



Studying neural circuits of decision-making in *Drosophila* larva

Tihana Jovanic

► To cite this version:

Tihana Jovanic. Studying neural circuits of decision-making in *Drosophila* larva. *Journal of Neurogenetics*, 2020, 34 (1), pp.162 - 170. 10.1080/01677063.2020.1719407 . hal-03400525

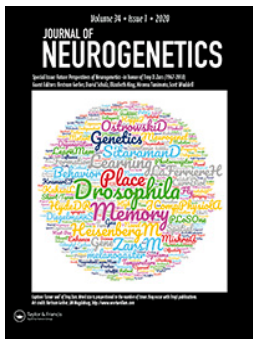
HAL Id: hal-03400525

<https://hal.science/hal-03400525>

Submitted on 25 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Studying neural circuits of decision-making in *Drosophila* larva

Tihana Jovanic

To cite this article: Tihana Jovanic (2020) Studying neural circuits of decision-making in *Drosophila* larva, Journal of Neurogenetics, 34:1, 162-170, DOI: [10.1080/01677063.2020.1719407](https://doi.org/10.1080/01677063.2020.1719407)

To link to this article: <https://doi.org/10.1080/01677063.2020.1719407>



Published online: 13 Feb 2020.



Submit your article to this journal [↗](#)



Article views: 178



View related articles [↗](#)



View Crossmark data [↗](#)

PERSPECTIVE



Studying neural circuits of decision-making in *Drosophila* larva

Tihana Jovanic^{a,b} 

^aUniversité Paris-Saclay, CNRS, Institut des Neurosciences Paris Saclay, Gif-sur-Yvette, France; ^bDecision and Bayesian Computation, UMR 3571 Neuroscience Department & USR 3756 (C3BI/DBC), Institut Pasteur & CNRS, Paris, France

ABSTRACT

To study neural circuits underlying decisions, the model organism used for that purpose has to be simple enough to be able to dissect the circuitry neuron by neuron across the nervous system and in the same time complex enough to be able to perform different types of decisions. Here, I lay out the case: (1) that *Drosophila* larva is an advantageous model system that balances well these two requirements and (2) the insights gained from this model, assuming that circuit principles may be shared across species, can be used to advance our knowledge of neural circuit implementation of decision-making in general, including in more complex brains.

ARTICLE HISTORY

Received 22 August 2019
Accepted 18 January 2020

KEYWORDS

Drosophila larva; neural circuits; decision-making

1. Introduction

Any organism has the capacity to do many different behaviors, that all serve a certain purpose; from finding food, mating to escaping predators or avoiding other dangers. Yet many of these behaviors cannot occur simultaneously as they are physically mutually exclusive. An organism therefore needs to select which behavior to express in a given moment. The capacity to select the best behavior to execute is essential as depending on the situation some behaviors are more advantageous than others. If this process goes awry it can have serious consequences: if we don't eat when we are hungry or stay in presence of dangers we may die. Decision-making is therefore a fundamental aspect of behavior that is shared across the animal kingdom and the underlying mechanisms have evolved to ensure that it serves the organisms best interest and often combine instinctual drives and higher-order adaptive functions (Adams, Watson, Pearson, & Platt, 2012; Kristan, 2008; Pearson, Watson, & Platt, 2014).

The decision-making process needs to be successful in two ways. First, in terms of the appropriateness of the selected behavior to the situation. To make a choice, an animal will therefore take into account both the environmental context as well as its internal physiological state (Palmer & Kristan, 2011). It means the nervous system needs to be able to integrate different types of contextual and/or internal-state information with the predicted outcome of a behavior. This supposes an organization of the neural architecture that is flexible and where these systems can interact. Second, decisions need to be successfully implemented: the selected behavior needs to be fully expressed, while the competing behaviors need to be fully suppressed. Mechanisms must therefore exist in the nervous system that ensure that one behavior is promoted others are being inhibited (Kovac &

Davis, 1977; Kupfermann & Weiss, 2001; Redgrave, Prescott, & Gurney, 1999). It is essential that this selection is full and complete so that the animals express clear and stable behaviors. Indeed, the etymology of the word decision stems from the Latin *decider* literally to cut off (for de-+ caedere 'to cut').

Ultimately, the problem of selecting a behavior would be very different if the nervous system were organized into discrete modules where specific sensory inputs generate behaviors executed by specific effectors (Kupfermann & Weiss, 2001). Given that behaviors use overlapping neural networks and effector systems, the problem of resolving conflicts between competing demands on these systems by competing drives is a key feature of behavior (Prescott, 2007; Redgrave *et al.*, 1999). Nervous systems therefore need to have mechanisms in place that can implement these competitive interactions successfully (Koyama & Pujala, 2018; Maass, 2000; Mink, 1996; Redgrave, Vautrelle, & Reynolds, 2011; Wang, 2008).

Studying cellular and synaptic mechanism of how the nervous system selects behaviors can be challenging in complex brains. Model systems with compact and numerically simple brains are more amenable for detailed characterization of the neural circuit mechanisms involved in competitive interactions (Baca, Marin-Burgin, Wagenaar, & Kristan, 2008; Gaudry & Kristan, 2009; Gillette & Brown, 2015; Jovanic *et al.*, 2016; Kovac & Davis, 1977; Kristan, 2008; Reyn *et al.*, 2014).

One such model organism is the fruitfly larva, that will be discussed in this article. The *Drosophila* larva has many advantages for dissecting neural circuits, with the possibility to combine the use of precise genetic tools in *Drosophila* for manipulating and monitoring neuronal activity, the ongoing reconstruction of the connectome using electron microscopy,

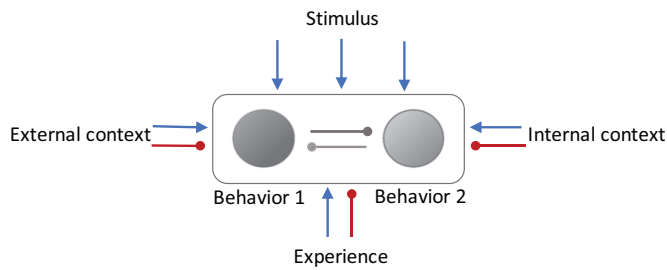


Figure 1. Schematics of flexible decisions. Sensory stimuli may evoke different behavior outputs. The different behavioral options mutually compete. These competitive interactions can be modulated by contextual information about the environment, animal's internal state and/or experience. Arrows represent excitation and lines with circles inhibition.

high-throughput assays for observing and quantifying behaviors with electrophysiology. These approaches have been used over the recent years to study the underlying mechanisms of multisensory processing (Ohyama *et al.*, 2015), motor control (Fushiki *et al.*, 2016; Heckscher *et al.*, 2015; Takagi *et al.*, 2017; Zarin, Mark, Cardona, Litwin-Kumar, & Doe, 2019; Zwart *et al.*, 2016), behavioral choice and sequence generation (Jovanic *et al.*, 2016) and learning and memory (Eichler *et al.*, 2017; Saumweber *et al.*, 2018). While the *Drosophila* larva is undoubtedly a powerful model system for neural circuit dissection, the relevance of the findings from a simple nervous system to other more complex brains is often debated. This concern is two-fold: on one side, the question is whether the circuit mechanisms found in the larva will be comparable to those that take place in vertebrate nervous systems given the neuroanatomical differences. On the other, it is debated whether the decision-making process in the larva is complex enough to bring any insight that would be useful to more sophisticated forms of decision-making. These are important and valid concerns as they influence the framework for defining the problem that is being studied, interpreting the results and drawing conclusions.

The term decision-making can be ambiguous and has been used to describe processes of variable complexity across the animal kingdom from *C.elegans* to humans (Barker & Baier, 2015; Constantinople, Piet, & Brody, 2019; Faumont, Lindsay, & Lockery, 2012; Gillette & Brown, 2015; Glimcher & Rustichini, 2004; Gold & Shadlen, 2007; Hung & Stopfer, 2018; Jarrell *et al.*, 2012; Kristan, 2012). Generally, decision-making is often thought as a high-level cognitive process that leads to choices by weighing the different options and their expected outcomes often over long periods of time. In reality though, there are many different types of decisions from very simple ones like for example which hand to use to open the door, or what to order from the menu, to more complex ones such as which job to take. (Kristan, 2008; Pearson *et al.*, 2014). In addition, many animals with very little cognition, like invertebrates, make choices of what to do (whether to eat or to escape for example) depending on both external and internal factors (Palmer & Kristan, 2011). Some of the mechanisms underlying different aspects of these different types of decisions will be different, but others will be shared. In addition, in some cases the simple decisions have also been referred to as behavioral choice

(Faumont *et al.*, 2012; Gaudry & Kristan, 2009; Jovanic *et al.*, 2016). Thus, the question of what exactly constitutes a decision has been a challenging one (Glimcher, 2004) and often the boundaries between the processes described by the terms like behavioral choice, action-selection and decision-making may be blurry and not always clearly distinguished.

For a purpose of this review, I will refer to decisions as events that involve a selection by the nervous system of a behavior based on various external and internal sensory information and an unambiguous commitment to that behavior (Figure 1). During decisions, the mapping from sensory inputs to motor outputs needs to be flexible for a complex and dynamic environment to be able to modulate decisions (Pearson *et al.*, 2014). The complexity of decisions will decrease with the number of inputs and alternatives available.

I will present the case for using *Drosophila* larva as a model system to study the neural circuit mechanisms of decision-making. I will outline the technical advantages for dissecting neural circuits specifically from the point of view of decision-making. I will then give examples of decisions in the larva. Finally, I will propose that some of the concerns that the neural circuit mechanisms of decision-making identified in the larva may not be relevant to other species with more complex forms of decisions-making may result from the conceptual frameworks that have been traditionally used to study this problem. I argue first, that much of larval behavior has been underestimated and have been thought as hard-wired and not flexible thus not bearing a choice component. Secondly, although the complexity of decision-making of the larva may be limited compared to the type of decisions made by animals with more complex brains, some of the basic principles of how decisions are implemented in the nervous system could be shared across the animal kingdom (Adams *et al.*, 2012). Similar computations could use similar circuit motifs as building blocks (Braganza & Beck, 2018; Koyama & Pujala, 2018). Thus, combining precise circuit dissection in a simple model system with investigating more complex forms of decision in complex brains could fill the gap in our knowledge about the neural circuit implementation of decision-making (Kristan, 2008).

2. Mapping neural circuits in the *Drosophila* larva

Using *Drosophila* larva for dissecting neural circuits has many advantages primarily due to the exquisite genetic tools that allow precise labeling of neurons and their interconnectivity as well as monitoring of neuronal activity. These precise tools can also be used to manipulate neuronal activity to determine how behaviors are affected using automated behavioral detection methods. With the help of light and more recently electron microscopy it is now possible to obtain brain-wide wiring diagrams, which opens up avenues for analysis of neural circuit at a new scale.

2.1. Identifying elements of decision circuits

In recent years, numerous sophisticated genetic tools that allow both the visualization and manipulation of activity of

different cell types often at single cell resolution have been developed (Simpson, 2009; Sivanantharajah & Zhang, 2015). These resources allow silencing/activating of neuronal activity in behaving animals and monitoring the changes that occur in their behavior. In order to quantify the changes in behaviors precisely, one needs to be able to detect behaviors unambiguously. Behavioral detection can be done either manually (Seeds *et al.*, 2014) or automatically (Aleman-Meza, Jung, & Zhong, 2015; Gershow *et al.*, 2012; Gomez-Marín, Partoune, Stephens, Louis, & Brembs, 2012; Kabra, Robie, Rivera-Alba, Branson, & Branson, 2013; Masson *et al.*, 2020). The capacity to successfully identify neurons is largely increased by joining the power of genetic tools with automated analysis methods. Automatic behavioral detection allows screening libraries of neuronal lines that would be labor-costly to do using manual annotations and therefore impossible to perform at the same scale as when using automated methods (Jovanic *et al.*, 2019; Masson *et al.*, 2020; Robie *et al.*, 2017; Triphan *et al.*, 2016). In addition, automated methods allow to quantify nuanced movements as well and the precise timing of the initiation of a movement, its duration as well as the frequency of a behavior in a population of genetically identical individuals which is essential for linking neural activity to specific aspects of behavior. These tools combined with amenability of fruit-flies larvae to perform large scale behavioral screens and *Drosophila* line libraries available have made it possible to uncover neurons relevant for particular behaviors (Kvon *et al.*, 2014; Pfeiffer *et al.*, 2008; 2010). This is a crucial step in mapping neural circuits, as it allows to identify its constitutive elements, whose relationships can further be tested using functional and structural connectivity studies.

To map circuits for decisions one approach is to identify a neuron that can bias decisions and then use it as a starting point to reconstruct its synaptic partners in electron microscopy (Jovanic *et al.*, 2016; Ohyama *et al.*, 2015). But how do we define a ‘decision-making’ neuron? Decision-making is a complex process that relies on the income of sensory information that is relayed by sensory pathways while their execution relies on the motor pathways. The decision outcome will thus be affected by changes in sensory processing or motor control neurons. It is therefore important to define a phenotype that we would assign to a ‘decision’ neuron in order to be able to discern neurons that are implicated in the decisions process from those involved in sensory processing or motor control. In an example, when multiple (two or more) options are available in response to a single sensory cue, a choice needs to be made of which one to pursue or execute. When a decision is made one behavior will be selected at the expense of other behaviors. Let’s take case of two behaviors A and B that are competing with each other. For each behavior, there is a neuron that is promoting that behavior and inhibiting the competing behavior. If we were to silence the activity of a neuron promoting behavior A and inhibiting behavior B, we will expect to have a decrease of behavior A and increase of behavior B. Conversely, if we activate the same neuron, the behavior A will increase and behavior B decrease. It follows, that a decision-making

phenotype would be one where the decrease in one behavior is accompanied by an increase of another or several other competing behaviors, suggesting that the neurons whose silencing results in such behavioral phenotypes, could promote one behavior and inhibit other behaviors when there are two or multiple possible options available (Masson *et al.*, 2020).

2.2. Mapping local circuits and brain-wide networks for decision-making

The challenge that the nervous system puts in front of us due to the complexity of interactions between the neurons in neural networks, both local and nervous system wide, is that understanding the principles of its workings requires us to be able to interpret the function of the neurons in the context of larger neural networks.

The elegance of genetic manipulation of neural circuit elements in *Drosophila* larva has recently been complemented by the power of connectomics (Eichler *et al.*, 2017; Heckscher *et al.*, 2015; Jovanic *et al.*, 2016; Ohyama *et al.*, 2015; Schneider-Mizell *et al.*, 2016; Zwart *et al.*, 2016) as well as functional imaging methods (He *et al.*, 2019; Heckscher, Lockery, & Doe, 2012; Karagyozyov, Mihovilovic Skanata, Lesar, & Gershow, 2018; Lemon *et al.*, 2015; Ohyama *et al.*, 2015; Pulver *et al.*, 2015; Simpson & Looger, 2018; Takagi *et al.*, 2017; Yao, Macara, Lelito, Minosyan, & Shafer, 2012; Zarin *et al.*, 2019). This combination of methods has proven to be a powerful toolkit for dissecting neural circuit mechanisms.

Once a ‘decision’ neuron has been identified we can locate this neuron in the electron microscopy volume and reconstruct its synaptic partners (Schneider-Mizell *et al.*, 2016). These connections can further be tested functionally for the neurons labeled in driver lines (Li *et al.*, 2014). The implication of the reconstructed partner neurons in decision-making can further be tested using functional studies.

In order to understand the underlying mechanisms of decision-making it is not enough to look at the local circuits. There are two main views of how is the decision-making process organized in the nervous system. According to centralized models of decision-making decisions are made in specialized decision-making centers based on highly-processed sensory information that arrives there and then is relayed to the motor planning areas for execution (Miller, Galanter, & Pribram, 1960). The non-centralized models say the decision-making process is distributed in the nervous system from the sensory to the motor side and it will be by competitive interactions between the different sensorimotor pathways that the decision will emerge (Cisek, 2007). It is therefore essential to be able to study the processes underlying decisions at the level of the entire nervous system. A compact brain such as the one of *Drosophila* larva can help us understand the organization of a decision-making process. In addition to mapping local circuits, in the larval brain, it is possible to trace the long-range projection neurons that connect the sites of competitions with distant regions of the nervous system. Determining the brain-wide

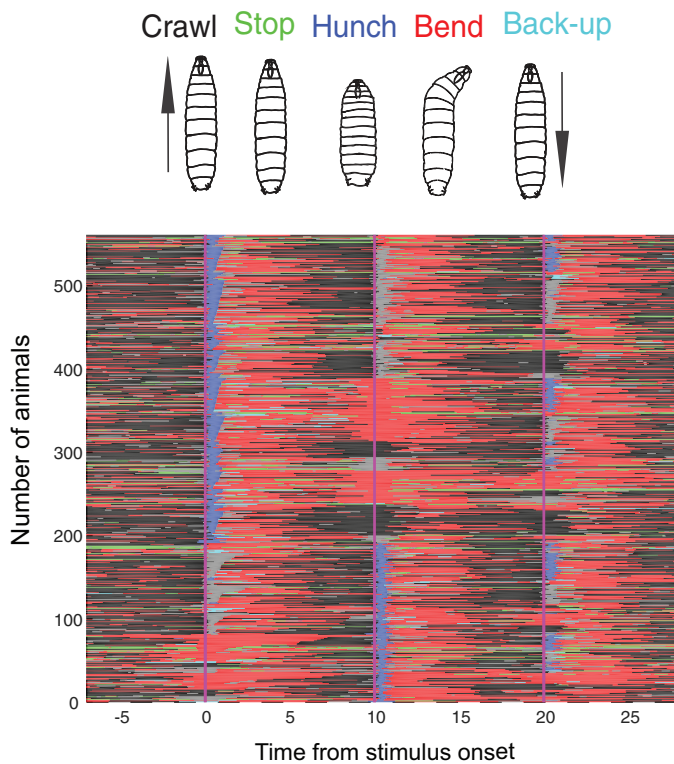


Figure 2. Ethogram of larval response to an air-puff. Each line is a larva. Blue-Hunch, Red-Bend, Crawl-Black, Green-Stop, Cyan-Back-up, Grey-Small motion. Different larvae respond differently. The behavioral detection used to detect the different actions is described in Masson *et al.* (2020). Repeated presentations of the same stimulus (here air-puff) result in different actions in the same larva.

connectivity of a ‘decision’ network using both structural and functional studies would help us determine whether decision-making is distributed or centralized and to which extent.

3. What decisions do *Drosophila* larvae make?

For all of these reasons, namely the precise labeling and manipulation of single neurons to brain-wide maps of neural network, *Drosophila* is powerful system to dissect neural circuits. But how relevant is it to understand the neural circuit structure and function underlying decisions in a small insect brain? What types of decisions do they make and is the nature of the underlying processes in these behaviors similar to the ones occurring during more complex decisions in more complex brains?

Generally, in its simplest form, a decision is the choice of the type of behavior to execute in response external and/or internal sensory information when there are multiple possibilities available. This can be a choice between to respond or not to respond or a choice between different types of actions (Gold & Shadlen, 2007). What will make decisions more or less complex will be the type and features of the sensory information, on one side and on the other, the number and types of behavioral options available (and the level of certainty about their respected outcomes) (Schleyer, Diegelmann, Michels, Saumweber, & Gerber, 2013). Finally, decisions will be modulated by the external context, larva’s internal, motivational, behavioral state and experience.

Drosophila larvae have innate preferences for some stimuli and can be repelled by others. For instance, in presence of appetitive odors the larvae will approach the source of odor (Fishilevich *et al.*, 2005; Gomez-Marín, Stephens, & Louis, 2011; Louis, Huber, Benton, Sakmar, & Vosshall, 2008). In presence of aversive or noxious stimuli they will use strategies to escape or avoid the unpleasant situation. This is true for examples, for responses to light that increase head-casting (Zhao *et al.*, 2017), high temperature that increases crawling speed, mechanosensory stimuli: vibration, air-puff, touch that induce hunching, bending, stopping and crawling backwards and in some cases, reorientation (Jovanic *et al.*, 2016; Ohyama *et al.*, 2013; Tsubouchi, Caldwell, & Tracey, 2012; Yan *et al.*, 2013; Zhang, Yan, Jan, & Jan, 2013) or noxious stimuli that can induce rolling (Burgos *et al.*, 2018; Ohyama *et al.*, 2015; Robertson, Tsubouchi, & Tracey, 2013) or full body contraction (Turner *et al.*, 2016). Thus, larvae respond to different types of stimuli with different actions. But even to a single stimulus they can respond differently especially if these stimuli are weak or ambiguous (Jovanic *et al.*, 2016; Tsubouchi *et al.*, 2012). The same stimulus may evoke different behaviors in different larvae or upon repeated presentation in the same larva (Jovanic *et al.*, 2016) (Figure 2). Thus, *Drosophila* larvae have a rich repertoire of flexible sensorimotor behaviors. The sensory neurons mediating these responses have been well characterized (Klein *et al.*, 2015; Merritt & Whittington, 1995; Sprecher, Cardona, & Hartenstein, 2011; Xiang *et al.*, 2010) and the downstream neural circuitry have started to be unraveled, revealing multilevel convergence architecture for enhanced action selection during responses to multimodal stimuli (Ohyama *et al.*, 2015), disinhibitory mechanisms of behavioral choice (Jovanic *et al.*, 2016; Zhao *et al.*, 2019), modular organization of escape behaviors (Burgos *et al.*, 2018) and a bistable inhibitory system for olfactory processing among others.

Many of the behaviors of *Drosophila* larvae can be thought of as approaching good things (appetitive behaviors) and avoiding bad things (aversive behaviors). Larvae orient themselves in different types of sensory gradients (Gepner, Mihovilovic Skanata, Bernat, Kaplow, & Gershow, 2015; Gershow *et al.*, 2012; Gomez-Marín *et al.*, 2011; Jovanic *et al.*, 2019; Kane *et al.*, 2013; Luo *et al.*, 2010; Schulze *et al.*, 2015; Tastekin *et al.*, 2018; 2015) and they will use different locomotor strategies to move in space up or down the gradients. In gradients of different modalities larvae use similar strategies: they modulate runs and turns (frequency and direction) to move away from unfavorable conditions and towards favorable conditions. Larvae performs these navigational decisions during chemotaxis (in odor gradients), phototaxis (light intensity gradients), thermotaxis (temperature gradients), anemotaxis (wind speed gradients), gradients of CO₂ and in combination of multiple possibly conflicting sensory gradients (Gepner *et al.*, 2015). Sensory neurons involved in sensing these gradients have by large been described and central neurons have started to be identified by manipulating their activity during various taxes (Gong *et al.*, 2010; Kane *et al.*, 2013; Slater, Levy, Chan, & Larsen,

2015; Tastekin *et al.*, 2015; 2018). It will be interesting to correlate the activity of these neurons to specific navigational strategies in behaving larvae (Karagyzov *et al.*, 2018) to better understand their role in navigational decision-making.

3.1. Modulation of decisions by the internal state

Animal's internal physiological state will affect animal's receptivity to its environment (Palmer & Kristan, 2011). For example, an animal that is hungry might ignore aversive external stimuli as it will be primarily motivated by searching for food and will thus continue foraging. A type of decision that will strongly be influenced by internal state are decisions related to feeding. When faced with the option to feed, several different decisions must be made: first whether or not to eat, what and how much to eat, and when to eat (Bjordal, Arquier, Kniazeff, Pin, & Léopold, 2014; Itskov & Ribeiro, 2013; Kudow *et al.*, 2017). In order to make appropriate choices the nervous system has to evaluate internal energy (nutrients) level and environmental information (presence of food or dangers) to make decisions whether, when and how to feed. The latter is achieved through a sophisticated olfactory and gustatory systems that allow to search and detect different types of food sources (Schleyer *et al.*, 2013). Satiation state influences the decisions what to eat (Itskov & Ribeiro, 2013) and olfactory and gustatory preferences are modified in hungry larvae. For example, normally *Drosophila* larvae exposed to pathogenic bacteria move away from the food. However, this evasion behavior was diminished in starved larvae that overcome the aversions of danger to look for food (Surendran, Hückesfeld, Wäschle, & Pankratz, 2017). Recently sensory neurons for sensing of the internal state in the larva have been described providing an entry point into elucidate molecular and circuit mechanisms underlying sensory processing of internal body state and how it affects behavioral choice (Qian, Kaplow, Lee, & Grueber, 2018). These decisions will be further modulated by other competing internal or external stimuli. Indeed larval food search behavior that involves risky diving into hydrogel illustrates larval decisions that balance risk against benefit (Kim, Alvarez, Lechuga, & Louis, 2017).

3.2. Contextual modulation of decisions

Decision can be modulated by the multisensory context. The presence of stimuli from multiple modalities that inform about the same object (i.e. a parasitoid wasp) will enhance action selection in the *Drosophila* larva (Ohyama *et al.*, 2015). Sometimes information from different sensory modalities can provide conflicting alternatives and the larva will need to make a choice between the two (Koseki *et al.*, 2016). Mechanisms must exist that ensure that the different circuits interact to make an adaptive choice.

Finally, the environmental context can also modulate decisions by modifying the behavioral options available (Cisek, 2007; Cisek & Kalaska, 2010). Different environments can provide different opportunities for expressing different behaviors. For example, in response to an air-puff

larvae will typically perform different avoidance action types on the surface of the agar plate (Figure 2) (Jovanic *et al.*, 2016; Masson *et al.*, 2020; Ohyama *et al.*, 2013). However, if in the agar surface we introduce small holes, larvae will dig into the agar in presence of air-puff more than in its absence (unpublished). The affordance provided by the holes as a potential place where the larva could dig into to protect itself from the wind changed larval behavior in response to an air-puff stimulus. Thus, decisions can be modulated by the environmental context in terms of opportunities for actions it provides.

3.3. Modulation of decision by the experience

Interactions between the animal and environment will result in memories that can be called to inform future decisions. Through this process larvae will learn that some cues might be associated with reward or punishment and will express this memory in the case it is useful for improving their situation (Schleyer *et al.*, 2013; Schleyer, Fendt, Schuller, & Gerber, 2018). The decision to behaviorally express an associative memory trace and the architecture of the chemobehavioral system have been reviewed in (Schleyer *et al.*, 2013). The connectome of the mushroom body, learning and memory center, in the larva has been reconstructed (Eichler *et al.*, 2017) and its functional architecture characterized (Saumweber *et al.*, 2018) which provides the basis for detailed functional studies of conditioned behavior (i.e. Eschbach *et al.* (2019)).

4. Relevance of studying decision-making in the *drosophila* larva

While the stimulus-response model has often been used to describe behaviors in simple model systems and thus defined them as hard-wired, lacking flexibility and not resulting from animals' choice but rather being an automated response to a stimulus, recent studies show that even the simplest behaviors may possess behavioral plasticity (Gorostiza, 2018) allowing the animal to modify their behavior responses according to environmental context, metabolic demands and physiological state.

Another important consideration is the fact that in the context of a lab, behavioral variability is controlled in order to be able to observe, measure and quantify the behavior or aspects of behaviors of interest and link it to underlying circuit mechanisms. Although this simplification is necessary to make causal links between circuit elements and specific aspects of decisions, it needs to be taken into account as this may make the behaviors seem more rigid and less plastic than they might actually be (Gorostiza, 2018). For example, typically it is necessary to devise paradigms that allow us to specifically reveal learning and memory effects on behavior and its underlying mechanisms. However, given the *Drosophila* larvae nervous system possesses the circuitry and molecular machinery to learn, its behavior might be shaped by prior exposure to similar stimuli information even in behavioral paradigms that are dominated by innate behaviors.

While it is obvious that the decision-making process in the *Drosophila* larva lacks the cognitive and emotional complexity of the one in us humans, or other vertebrates, I believe it is fair to acknowledge that the various examples of larval *Drosophila* behaviors from the previous sections illustrate decision-making of various complexity: whether it is to select different types of escape responses (Jovanic *et al.*, 2016; Ohyama *et al.*, 2015) or to make cost benefit decisions (Kim *et al.*, 2017). These rather simple decisions share some of the features of the decision-making problem in general. For example, even in the simplest cases of decision-making, there will be competing demands on the effector systems and therefore an organization in the nervous system needs to be in place to successfully and accurately resolve these conflicts. In addition, the mapping between sensory information and motor output needs to be flexible to enable the multisensory context, state and experience to all modulate decisions (Figure 1).

The principles of how competitive interactions of flexible decisions are implemented at the level of neural circuits may thus be studied in the larva, but how likely it is that the neural architectures for decision-making in the larva will be relevant to decisions in more complex vertebrate brains? Connectivity patterns that give rise to particular neuronal dynamics have been shown to appear in multiple nervous systems. One such motif is mutual inhibition of inhibition found in multiple species to implement competitive interactions and similar computations (Goddard, Mysore, Bryant, Huguenard, & Knudsen, 2014; Jovanic *et al.*, 2016; Koyama *et al.*, 2016; Koyama & Pujala, 2018). This concept of circuit motifs as building blocks of elementary computations that are reused to implement similar functions across brain areas and nervous systems (Koyama & Pujala, 2018) offers a level of abstraction that can be used to extract the principles of circuit function. These can be further applied to many different nervous systems. In this view, understanding the detailed mechanisms of how choice is made between conflicting drives and how the constantly incoming information about the environment and the internal state feed into this process, regardless of the complexity of the selection problem, may provide a solid principled knowledge about decision-making and open new avenues, establish new concepts and offer a new interpretation of existing experimental data (Kristan, 2008).

Over the recent years a view has emerged that decisions are made through competitive interactions of sensorimotor pathways. In the view of this interactive model of decision-making, simultaneous processes of sensorimotor control and action selection are distributed in the nervous system (Alcaraz *et al.*, 2015; Cisek, 2007; Cisek & Kalaska, 2010). It is thus essential to be able to study its neural implementation in a brain-wide manner. Understanding decision-making in a compact brain like the one of the fruitfly larva where such endeavor is feasible with cellular and synaptic resolution, can offer a roadmap for elucidating the mechanisms in more complex brains by giving us clues where to look and what kind of structures to expect. Compared to the classical view of decision-making as a series of processing

stages, the interactive framework sheds light on decisions in a new way. While this framework may not apply to all decisions equally, it may allow us to bridge the gap between the view that invertebrate behaviors are hard-wired innate routines, each being triggered by a combination of external stimuli and internal drivers, while more complex organisms go through deliberate decision-making composed of several stages of computation before taking any external actions.

Acknowledgements

The author thanks Daniel Vasiliauskas for comments on this manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 798050 and from the ANR (agence nationale de la recherche): ANR-17-CE37-0019.

ORCID

Tihana Jovanic  <http://orcid.org/0000-0002-0525-8620>

References

- Adams, G.K., Watson, K.K., Pearson, J., & Platt, M.L. (2012). Neuroethology of decision-making. *Current Opinion in Neurobiology*, 22, 982–989. doi:10.1016/j.conb.2012.07.009
- Alcaraz, F., Marchand, A.R., Vidal, E., Guillou, A., Faugère, A., Coutureau, E., & Wolff, M. (2015). Flexible use of predictive cues beyond the orbitofrontal cortex: role of the submedial thalamic nucleus. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 35, 13183–13193. doi:10.1523/JNEUROSCI.1237-15.2015
- Aleman-Meza, B., Jung, S.-K., & Zhong, W. (2015). An automated system for quantitative analysis of *Drosophila* larval locomotion. *BMC Developmental Biology*, 15, 11. doi:10.1186/s12861-015-0062-0
- Baca, S.M., Marin-Burgin, A., Wagenaar, D.A., & Kristan, W.B. Jr. (2008). Widespread inhibition proportional to excitation controls the gain of a leech behavioral circuit. *Neuron*, 57, 276–289. doi:10.1016/j.neuron.2007.11.028
- Barker, A.J., & Baier, H. (2015). Sensorimotor decision making in the zebrafish tectum. *Current Biology*, 25, 2804–2814. doi:10.1016/j.cub.2015.09.055
- Björndal, M., Arquier, N., Kniazeff, J., Pin, J.P., & Léopold, P. (2014). Sensing of amino acids in a dopaminergic circuitry promotes rejection of an incomplete diet in *Drosophila*. *Cell*, 156, 510–521. doi:10.1016/j.cell.2013.12.024
- Braganza, O., & Beck, H. (2018). The circuit motif as a conceptual tool for multilevel neuroscience. *Trends in Neurosciences*, 41, 128–136. doi:10.1016/j.tins.2018.01.002
- Burgos, A., Honjo, K., Ohyama, T., Qian, C.S., Shin, G. J.-e., Gohl, D.M., ... Grueber, W.B. (2018). Nociceptive interneurons control modular motor pathways to promote escape behavior in *Drosophila*. *eLife*, 7, 1557. doi:10.7554/eLife.26016

- Cisek, P. (2007). Cortical mechanisms of action selection: the affordance competition hypothesis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 362, 1585–1599. doi:10.1098/rstb.2007.2054
- Cisek, P., & Kalaska, J.F. (2010). Neural Mechanisms for Interacting with a World Full of Action Choices. *Annual Review of Neuroscience*, 33, 269–298. doi:10.1146/annurev.neuro.051508.135409
- Constantinople, C.M., Piet, A.T., & Brody, C.D. (2019). An analysis of decision under risk in rats. *Current Biology*, 29, 2066–2074.e5. doi:10.1016/j.cub.2019.05.013
- Eichler, K., Li, F., Litwin-Kumar, A., Park, Y., Andrade, I., Schneider-Mizell, C.M., ... Cardona, A. (2017). The complete connectome of a learning and memory centre in an insect brain. *Nature*, 548, 175–182. doi:10.1038/nature23455
- Eschbach, C., Fushiki, A., Winding, M., Schneider-Mizell, C.M., Shao, M., & Arruda, R. (2019). Multilevel feedback architecture for adaptive regulation of learning in the insect brain. *bioRxiv*, 13, 1557–1544. doi:10.1101/649731
- Faumont, S., Lindsay, T.H., & Lockery, S.R. (2012). Neuronal microcircuits for decision making in *C. elegans*. *Current Opinion in Neurobiology*, 22, 580–591. doi:10.1016/j.conb.2012.05.005
- Fishilevich, E., Domingos, A.I., Asahina, K., Naef, F., Vosshall, L.B., & Louis, M. (2005). Chemotaxis behavior mediated by single larval olfactory neurons in *Drosophila*. *Curbio*, 15, 2086–2096. doi:10.1016/j.cub.2005.11.016
- Fushiki, A., Zwart, M.F., Kohsaka, H., Fetter, R.D., Cardona, A., Nose, A., & Griffith, L.C. (2016). A circuit mechanism for the propagation of waves of muscle contraction in *Drosophila*. *eLife*, 5, e13253. doi:10.7554/eLife.13253
- Gaudry, Q., & Kristan, W.B. (2009). Behavioral choice by presynaptic inhibition of tactile sensory terminals. *Nature Publishing Group*, 12, 1450–1457. doi:10.1038/nn.2400
- Gepner, R., Mihovilovic Skanata, M., Bernat, N.M., Kaplow, M., & Gershow, M. (2015). Computations underlying *Drosophila* phototaxis, odor-taxis, and multi-sensory integration. *eLife*, 4, e06229. doi:10.7554/eLife.06229
- Gershow, M., Berck, M., Mathew, D., Luo, L., Kane, E.A., Carlson, J.R., & Samuel, A.D.T. (2012). Controlling airborne cues to study small animal navigation. *Nature Methods*, 9, 290–296. doi:10.1038/nmeth.1853
- Gillette, R., & Brown, J.W. (2015). The sea slug, *Pleurobranchaea californica*: a signpost species in the evolution of complex nervous systems and behavior. *Integrative and Comparative Biology*, 55, 1058–1069.
- Glimcher, P.W. (2004). *Decisions, uncertainty, and the brain*. Cambridge, MA: MIT Press.
- Glimcher, P.W., & Rustichini, A. (2004). Neuroeconomics: the consilience of brain and decision. *Science*, 306, 447–452. doi:10.1126/science.1102566
- Goddard, C.A., Mysore, S.P., Bryant, A.S., Huguenard, J.R., & Knudsen, E.I. (2014). Spatially reciprocal inhibition of inhibition within a stimulus selection network in the avian midbrain. *PloS One*, 9, e85865. doi:10.1371/journal.pone.0085865
- Gold, J.I., & Shadlen, M.N. (2007). The neural basis of decision making. *Annual Review of Neuroscience*, 30, 535–574. doi:10.1146/annurev.neuro.29.051605.113038
- Gomez-Marin, A., Partoune, N., Stephens, G.J., Louis, M., & Brembs, B. (2012). Automated tracking of animal posture and movement during exploration and sensory orientation behaviors. *PloS One*, 7, e41642. doi:10.1371/journal.pone.0041642
- Gomez-Marin, A., Stephens, G.J., & Louis, M. (2011). Active sampling and decision making in *Drosophila* chemotaxis. *Nature Communications*, 2, 441. doi:10.1038/ncomms1455
- Gong, Z., Liu, J., Guo, C., Zhou, Y., Teng, Y., & Liu, L. (2010). Two pairs of neurons in the central brain control *Drosophila* innate light preference. *Science*, 330, 499–502. doi:10.1126/science.1195993
- GORostiza, E.A. (2018). Does cognition have a role in plasticity of “innate behavior?” a perspective from *Drosophila*. *Frontiers in Psychology*, 9, 1502. doi:10.3389/fpsyg.2018.01502
- He, L., Gulyanov, S., Mihovilovic Skanata, M., Karagoyozov, D., Heckscher, E.S., Krieg, M., ... Tracey, W.D. (2019). Direction selectivity in *Drosophila* proprioceptors requires the mechanosensory channel Tmc. *Current Biology*, 29, 945–956.e3. doi:10.1016/j.cub.2019.02.025
- Heckscher, E.S., Lockery, S.R., & Doe, C.Q. (2012). Characterization of *Drosophila* larval crawling at the level of organism, segment, and somatic body wall musculature. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 32, 12460–12471. doi:10.1523/JNEUROSCI.0222-12.2012
- Heckscher, E.S., Zarin, A.A., Faumont, S., Clark, M.Q., Manning, L., Fushiki, A., ... Doe, C.Q. (2015). Even-skipped(+) interneurons are core components of a sensorimotor circuit that maintains left-right symmetric muscle contraction amplitude. *Neuron*, 88, 314–329. doi:10.1016/j.neuron.2015.09.009
- Hung, Y.-S., & Stopfer, M. (2018). Decision making: how fruit flies integrate olfactory evidence. *Current Biology*, 28, R757–R759. doi:10.1016/j.cub.2018.05.065
- Itskov, P.M., & Ribeiro, C. (2013). The dilemmas of the gourmet fly: the molecular and neuronal mechanisms of feeding and nutrient decision making in *Drosophila*. *Frontiers in Neuroscience*, 7, 12. doi:10.3389/fnins.2013.00012
- Jarrell, T.A., Wang, Y., Bloniarz, A.E., Brittin, C.A., Xu, M., Thomson, J.N., ... Emmons, S.W. (2012). The connectome of a decision-making neural network. *Science*, 337, 437–444. doi:10.1126/science.1221762
- Jovanic, T., Schneider-Mizell, C.M., Shao, M., Masson, J.-B., Denisov, G., Fetter, R.D., ... Zlatić, M. (2016). Competitive disinhibition mediates behavioral choice and sequences in *Drosophila*. *Cell*, 167, 858–870.e19. doi:10.1016/j.cell.2016.09.009
- Jovanic, T., Winding, M., Cardona, A., Truman, J.W., Gershow, M., & Zlatić, M. (2019). Neural substrates of *Drosophila* larval anemotaxis. *Current Biology*, 29, 554–566.e4. doi:10.1016/j.cub.2019.01.009
- Kabra, M., Robie, A.A., Rivera-Alba, M., Branson, S., & Branson, K. (2013). JAABA: interactive machine learning for automatic annotation of animal behavior. *Nature Methods*, 10, 64–67. doi:10.1038/nmeth.2281
- Kane, E.A., Gershow, M., Afonso, B., Larderet, I., Klein, M., Carter, A.R., ... Samuel, A.D.T. (2013). Sensorimotor structure of *Drosophila* larva phototaxis. *Proceedings of the National Academy of Sciences of the United States of America*, 110, E3868–77. doi:10.1073/pnas.1215295110
- Karagoyozov, D., Mihovilovic Skanata, M., Lesar, A., & Gershow, M. (2018). Recording neural activity in unrestrained animals with three-dimensional tracking two-photon microscopy. *Cell Reports*, 25, 1371–1383.e10. doi:10.1016/j.celrep.2018.10.013
- Kim, D., Alvarez, M., Lechuga, L.M., & Louis, M. (2017). Species-specific modulation of food-search behavior by respiration and chemosensation in *Drosophila* larvae. *eLife*, 6, 356. doi:10.7554/eLife.27057
- Klein, M., Afonso, B., Vonner, A.J., Hernandez-Nunez, L., Berck, M., Tabone, C.J., ... Samuel, A.D.T. (2015). Sensory determinants of behavioral dynamics in *Drosophila* thermotaxis. *Proceedings of the National Academy of Sciences of the United States of America*, 112, E220–9. doi:10.1073/pnas.1416212112
- Koseki, N., Mori, S., Suzuki, S., Tonooka, Y., Kosugi, S., Miyakawa, H., & Morimoto, T. (2016). Individual differences in sensory responses influence decision making by *Drosophila melanogaster* larvae on exposure to contradictory cues. *Journal of Neurogenetics*, 30, 288–296. doi:10.1080/01677063.2016.1202949
- Kovac, M.P., & Davis, W.J. (1977). Behavioral choice: neural mechanisms in *Pleurobranchaea*. *Science*, 198, 632–634. doi:10.1126/science.918659
- Koyama, M., Minale, F., Shum, J., Nishimura, N., Schaffer, C.B., & Fetcho, J.R. (2016). A circuit motif in the zebrafish hindbrain for a two alternative behavioral choice to turn left or right. *eLife*, 5, 451. doi:10.7554/eLife.16808
- Koyama, M., & Pujala, A. (2018). Mutual inhibition of lateral inhibition: a network motif for an elementary computation in the brain. *Current Opinion in Neurobiology*, 49, 69–74. doi:10.1016/j.conb.2017.12.019

- Kristan, W.B. (2008). Neuronal decision-making circuits. *Current Biology*, 18, R928–R932. doi:10.1016/j.cub.2008.07.081
- Kristan, W.B. Jr. (2012). Decision points: the factors influencing the decision to feed in the medicinal leech. *Frontiers in Neuroscience*, 6, 101. doi: 10.3389/fnins.2012.00101/abstract
- Kudow, N., Miura, D., Schleyer, M., Toshima, N., Gerber, B., & Tanimura, T. (2017). Preference for and learning of amino acids in larval *Drosophila*. *Biology Open*, 6, 365–369. doi:10.1242/bio.020412
- Kupfermann, I., & Weiss, K.R. (2001). Motor program selection in simple model systems. *Current Opinion in Neurobiology*, 11, 673–677. doi:10.1016/S0959-4388(01)00267-7
- Kvon, E.Z., Kazmar, T., Stampfel, G., Yáñez-Cuna, J.O., Pagani, M., Schernhuber, K., ... Stark, A. (2014). Genome-scale functional characterization of *Drosophila* developmental enhancers in vivo. *Nature*, 512, 91–95. doi:10.1038/nature13395
- Lemon, W.C., Pulver, S.R., Höckendorf, B., McDole, K., Branson, K., Freeman, J., & Keller, P.J. (2015). Whole-central nervous system functional imaging in larval *Drosophila*. *Nature Communications*, 6, 7924–7916. doi:10.1038/ncomms8924
- Li, H.-H., Kroll, J.R., Lennox, S.M., Ogundeyi, O., Jeter, J., Depasquale, G., & Truman, J.W. (2014). A GAL4 driver resource for developmental and behavioral studies on the larval CNS of *Drosophila*. *Cell Reports*, 8, 897–908. doi:10.1016/j.celrep.2014.06.065
- Louis, M., Huber, T., Benton, R., Sakmar, T.P., & Vosshall, L.B. (2008). Bilateral olfactory sensory input enhances chemotaxis behavior. *Nature Neuroscience*, 11, 187–199. doi:10.1038/nn2031
- Luo, L., Gershow, M., Rosenzweig, M., Kang, K., Fang-Yen, C., Garrity, P.A., & Samuel, A.D.T. (2010). Navigational decision making in *Drosophila* thermotaxis. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 30, 4261–4272. doi:10.1523/JNEUROSCI.4090-09.2010
- Maass, W. (2000). On the computational power of winner-take-all. *Neural Computation*, 12, 2519–2535. doi:10.1162/089976600300014827
- Masson, J.-B., Laurent, F., Cardona, A., Barre, C., Skatchkovsky, N., & Truman, J.W. (2020). Identifying neural substrates of competitive interactions and sequence transitions during mechanosensory responses in *Drosophila*. *Plos Genetics*. (in press)
- Merritt, D.J., & Whittington, P.M. (1995). Central projections of sensory neurons in the *Drosophila* embryo correlate with sensory modality, soma position, and proneural gene function. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 15, 1755–1767. doi:10.1523/JNEUROSCI.15-03-01755.1995
- Miller, G.A., Galanter, E., & Pribram, K.H. (1960). *Plans and the structure of behavior*. New York: Holt, Rinehart and Winston, Inc.
- Mink, J.W. (1996). The basal ganglia: focused selection and inhibition of competing motor programs. *Progress in Neurobiology*, 50, 381–425. doi:10.1016/S0301-0082(96)00042-1
- Ohyama, T., Jovanic, T., Denisov, G., Dang, T.C., Hoffmann, D., Kerr, R.A., & Zlatić, M. (2013). High-throughput analysis of stimulus-evoked behaviors in *Drosophila* larva reveals multiple modality-specific escape strategies. *PloS One*, 8, e71706. doi:10.1371/journal.pone.0071706
- Ohyama, T., Schneider-Mizell, C.M., Fetter, R.D., Aleman, J.V., Franconville, R., Rivera-Alba, M., ... Zlatić, M. (2015). A multilevel multimodal circuit enhances action selection in *Drosophila*. *Nature*, 520, 633–639. doi:10.1038/nature14297
- Palmer, C.R., & Kristan, W.B. (2011). Contextual modulation of behavioral choice. *Current Opinion in Neurobiology*, 21, 520–526. doi:10.1016/j.conb.2011.05.003
- Pearson, J.M., Watson, K.K., & Platt, M.L. (2014). Decision making: the neuroethological turn. *Neuron*, 82, 950–965. doi:10.1016/j.neuron.2014.04.037
- Pfeiffer, B.D., Jenett, A., Hammonds, A.S., Ngo, T.-T.B., Misra, S., Murphy, C., ... Rubin, G.M. (2008). Tools for neuroanatomy and neurogenetics in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 9715–9720. doi:10.1073/pnas.0803697105
- Pfeiffer, B.D., Ngo, T.-T.B., Hibbard, K.L., Murphy, C., Jenett, A., Truman, J.W., & Rubin, G.M. (2010). Refinement of tools for targeted gene expression in *Drosophila*. *Genetics*, 186, 735–755. doi:10.1534/genetics.110.119917
- Prescott, T.J. (2007). Forced moves or good tricks in design space? Landmarks in the evolution of neural mechanisms for action selection. *Adaptive Behavior*, 15, 9–31. doi:10.1177/1059712306076252
- Pulver, S.R., Bayley, T.G., Taylor, A.L., Berni, J., Bate, M., & Hedwig, B. (2015). Imaging fictive locomotor patterns in larval *Drosophila*. *Journal of Neurophysiology*, 114, 2564–2577. doi:10.1152/jn.00731.2015
- Qian, C.S., Kaplow, M., Lee, J.K., & Grueber, W.B. (2018). Diversity of internal sensory neuron axon projection patterns is controlled by the POU-domain protein Pdm3 in *Drosophila* larvae. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 38, 2081–2093. doi:10.1523/JNEUROSCI.2125-17.2018
- Redgrave, P., Prescott, T.J., & Gurney, K. (1999). The basal ganglia: a vertebrate solution to the selection problem? *Neuroscience*, 89, 1009–1023. doi:10.1016/S0306-4522(98)00319-4
- Redgrave, P., Vautrelle, N., & Reynolds, J.N.J. (2011). Functional properties of the basal ganglia's re-entrant loop architecture: selection and reinforcement. *Nsc*, 198, 138–151. doi:10.1016/j.neuroscience.2011.07.060
- Reyn, V. C.R., Breads, P., Peek, M.Y., Zheng, G.Z., Williamson, W.R., & Yee, A.L. (2014). A spike-timing mechanism for action selection. *Nature Neuroscience*, 17, 962–970. doi:10.1038/nn.3741
- Robertson, J.L., Tsubouchi, A., & Tracey, W.D. (2013). Larval defense against attack from parasitoid wasps requires nociceptive neurons. *PloS One*, 8, e78704. doi:10.1371/journal.pone.0078704
- Robie, A.A., Hirokawa, J., Edwards, A.W., Umayam, L.A., Lee, A., Phillips, M.L., ... Branson, K. (2017). Mapping the neural substrates of behavior. *Cell*, 170, 393–406.e28. doi:10.1016/j.cell.2017.06.032
- Saumweber, T., Rohwedder, A., Schleyer, M., Eichler, K., Chen, Y.-c., Aso, Y., ... Gerber, B. (2018). Functional architecture of reward learning in mushroom body extrinsic neurons of larval *Drosophila*. *Nature Communications*, 9, 1104. doi:10.1038/s41467-018-03130-1
- Schleyer, M., Diegelmann, S., Michels, B., Saumweber, T., & Gerber, B. (2013). Decision making in larval *Drosophila*. *Handbook of Behavioral Neuroscience*, 22, 41–55. doi:10.1016/B978-0-12-415823-8.00005-8
- Schleyer, M., Fendt, M., Schuller, S., & Gerber, B. (2018). Associative learning of stimuli paired and unpaired with reinforcement: evaluating evidence from maggots, flies, bees, and rats. *Frontiers in Psychology*, 9, 1494. doi:10.3389/fpsyg.2018.01494
- Schneider-Mizell, C.M., Gerhard, S., Longair, M., Kazimiers, T., Li, F., Zwart, M.F., ... Cardona, A. (2016). Quantitative neuroanatomy for connectomics in *Drosophila*. *eLife*, 5, 1133. doi:10.7554/eLife.12059
- Schulze, A., Gomez-Marín, A., Rajendran, V.G., Lott, G., Musy, M., Ahammad, P., ... Louis, M. (2015). Dynamical feature extraction at the sensory periphery guides chemotaxis. *eLife*, 4, 1129. doi:10.7554/eLife.06694
- Seeds, A.M., Ravbar, P., Chung, P., Hampel, S., Midgley, F.M., Mensh, B.D., & Simpson, J.H. (2014). A suppression hierarchy among competing motor programs drives sequential grooming in *Drosophila*. *eLife*, 3, e02951.
- Simpson, J.H. (2009). Mapping and manipulating neural circuits in the fly brain. *Advances in Genetics*, 65, 79–143. doi:10.1016/S0065-2660(09)65003-3
- Simpson, J.H., & Looger, L.L. (2018). Functional imaging and optogenetics in *Drosophila*. *Genetics*, 208, 1291–1309. doi:10.1534/genetics.117.300228
- Sivanantharajah, L., & Zhang, B. (2015). Current techniques for high-resolution mapping of behavioral circuits in *Drosophila*. *Journal of Comparative Physiology. a, Neuroethology, Sensory, Neural, and Behavioral Physiology*, 201, 895–909. doi:10.1007/s00359-015-1010-y
- Slater, G., Levy, P., Chan, K.L.A., & Larsen, C. (2015). A central neural pathway controlling odor tracking in *Drosophila*. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 35, 1831–1848. doi:10.1523/JNEUROSCI.2331-14.2015
- Sprecher, S.G., Cardona, A., & Hartenstein, V. (2011). The *Drosophila* larval visual system: high-resolution analysis of a simple visual

- neuropil. *Developmental Biology*, 358, 33–43. doi:[10.1016/j.ydbio.2011.07.006](https://doi.org/10.1016/j.ydbio.2011.07.006)
- Surendran, S., Hückesfeld, S., Wäschle, B., & Pankratz, M.J. (2017). Pathogen-induced food evasion behavior in *Drosophila* larvae. *The Journal of Experimental Biology*, 220, 1774–1780. doi:[10.1242/jeb.153395](https://doi.org/10.1242/jeb.153395)
- Takagi, S., Cocanougher, B.T., Niki, S., Miyamoto, D., Kohsaka, H., Kazama, H., ... Nose, A. (2017). Divergent connectivity of homologous command-like neurons mediates segment-specific touch responses in *Drosophila*. *Neuron*, 96, 1373–1387.e6. doi:[10.1016/j.neuron.2017.10.030](https://doi.org/10.1016/j.neuron.2017.10.030)
- Tastekin, I., Khandelwal, A., Tadres, D., Fessner, N.D., Truman, J.W., Zlatić, M., ... Louis, M. (2018). Sensorimotor pathway controlling stopping behavior during chemotaxis in the *Drosophila melanogaster* larva. *eLife*, 7, 2452. doi:[10.7554/eLife.38740](https://doi.org/10.7554/eLife.38740)
- Tastekin, I., Riedl, J., Schilling-Kurz, V., Gomez-Marin, A., Truman, J.W., & Louis, M. (2015). Role of the subesophageal zone in sensorimotor control of orientation in *Drosophila* larva. *Current Biology*, 25, 1448–1460. doi:[10.1016/j.cub.2015.04.016](https://doi.org/10.1016/j.cub.2015.04.016)
- Triphan, T., Nern, A., Roberts, S.F., Korff, W., Naiman, D.Q., & Strauss, R. (2016). A screen for constituents of motor control and decision making in *Drosophila* reveals visual distance-estimation neurons. *Scientific Reports*, 6. Article number: 27000. doi:[10.1038/srep27000](https://doi.org/10.1038/srep27000)
- Tsubouchi, A., Caldwell, J.C., & Tracey, W.D. (2012). Dendritic filopodia, ripped pocket, NOMPC, and NMDARs contribute to the sense of touch in *Drosophila* larvae. *Current Biology*, 22, 2124–2134. doi:[10.1016/j.cub.2012.09.019](https://doi.org/10.1016/j.cub.2012.09.019)
- Turner, H.N., Armengol, K., Patel, A.A., Himmel, N.J., Sullivan, L., Iyer, S.C., ... Cox, D.N., et al. (2016). The TRP channels Pkd2, NompC, and Trpm act in cold-sensing neurons to mediate unique aversive behaviors to noxious cold in *Drosophila*. *Current Biology*, 26, 3116–3128. doi:[10.1016/j.cub.2016.09.038](https://doi.org/10.1016/j.cub.2016.09.038)
- Wang, X.-J. (2008). Decision making in recurrent neuronal circuits. *Neuron*, 60, 215–234. doi:[10.1016/j.neuron.2008.09.034](https://doi.org/10.1016/j.neuron.2008.09.034)
- Xiang, Y., Yuan, Q., Vogt, N., Looger, L.L., Jan, L.Y., & Jan, Y.-N. (2010). Light-avoidance-mediating photoreceptors tile the *Drosophila* larval body wall. *Nature*, 468, 921–926. doi:[10.1038/nature09576](https://doi.org/10.1038/nature09576)
- Yan, Z., Zhang, W., He, Y., Gorczyca, D., Xiang, Y., Cheng, L.E., ... Jan, Y.N., et al. (2013). *Drosophila* NOMPC is a mechanotransduction channel subunit for gentle-touch sensation. *Nature*, 493, 221–225. doi:[10.1038/nature11685](https://doi.org/10.1038/nature11685)
- Yao, Z., Macara, A.M., Lelito, K.R., Minosyan, T.Y., & Shafer, O.T. (2012). Analysis of functional neuronal connectivity in the *Drosophila* brain. *Journal of Neurophysiology*, 108, 684–696. doi:[10.1152/jn.00110.2012](https://doi.org/10.1152/jn.00110.2012)
- Zarin, A.A., Mark, B., Cardona, A., Litwin-Kumar, A., & Doe, C.Q. (2019b). A multilayer circuit architecture for the generation of distinct locomotor behaviors in *Drosophila*. *eLife*, 8, e51781. doi:[10.7554/eLife.51781](https://doi.org/10.7554/eLife.51781)
- Zhang, W., Yan, Z., Jan, L.Y., & Jan, Y.-N. (2013). Sound response mediated by the TRP channels NOMPC, NANCHUNG, and INACTIVE in chordotonal organs of *Drosophila* larvae. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 13612–13617. doi:[10.1073/pnas.1312477110](https://doi.org/10.1073/pnas.1312477110)
- Zhao, W., Gong, C., Ouyang, Z., Wang, P., Wang, J., Zhou, P., ... Gong, Z., et al. (2017). Turns with multiple and single head cast mediate *Drosophila* larval light avoidance. *PloS One*, 12, e0181193. doi:[10.1371/journal.pone.0181193](https://doi.org/10.1371/journal.pone.0181193)
- Zhao, W., Zhou, P., Gong, C., Ouyang, Z., Wang, J., Zheng, N., & Gong, Z. (2019). A disinhibitory mechanism biases *Drosophila* innate light preference. *Nature Communications*, 10, 124. doi:[10.1038/s41467-018-07929-w](https://doi.org/10.1038/s41467-018-07929-w)
- Zwart, M.F., Pulver, S.R., Truman, J.W., Fushiki, A., Fetter, R.D., Cardona, A., & Landgraf, M. (2016). Selective inhibition mediates the sequential recruitment of motor pools. *Neuron*, 91, 615–628. doi:[10.1016/j.neuron.2016.06.031](https://doi.org/10.1016/j.neuron.2016.06.031)