Quantifying Behavior To Solve Sensorimotor Transformations: Advances from Worms and Flies

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Abstract

The development of new computational tools has recently opened up the study of natural behaviors at a precision that was previously unachievable. These tools permit a highly quantitative analysis of behavioral dynamics at timescales that are well matched to the timescales of neural activity. Here we examine how combining these methods with established techniques for estimating an animal's sensory experience presents exciting new opportunities for dissecting the sensorimotor transformations performed by the nervous system. We focus this review primarily on examples from *Caenorhabditis elegans* and *Drosophila melanogaster* – for these model systems, computational approaches to characterize behavior, in combination with unparalleled genetic tools for neural activation, silencing, and recording, have already proven instrumental for illuminating underlying neural mechanisms.

Introduction

Behavior is dynamic, complex, and seemingly noisy – this presents challenges for both quantifying it and connecting it with the underlying neural activity that generates it [1]. One challenge with quantifying behavior is defining it – while neural activity can be measured in spikes or fluctuations of membrane voltage, scientists have yet to agree on a definition of what constitutes 'behavior'. For example, a survey of ethologists [2] yielded largely inconsistent definitions. The respondents agreed with incompatible statements such as both 'only animals behave' and 'algae chemotaxis is behavior', 'behavior is always executed through muscular activity' but 'sponges behave' (without muscles), and 'a person deciding to do nothing is behaving' (without using muscles). One way to make progress on this question may then be to simply quantify as much as possible about what an animal does (what we here refer to as 'behavior'). Thanks to developments in computer science and the increasing availability of high resolution, high-frame rate cameras, we are now able to capture and quantify orders of magnitude more data about animal movements and actions than was possible even a decade ago. Faced with these new large datasets, there has been a recent explosion of interest in developing algorithms to automate the classification of

behavior. Rather than focus on summary statistics, these algorithms provide the ability to precisely measure individual-level variation in behavior, as it evolves over time. In addition, these methods represent a powerful alternative to human classification, which is typically slow, difficult to reproduce, and often introduces unwanted biases [3,4]. These automated tools further provide consistency when analyzing phenotypes that result from genetic and neural perturbations [4].

In parallel, new computational frameworks now allow scientists to estimate the sensory experience of the animal under study. That is, both the input and output space of the nervous system can be extensively sampled. These data can then be used to build models that not only predict behavior (keeping in mind that an animal's own behavior affects its sensory experience), but also identify the internal computations that the nervous system performs. These capabilities become even more powerful when combined with a simple nervous system and genetic toolkit that facilitate testing predictions from models via targeted neural manipulations and recordings (e.g., determining the specific neurons involved in a given behavior and over what timescales). We therefore focus our review on new methods for behavioral quantification in worms and flies, delving into how to use quantitative behavioral analysis in combination with genetic tools to map *full* sensorimotor pathways, from neurons that process sensory information all the way to neurons that coordinate behavior (Figure 1).

Automated Methods to Quantify Behavior

The most widely used automated methods take raw recordings – consisting of video, audio, or other measurements – and segment them into behaviors that are pre-defined by the experimenter (Figure 2Aii and 2Bii). For example, tracking software can find the location of animals in raw video frames by identifying pixels that differ from the background. The trajectory of each individual animal is then tracked across time, providing additional features of the animal's behavior such as its velocity and changes in orientation [5–8]. More complex species-specific behaviors can be identified by adding additional assumptions. For instance, a fruit fly has two wings that can be recognized by virtue of being more transparent than the body – quantification of wing position permits identification of, for example, aggressive wing threats[9]. Other forms of raw data - such as acoustic recordings - can also be automatically segmented, for example by using a pre-defined template to identify when an animal is emitting a specific acoustic signal[10] (Figure 2A). While effective at classifying specific behaviors, these methods are not flexible. In contrast, the Janelia Automatic Animal Behavior Annotator (JAABA)[11] starts with video clips segmented using some of the aforementioned tools, but then allows the user to select video frames that correspond to a particular behavior (e.g., wing grooming) (Figure 2B). The program automatically 'learns' to identify all other frames with similar features. This class of machine learning algorithm can be easily trained on almost any type of data.

Ultimately though, one would like to identify *all* the actions that an animal performs (e.g. singing, running, or reaching) without needing the experimenter to painstakingly define one after another. The first step in this process is to identify some simpler representation of an animal's posture. The power of this analysis was first shown in *C. elegans*, an animal that can be described as a curved 1-dimensional line. Performing a dimensionality reduction

technique known as principal component analysis (PCA) revealed that only four dimensions account for the overwhelming majority of a worm's postures [12]. How the animal's posture changes in each of these dimensions is sufficient to explain a wide variety of behaviors, from foraging to thermotaxis[12,13], and has led to the discovery of new behaviors [14] as well as identified novel roles for genes in behavior[15]. Similar approaches that use automated tools to more closely quantify posture have been used to characterize larval zebrafish [16] and larval *Drosophila* [17] behavior.

While the aforementioned methods provide a description of an animal's posture at any given moment in time, they lack information about the context in which those postures were used. Converting postures (e.g., 'straight forearm' or 'extended elbow') into actions (e.g., 'reaching') requires some way of clustering together stereotyped sequences of postures that are repeatedly seen with only minor variations. Methods to accomplish this range from embedding postures into a low-dimensional space to find 'clumps' of similar sequences[18] to identifying when one posture is predictable from previous postures[19], though other methods exist[15,17,20,21]. What is exciting about these 'unsupervised' algorithms is that they offer not only ever-more-precise quantification of what an animal is doing at each moment in time, but also reveal the underlying structure of behavior (e.g., which behaviors are sub-programs of other behaviors or which behaviors co-occur), and offer the potential for discovering completely new behaviors. These methods are not limited to single animals either: additional behaviors may fall out of clustering data from multiple animals engaged in social behaviors[22,23]. In addition, one can ascend another level of timescales and cluster actions into 'sequences of actions'. Doing so in *Drosophila* reveals long timescale structure in behavior that does not fall out of the simple transition probabilities between actions[24].

These methods provide the foundation for dissecting the underlying neural circuit activity. With sophisticated tools for behavioral quantification in hand, we can ask which neurons are *necessary* and which *sufficient* to drive a particular behavior by using the powerful genetic toolkits available for *C. elegans* and *Drosophila* [25,26]. Even single neurons and cell types can be targeted, providing a level of circuit dissection simply unavailable in larger organisms. For example, one study screened the individual behaviors of more than 37,000 *Drosophila* larvae, each expressing Channelrhodopsin in one of roughly 1,000 different neural populations [27]. Using a behavioral tracker, the authors identified behaviors driven by activation of a neural population and then built maps connecting specific neurons to each behavior. Rapidly screening so many animals would not have been possible without automated methods. A more recent study expanded this approach to adult *Drosophila*, automatically cataloging the neural correlates of behaviors using 400,000 flies and targeting 2,204 different neuronal populations for activation [28]. Other studies in adult flies have used similar automated strategies to identify, for example, the connection between specific visual neurons and behavior [29] or that the same subset of neurons can induce different behaviors (courtship vs. aggression), depending on levels of neural activation [30]. In *C. elegans*, automated behavioral classification has been used to distinguish between modes of foraging behavior, and then connect these modes with specific neuromodulatory pathways [31], or to identify the specific neural subsets involved in discrete aspects of random search behavior [32]. Automated behavioral analysis can also lead to a more precise manipulation of neural activity [33].

Linking Sensation to Action

Although these new methods for behavioral quantification offer a greater understanding of what the animal is *doing*, they are in general agnostic to the sensory experience of the animal. Bringing together highly quantitative readouts of both behavior and dynamic sensory stimuli will enable mapping complete sensorimotor transformations [34].

One way to begin is to simply activate sensory neurons directly and quantify which behaviors are produced as a result [29,35–37] (Figure 3A). By varying the activity of sensory neurons and observing the motor output, one can directly compute the mapping the animal uses for behavior. However, animals that are naturally behaving receive a diverse range of sensory inputs. Establishing which inputs drive behavior can be challenging as natural sensory spaces not only contain both temporal and spatial correlations, they can also be altered by an animal's own behavior. This means that simply examining the stimuli that precede or co-occur with a particular behavior is insufficient. Instead, newer computational techniques are needed to estimate relevant sensory features in spite of these correlations, by finding those most linked to the output[38–40]. These methods, in general, fit a linear filter that processes an input before being passed through a nonlinear function [41]. The linear filter (Figure 3, column iii) describes which temporal features of the inputs drive the output. Though these methods were initially developed to predict neural activity from complex sensory stimuli, they can be extended to predict behavior. This has now been done in a few cases using *C. elegans* and *Drosophila*, highlighted below.

For example, when *C. elegans* leave a patch of food they will forage for new food by searching a local area before switching to a more global search (Figure 3B). How does this search strategy - this particular sequence of actions - change based on the animal's sensory experience? The sensory environment can be estimated by measuring the concentration of food and position of the animal's nose, while the motor output can be measured using automated tools described above to track the worm's trajectory[42,43] (similar measurements have been made using *Drosophila* larvae in an olfactory environment [44]). Sensorimotor filters obtained from these data reveal a role for long timescale integration (~25 minutes) in guiding foraging decisions. Identifying these transformations enables finding the neurobiological underpinnings. In this case, the temporal width of the filter is determined by the concentration of the CREB molecule in a single pair of interneurons.

Social interactions add another layer of complexity, where the behavioral output of one animal serves as input to the sensorimotor processes of another animal. *Drosophila* courtship was previously thought to be comprised of a static sequence of actions, but measurement of the movements of two flies along with the song that the male produces identified a novel role for sensory feedback (e.g., the movements of the female) in song patterning decisions (e.g., choosing which song mode to produce at each moment in time) [45] (Figure 3C). Although courtship involves a dynamic feedback loop between two animals, the loop can be 'broken' by presenting controlled artificial stimuli. For example, the motion of an apparent female can modify the amplitude of song pulses produced by a tethered, walking male[46]. Similar analyses of the sensory cues that drive behavior can also be performed in flying animals, though this has the added complexity of requiring more degrees of freedom to

explain sensorimotor transformations in a 3D environment[47–49]. These studies that rely on the precise measurement of behavior reveal that events that were previously considered 'random' or 'noisy' are, in fact, carefully orchestrated sensorimotor processes (though note that this careful orchestration may also include some beneficial stochasticity, for example in creating variable behavioral responses to reliable sensory stimuli [50,51]).

Dissecting Circuits for Sensorimotor Behaviors

How exactly does sensation lead to changes in behavior? An early example of solving this problem comes from the careful measurement of turning responses to visual motion [52,53]. These measurements suggested models with a clear prediction: motion should be detected via correlated activation of two nearby units coupled by a delay (such that activation of one unit followed by activation of the other would appear as motion). Building on these models, recent studies using *Drosophila* and its genetic and neural circuit toolkit, have identified pairs of visual neurons that implement precisely this algorithm: one pair for correlated increases in luminance and one pair for correlated decreases [54]. Further quantification of the sensory environment that flies experience in nature has revealed more accurate descriptions of both visuomotor behavior and the underlying circuitry [55,56]. These examples highlight the value of starting with a detailed model of a sensorimotor process inspired by quantitative behavioral data in order to solve neural circuit mechanisms.

For some behaviors, it may not be as obvious which neural pathways are involved – in this case, genetic and neural circuit manipulations combined with quantitative models of behavior can provide insights. For example, prior to the quantification of *Drosophila* courtship described in the previous section, there was little reason to examine neural coding in visual pathways when considering the neural basis for courtship song patterning [45]. However, the modeling made the key prediction that distance between two individuals is a major predictor of song choice (pulse versus sine), which was confirmed with mutations that rendered the male fly blind. Similarly, in a separate study, high-resolution behavioral tracking of groups of *Drosophila* presented with an aversive odor identified a social component to odor avoidance. By silencing and activating leg mechanosensory neurons, the authors found a surprising role for touch receptors in collective responses to odor stimuli [57].

Beyond identifying which neurons contribute to behavior, we would also like to identify the patterns of neural activity that are responsible for driving such dynamic behavior. To gain cellular-resolution access to the nervous system, it is often necessary to head-fix or immobilize the animal – while this limits behavior, it is still possible to identify neural correlates of sensorimotor processing from such recordings using either electrophysiology or imaging of calcium sensors [58]. For example, worms are typically restrained in a microfluidic chip for calcium imaging [59]. In such a preparation, whole-brain imaging revealed that the neural dynamics are highly constrained to only a few main modes of activity, each of which corresponds to distinct actions [60]. In contrast, head-fixed flies can be allowed to fly [61] or to walk on an air-supported treadmill [62]. Stimuli can then be presented in closed-loop with the fly's motion, and the nervous system can be imaged at high resolution in such virtual environments. Employing such a setup has already revealed

many of the sensorimotor processes that underlie spatial navigation in *Drosophila* [63,64]. Recording neural activity during behavior also facilitates finding neural responses that co-vary with behavior on short timescales (such as within a single trial). For instance, visual cells in the *Drosophila* eye responsive to wide-field horizontal or vertical motion also encode information about the velocity of the animal's self-motion to compensate for the expected effect on the incoming visual signal [65,66].

Remarkably, we are already able to record from the majority of the neurons, at cellular resolution, in two animals during free behavior, *C. elegans* [67,68] and another invertebrate, *Hydra* [69], though progress is being made toward similar capabilities in adult *Drosophila* [70,71], larval *Drosophila* [72], and larval zebrafish [73]. For *C. elegans*, real-time quantification of movement drives the motion of a motorized stage that re-positions the worm directly under the neural imaging scope [67,68]. Such experiments can connect population neural activity with natural behaviors, such as forward or backward motion, or turning, and can even track activity over long-term behavioral state changes [74]. Due to the complexity of the neural dynamics, dimensionality reduction techniques such as PCA can be used to identify subnetworks that co-vary in their activity and are implicated in certain behaviors.

In addition to worms and flies, the larval zebrafish also offers a compact nervous system along with an advanced genetic toolkit to facilitate studies of sensorimotor processes [75]. Automated behavioral tools (though fully supervised) have also been used to track fish and study locomotion [76], prey capture [77], or social behaviors [78]. Most importantly, facilitated by its transparency, methods have been developed to rapidly image neural activity at cellular-resolution from the entire brain during fictive behavior [79]. This has led to breakthroughs in, for example, dissecting the circuits underlying the optomotor response [80] or locomotor learning [81] in the zebrafish. The investigation of whole-brain activity is arguably more mature in zebrafish than in either *C. elegans* or *Drosophila*. The next few years should hopefully lead to a convergence between studies of these organisms, with the behavioral quantification tools developed in the latter species applied to the former and the circuit dissection philosophy of zebrafish brought to bear on worms and flies.

Future Directions

As outlined above, recent advances in behavioral quantification facilitate solving the underlying neural circuit mechanisms. However, several challenges remain. First, the methods described above require animals to be placed in artificial environments in order to cleanly segment videos. However, placing animals in richer environments would provide better access to the computations that brains evolved to solve. Methods that make 'unsupervised' behavioral classification more compatible with recordings taken in natural settings would expand the dictionary of behaviors that can be identified. Second, most behavioral quantification methods capture video of animals from a single vantage point, but characterization of the full repertoire of animal behavior will likely require combining data from multiple cameras and from other types of recordings (e.g., muscle activity [82] or leg kinematics [83]). Third, as we advocate in this review, behavioral quantification should be intertwined with studies of the sensory feedback that drives or modulates behavior. One

current limitation is that experimenters pre-define the features of the sensory space to feed into models. It should be possible to identify relevant sensory features in an 'unsupervised' fashion [84], similar to new methods for behavioral analysis. Fourth, current models of sensorimotor transformations often assume an animal does not switch between behavioral states despite the fact that we know that internal state plays an important role in behavior [85–87]. Models that can capture longer timescales in behavior or 'hidden states' (e.g., using hidden Markov models) could identify the influence of these internal states and will facilitate finding their neural correlates. Finally, many of the tools described in this review characterize the relationships between animal postures and their environments as linear, when in fact they may be highly nonlinear. The use of machine learning techniques, including deep learning, to introduce nonlinearities into the relationships between postures should produce more precise descriptions of behavior [88–90].

The models described in this review are also largely descriptive in nature. However, models that are *generative*, or able to generate simulated behavior, can provide a set of underlying rules that the nervous system may implement [91]. These rules could then be used to develop a theory of *why* behaviors are generated in the way that they are [1]. Such a comprehensive theory will lead to better measures of behavior that can ultimately be related back to the neural activity that generates it. Nonetheless, the recent explosion of methods for behavioral quantification, combined with the unparalleled genetic and neural circuit toolkits of worms and flies, has yielded exciting results with regard to the cellular mechanisms underlying both sensorimotor transformations and the generation of behavior. These studies reveal the promise of these model systems in providing a sophisticated understanding of the fundamental principles that shape behavior.

Acknowledgments

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Highlights

- New automated methods permit characterization of the full repertoire of an animal's behavior.

- Quantification of dynamic sensory stimuli in combination with behavioral analysis will facilitate identification of the computations the nervous system performs to transform sensory responses into motor outputs.

- Extensive genetic tools and a simple nervous system make worms and flies attractive model systems for harnessing the full power of these new computational methods to solve the mechanisms underlying sensorimotor transformations.
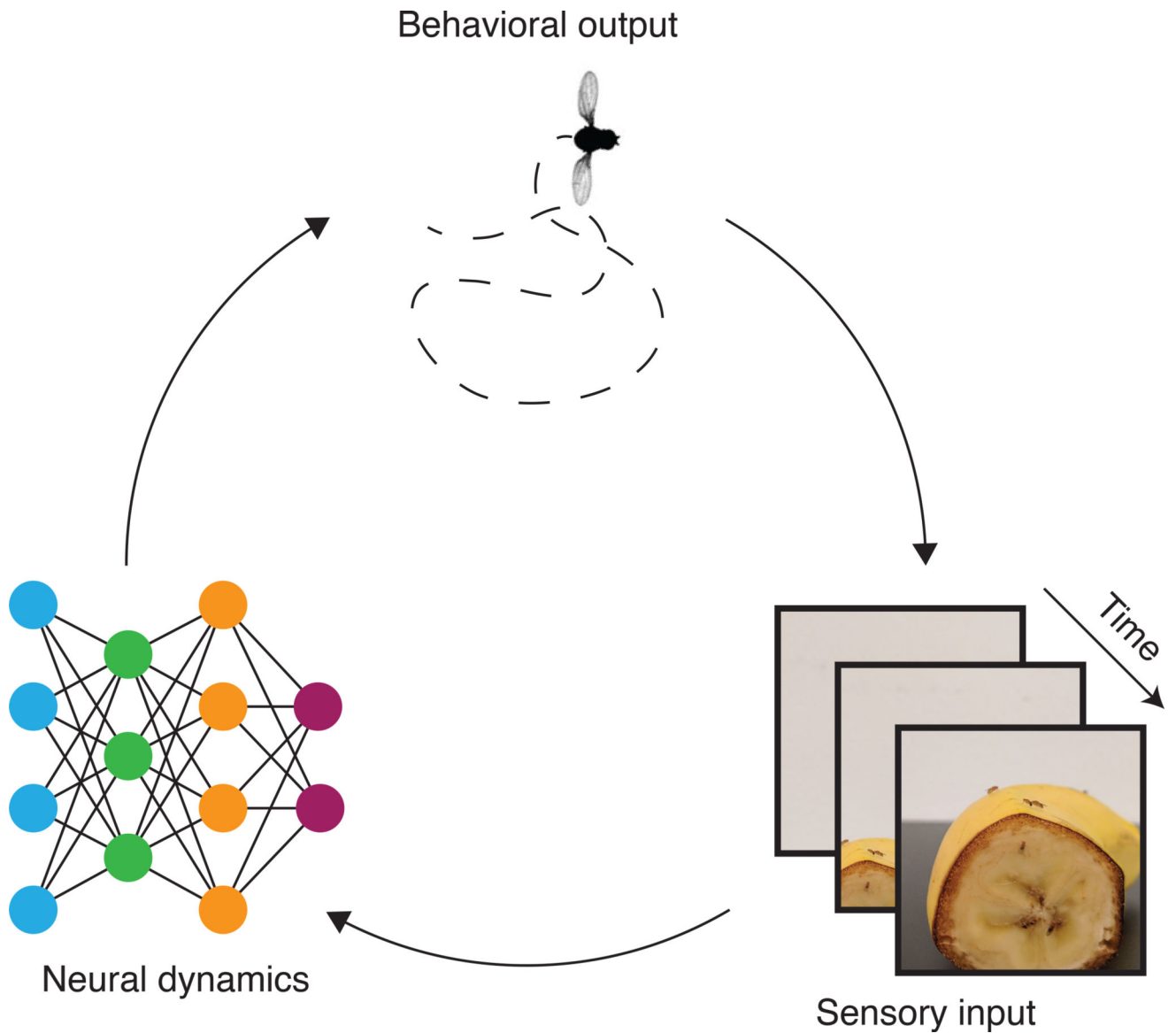
Figure 1. Mapping full sensorimotor pathways

Solving the sensorimotor transformations that nervous systems perform requires quantification of sensory inputs, neural dynamics and behavioral outputs. Sensory inputs influence neural activity which drives behavior, which in turn can change the sensory input that an animal receives (image of fly adapted from Muijres et al 2014).
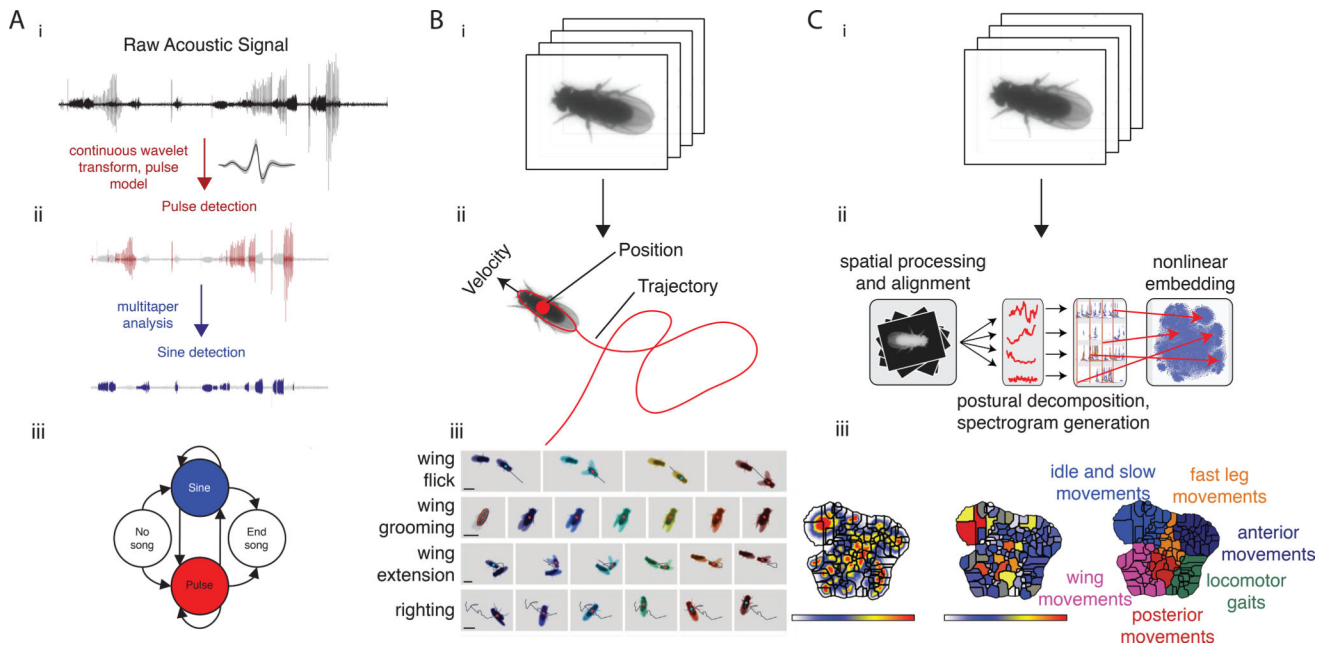
Figure 2. Automated algorithms for quantifying behavior

(**A**) A fully supervised algorithm for segmenting acoustic behavior begins by (i) taking raw audio recordings, (ii) using an algorithm to identify salient features - here, 'pulse' and 'sine' types of song - and then inferring (iii) the longer-term bout structure (consisting of alternating trains of pulse and sine song) (modified from Arthur et al. 2013). (**B**) Starting with video data (i), other supervised algorithms (ii) find salient features such as center of mass (position), velocity, trajectory, and so on. (iii) A semi-supervised machine learning algorithm (JAABA) can use these features to identify discrete actions defined by the experimenter (modified from Kabra et al. 2013). (**C**) Largely unsupervised methods attempt to identify *all* behaviors from raw video data (i). (ii) One such method takes a set of aligned images from movies of flies and decomposes the dynamics into a low-dimensional basis set. Time series are produced by projecting the original pixel values onto this basis set, and these trajectories are then embedded into two dimensions (using t-SNE). (iii) Each position in the behavioral map corresponds to a unique set of postural dynamics, with nearby points representing similar motions (modified from Berman et al 2014). Maps are built by computing the probability of being embedded in this point in 2D space (left), then clustered with a watershed algorithm into discrete actions (middle) before identifying what general behaviors large regions of space belong to (right).
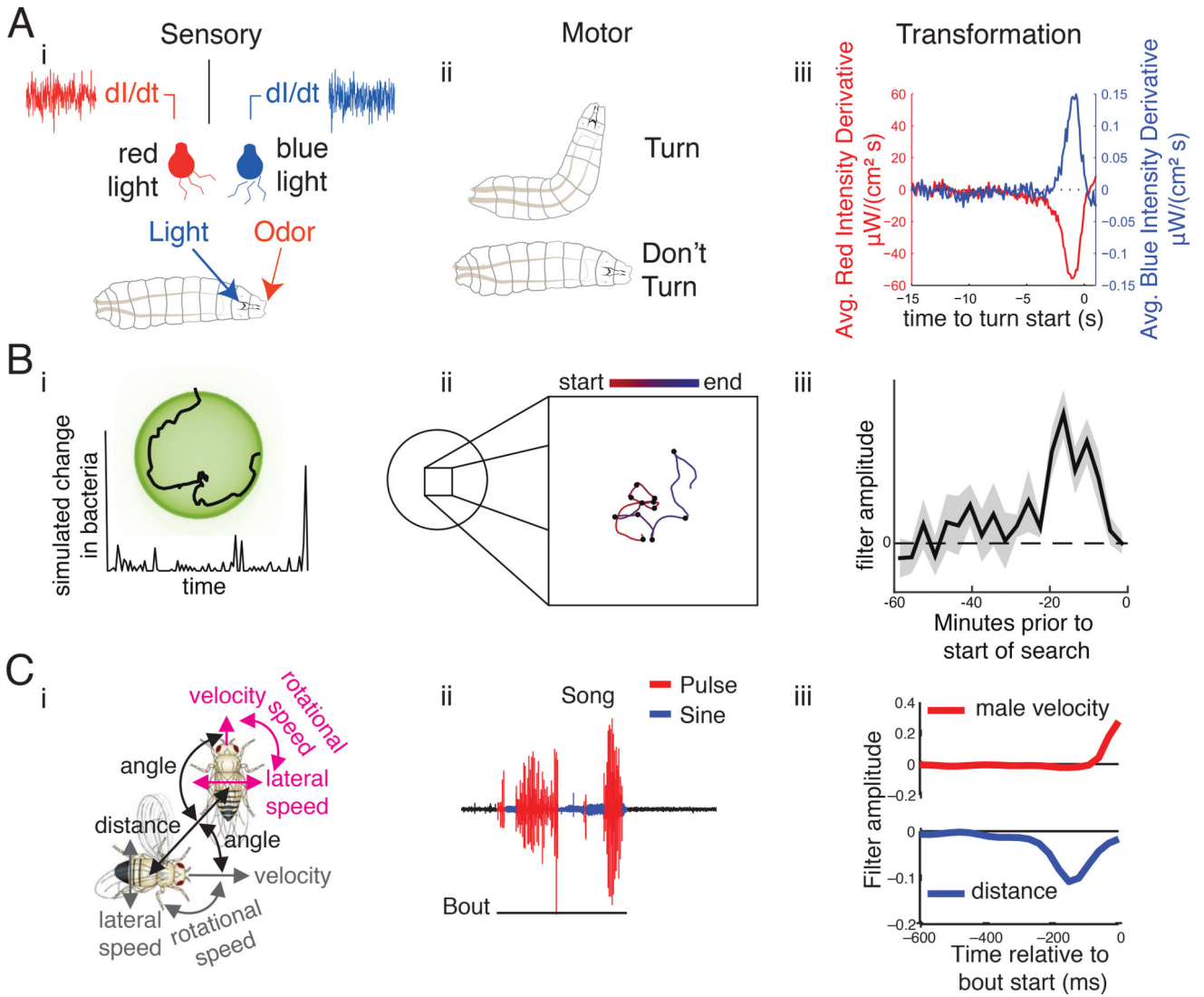
Figure 3. Inferring sensorimotor transformations from behavioral data

(**A**) Visual and olfactory pathways can be driven by two different colors of light use Gaussian white noise patterns (i). On the basis of this activation, Drosophila larvae will decide whether or not to turn (ii). (iii) A reverse-correlation analysis finds the filters that transform sensory neuron activation into behavioral choices (modified from Gepner et al 2015). (**B**) (i) *C. elegans* that are on a food patch will explore (insert) and experience changes in food concentration. (ii) When they are exploring off-food, they will explore a small area by emitting a certain number of large-angle turns (represented here as black dots) to interrupt their forward locomotion and keep them in a small area. (iii) This sensory experience and turning output can be linked by a linear filter that keeps track of ~25 minutes of experience (modified from Calhoun et al 2015). (**C**) (i) *Drosophila* males will court a female by observing features such as the distance between the male and female and her velocity and (ii) will produce a dynamic song via wing vibration. (iii) The type of song that

is emitted can be predicted based on the male's own velocity and the inter-fly distance, for instance (modified from Coen et al 2014).