviors of large animals and humans, as clearly seen during the foraging of honeybees, that include learning and memory, communication skills, and exceptionally well-orchestrated task or labor divisions etc. At this juncture, to begin study the underlying mechanisms, from the genetic aspects IEGs stand out to be the ultimate choice. Because IEGs are the first activated genes linking membrane events and nucleus, even without the requirement for de novo protein synthesis (Bahrami & Drablos, 2016; Fowler et al., 2011). They are also stimulated by cell-extrinsic and cell-intrinsic signals (Bahrami & Drablos, 2016; Loebrich & Nedivi, 2009). Moreover, their roles in the process of learning and memory formation and their important role in phenotypic changes as well as everyday brain function had well been demonstrated (Khan et al., 2025; Loebrich & Nedivi, 2009; Minatohara et al., 2015). Thus, the alteration in the IEGs expression level has been considered to be occurring naturally or generally in response to synaptic activity and the first step towards long-term activity-dependent plasticity (Loebrich & Nedivi, 2009; Singh & Chanu, 2024). It may be noted that different types of stimulations induce different sets of IEGs (Bepari et al., 2012; Dahmen et al., 1997; Lacar et al., 2016; Xu et al., 2019), indicating that different types of IEGs could link to different behaviors. Meanwhile *Egr-1* being one of most widely studied IEGs, remains to be gene of choice to start with and examine its role in honeybee foraging. In this study we aimed to investigate expression pattern of *Egr-1* from the start of foraging towards the subsequent trips as the foraging continued. As we were interested to examine the *Egr-1* level across the foraging trips we collected the bees at different trips and have examined the gene expression level. We did find higher expression of *Egr-1* in the subsequent trips compare to the start of foraging. Moreover, the level of before-foraging is higher than the level of after-foraging. This finding indicates the role of *Egr-1* in the motivation of food-reward foraging continuation and suggest a possible link with the communication and interaction among foragers. It may be noted that our previous studies demonstrated upregulation of *Egr-1* during the entire time of foraging while the level of *Egr-1* varied from the start to the end of foraging (Singh & Chanu, 2024) which reveals a role of *Egr-1* on foraging and communication in honeybees supporting the present finding. We have also found differential expression of *Egr-1* pre-mRNA, which showed higher expression in all the trips and after foraging compared to the level at before-foraging. This study highlights, both *Egr-1* pre-mRNA and *Egr-1* are highly reliable neural markers that can be used in finding associated genes or proteins and cellular processing that regulates different brain functions and behaviors that may be translated to higher animals and humans which requires further intelligent experimental designs.

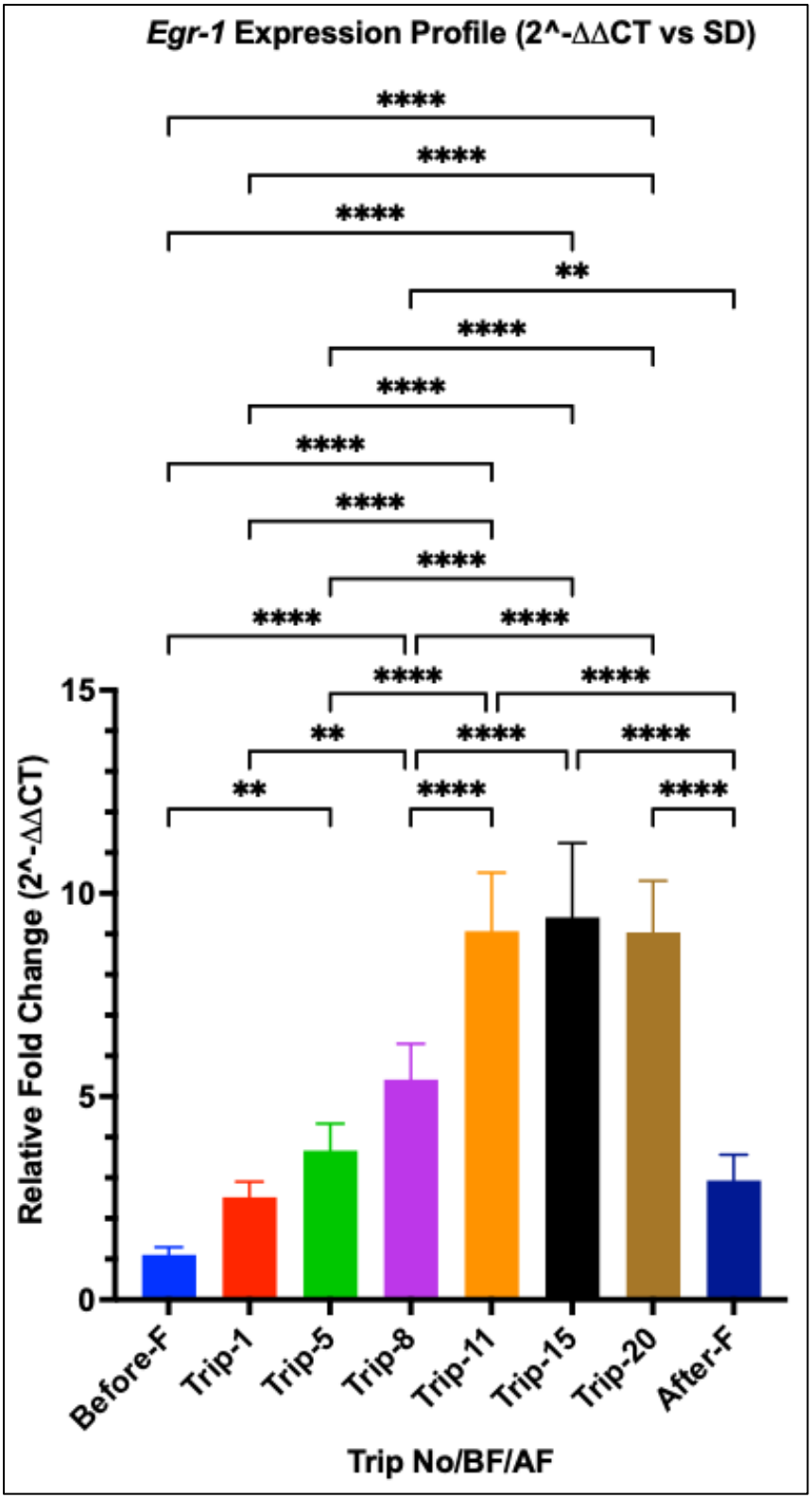

Figure 1 *Egr-1* expression profile during honeybee foraging, and before/after-foraging. Bar graphs with standard deviation (SD) of relative fall change (2^⁻ΔΔCT) at different collection points are presented in different colors, and F stands for foraging. Number of samples in each group are before-foraging (BF): n=7, 1st-trip: n=7, 5th-trip: n=5, 8th-trip: n=5, 11th-trip: n=6, 15th-trip: n=6, 20th-trip: n=5, after-foraging (AF): n=6. Statistical difference between adjacent groups were analyzed using One-way ANOVA with Tukey's multiple comparisons test. The p values greater than 0.05 are considered statically not significant and * indicates P< 0.05, ** indicates P< 0.01, *** indicates P < 0.001, **** indicates P < 0.0001

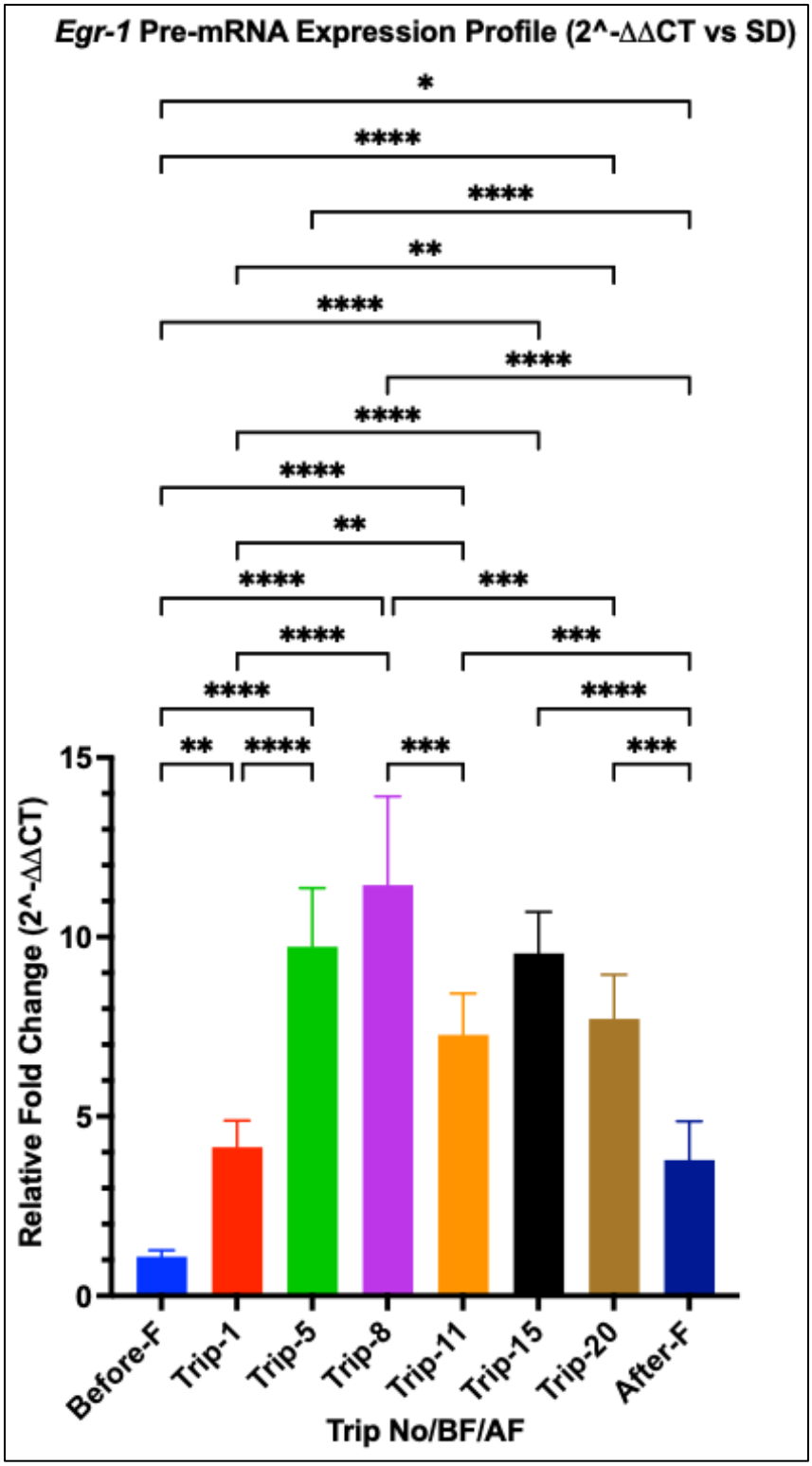

Figure 2 *Egr-1* pre-mRNA expression profile during honeybee foraging, and before/after-foraging. Bar graphs with standard deviation (SD) of relative fall change ($2^{-}\Delta\Delta CT$) at different collection points are presented in different colors and F stands for foraging. Number of samples in each group are before-foraging (BF): n=7, 1st-trip: n=7, 5th-trip: n=5, 8th-trip: n=5, 11th-trip: n=6, 15th-trip: n=6, 20th-trip: n=6, after-foraging (AF): n=6. Statistical difference between adjacent groups were analyzed using One-way ANOVA with Tukey's multiple comparisons test. The p values greater than 0.05 are considered statically not significant and * indicates P< 0.05, ** indicates P< 0.01, *** indicates P < 0.001, **** indicates P < 0.0001

Table 1 One-way Anova with Tukey's multiple comparisons test results for *Egr-1*

Comparing Groups	Mean Diff.	95.00% CI of Diff.	P Figure	P Value
Before-F vs. Trip-1	-1.422	-3.409 to 0.5653	ns	0.3221
Before-F vs. Trip-5	-2.564	-4.552 to -0.5772	**	0.0043
Before-F vs. Trip-8	-4.316	-6.303 to -2.329	****	<0.0001
Before-F vs. Trip-11	-7.969	-9.857 to -6.081	****	<0.0001
Before-F vs. Trip-15	-8.307	-10.19 to -6.418	****	<0.0001
Before-F vs. Trip-20	-7.935	-9.922 to -5.948	****	<0.0001
Before-F vs. After-F	-1.834	-3.722 to 0.05441	ns	0.0622
Trip-1 vs. Trip-5	-1.142	-3.289 to 1.004	ns	0.6817
Trip-1 vs. Trip-8	-2.894	-5.041 to -0.7479	**	0.0025
Trip-1 vs. Trip-11	-6.547	-8.602 to -4.492	****	<0.0001
Trip-1 vs. Trip-15	-6.885	-8.940 to -4.830	****	<0.0001
Trip-1 vs. Trip-20	-6.513	-8.659 to -4.367	****	<0.0001
Trip-1 vs. After-F	-0.4118	-2.467 to 1.643	ns	0.9979
Trip-5 vs. Trip-8	-1.752	-3.898 to 0.3946	ns	0.1811
Trip-5 vs. Trip-11	-5.404	-7.459 to -3.349	****	<0.0001
Trip-5 vs. Trip-15	-5.742	-7.797 to -3.687	****	<0.0001
Trip-5 vs. Trip-20	-5.37	-7.517 to -3.224	****	<0.0001
Trip-5 vs. After-F	0.7307	-1.324 to 2.786	ns	0.9429
Trip-8 vs. Trip-11	-3.653	-5.708 to -1.598	****	<0.0001
Trip-8 vs. Trip-15	-3.99	-6.045 to -1.935	****	<0.0001
Trip-8 vs. Trip-20	-3.619	-5.765 to -1.472	****	<0.0001
Trip-8 vs. After-F	2.482	0.4275 to 4.537	**	0.0089
Trip-11 vs. Trip-15	-0.3378	-2.297 to 1.622	ns	0.9992
Trip-11 vs. Trip-20	0.03391	-2.021 to 2.089	ns	>0.9999
Trip-11 vs. After-F	6.135	4.176 to 8.094	****	<0.0001
Trip-15 vs. Trip-20	0.3717	-1.683 to 2.427	ns	0.9989
Trip-15 vs. After-F	6.473	4.513 to 8.432	****	<0.0001
Trip-20 vs. After-F	6.101	4.046 to 8.156	****	<0.0001

Note: ns represents not significant and p values less than 0.05 are considered not significant

Table 2 One-way Anova with Tukey's multiple comparisons test results for *Egr-1* pre-mRNA

Comparing Groups	Mean Diff.	95.00% CI of Diff.	P Figure	P Value
Before-F vs. Trip-1	-3.053	-5.493 to -0.6135	**	0.006
Before-F vs. Trip-5	-8.637	-11.08 to -6.197	****	<0.0001
Before-F vs. Trip-8	-10.35	-12.79 to -7.914	****	<0.0001
Before-F vs. Trip-11	-6.177	-8.495 to -3.859	****	<0.0001

Before-F vs. Trip-15	-8.45	-10.77 to -6.132	****	<0.0001
Before-F vs. Trip-20	-6.628	-8.946 to -4.310	****	<0.0001
Before-F vs. After-F	-2.695	-5.013 to -0.3764	*	0.0132
Trip-1 vs. Trip-5	-5.583	-8.219 to -2.948	****	<0.0001
Trip-1 vs. Trip-8	-7.301	-9.936 to -4.666	****	<0.0001
Trip-1 vs. Trip-11	-3.124	-5.647 to -0.6005	**	0.0068
Trip-1 vs. Trip-15	-5.397	-7.920 to -2.874	****	<0.0001
Trip-1 vs. Trip-20	-3.575	-6.098 to -1.051	**	0.0013
Trip-1 vs. After-F	0.3587	-2.165 to 2.882	ns	0.9998
Trip-5 vs. Trip-8	-1.718	-4.353 to 0.9177	ns	0.4397
Trip-5 vs. Trip-11	2.46	-0.06366 to 4.983	ns	0.0606
Trip-5 vs. Trip-15	0.1862	-2.337 to 2.709	ns	>0.9999
Trip-5 vs. Trip-20	2.009	-0.5145 to 4.532	ns	0.2055
Trip-5 vs. After-F	5.942	3.419 to 8.465	****	<0.0001
Trip-8 vs. Trip-11	4.177	1.654 to 6.700	***	0.0001
Trip-8 vs. Trip-15	1.904	-0.6193 to 4.427	ns	0.2618
Trip-8 vs. Trip-20	3.726	1.203 to 6.250	***	0.0007
Trip-8 vs. After-F	7.66	5.136 to 10.18	****	<0.0001
Trip-11 vs. Trip-15	-2.273	-4.679 to 0.1325	ns	0.0757
Trip-11 vs. Trip-20	-0.4508	-2.857 to 1.955	ns	0.9987
Trip-11 vs. After-F	3.482	1.077 to 5.888	***	0.001
Trip-15 vs. Trip-20	1.823	-0.5833 to 4.228	ns	0.2574
Trip-15 vs. After-F	5.756	3.350 to 8.162	****	<0.0001
Trip-20 vs. After-F	3.933	1.527 to 6.339	***	0.0002

Note: ns represents not significant and p values less than 0.05 are considered not significant

5. Conclusion

Desigining experiments for understanding the biology of behavior is an extremely challenging research. Most study designs approach in creating different artificial conditions and the question remains is how far those findings can be applicable to the natural phenomenon. In such challanging situation, honeybee provides an incredible model system allowing us to address some fundamental questions and get answers, that may be applicable to the natural scenarios. Because, honeybee behaviors can be studied and examined in a semi natural environment, and can be performed with a little or negligible disturbace to their normal behavior. At this juncture, using immediate early gene is an appropriate choice, to find the underlying regulatory genes of behavior, such as foraging behavior. Thus outcome or the results in this study, may have a promising suggestions that can be applied across animal kindom beyond insects. Besides, honeybee behavior poses a highly similar social behaviors of social animals and humans. And immediate early gene *Egr-1* may be used as neural marker to investige and find the molecular and cellular pathways that regulates different types of behaviors.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interests.

References

[1] Bahrami, S., & Drablos, F. (2016). Gene regulation in the immediate-early response process. Adv Biol Regul, 62, 37-49. https://doi.org/10.1016/j.jbior.2016.05.001

[2] Bepari, A. K., Watanabe, K., Yamaguchi, M., Tamamaki, N., & Takebayashi, H. (2012). Visualization of odor-induced neuronal activity by immediate early gene expression. BMC Neurosci, 13, 140. https://doi.org/10.1186/1471-2202-13-140

[3] Chittka, L., & Geiger, K. (1995). Can honey bees count landmarks? Animal Behaviour, 49(1), 159-164. https://doi.org/https://doi.org/10.1016/0003-3472(95)80163-4

[4] Clayton, D. F. (2000). The genomic action potential. Neurobiol Learn Mem, 74(3), 185-216. https://doi.org/10.1006/nlme.2000.3967

[5] The Colony and Its Organization, Mid-Atlantic Apiculture Research Extension Consortium, canr.udel.edu. WordPress

[6] Dahmen, N., Fehr, C., Reuss, S., & Hiemke, C. (1997). Stimulation of immediate early gene expression by desipramine in rat brain. Biol Psychiatry, 42(5), 317-323. https://doi.org/10.1016/S0006-3223(96)00361-7

[7] Fowler, T., Sen, R., & Roy, A. L. (2011). Regulation of primary response genes. Mol Cell, 44(3), 348-360. https://doi.org/10.1016/j.molcel.2011.09.014

[8] Frisch, K. (1965). Tanzsprache und Orientierung der Bienen. Berlin; Heidelberg. Springer Verlag. https://doi.org/10.1007/978-3-642-94916-6

[9] Hunt, K. L., & Chittka, L. (2015). Merging of long-term memories in an insect. Curr Biol, 25(6), 741-745. https://doi.org/10.1016/j.cub.2015.01.023

[10] Khan, Z. U., Carretero-Rey, M., de Leon-Lopez, C. A. M., & Navarro-Lobato, I. (2025). Memory-Associated Immediate Early Genes: Roles in Synaptic Function, Memory Processes, and Neurological Diseases. Mol Neurobiol, 62(12), 15885-15915. https://doi.org/10.1007/s12035-025-05203-x

[11] Kiya, T., Ugajin, A., Kunieda, T., & Kubo, T. (2012). Identification of kakusei, a nuclear non-coding RNA, as an immediate early gene from the honeybee, and its application for neuroethological study. Int J Mol Sci, 13(12), 15496-15509. https://doi.org/10.3390/ijms131215496

[12] Lacar, B., Linker, S. B., Jaeger, B. N., Krishnaswami, S. R., Barron, J. J., Kelder, M. J. E., Parylak, S. L., Paquola, A. C. M., Venepally, P., Novotny, M., O'Connor, C., Fitzpatrick, C., Erwin, J. A., Hsu, J. Y., Husband, D., McConnell, M. J., Lasken, R., & Gage, F. H. (2016). Nuclear RNA-seq of single neurons reveals molecular signatures of activation. Nat Commun, 7, 11022. https://doi.org/10.1038/ncomms11022

[13] Loebrich, S., & Nedivi, E. (2009). The function of activity-regulated genes in the nervous system. Physiol Rev, 89(4), 1079-1103. https://doi.org/10.1152/physrev.00013.2009

[14] Lutz, C. C., & Robinson, G. E. (2013). Activity-dependent gene expression in honey bee mushroom bodies in response to orientation flight. J Exp Biol, 216(Pt 11), 2031-2038. https://doi.org/10.1242/jeb.084905

[15] Minatohara, K., Akiyoshi, M., & Okuno, H. (2015). Role of Immediate-Early Genes in Synaptic Plasticity and Neuronal Ensembles Underlying the Memory Trace. Front Mol Neurosci, 8, 78. https://doi.org/10.3389/fnmol.2015.00078

[16] Naeger, N. L., & Robinson, G. E. (2016). Transcriptomic analysis of instinctive and learned reward-related behaviors in honey bees. J Exp Biol, 219(Pt 22), 3554-3561. https://doi.org/10.1242/jeb.144311

[17] Pan, L., Zhong, S., Xu, T., Chen, W., & Zeng, Z. (2024). The Honey Bee Colony's Criterion for Candidate Selection: "Ongoing" or "One-Shot"? Animals (Basel), 14(11). https://doi.org/10.3390/ani14111535

[18] Perez-Cadahia, B., Drobic, B., & Davie, J. R. (2011). Activation and function of immediate-early genes in the nervous system. Biochem Cell Biol, 89(1), 61-73. https://doi.org/10.1139/O10-138

[19] Singh, A. S., & Chanu, M. T. (2021). Honey Bee Products: Honey And Royal Jelly And Their Nutritional And Medicinal Values To Humans. British Journal of Biomedical Research. https://doi.org/10.24942/bjbmr.2021.815

[20] Singh, A. S., & Chanu, M. T. (2022). Combined role of immediate early genes Egr-1, Hr-38 and Kakusei in the foraging behavior and learning in honeybees. World Journal of Advanced Research and Reviews, 16(2), 458–465. https://doi.org/https://doi.org/10.30574/wjarr.2022.16.2.1169

[21] Singh, A. S., & Chanu, M. T. (2024). Immediate Early Genes Egr-1, Hr38 and Kakusei Function Throughout the Entire Foraging Period: A Study on Honeybees. www.preprint.org. https://doi.org/doi:10.20944/preprints202501.0047.v1

[22] Singh, A. S., Shah, A., & Brockmann, A. (2018). Honey bee foraging induces upregulation of early growth response protein 1, hormone receptor 38 and candidate downstream genes of the ecdysteroid signalling pathway. Insect Mol Biol, 27(1), 90-98. https://doi.org/10.1111/imb.12350

[23] Singh, A. S., & Takhellambam, M. C. (2021). A Method to Study Honey Bee Foraging Regulatory Molecules at Different Times During Foraging. Front Insect Sci, 1, 723297. https://doi.org/10.3389/finsc.2021.723297

[24] Singh, A. S., Takhellambam, M. C., Cappelletti, P., & Feligioni, M. (2020). Immediate early gene kakusei potentially plays a role in the daily foraging of honey bees. PLoS One, 15(5), e0222256. https://doi.org/10.1371/journal.pone.0222256

[25] Sommerlandt, F. M. J., Brockmann, A., Rossler, W., & Spaethe, J. (2019). Immediate early genes in social insects: a tool to identify brain regions involved in complex behaviors and molecular processes underlying neuroplasticity. Cell Mol Life Sci, 76(4), 637-651. https://doi.org/10.1007/s00018-018-2948-z

[26] Wojciechowski, M., Lowe, R., Maleszka, J., Conn, D., Maleszka, R., & Hurd, P. J. (2018). Phenotypically distinct female castes in honey bees are defined by alternative chromatin states during larval development. Genome Res, 28(10), 1532-1542. https://doi.org/10.1101/gr.236497.118

[27] Xu, C., Li, Q., Efimova, O., Jiang, X., Petrova, M., A, K. V., Kolosov, P., Aseyev, N., Koshkareva, K., Ierusalimsky, V. N., Balaban, P. M., & Khaitovich, P. (2019). Identification of Immediate Early Genes in the Nervous System of Snail Helix lucorum. eNeuro, 6(3). https://doi.org/10.1523/ENEURO.0416-18.2019