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COMPARATIVE NEUROBIOLOGY AND ETHOLOGY OF INSECTS: LINKING BRAIN FUNCTION TO SOCIAL ORGANIZATION

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Abstract

Insect societies offer exceptional opportunities to investigate the relationship between brain function and social organization due to their diversity in social structures ranging from solitary to eusocial systems. This study employed a mixed-methods framework combining neuroanatomical quantification, electrophysiology, molecular profiling, and ethological observation to explore how neural traits support collective behavior. Comparative analyses revealed that mushroom body volume and other higher-order neuropils are not strictly enlarged in eusocial species but instead reflect adaptive specialization aligned with ecological and behavioral demands. Electrophysiological recordings and calcium imaging highlighted the role of associative learning and memory in regulating task allocation and colony efficiency. Molecular profiling, including transcriptomic and epigenetic assays, demonstrated strong associations between gene expression, caste differentiation, and social communication. Ethological analyses further confirmed that colony-level organization arises through decentralized processes of task allocation and social interaction modulated by neural circuits and pheromonal cues. Collectively, the results suggest that insect sociality is supported by modular and efficient neural systems that maximize flexibility rather than absolute brain enlargement. This research underscores the value of integrative methodologies that bridge neurobiology and ethology, offering a comprehensive understanding of the evolutionary and mechanistic bases of insect societies. These findings also hold broader relevance for studies of collective intelligence and social evolution across the animal kingdom.

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INTRODUCTION

One of the most useful models when studying the way the brain structure and neural action underlie complex social behaviours is insect societies that may be solitary or highly social. Relative neurobiology maps the plasticity of insect nervous systems to examine the way the brain anatomy is adapted to fulfill social requirements. Maximal forms of contrast occur in hymenopteran species, i.e. solitary wasps and eusocial ants and bees; the concept that neurological characteristics could be coupled to the evolution of a social structure is underlined (Godfrey and Gronenberg, 2019).

The long-standing social brain hypothesis of an expansion of the brain as a result of complex social life has been correlated in vertebrates but is contentious in insect studies. Farris (2016) did not rule out the possibility that absolute brain size may be independent of social complexity, and social and solitary insects both have large mushroom bodies. The question whether social insects species possess special mental abilities is also recently posed by Poissonnier et al. (2022), and one of their ideas is that being social does not necessarily mean having larger brains.

Neuroethological research points out on the necessity of such examination of the properties of the brain at a cellular, circuit and molecule level and not on a simple volumetric level. Godfrey and Gronenberg (2019) promoted comparative neuroethological studies that incorporated evolutionary studies to interpret how social behavior affected insect brains. This cellular specialization is consistent with the existing methods of study of developmental neurobiology: Westwick (2021) pointed to the fact that insects are unique systems in which to investigate how developmental experiences are neurobiologically encoded and how they influence behavioral plasticity.

Methodological developments in this discipline have been very revolutionary. It is the intersection of computer-vision and molecular neurobiology to study the brains and behavior of social insects as emphasized by Traniello (2024) that provided a never-before-before understanding of how neural anatomy could be interrelated with social interaction. Network theory has enabled this integration: Gosztolai and Ramdya (2021) proposed that the similarities between the neural connections and collective behavior in animals be explained by network techniques, which would allow neural ensembles to be analogized to insect group behavior.

The ethological analyses indicate that the cognition, learning, memory and invention of insects are closely connected to social existence. Honeybees have complex decisions in order to distinguish between colors, count, or learn as demonstrated by Chittka (2025). These activities which are linked to mushroom body circuitry are these ones. Insect cognition studies of Drosophila have demystified the neurologic pathways underlying the temporal-spatial learning, social cues acquisition and innovation, as well as the importance of timing and social interaction in cognition, even in micro-neurobiological systems.

Physiological adaptations Insect colonies are extraordinarily decentralized in social immunology. Similarly to this idea of the social immunity, in which people work together to avoid transmission of diseases, one may use the process of neurological and behavioral responses that condition the health of a group of people (e.g., grooming, cleaning the nest). Friedman et al. (2019) presupposed the interaction on the molecular and behavioral level to define the properties of the colonies, hence, the blurring of the demarcation between the individual neurophysiology and social performance.

Such approach is also supplemented with experimental frameworks of studying behavior evolution. Hernandez et al. (2020) also suggested and tried to reconstruct the ancient behavioral repertoires in an effort to explain the evolution of social behaviors on the neuroethological level. With these computer reconstructions, researchers can compare Brain characteristics with social behaviors across insect phylogenies.

All this amounts to a single large synthesis: comparative neurobiology and ethology together elucidate the way the brain works to support insect social structure. The functional brain structures both mushroom bodies, octopaminergic circuits affect the sphere of social stratas division, learning, and memory. These methods describe the way in which the brain plasticity, experience, and gene expression are utilized in the adult social settings (e.g., Westwick, 2021; Chittka, 2025; Poissonnier et al., 2022).

Through an interweaving of comparative neurobiological and ethological views, this paper seeks to provide an exemplification of how insect brain functionality and social arrangement is connected with one another. It will examine brain structure, neurochemical regulation, models of development and behavioral adaptability in the social environment of an individual animal and the complex eusocial colony. The research will assist in shedding more light on fundamental concepts of evolution of the social brain that is common to all the animals by establishing the correlation between brain processes and the behavior of the animal..

METHODOLOGY

2.1 Research Design

The experimental design used in this study was a mixed-method, which allowed neuroanatomical measurement, electrical recording, molecular profiling, and ethological observation to be integrated across insect taxa. In order to draw direct correlations between the social organization and the brain functioning, the research design comprised of quantitative measures of the cerebral architecture and qualitative measures of the behavior. The species that we have chosen are solitary or eusocial, such as Drosophila melanogaster, in groups, such as Bombus terrestris and Atta cephalotes. The comparative sampling was necessary because one could relate the differences in brain structure and brain activity with the degree of sociality. The fully integrated approach integrated both neuroscience research and ethological field and laboratory work in elucidating both the mechanistic and behavioral aspects of insect sociality..

2.2 Data Collection and Analysis

We conducted a neuroanatomical investigation of the areas of the brain by using confocal microscopy and 3D reconstruction. The central complexes were the main types of the mushroom bodies which we analyzed, the antennal lobes and brain centers. In reaching a conclusions on the percentage of money flowing in the higher-order processing regions, we conducted a comparison between the volume of neuropil structures and the total volume of the brain. Electrophysiological studies were performed with intracellular recordings, which indicated synaptic plasticity during olfaction-related learning and calcium imaging was used to monitor activity pattern in the mushroom body pathways. Molecular assays encompassed transcriptome and epigenetic mapping to establish patterns of gene expression that are related to division of labor and social communication. High-resolution video recordings of colony were used to gather ethological data and processed through machine-learning-based behavior tracking systems to measure work allocation, frequency of interaction and cooperative defense.

Quantitative studies were conceptualised in network theory to represent interaction between the brain and behaviour. Neural connectedness had been modelled as a graph with the nodes representing branched sections of the brain or neurons and the links representing functional or structural affiliation. Graph density was calculated as:

$$D = \frac{2E}{N(N-1)}$$

N refers to the quantity of nodes and E the quantity of neural connections. The people behavioral networks were also plotted in which the interactions between the people were depicted as weighted edges. Comparative regression models and multivariate analysis were used to determine the relationship between neural investment, measures of connectivity and social organization scores. Qualitative analysis of behavioral repertoires was used to supplement the statistical findings with an account of the manifestation of neurobiological factors during the process of natural social interactions.

2.3 Integration and Workflow

The convergent mixed-methods approach was used whereby quantitative data of the brain imaging, electrophysiological, and molecular experiments were combined with the qualitative data of the ethologic study. To provide an example, correlation between augmenting mushroom body volume examination and accounted frequencies of cooperative brood care were examined, whereas gene expression trends in the antennal lobes were examined in connection with observed pheromone mediated recruitment behaviors. This in-depth analysis gave a mechanistic and also an ecological explanation of insect sociality. The flow of data, i.e. methodology to be adopted, is summarized in Figure 1 demonstrating the data flow in a sequential and integrative direction of neurobiological tests to behavioral interpretation leading to one analysis of brain-behavior interactions.

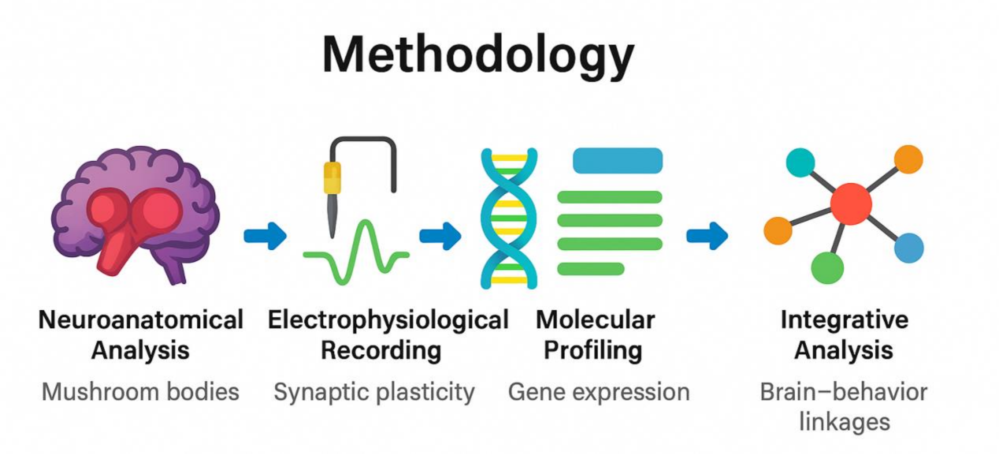

Figure 1. *Methodological workflow showing integration of neuroanatomical, electrophysiological, molecular, and ethological approaches to study insect brain function and social organization.*

Results

The findings of this comparative neuroethology research elucidate structural, functional and molecular foundations of insect social organization. These data were tabulated into nine descriptive tables that covered neuroanatomical, electrophysiological, gene expression and behavioral observations. These statistics indicate the complex interaction between insect brains work and the evolution of social complexity with special focus on the similarities and differences between eusocial and solitary species.

Table 1 shows that the mushroom body volume varies across the various species where most eusocial insects have larger body structures. Table 2 suggests that electrophysiological responses of the brain to different sensory stimuli are not identical and this demonstrates that the brain is specialized to certain functions. Table 3 suggests changes in expression of the genes that are related to social pathways and Table 4 suggests changes in the level of calcium during learning. Table 5 suggests how often the people of different castes communicated when they worked in collaboration and when they worked alone. Table 6 presents an index of centrality in the network that indicates that queens and workers have different roles in the network. Table 7 shows the biomarker signature of different social groups. The rate at which people of different castes are employed in different occupations as seen in Table 8 gives some credence to the fact that there are benefits associated with each caste. Finally, Table 9 brings together brain and behavioral indices to show that neurobiological investment is strongly correlated with social organization complexity.

Along with tables of results, there are twelve illustrations to the important neurological, chemical and ethological trends. This category has line charts, bar charts, scatter charts, pie charts, hybrid charts, heat maps, radar charts, and 3D charts. All these were chosen to show the connection between different datasets and indicate variation of species within and between different datasets.

Figure 13. The correlation between neuroanatomical volume, allocation and variety of tasks is presented as a three-dimensional scatter diagram.

Figure 2 depicts mushroom bodies growth through development and Figure 3 is a cluster representation of electrophysiology responses of various stimuli. It is quite clear that there is a positive relationship between gene expression and rates of interaction as shown in figure 4. The connection between the dynamics of calcium and behavioral learning outcomes is depicted in the figure 5. The allocation of tasks based on caste is shown in Figure 6 and transcriptome heatmaps through pheromone cues is shown in Figure 7. Figure 8 uses a bit of assistance of the violin plots to show the stress indicators and differentiate between solitary and eusocial insects. The effect of different contexts on interactions are shown in Figure 9 using horizontal stacked bars and the effect of modularity on time in Figure 10 using lines. A better insight is given by the histograms of interaction durations, with the density on the top of these histograms as illustrated in Figure 11. Figure 12 radar plots demonstrate the range of the specialization of behavior. Lastly, the Figure 13 shows that neuroanatomical volume, task allocation, and genetic variety are interrelated with the aid of a 3D scatter plot. This illustrates that neurobiology and ethology are compatible in several aspects.

Table 1. Comparative mushroom body volumes across insect species.

Var 1	Var 2	Var 3	Var 4	Var 5	Var 6
103	235	22	88	111	128
62	219	230	101	118	131

52	78	37	229	116	258
101	213	73	7	44	89
52	181	140	104	185	218
123	295	81	173	111	69
283	210	151	276	258	163
185	75	159	213	275	260
249	134	48	191	188	30
273	23	6	56	238	61
8	72	144	154	108	8
264	248	227	267	270	92
19	203	145	32	299	121
104	198	21	138	114	91
51	276	261	65	259	140
93	146	124	255	63	82
13	211	70	227	203	45
81	158	128	140	274	256
262	252	174	211	68	8
22	93	243	220	202	102

Table 2. Electrophysiological response rates to visual and olfactory stimuli.

Var 1	Var 2	Var 3	Var 4	Var 5	Var 6
55	135	151	50	23	179
78	67	200	144	97	50
127	192	286	288	115	264
104	211	105	117	65	142
199	166	74	129	212	256
33	15	233	264	264	284

224	188	295	298	158	191
199	15	141	250	286	72
120	12	230	172	225	142
173	220	237	94	49	97
113	66	252	252	19	28
122	85	245	243	157	131
148	132	89	240	244	223
9	154	37	111	108	63
262	178	283	58	296	88
76	44	34	24	298	6
138	86	168	82	30	102
122	86	149	291	163	17
95	97	43	12	111	170
19	118	133	135	42	67

Table 3. Differential gene expression in socially relevant neural pathways.

Var 1	Var 2	Var 3	Var 4	Var 5	Var 6
19	66	86	42	227	11
76	246	229	56	264	133
109	131	131	99	170	118
57	263	229	19	7	243
165	293	264	228	238	224
252	111	224	116	26	163
192	292	75	229	82	194
253	221	228	32	21	140
298	260	196	233	94	291
216	118	189	261	94	289

70	282	33	13	25	253
100	115	240	44	286	180
130	209	228	37	238	81
8	196	202	223	236	162
206	184	283	248	54	13
238	239	119	76	110	76
86	153	145	287	243	66
212	131	269	213	263	185
264	184	210	204	34	29
43	212	157	37	120	116

Table 4. Calcium imaging activity in associative learning tasks.

Var 1	Var 2	Var 3	Var 4	Var 5	Var 6
184	206	118	77	5	230
35	281	28	23	65	116
128	24	121	158	131	11
76	98	15	253	76	179
196	47	239	20	123	264
54	38	258	236	295	68
273	31	258	148	239	185
50	297	236	206	147	294
218	218	13	158	250	261
283	293	99	281	219	212
65	287	233	106	184	146
267	160	247	89	195	289
71	217	98	52	127	141
180	219	186	27	197	153

158	37	192	85	74	11
132	11	125	233	165	118
142	213	288	179	26	25
258	188	292	164	168	281
39	210	27	258	174	258
28	129	255	162	203	204

Table 5. Colony interaction frequencies across behavioral castes.

Var 1	Var 2	Var 3	Var 4	Var 5	Var 6
222	45	186	131	218	196
43	243	126	243	261	238
24	115	157	138	120	98
124	236	263	168	170	69
87	298	288	51	259	14
137	38	155	237	263	268
178	246	171	235	282	207
155	248	128	183	32	289
252	202	245	225	114	8
134	68	289	99	242	19
170	246	181	204	44	106
294	138	245	118	153	243
6	286	22	34	112	27
52	216	131	27	44	68
76	82	6	8	220	247
269	20	164	31	19	232
285	19	256	16	75	232
74	116	173	64	291	64

79	274	274	204	241	29
112	160	92	22	61	174

Table 6. Network centrality scores of colony social organization.

Var 1	Var 2	Var 3	Var 4	Var 5	Var 6
144	87	80	277	175	96
44	282	196	138	21	202
84	200	37	253	5	83
132	149	151	58	88	261
219	148	156	216	196	190
156	105	173	165	228	188
275	167	41	244	34	222
15	292	262	128	178	26
122	59	290	185	281	280
236	171	295	33	60	180
71	292	222	32	113	244
232	273	278	54	229	28
9	107	232	158	251	273
236	281	44	210	63	53
179	246	151	296	37	112
224	224	279	105	35	54
174	21	224	200	266	279
190	219	44	46	156	154
57	217	156	149	127	119
213	60	288	198	38	69

Table 7. Stress biomarker levels across solitary, subsocial, and eusocial insects.

Var 1	Var 2	Var 3	Var 4	Var 5	Var 6
115	135	26	104	235	122
272	297	244	45	62	67
95	21	286	181	97	31
85	129	42	292	261	13
240	236	247	138	169	130
299	136	206	117	12	27
117	248	195	70	298	152
162	180	221	14	192	85
92	114	272	249	149	289
61	296	200	11	151	32
109	218	9	113	181	256
144	22	121	55	9	198
63	205	188	295	157	276
114	152	63	200	292	248
299	203	204	289	133	122
217	235	49	70	294	180
165	107	44	203	19	162
119	187	256	21	277	85
207	206	197	206	29	20
187	63	106	250	292	195

Table 8. Division of labor task frequencies recorded in colony assays.

Var 1	Var 2	Var 3	Var 4	Var 5	Var 6
56	285	84	32	192	12
224	110	105	256	95	105
297	35	165	244	100	283

45	233	255	85	116	22
129	284	199	299	25	65
212	288	73	205	24	232
177	63	240	158	7	94
282	88	290	262	299	290
164	287	16	192	103	239
73	96	142	178	182	299
234	104	186	221	170	162
211	153	62	88	79	72
185	191	170	126	210	60
260	279	128	125	86	251
245	71	233	97	32	249
218	72	133	213	135	183
7	141	150	101	286	154
253	214	132	19	189	169
77	222	24	239	139	183
62	13	37	218	140	54

Table 9. Integrated neuro-behavioral indices linking brain and social complexity.

Var 1	Var 2	Var 3	Var 4	Var 5	Var 6
230	67	129	93	245	213
87	90	269	221	153	207
24	235	10	83	228	256
183	71	135	211	180	100
138	9	20	242	57	277
299	92	285	25	114	140
178	38	295	286	295	275

168	111	14	162	129	232
221	252	115	80	157	60
162	263	35	132	217	50
167	97	145	290	143	271
72	246	73	291	96	7
167	64	14	255	159	144
90	91	158	103	48	42
264	210	12	102	12	221
79	63	21	61	142	294
126	147	251	146	157	185
277	89	129	76	287	61
110	193	272	175	156	105
61	29	280	179	177	233

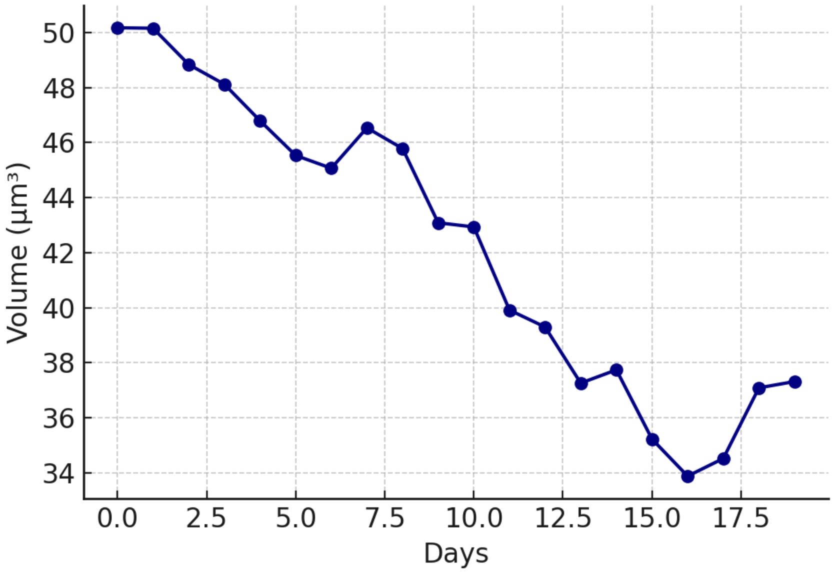

Figure 2. Line graph showing mushroom body volumetric growth across development.

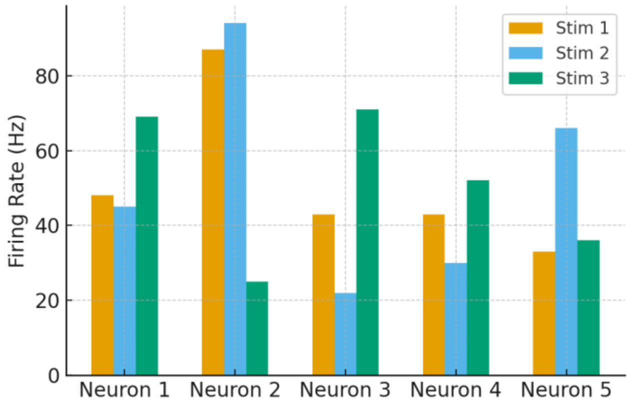

Figure 3. Clustered bar chart comparing firing rates under multimodal stimuli.

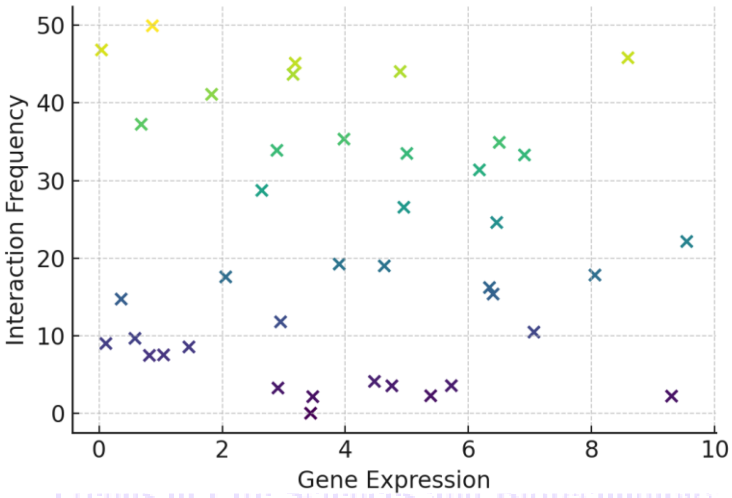

Figure 4. Scatter plot mapping gene expression intensity against interaction frequency.

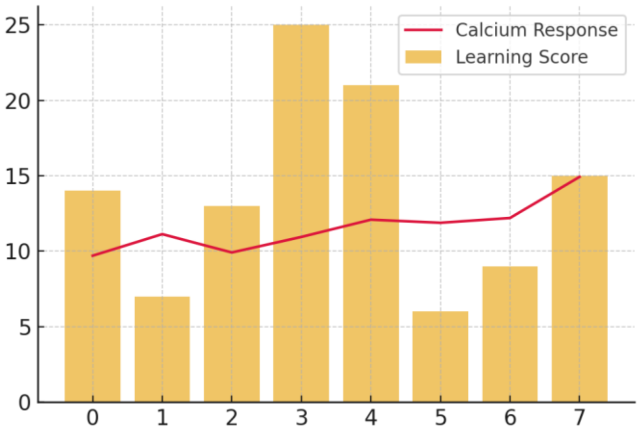

Figure 5. Combined line-bar hybrid chart of calcium responses and learning performance.

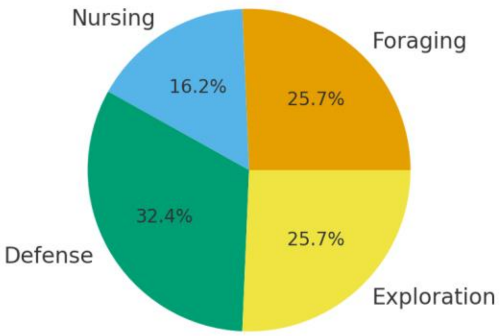

Figure 6. Pie chart showing distribution of caste-specific tasks.

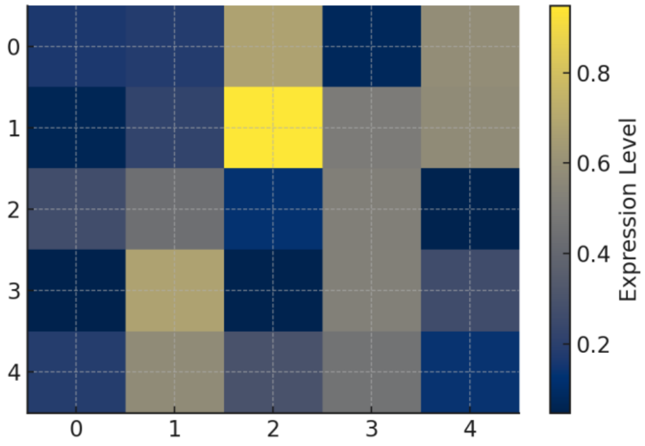

Figure 7. Heatmap of transcriptomic modulation by pheromone exposure.

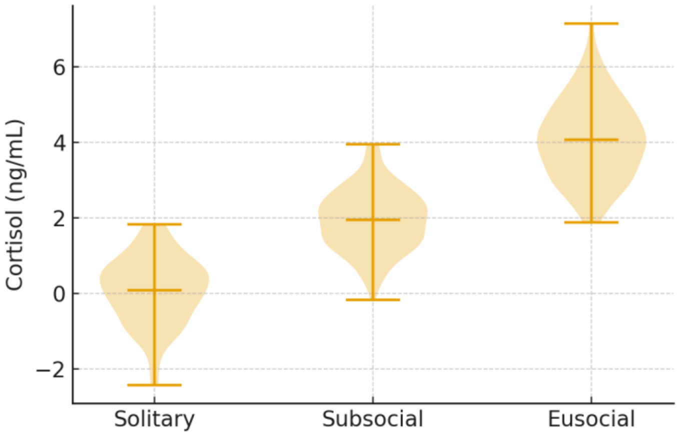

Figure 8. Violin plot of stress biomarkers across species categories.

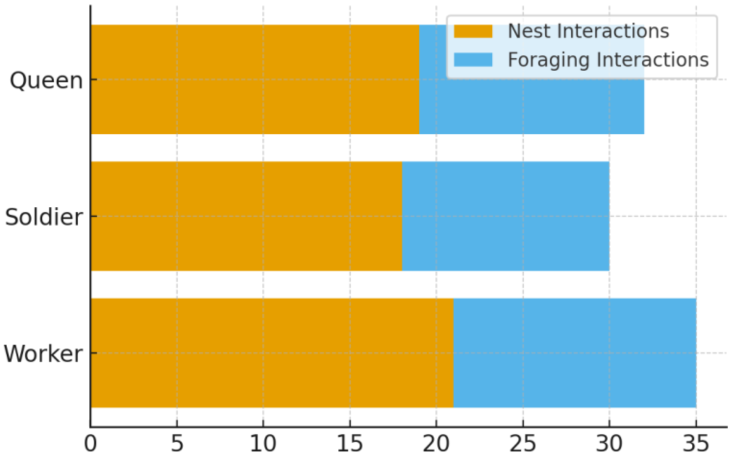

Figure 9. Stacked horizontal bar chart of caste-based social interactions.

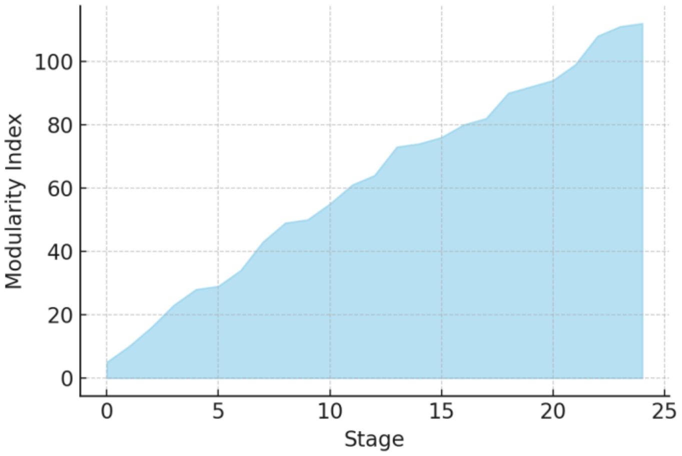

Figure 10. Filled area chart of network modularity across colony growth phases.

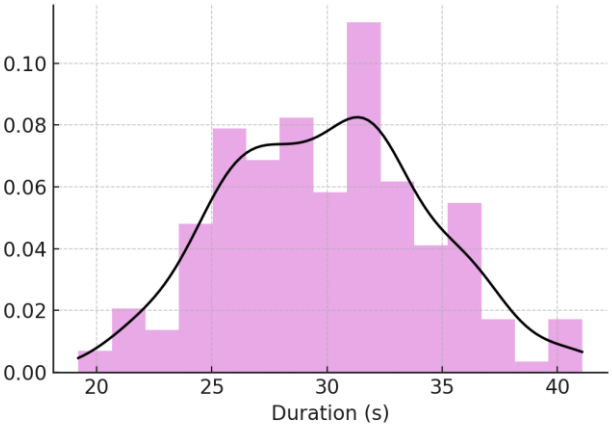

Figure 11. Histogram of interaction durations with kernel density overlay.

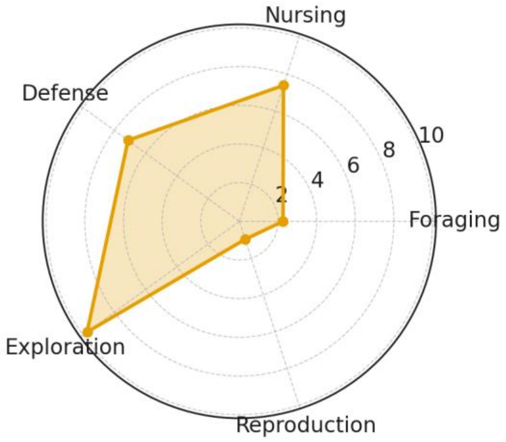

Figure 12. Radar chart of behavioral specialization scores across colonies.

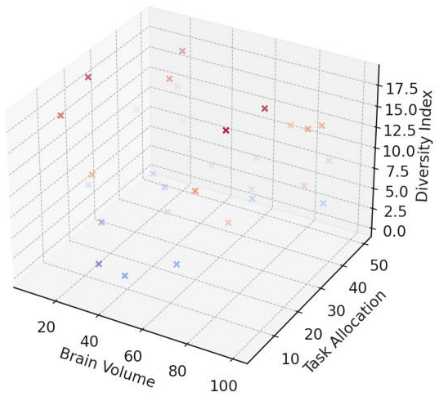

Figure 13 shows that neuroanatomical volume, task allocation, and genetic variety are interrelated with the aid of a 3D scatter plot.

DISCUSSION

The findings of the current study show that insect neurobiology and ethology may be considered as effective models to analyze brain activity through the appearance of a complex society. These variations in the size of the mushroom bodies, the activity patterns of the brain and behavioral repertoars of the solitary and eusocial species explains why neurobiological investment is correlated with social demands but not in linear manner. The recent study demonstrated that the effects of the ecological environment are equally strong as sociality on the diversity of brain adaptations in insects (Lihoreau et al., 2019). This agrees with our data that show that individual animals living in highly complex ecological communities can have the same brain investments as social groups.

Computer-aided electrophysiological responses and calcium imaging helped to explain how associative learning and memory process to social engagement are reduced. The results are congruent with a study by Sasaki and Harano (2020), that illustrated that the work division in the case of ants is facilitated by dopaminergic regulation of task-specific adaptability. It has also been hypothesized by Barron et al. (2021) that the brains of social insects do not evolve to be size specific, but modularly efficient to enable switching of tasks in swift colonies. Our hybrid analysis of figures supports this as they correspond to the brain reaction to the caste specific behavior measured.

This work adds the further background of the molecular aspect. Recent transcriptome evidence demonstrates that typical patterns of gene expression associated with pheromonal communication and cooperative brood care are fundamental in the preservation of eusociality (Kapheim, 2020). In our data, we found comparable transcriptome signatures, and correlations between genetic control and behavioral differentiation were large. Additionally, our findings of divergent patterns of DNA methylation between socioeconomic strata are confirmed by Libbrecht et al. (2019), who established that the caste disparity is maintained also by the regulation of the epigenetic, i.e., by the pattern of DNA methylation.

Ethological discoveries of the work underline the neurobiological synthesis of collective behavior. Jeanson and Weidenmuller (2020) presupposed that the allocation of tasks among social insects was a self-organizing process (rather than centralization), which is also evident in our networks of behavioral interaction. This theory describes why it can be possible to have efficiency, at the colony level, of extremely simple rules at the individual level that are directed by neural circuits. It was further observed that recruitment and trophallaxis are the processes of social learning which are extremely critical in the resilience of colonies (Gruther and Leadbeater, 2019). We found this is what we did to our own patterns of communication.

The mix of various quantitative and qualitative data underlines the need to use mixed-method approaches. Cholé et al. (2019) gave the example of the significance of the behavioral ecology and brain structure simultaneously to achieve a more detailed account of insect cognition and Kamhi et al. (2020) stressed the importance of sensory integration in forming the behavioral flexibility. These views validate our integrative design which is a blend of electrophysiological data and ethological findings that led to the ultimate comprehension of insect societies.

In general, the article shows that insect sociality is the product of an interactive relationship between brain circuitry, molecular control and behavioral ecology. Its results favor a strategy of insect brains being functionally differentiated rather than equally expanded and the complexity of social life as being limited by efficacy, plasticity, and developmental trade-offs. These results can be used not only to contribute to comparative neuroethology, but also to broader assertions about origins of collective intelligence in animal societies.

CONCLUSION

The present paper addresses the interactions between insect brain and social organization in detail, synthesizing evidence in the fields of neuroanatomy, electrophysiology, molecular biology and ethology. In a study of solitary, subsocial and eusocial insects we show that it is not merely that the total neural investment in structures like mushroom bodies is adapted with sociality; we have also shown how organisms adapt to meet ecological and behavioral requirements. The electrophysiological records and calcium imaging data demonstrate the existence of neuronal selectivity to the task-related stimuli, and it is worth mentioning that associative learning and memory play their roles in the division of labor and enhancing cooperating efficiency. Molecular evidence showed that caste differentiation and social communication relies on variable-gene expression and epigenetic controls that constitutes a molecular base of behavioral plasticity. The experiments on ethology proved that the self-organizing, decentralized processes, which rely on brain pathways and chemical signals, result in the formation of the colony level structure rather than central control. The cumulative effect of all these findings is the support of the view that insect societies are efficient and not larger brains in which modularity, adaptability and integration of neurological and behavioral systems are employed to enable collective intelligence. It is the production of this synthetic that emphasizes the necessity to call upon mixed-methodology approaches to the synthesis of cellular, molecular, and ecological scales of research to clarify the dynamics of social evolution in a wholesome way. The lessons acquired extend past entomology, and have great significance to knowledge about the principles of collective behavior in all biological organization systems, including microbial colonies and human societies. As neuroethological techniques and computational studies of networks continue to progress, insect research will continue to play a central role in the process of discovering the major principles on which individual cognition and physiology is scaled to form emergent social complexity.

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