CAPSTONE

Bring more bugs into the lab! A review on the use of social invertebrate species as models in the study of learning and memory

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INTRODUCTION

The study of mammalian learning and memory is a field relying on the use of various animal models, due in part to the unethical nature of human experimentation and nonhuman models providing faster development and simpler or more accessible nervous systems. However, nonhuman primates, the closest mammalian analogue to humans, are expensive to care for and take a long time to reach adulthood[1]. More common are small animal models such as mice and rats, which are less expensive to acquire and raise, and have a vast spectrum of genetic modifications to create robust models of specific disorders[2]. However, using only a select few models over and over in research can create artificial boundaries, so common model species should be complemented by studies using uncommon models. To this end, various invertebrate species are suitable models for research. The animals themselves require small setups and minimal care. In addition to this low-intensity maintenance, invertebrate brains have structural analogues that link them to brain structures in many other species, including humans. Most notable is the link between a highly distinct region in invertebrates, the mushroom bodies in bees or hemiellipsoid bodies in crabs, and the cerebral cortex in vertebrates[3,4]. This provides an anatomical basis for the use of these animals in neurological studies on learning. In addition to analogous brain structures, invertebrates have biomarkers useful for cognitive studies of anxiety-like and depression-like behaviors. Crabs and bees have biogenic amines such as serotonin and dopamine, as well as a third amine, octopamine, that is analogous to human noradrenaline. Assessment of these biogenic amines in invertebrates has shown changes in response to various stimuli that simulate the same changes in humans[5]. A study on social harassment in crayfish resulted in a rise in hemolymphic serotonin levels and anxiety-like behaviors; both were attenuated with a common antianxiety drug[6]. In humans, stressful situations such as harassment result in abnormal, fluctuating serotonin levels and short-term anxiety[7]. Both depression or anxiety, along with other mood disorders, have an impact on behavior, cognition, and learning in humans, so having measurable biomarkers of analogous chemical reactions is useful in learning studies that examine emotional impacts[8]. The crayfish, with many features analogous to results seen in human studies, are useful models for anxiety and depression. With plenty of reason for using invertebrate models, current studies in a wide range of fields utilize the fruit flies *D. melanogaster* and the nematodes *C. elegans*. There are uses for *D. melanogaster* in genetics, development, and aging, as well as learning and behavior[9]. Similarly, *C. elegans* are used in neural development research, as they have accessible and plain nervous systems[10]. However, both of these species are limited in their uses. The simple nervous system of *C. elegans* is incapable of firing action potentials in the same way more complex neurons can[11]. Conversely, while fruit flies have a wide range of uses within studies on learning, due to their fast speeds and small size in conjunction with their small setups, factoring in the social behaviors of a fruit fly colony is difficult. The mechanisms that regulate their social grouping activity are not easily accessed or recorded, making the social habits of *D. melanogaster* difficult to classify and control for in an experiment[12]. However, the social behaviors of a model organism are an important variable to account for, especially when humans are also a social species[13]. In order to include social behaviors, a wider range of invertebrate species with well-studied and defined social behaviors should be used. There are previous studies done that used social invertebrates to examine a wide range of certain traits, all associated with learning and memory[14]. The body of research provides a range of evidence that these social invertebrates display long-term memory in conjunction with associative learning, egocentric comprehension of their environment, and context-dependant learning[15,16,17]. This review article will discuss and encourage the use of three groups of social invertebrates that are viable models for the study of learning and memory, specifically honeybees of the genus *Apis*, homing ants of the genus *Cataglyphis*, and the herbivorous crab *Neohelice granulata*, also referred to as *Chasmagnathus granulata*.

BEES, ASSOCIATIVE LEARNING, AND SLEEP DEPRIVATION

Invertebrates go through stages of sleep, wakefulness, and sudden arousal. Crayfish show slow brain waves during sleep periods that are similar to REM slow brain waves in vertebrates; scorpions display low responsiveness and heart rate in varying stages during sleep-like behaviors[18,19]. Honeybees, however, show evidence of a capability to sleep that is more similar to avian or mammalian sleep patterns. The bees have fluctuations in overall neuronal responses to stimuli that synchronize with a typical circadian rhythm and their sleep-wake cycles can be reliably monitored by recordings of their antennal movements[20, 21]. In mammals, sleep is essential for memory consolidation, a two-stage system redistributing initially recorded memories to long-term storage throughout the pallium[22]. Sleep is also essential to avian memory consolidation, although it follows an unknown, alternate consolidation pathway that is thought to end within the avian pallium[23]. Do honeybees, with their circadian rhythms and mushroom bodies functioning as analogs to mammalian sleep patterns and vertebrate cortices, also utilize sleep for memory consolidation[24]?

To show the link between learning and sleep in bees, Hussaini et al. approached this question using a sleep deprivation study, exploring the effects of not sleeping on learning in bees. Two types of learning were examined, acquisition learning and extinction learning. By pairing a sugar reward with a neutral odor, the bees were trained into a proboscis extension response to the odor. This is an example of acquisition learning, a strong memory trace. After one night of sleep deprivation post training, the bees were tested to determine if they still responded to the odor without the sugar. Interestingly enough, the sleep deprivation did not attenuate the proboscis extension response. Repeating the initial training, the bees then underwent extinction training the following day, exposing them to the odor without the reward. This is an example of extinction learning, a weaker memory trace where the bee suppresses the previously learned memory, something that is considered as a new memory of an inhibitory response[25]. This time, the sleep deprived bees performed significantly worse on retention trials than rested bees after extinction trials. The way each form of learning was differentially affected by sleep deprivation was also seen in mammals. In some mammalian studies, strong conditioning is not affected by sleep deprivation, while the weaker extinction memory or spatial learning is affected[26, 27]. Sleep is essential to forming new memories in bees, especially during weak forms of learning such as extinction learning.

ANTS, EGOCENTRIC MAPPING, AND NAVIGATIONAL MEMORY

For successful navigation, organisms need some way of recalling their spatial orientation in relation to the world around them. In mammals, each individual forms a cognitive map using their hippocampus to store landmark memories, while different parts of their cortex form egocentric memories.[28] In this way, humans constantly update their internal maps with information of where their body is within the environment[29]. This kind of goal-directed navigation is also seen within invertebrates, especially in species that forage outward from a home nest such as honeybees or ants[30]. The homing desert ant, *Cataglyphis*, is an exceptional model for navigational learning and memory. In addition to using visual landmarks, these ants have an additional way of navigating when the desert shifts and landmarks change. They create an egocentric map, combining information from their internal odometer and the polarization of the sky above them in order to determine their exact location relative to their nestbox[31].

To show clearly the desert ant's ability to form egocentric, updating maps over the more common landmark memory, Andel and Wehner teach ants to follow obvious landmarks from their nest-box to a feeder, then displace the ants to a landmark-free pathway[16]. The ants did not search for missing landmarks, but neither did they simply repeat the pathway back to the nest without interruption. They walked directly towards the nest-box for a short distance, then began systematic searching patterns. Each ant searched further in certain directions and in alternate patterns, although most navigated successfully back to the nest-box in the end. Then, ants would be allowed to run all the way home along the landmark path, then picked up and placed back at the feeder in order to perform a second run home. Once they reached the nest-box a second or third time, the ants were displaced into the landmark-free pathway. Each ant started their path following the landmarks down the wrong way, but they soon corrected their paths towards home. The further away the ants had been displaced, the sooner the ant would begin searching for familiar ground. In humans, the same kind of procedural egocentric mapping is created simultaneously with landmark memory formation. If landmarks are not available, or the landmarks are moved, humans will be able to correctly retrace their route back home based on the navigational, rather than landmark memory[32]. This same response is seen by Andel and Wehner. Once the ant's landmark memories no longer match the route home, they prioritize their internal maps for navigational tasks.

CRABS, LONG-TERM HABITUATION, AND CONTEXT-DEPENDANT LEARNING

The herbivorous crab *Neohelice granulata* is the sixth most studied crab species, with a toolbox of varied behavioral, pharmacological, electrophysiological and molecular methods established for examining neurophysiological changes in the crab's responses to various stimuli[33,34]. A common experimental paradigm is training crabs to suppress their flee response from a simulated overhead predator. The pattern and timing of this training can result in a dramatic difference in habituation. Exposing the crab to 15 training trials in one minute results in short-term habitation, while repeating 15 trials over the span of several hours results in long-term habituation that can last for at least five days without any additional training[15]. In this way, long-term or short-term potentiation of the crab's flee response may be activated without using invasive stimuli. The crabs do not simply memorize the stimuli and respond to that exact stimulus, which indicates simpler stimulus-specific learning. Instead, the trained responses of the crabs are attenuated when exposed to the stimulus within a new, unfamiliar environment[35]. By only trusting the visual stimulus in certain environments, these crabs pair a specific stimulus with a specific context in a display of context-dependant learning. Training the crab to generalize or differentiate similar stimuli in the same escape response paradigm has shown changes within the crab's lobula giant neurons, brain areas responsible for long-term memory storage of visual stimuli in crustaceans[36]. Hemiellipsoid bodies, brain structures similar to the vertebrate cortex, also appear to be involved in the crab's ability to associate place memories with the stimulus they are exposed to[3,36]. In this way, the way crabs learn utilizes one brain structure for storage of the memories and a cortex analogue for associating place memory to a stimuli. This is similar to the dual-process model of recognition memory, a suggested theory of the way humans form associations between place memories and cues. While this theory is still highly debated, animal studies using rodents imply that the process of recognizing stimuli is subdivided neuroanatomically between the cortex for processing and association and another brain region, likely the hippocampus, for generalizing or differentiating a presented stimulus[37,38]. These crabs may be a very simple animal model that supports the dual-process model of recognition memory, with their hemiellipsoid bodies and lobula giant neurons functioning as the two regions involved in the recognition process.

CONCLUSION

The simplistic nature of an invertebrate brain compared to a mammal brain allows for easier pinpointing of the underlying mechanisms when studying learning and memory, while also similar enough in structure to display analogous functions. While the invertebrate species discussed in this review display evidence of complex long-term memory formation, each species of interest lends themselves to specific neuroscientific specialties. Honeybees display clear and trackable sleep patterns useful in studies on the memory traces that are affected by sleep deprivation, as well as associative learning with sugar rewards. Homing ants display an egocentric comprehension of their environment, ideal for studies into spatial memory and the retrieval of navigational memories. *Neohelice* crabs are capable of context-dependant learning, with established methods of recording the neurophysiological changes the crab undergoes. Each of these species have unique traits beneficial to neuroscientific studies on learning and memory, and should be used to precede or supplement the use of vertebrate models. Future research should examine invertebrate and murine models simultaneously in order to determine the similarity and accuracy of their relations to the human disorders they are being used to investigate.

REFERENCES

1. Jennings, M., Prescott, M. J., & Joint Working Group on Refinement (Primates). (2009). Refinements in husbandry, care and common procedures for non-human primates: Ninth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals*, *43*(1_suppl), 1-47.
2. Brenowitz, E. A., & Zakon, H. H. (2015). Emerging from the bottleneck: benefits of the comparative approach to modern neuroscience. *Trends in neurosciences*, *38*(5), 273-278.
3. Maza, F. J., Sztarker, J., Shkedy, A., Peszano, V. N., Locatelli, F. F., & Delorenzi, A. (2016). Context-dependent memory traces in the crab's mushroom bodies: Functional support for a common origin of high-order memory centers. *Proceedings of the National Academy of Sciences*, *113*(49), E7957-E7965.
4. Tomer, R., Denes, A. S., Tessmar-Raible, K., & Arendt, D. (2010). Profiling by image registration reveals common origin of annelid mushroom bodies and vertebrate pallium. *Cell*, *142*(5), 800-809.
5. Bateson, M., Desire, S., Gartside, S. E., & Wright, G. A. (2011). Agitated honeybees exhibit pessimistic cognitive biases. *Current biology*, *21*(12), 1070-1073.
6. Bacqué-Cazenave, J., Cattaert, D., Delbecque, J. P., & Fossat, P. (2017). Social harassment induces anxiety-like behaviour in crayfish. *Scientific reports*, *7*, 39935.
7. Stein, D. J., & Stahl, S. (2000). Serotonin and anxiety: current models. *International clinical psychopharmacology*, *15*, S1-6.
8. Murphy, F., Smith, K., Cowen, P., Robbins, T., & Sahakian, B. (2002). The effects of tryptophan depletion on cognitive and affective processing in healthy volunteers. *Psychopharmacology*, *163*(1), 42-53.
9. Jennings, B. H. (2011). Drosophila–a versatile model in biology & medicine. *Materials today*, *14*(5), 190-195.

10. Kosinski, R. A., & Zaremba, M. (2007). DYNAMICS OF THE MODEL OF THE CAENORHABDITIS ELEGANS NEURAL NETWORK. *Acta Physica Polonica B*, *38*(6).
11. Clare, J. J., Tate, S. N., Nobbs, M., & Romanos, M. A. (2000). Voltage-gated sodium channels as therapeutic targets. *Drug discovery today*, *5*(11), 506-520.
12. Simon, A. F., Chou, M. T., Salazar, E. D., Nicholson, T., Saini, N., Metchev, S., & Krantz, D. E. (2012). A simple assay to study social behavior in Drosophila: measurement of social space within a group 1. *Genes, Brain and Behavior*, *11*(2), 243-252.
13. Young, S. N. (2008). The neurobiology of human social behaviour: an important but neglected topic. *Journal of psychiatry & neuroscience: JPN*, *33*(5), 391.
14. Glanzman, D. L. (2010). Common mechanisms of synaptic plasticity in vertebrates and invertebrates. *Current Biology*, *20*(1), R31-R36.
15. Hussaini, S. A., Bogusch, L., Landgraf, T., & Menzel, R. (2009). Sleep deprivation affects extinction but not acquisition memory in honeybees. *Learning & memory*, *16*(11), 698-705.
16. Andel, D., & Wehner, R. (2004). Path integration in desert ants, Cataglyphis: how to make a homing ant run away from home. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1547), 1485-1489.
17. Hermitte, G., Pedreira, M. E., Tomsic, D., & Maldonado, H. (1999). Context Shift and Protein Synthesis Inhibition Disrupt Long-Term Habituation after Spaced, but Not Massed, Training in the CrabChasmagnathus. *Neurobiology of learning and memory*, *71*(1), 34-49.
18. Mendoza-Angeles, K., Cabrera, A., Hernández-Falcón, J., & Ramón, F. (2007). Slow waves during sleep in crayfish: A time–frequency analysis. *Journal of neuroscience methods*, *162*(1-2), 264-271.
19. Tobler, I., & Stalder, J. (1988). Rest in the scorpion—a sleep-like state?. *Journal of Comparative Physiology A*, *163*(2), 227-235.
20. Kaiser, W., & Steiner-Kaiser, J. (1983). Neuronal correlates of sleep, wakefulness and arousal in a diurnal insect. *Nature*, *301*(5902), 707.
21. Sauer, S., Kinkelin, M., Herrmann, E., & Kaiser, W. (2003). The dynamics of sleep-like behaviour in honey bees. *Journal of Comparative Physiology A*, *189*(8), 599-607.
22. Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiological reviews*, *93*(2), 681-766.
23. Rattenborg, N. C., Martinez-Gonzalez, D., Roth, T. C., & Pravosudov, V. V. (2011). Hippocampal memory consolidation during sleep: a comparison of mammals and birds. *Biological Reviews*, *86*(3), 658-691.
24. Menzel, R. (2013). In search of the engram in the honeybee brain. In *Handbook of Behavioral Neuroscience* (Vol. 22, pp. 397-415). Elsevier.
25. Eisenhardt, D., & Menzel, R. (2007). Extinction learning, reconsolidation and the internal reinforcement hypothesis. *Neurobiology of learning and memory*, *87*(2), 167-173.
26. Fu, J., Li, P., Ouyang, X., Gu, C., Song, Z., Gao, J., ... & Hu, B. (2007). Rapid eye movement sleep deprivation selectively impairs recall of fear extinction in hippocampus-independent tasks in rats. *Neuroscience*, *144*(4), 1186-1192.
27. Silvestri, A. J. (2005). REM sleep deprivation affects extinction of cued but not contextual fear conditioning. *Physiology & behavior*, *84*(3), 343-349.
28. Squire, L. R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychological review*, *99*(2), 195.
29. Save, E., & Moghaddam, M. (1996). Effects of lesions of the associative parietal cortex on the acquisition and use of spatial memory in egocentric and allocentric navigation tasks in the rat. *Behavioral neuroscience*, *110*(1), 74.
30. Goldschmidt, D., Manoonpong, P., & Dasgupta, S. (2017). A neurocomputational model of goal-directed navigation in insect-inspired artificial agents. *Frontiers in neurorobotics*, *11*, 20.
31. Wehner, R., & Srinivasan, M. V. (2003). Path integration in insects. *The neurobiology of spatial behaviour*, 9-30.
32. Broadbent, H. J., Farran, E. K., & Tolmie, A. (2015). Sequential egocentric navigation and reliance on landmarks in Williams syndrome and typical development. *Frontiers in psychology*, *6*, 216.
33. Spivak, E. D. (2010). The crab Neohelice (= Chasmagnathus) granulata: an emergent animal model from emergent countries. *Helgoland Marine Research*, *64*(3), 149.
34. Tomsic, D., & Romano, A. (2013). *Invertebrate Learning and Memory: Chapter 26. A Multidisciplinary Approach to Learning and Memory in the Crab Neohelice (Chasmagnathus) granulata* (Vol. 22). Elsevier Inc. Chapters.
35. Tomsic, D., Pedreira, M. E., Romano, A., Hermitte, G., & Maldonado, H. (1998). Context-us association as a determinant of long-term habituation in the crabChasmagnathus. *Animal Learning & Behavior*, *26*(2), 196-209.
36. Sztarker, J., & Tomsic, D. (2011). Brain modularity in arthropods: individual neurons that support “what” but not “where” memories. *Journal of Neuroscience*, *31*(22), 8175-8180.
37. Mandler, G. (2008). Familiarity breeds attempts: A critical review of dual-process theories of recognition. *Perspectives on Psychological Science*, *3*(5), 390-399.
38. Brown, M. W., & Aggleton, J. P. (2001). Recognition memory: what are the roles of the perirhinal cortex and hippocampus?. *Nature Reviews Neuroscience*, *2*(1), 51.