How to Build a Behavior

Gordon J. Berman1,∗
1Department of Biology, Emory University, 1510 Clifton Road, Atlanta, GA 30322, USA

Abstract

How are complicated behavioral sequences executed? In this issue of Neuron, Duistermars et al. (2018) deconstruct neural control schemes underlying threats in flies, finding a small collection of neurons in which varying levels of activation lead to the performance of different movements.

Speaking from experience as someone who writes about animal behavior for a living, a common problem emerges almost every time I sit in front of the computer to type: there are no good synonyms for the word behavior. There are words that appropriate aspects of the concept and can suffice if one desperately wishes to avoid using the word ‘behavior’ four times in as many sentences—motion, movement, dynamics, pattern, activity, action, choice, gait, decision—but none of these quite capture the essence of what one wishes to describe. Why is this? Likely because the word ‘behavior’ is usefully vague. Is behavior the twitch of a muscle or the movement of a hand, a discrete action or something involving longer timescale modulation (e.g., foraging)? This nebulousness sanctions us to describe the things animals do as a property that spans across many length scales, timescales, and levels of descriptive complexity with a single word. It is a useful catch-all that allows us to lump together all the things we ascribe to an animal’s movements and interactions with the world.

What happens, though, when we try to link behavior (whatever that means) to its neural underpinnings? The benefits of such vagueness quickly vanish, dissolving into an unsatisfying slurry of unconvincing correlations and halfhearted interpretations. Understanding the neural control of behavior usually requires picking length and timescales to study, something typically achieved by engraining particular behavioral scales into the experiment (e.g., lever pressing, turning in a maze, reaching for an object). If attempting to understand natural behavior, however, biology does not ordinarily allow for such conveniences. When searching for food, attracting a mate, or driving a car, an animal’s brain is coordinating activities across many scales, adding and subtracting pieces of movement, adapting based on sensory information, and deciding whether to start doing something altogether different. There is no natural timescale to select, so deciding what features of the neural activity to measure or analyze becomes a matter of lucky guessing at best or an ill-posed problem at worst. Moreover, it is unclear whether the timescales of neural activity must match those of the behavior (e.g., insect indirect flight muscles, which can be controlled at a longer timescale than an individual wing beat).

∗Correspondence: gordon.berman@emory.edu.
So how do we proceed? In this issue of *Neuron*, Duistermars et al. use aggressive threat displays in the vinegar fly *Drosophila melanogaster* as a means of uncovering how the nervous system controls behavior at a variety of scales, from the movements of individual appendages to the performance of a complicated behavioral sequence (Duistermars et al., 2018). Aggression is a common behavior across many animals, as it can assist in defending or acquiring resources such as territory, food, or potential mates. In many cases, aggressive interactions take the form of a threat display, in which the animal telegraphs—with varying degrees of truthfulness—an ability to physically best another individual. Sturtevant, for example, noticed that male flies “may sometimes be seen to spread their wings, run at each other, and apparently butt heads” (Sturtevant, 1915).

But what triggers this behavior? And is aggression a fundamental behavior controlled by a single neural switch, or is it a combination of separate commands, methodically coordinated and organized? Or is it somewhere in between, more a dimmer knob than a switch to be flipped on or off? Through careful behavioral analysis, experimental manipulations, and targeted neuronal activations, Duistermars et al. (2018) ask precisely this question, attempting to ascertain whether threat sequences represent a distinct motor sequence and, if so, how they are controlled by the fly’s nervous system.

To understand such motor sequences, though, one first needs to precisely and quantitatively define the behaviors one wishes to study (Berman, 2018). To achieve this goal, Duistermars et al. (2018) use supervised machine learning techniques to identify threat bouts between pairs of male flies. Additionally, manually defined thresholds on a fly’s wing angle, as well as its body-frame velocity and angular velocity, are used to define subsections of an individual bout, resulting in a set of five motor elements: wing pumps, wing elevations, charges, turns, and periods of immobility. Analyzing hundreds of bouts, Duistermars et al. (2018) observe that these motor elements can be used independently or in combination during any particular threat instance, providing a hint that aggressive threats may be made up of individually addressable modules.

To show that a fly’s control system is indeed modular, however, Duistermars et al. (2018) dig further, seeing what aspects of threats are triggered by which types of stimuli. To disassociate visual cues from olfactory ones, they place a male fly in an arena containing a dead male fly (olfactory) and/or a moving dummy (visual) that can elicit courtship-like social behaviors in flies (Agrawal et al., 2014). While flies do not perform many threats with just one of these two stimuli, strong threat responses were generated with both present. In addition, genetic ablation of several of the flies’ olfactory receptors prevented threats from being performed, demonstrating the necessity of both stimuli to elicit threat responses.

Understanding the behavioral components within and the sensory inputs required for threat behavior, Duistermars et al. (2018) next identify the neurons underlying this response, starting with a previously identified GAL4-UAS line that was shown to be necessary and sufficient for aggressive responses (Hoopfer et al., 2015). They then narrow down the identified neurons using an intersectional genetic approach to find two classes of neurons in the brain (collectively named SplitThr) that can induce threat responses when thermogenetically excited. Activating these neurons induced threat behavior that, while not
identical to wild-type responses, was surprisingly similar. In addition, thermogenetically activating Split<sub>Thr</sub> neurons allowed for the performance of threats to a moving dummy, even in absence of a dead fly to provide chemosensory cues. Furthermore, two somewhat unexpected results emerged in these experiments. First, the number of threat bouts increased with increasing temperature (and thus, increasing thermogenetic activation). Second, Split<sub>Thr</sub> activation, while producing threats, did not result in wing extensions or charges. Together, these results suggest that behavioral control in aggression may be implemented through changing the level of activation in Split<sub>Thr</sub> neurons.

Exploring these findings further, Duistermars et al. (2018) also performed optogenetic activation on the Split<sub>Thr</sub> neurons using Chrimson, a red-shifted channelrhodopsin (Klapoetke et al., 2014). Modulating the light intensity and stimulation frequency during these experiments, they indeed found a dependence through varying signaling in these neurons. For example, low-intensity stimulation reproduced results seen in the thermogenetic experiments, with no threats present in absence of a dummy. At higher intensities, though, Duistermars et al. (2018) noticed that, surprisingly, no dummy (or dead fly) was necessary to induce threats. Moreover, as the stimulation frequency was increased, the fly performed a wider array of threat display components (see Figure 1), starting at turns and charges at low frequencies (~5 Hz) and including wing elevations and pumps as well at higher frequencies (~20 Hz). Across all the levels of activation, though, sequences of behavior remained intact, always following a typical sequence pattern.

Performing additional genetic intersections, Duistermars et al. (2018) were able to find a very small collection of neurons (~3 per half-brain, located in the anterior inferior protocerebellum, or AIP) that were necessary for threat productions in aggressive flies and sufficient to induce aggression in non-aggressive flies. Based on the results shown in this article, the activity of these neurons in the AIP could be a key component of this process for the fly. Less clear, however, is whether they are directly integrating sensory information, and thus directly reporting the state of the external world, or whether sensory input is added downstream of AIP, thus making AIP a reporter of the animal’s internal state. Further neuroimaging experiments are likely required to disambiguate between these models.

Put together, the results in this article provide evidence that although a relatively small number of neurons are needed to induce aggressive behavior, the repertoire of actions (and perhaps the level of intended threat to communicate) chosen can be modulated in an activation-dependent manner. This tunability has been previously suggested in game theoretic models of aggression (e.g., “badges of dominance”), where continuously tunable threat signals are used to explain variability in a population (Maynard Smith and Harper, 1988). In addition, the observed threshold dependence in the neural control of behavior is reminiscent of recent results in Drosophila courtship behavior, where different levels of activation were found to drive species-specific song outputs (Ding et al., 2017), as well as previous results showing that activating the same set of neurons could engender either aggressive or courtship behavior in flies (Hoopfer et al., 2015).

Thus, this story evokes much of Tinbergen’s ethological theories, observing that a single internal state, “reproductive instinct,” could lead to increased levels of courting, fighting,
nesting, or other related behaviors, all dependent on the sensory stimuli presented to the animal and its own internal state (Tinbergen, 1951). Competing with these types of states, though, are other, perhaps more mundane, drives like hunger, thirst, sleep, or thermoregulation. Understanding how this hierarchical set of internal states compete likely requires an accordingly hierarchical picture of neural dynamics (Dawkins, 1976). How an organism uses its nervous system to switch between actions, tasks, and longer timescale behaviors has been a motivating question in ethology since its founding. Now, however, using sophisticated tools for measuring and manipulating neural activity and behavior in freely behaving animals, we are starting to generate a picture of how these processes may occur, bringing new power to old theories and rapid progress toward understanding how behaviors adapt and evolve.

REFERENCES


Figure 1. Behavioral Modularity Is Generated through Continuous Changes in Neural Activity
Low-intensity optogenetic activation reproduced results seen in the thermogenetic experiments, with no threats present in absence of a dummy. As the stimulation frequency was increased, the fly performed a wider array of threat display components, starting at turns and charges at low frequencies (~5 Hz) and including wing elevations and pumps as well at higher frequencies (~20 Hz).