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Understanding the Circuit Mechanisms of Multisensory Integration Using Drosophila as a Model System

Huda Zwaine
hudazwaine11@gmail.com

ABSTRACT

Animals rarely experience the world through only one sense at a time. The brain has to determine whether information from different senses is coming from the same event and, if so, integrate it (multisensory integration [MSI]) to generate an appropriate response. The circuit mechanisms of MSI are difficult to study in vertebrate brains where multisensory neurons and regions can be identified but synaptic-resolution architecture of whole circuits is unavailable.

This study presents a literature review of recent papers on multisensory integration in the larva of the fruit fly, *Drosophila melanogaster*. *Drosophila* larva is a good model for these questions because many experimental strategies can be applied at once. Recently, the larval connectome which gives a synapse-level map of the nervous system has been generated. Researchers could then trace sensory pathways to find where information from different modalities converges . Powerful genetic tools (e.g. GAL4-lines) can be used to label, activate or silence particular candidate neurons. Finally, calcium imaging and optogenetics can be used to test whether these neurons really respond to multiple sensory signals and if their activity is necessary or sufficient for a behavior.

Overall, the review of the existing literature suggests that MSI does not occur late in sensory processing, in one brain region. It seems sensory information is converging at many highly recurrent multimodal points along neural circuits in the brain. Many neurons may also receive direct or indirect information from several modalities and are not completely unimodal. This organization would allow nervous systems to analyse sensory information at different stages and adapt behaviour so that it responds accordingly.

INTRODUCTION

Animals rarely experience the world through only one sense at a time. For example, a predator attack could not only produce mechanical, visual, olfactory and nociceptive signals but also food can give us a mix of smell, taste, sound and visual signals. The brain needs to figure out whether information from the different senses is coming from the same event and put them together so it can make an appropriate response. This is multisensory integration (MSI). At the same time, combining sensory information can

also help animals to identify meaningful things or things that might not be happening naturally and to avoid responding to random sensory noise and to make more accurate behavioral decisions (Ohyama et al. 2015). Now the question is how does the brain integrate information from many sensory modalities?

Early research demonstrated how multisensory integration can vary how strongly each neuron responds to sensory information. Meredith and Stein found neurons that responded differently to two sensory stimuli when presented together than when either stimulus was presented alone. In some cases, the combined response was stronger than what would be expected from individual responses (Stein and Meredith 1993). This showed that sensory pathways do not always process information independently. But there are still many important questions about MSI that were not answered until years later. Where do different sensory pathways first converge? Does integration happen early in sensory processing, later at a behavioral decision point, or at several stages? Is the integration performed by individual neurons or by a larger network? Most importantly, how does this neural activity produce an actual change in behavior (Ohyama et al. 2015)?

These questions are highly difficult to study in vertebrate brains. Scientists are able to identify multisensory neurons and brain regions in mammals, but it is much harder to trace the whole circuits that produce these responses (Stein and Meredith 1993). Vertebrate brains are extremely large and complex and researchers do not have complete synaptic-resolution wiring diagrams yet (Luo 2020). It's also very hard to find out which neuron is the same in different animals. It is hard to connect circuit structure, neural activity, and behavior in one system. Finding a neuron that responds to two senses does not necessarily reveal which pathways were involved in those inputs, where the information was integrated, or whether that neuron was the cause of that behavior (Ohyama et al. 2015).

The fruit fly Drosophila melanogaster, particularly the larva, serves as a good model to answer these questions (Ohyama et al. 2015). Its nervous system is much smaller than a vertebrate brain, but it still has a lot of behaviors like crawling, turning, feeding and nociceptive rolling (Ohyama et al. 2015). Many of the larval neurons are also stereotyped and genetically identifiable and tractable, so researchers can target the same neuron type across different animals (Pfeiffer et al. 2008; Ohyama et al. 2015) .

What makes Drosophila suitable and useful here is that many experimental strategies can be applied at once. The larval connectome reconstructed by Winding et al. (2023) provides a synapse-level map of the nervous system. Researchers can then trace sensory pathways and locate where information from various modalities may converge (Winding et al. 2023). Genetic tools such as GAL4 and split-GAL4 can then be used to label, activate, or silence particular candidate neurons (Pfeiffer et al. 2008). Finally, calcium imaging and optogenetics can investigate whether these neurons really respond to multiple sensory signals and if their activity is necessary or sufficient for a particular behavior (Simpson and Looger 2018). This means that Drosophila research can turn from anatomical predictions to direct causal tests. A connectome might indicate that several sensory pathways touch the same neuron, but anatomy alone can't prove that functional integration occurs. Researchers then need to record the neuron's activity during different sensory stimuli and manipulate it to understand its role in behavior (Simpson and Looger 2018). The

larval escape circuit is especially relevant because various harmful stimuli can trigger related defense behaviors through pathways that are experimentally accessible (Ohyama et al. 2015; Burgos et al. 2018).

How the brain combines information from different senses to navigate behavior, and what Drosophila can tell us about the circuits responsible, becomes evident here. MSI is not just a late step in a single brain region. Rather, it seems that sensory information is converging at many highly recurrent multimodal points along neural circuits in the brain (Ohyama et al. 2015; Winding et al. 2023). Many neurons potentially also receive direct or indirect information from several modalities and are not completely unimodal (Winding et al. 2023). This organization would enable the nervous system to analyze sensory information at different stages and adapt behavior in such a way to respond depending on the situation (Ohyama et al. 2015). By examining the larval escape circuit and evidence from whole-brain connectomics and functional experiments, this paper will tackle the main principles that are emerging, as well as important questions that have not yet been resolved.

METHODS

This study provides a literature review to determine the neural circuit mechanisms of multisensory integration in *Drosophila melanogaster*. Studies relevant to this research were found in the academic database (PubMed) by searching for keywords: multisensory integration and *Drosophila melanogaster* larva. Nine sources were found and included in the literature review.

RESULTS

The sections below present the results of the literature review and critically discuss the research findings on how multiple sensory modalities are integrated in neural circuits to guide behaviour, comparing methodologies (e.g., connectomics, functional circuit analysis, behavioural neuroscience and computational approaches) and evaluating experimental findings.

The key findings and section headings were determined by identifying the main themes that appeared across the nine sources. These themes included principles of multisensory integration revealed by studies of an escape circuit, a navigation circuit, cross-modal inhibition, and finally findings on multisensory convergence from the larval connectome. The findings were grouped under these themes to compare evidence from connectomics, functional circuit analysis, behavioural neuroscience, and computational approaches.

1. The larval escape response as a model of multisensory integration

One of the most circuit-level examples of multisensory integration in *Drosophila* relates to the larval escape response. Larvae encounter many kinds of potentially dangerous threats, including mechanical contact, vibration and activation of nociceptive pathways. Depending on what sensory information is available, they might stop, bend, increase their crawling speed or perform a corkscrew-like rolling

response (Ohyama, T. et al., 2015; Burgos, A. et al., 2018). Rolling is important because it gives the larva the means to escape quickly from an imminent threat, such as predator attack (Ohyama, T. et al., 2015).

Mechanosensory and nociceptive information are combined to determine what escape behaviour the larva chooses. To investigate this, researchers activated class IV multidendritic arborisation neurons, also known as md-da neurons, which are nociceptors and are required for nociceptive rolling. They also subjected the larvae to vibration, which activated mechanosensory pathways. Each stimulus provided individually led to some defensive behaviour. But when nociceptive activation and vibration were combined, the probability of rolling increased significantly (Ohyama, T. et al., 2015).

This result was not merely the addition of two independent behavioural responses. The combined stimulation made rolling greater, as it created a synergistic effect between the modalities. Mechanosensory information thus affected how effectively nociceptive input elicited the strongest escape response. Biologically, this could mean that the nervous system would perceive evidence from multiple sensory channels as a more reliable signal of danger than either weak signal alone (Ohyama, T. et al., 2015).

Importantly, this result shows that multisensory integration affects action selection over sensory detection. The larva didn't simply produce a larger quantity of the same behaviour when it received two cues. Rather, the combination had an effect on the probability that it would choose rolling rather than fast crawling. Thus, multisensory processing was related to the choice between the competing behavioural outputs (Ohyama, T. et al., 2015).

However, that behavioural synergy alone cannot tell where integration happens. The two pathways may just rest on the same interneuron, stay separate until we go through a later decision process or interact at different points in the circuit. Understanding the circuit mechanism required anatomical reconstruction but also recording and causal manipulation (Ohyama, T. et al., 2015).

2. Early convergence in Basin interneurons

To identify possible points of convergence, Ohyama et al. combined behavioural experiments with genetic manipulation and electron microscopy reconstruction (Ohyama, T. et al., 2015). An activation screen revealed a group of interneurons called Basin neurons and four types - Basin-1, Basin-2, Basin-3 and Basin-4 - were reconstructed from electron microscopy {Ohyama, T. et al., 2015}.

The wiring diagram demonstrated that Basin-2 and Basin-4 were receiving substantial synaptic input from both nociceptive class IV da neurons and mechanosensory chordotonal neurons. These cells can be described as first-order multisensory interneurons because they receive convergent input from distinct sensory pathways simultaneously. This anatomical evidence that convergence between different sensory modalities starts early in the sensory processing hierarchy instead of at a higher-order brain centre, is useful {Ohyama, T. et al., 2015}.

The difference between anatomical convergence and functional integration must be kept in mind. A wiring diagram can show that inputs from two sensory pathways form synapses with the same neuron, but it cannot independently demonstrate how those inputs interact or whether the neuron is necessary for the natural behaviour. The authors therefore checked whether inputs from these two modalities are also functionally integrated by Basins. They activated either nociceptive neurons alone (by puffing ATP onto the nervous system of a semi-dissected animal that expressed the ATP-gated ion-channel P2X2), or mechanosensory neurons alone by providing vibration, or both, while imaging activity in Basins using a calcium indicator of neural activity {Ohyama, T. et al., 2015 ; chen et al}. They found that Basin-4 responded more to the combination of stimuli than to either stimulus alone – and the response was more than a linear sum {Ohyama, T. et al., 2015} , demonstrating synergistic multisensory integration early in the sensory processing hierarchy. However, they found that the 4 basin types didn't integrate sensory information in the same way. Where Basin-4 neurons were not four identical versions of the same integrator and Basin-1 and Basin-3 were receiving strong chordotonal input but little direct class IV da input, so these are mostly mechanosensory neurons, whereas Basin-2 and Basin-4 received a lot of input from both pathways {Ohyama, T. et al., 2015}, so they are multisensory neurons.

The authors checked how two of these Basins, for which they had a GAL4-line, contribute to behaviour. Activating Basin-4 leads to rolling, while activating Basin-1 would increase the crawling speed and not produce rolling {Ohyama, T. et al., 2015}. Silencing Basin neurons reduced the probability of rolling in response to combined stimulation {Ohyama, T. et al., 2015}, which indicates that Basin neurons were not only sufficient but were also involved in generating the rolling behaviour in response to stimuli. Taken together, connectomic and manipulation experiments show Basin-4 neurons as an early site of mechano-nociceptive convergence {Ohyama, T. et al., 2015}.

3. Later convergence in pathways controlling rolling

Early multisensory convergence in Basin-4 (and Basin-2) neurons did not take into account the entire circuit. If integration happened only in the Basin-4 (and -2), the two modalities would be combined once and then sent downstream as one signal. Instead, the reconstructed circuit showed that mechanosensory and nociceptive pathways also can interact again at later processing stages {Ohyama, T. et al., 2015}. One important downstream cell type is the Goro neuron. Goro neurons are called command-like neurons because their activation can lead to rolling, and if inactivated, there is a much smaller probability of rolling {Ohyama, T. et al., 2015} to occur. Calcium imaging determined that activating all Basins together, or just the multisensory Basin-4 could activate Goro neurons {Ohyama, T. et al., 2015}, so these are two parts of the circuit that are in a functional relationship. Electron-microscopy reconstruction also revealed pathways connecting those Basin neurons to the Goro neurons {Ohyama, T. et al., 2015}.

However, Goro neuron information from the multisensory Basin-4 converged again with the mechanosensory Basin-1, at multiple levels in the circuit. Combined activation of basin-4 and basin-1 evoked more rolling and more activity in Goro, than Basin-4 activation alone {Ohyama, T. et al., 2015}.

Repeated convergence can also explain why the system is sensitive and selective at the same time. For example, a weak cue might recruit part of the network but not enough to start rolling. When

mechanosensory and nociceptive signals interact, their effects can play out at different stages throughout the circuit, which could cause a higher likelihood that the rolling network in the downstream stage will achieve the level of activity required to initiate the behaviour and reach that threshold. The modelling by Ohyama et al. suggested that multilevel convergence could make a circuit more sensitive to weak combinations of stimuli than a circuit where the information is integrated at only one stage (Ohyama et al. 2015). Additionally, the escape response is divided into related motor modules. Goro activation can lead to rolling, but does not recreate every component of the natural escape sequence {Ohyama, T. et al., 2015}. Other neurons that lie downstream of the Basins, like the A00c neurons, which can contribute to increases in crawling speed. From this we can draw that the circuit does not depend on one neuron which specifies all of the behaviour. Instead, different pathways could control different components that can be recruited in different combinations {Ohyama, T. et al., 2015}.

Burgos et al. (2018) also further showed that nociceptive interneurons control modular motor pathways which are involved in escape. This organization might allow the nervous system to produce different defensive responses according to the intensity and combination of the received sensory inputs. A mild signal can happen to increase crawling speed without rolling; however, stronger or combined signals may lead to rolling making a more complete series of escape events {Burgos, A. et al., 2018}. The word "command-like" needs to be carefully interpreted. Goro activation can lead to rolling in the experiments, but it does not mean that Goro neurons control the whole sensory decision {Ohyama, T. et al., 2015}. As mentioned earlier, their activity is not limited to Goro neurons because all the signals coming from the upstream networks are controlled by more than one, but also the whole behaviour consists of other neurons that will control the speed, movement sequence, and direction {Ohyama, T. et al., 2015}.. Goro neurons are better understood as fundamental control points in a distributed sensorimotor network.

4. Multisensory integration during navigation.

The escape circuit demonstrates how two threatening signals could synergistically interact, but multisensory processing is not only limited to escape or to a reflex enhancement. Gepner et al. (2015) further investigated how visual and olfactory information could affect navigation in Drosophila larvae. {Gepner, R. et al., 2015}

Larval navigation consists of relatively straight runs that are sometimes interrupted by turns in response to an unfavourable change in the sensory conditions (e.g. concentration of attractive odour is reduced). A turn gives the larva the ability to sample other directions via head sweeps before beginning a new run again {Gepner, R. et al., 2015}.

Both visual and olfactory signals seem to be combined before the final non-linear decision is reached that determines if the larva turns. To investigate this the researchers presented temporally changing visual stimuli and "fictive" olfactory signals which are optogenetically generated {Gepner, R. et al., 2015}. By expressing the light-sensitive channel CsChrimson {Chen, T. W. et al., 2013} in the chosen olfactory receptor neurons, they could then manipulate olfactory neuronal activity with red light and at the same time, use blue light to independently control the visual environment. They then used reverse-correlation and a Linear-Nonlinear-Poisson model to connect the recent sensory history to the chances that the larva would produce a turn {Gepner, R. et al., 2015}.

The results did not support a model where the two modalities independently produced turns through completely separate pathways. Instead, the larvae showed to combine filtered visual and olfactory signals upstream of the final nonlinear decision to produce a turn {Gepner, R. et al., 2015}. A model that combines the two signals linearly predicted responses to multisensory stimulation better than one where the two modalities just independently controlled turning {Gepner, R. et al., 2015}.

Here a different integration type is represented compared to the synergistic enhancement outlined by Ohyama et al. (2015) . In the escape circuit, simultaneous mechanosensory and nociceptive cues significantly and disproportionately enhanced the chances of rolling to occur. In the navigation, the visual and olfactory evidence could have been combined into a common variable linearly that can influence the chances of a turn being produced. The output still went through a nonlinear behavioural decision, but sensory signals being combined before that decision was made is what is indicated by the evidence {Gepner, R. et al., 2015}.

The study is important because it further highlights how MSI could support not only rapid escape actions, but also could potentially support continuous navigational decisions. It also shows a computational description of integration through asking how those sensory signals are mathematically combined to be able to then predict a certain behaviour. But it doesn't identify the synaptic pathways or individual neurons that do this computation. The optogenetic olfactory signal also recreates the receptor-neuron activation only, rather than every physical and temporal property of that odour. Consequently, the work provides strong behavioural and computational evidence for integration, but anatomical implementation of the proposed linear combination is still not answered yet {Gepner, R. et al., 2015}.

This aligns with the strengths of Ohyama et al. (2015). Gepner et al. describe the shift from sensory evidence to behavioural chance in detail and they do not answer or discuss the underlying circuit. Ohyama et al. identify specific neurons and synaptic pathways but also put more focus on a particular decision of escape. The results of these studies demonstrate why understanding MSI will require explanation at many levels including: the behaviour computation, the neurons that perform that computation and the connections that are between those neurons..

For this purpose experiments that combine anatomical reconstruction, functional imaging of neural activity, activation and silencing, as well as modelling will be required in the future.

5. Cross-modal inhibition and sensory gating

Multisensory integration does not always enhance or maximize a response. Sometimes, input from one modality may suppress another pathway. Furthermore, Pan et al. (2023) has shown this through a cross-modal gating mechanism in the nociceptive system of the larva.

When the nociceptive input was weak, gentle mechanical stimulation decreased larval nociceptive responses. Low-threshold mechanosensory neurons inhibited the transmission to the A08n neurons, which are important second-order neurons that are in the nociceptive pathway. To mediate the presynaptic inhibition metabotropic GABA B receptors located at nociceptor terminals were used {Pan, G. et al., 2023}.

More Importantly, this inhibition didn't completely block all the nociceptive information ; It was useful when the nociceptor input was weak, but strong noxious input could still be received in the circuit. Therefore, Gentle mechanical information acted as a gate that could block weak nociceptive signals without disabling the defensive behaviour completely {Pan, G. et al., 2023}.

This research serves as a great contrast to Ohyama et al. (2015). In the Ohyama circuit, nociceptive input and vibration could interact to then enhance rolling. In the Pan study, however, low-threshold mechanical input (such as gentle touch) caused transmission of weak nociceptive information to get inhibited. So, with respect to nociception, mechanical stimulation doesn't have one fixed effect. Its effects depend on the intensity of the nociceptive input, the type of mechanosensory stimulation and where that interaction occurs in the circuit {Ohyama, T. et al., 2015; Pan, G. et al., 2023}. This further highlights how different multisensory neurons integrated input in different ways. The Integration could be enhancement, suppression, thresholding, and gating.. Enhancement could help increase sensitivity to combinations that are likely to indicate danger; on the other hand, inhibition could prevent weak or irrelevant nociceptive activity from unnecessarily triggering an escape response that is energetically costly.

A more specific cellular mechanism than what could be inferred from behavioural suppression alone was provided from Pan et al.. They showed how a single pathway could influence the amount of information transmitted by another pathway before that information even reaches a second-order nociceptive neuron through Identifying GABA B -receptor-mediated presynaptic inhibition. This serves as a clear example of one modality altering the gain of another pathway and then not just converging together at a common output neuron {Pan, G. et al., 2023}.

But the experiments do not mean that every natural nociceptive decision is determined by this gate. Behaviour arises from the additional downstream processing, and laboratory stimulation cannot recreate every combination of vibration and natural touch. The study lays out a specific mechanism for cross-modal modulation, but its contribution must still be noted as just a single component of the grand scheme of order which is the large spread out circuit.

6. Evidence from the whole-brain connectome

Several types of integration within specific behaviours are highlighted by the detailed studies above; however, they raise a general question: are these circuits unusual exceptional cases or is distributed convergence considered a general organisational feature of the larval nervous system?

The brain and ventral nerve cord of a first-instar Drosophila larva at synaptic resolution was reconstructed by Winding et al. (2023). This had 3,016 neurons and about 548,000 synapses, which enabled the study of sensory inputs, interneurons, descending pathways and motor-related outputs within the same network. {Winding, M. et al., 2023}.

The connectome was not a simple collection of sensory channels. Processing begins with receptor neurons that cater to specific modalities, but downstream pathways branch and form connections with other modalities. As information propagates through the network, neurons can get direct or indirect input from

multiple modalities. This anatomical framework then allows multisensory interactions to happen at multiple levels rather than at only a single universal integration center {Winding, M. et al., 2023}.

At different distances from their receptor, different sensory pathways converged. For instance some met after a few synaptic steps, while others remained partially separated until later convergence happened.

Another way in which multisensory integration is achieved is through feedback connections from multisensory neurons that mediate action-selection and learning, back onto neurons that mediate early stages of sensory processing. The connectome showed a high degree of feedback connections, with about 41% of the neurons getting involved with recurrent pathways. An organisation like that indicates that neural processing can't be understood as sensory information just moving through a fixed feedforward chain. Therefore, this could potentially allow sensory processing to depend on previous activity and learning {Winding, M. et al., 2023}, since activity can circulate through a network or be affected by feedback.

Dopaminergic neurons are in general responsible for learning, value assignment and reinforcement. Connections between sensory pathways and those dopaminergic systems are especially important. Multimodal exposure to these neurons might give the ability for an event to be evaluated on the basis of its combined sensory meaning rather than just requiring each sensory feature to be learned entirely in isolation. MSI might therefore affect not just the animal's immediate response but also how they later change their behaviour after a certain experience {Winding, M. et al., 2023}.

However, the connectome can only give anatomical possibilities, not firm proof of functional integration. The existence of a synapse shows that communication is structurally possible but it does not indicate that the connection is active during a specific behaviour. The number of synapses also does not determine physiological strength, as excitatory, inhibitory and neuromodulatory inputs can have various effects and they are not always integrated linearly. Furthermore, the reconstructed animal only presents one individual at a particular stage of its development {Winding, M. et al., 2023}. In the future, obtaining connectomes of multiple individuals at multiple life stages could reveal which circuit features are common, and which ones are different across individuals.

The connectome can be most powerful when it produces specific hypotheses (e.g., predicting a convergence neuron that can be recorded and manipulated) rather than when connectivity alone is directly taken as proof that integration has already been done. The validity of those connectomic predictions must then be tested using tools like electrophysiology, calcium imaging, genetic manipulation and behavioural experiments.

DISCUSSION

Emerging circuit principles

All these studies indicate that MSI in Drosophila cannot be described by one universal circuit or a certain computational rule. Nevertheless, several principles emerge.

First, convergence could start early, where for instance, Basin-2 and Basin-4 receive direct input from different sensory pathways. Pan et al., on the other hand, demonstrates that one of these modalities can control nociceptive transmission near the terminals of the primary sensory neurons. So sensory interactions could occur early near the start of processing instead of occurring only in late, higher-order centres {Ohyama, T. et al., 2015; Pan, G. et al., 2023}.

Second, different computations are used by different behaviours. The mechano-nociceptive escape circuit is synergistically enhanced; the navigational system combines visual and olfactory information before a nonlinear turning decision, and weak nociceptive transmission was inhibited via cross-modal gating through gentle mechanosensory stimulation {Gepner, R. et al., 2015; Ohyama, T. et al., 2015; Pan, G. et al., 2023}. MSI therefore does not just "add senses together": it could include any of the following: enhancement, weighted combination, and also inhibition…

Third, integration can occur at multiple levels within the circuit. For example, mechanosensory and nociceptive signals converge in first-order interneurons and have the ability to interact again through downstream pathways that lead into command-like and motor-related neurons, which are involved in the physical response. This multi-level repeated convergence may increase the sensitivity to meaningful combinations and link sensory evidence directly with action selection {Ohyama, T. et al., 2015}.

Fourth, integration is scattered across many networks. Basin-4, A08n, and Goro and other neurons are essential nodes in the circuit, but none should be considered an isolated centre that is responsible for the entire computation. Sensory convergence, action selection, thresholding, and translation of this into a motor output are all based on different interconnected neuronal populations. The whole brain connectome indicates that this distributed organization might be found in a big part of the larval nervous system {Winding, M. et al., 2023}.

Lastly, a sensory signal's impact could change based on the context: for example, the same specific sensation could either block or boost pain depending on the active pathway, where those signals converge and how intense that pain is. So, a sense alone does not have the ability to determine how we react. Instead, the final behavior depends completely on which other sensory modalities are present at the time, as well as previous experience. {Ohyama, T. et al., 2015; Gepner, R. et al., 2015; Pan, G. et al., 2023}

Limitations and unresolved questions

Even though we have results, there are many questions that still remain yet to be resolved.

Despite vibration, gentle touch, and painful mechanical pressure being mechanical signals, they don't always use the same receptors to reach the nervous system or produce the same effects. So comparisons across studies need to take into account that different types of stimulation activate different neuronal populations. Future research needs to figure out how specific mechanosensory pathways interact with pain, visual, and smell systems during the grand broader range of natural reality.

One more limitation is the association of anatomical connectivity with physiological effects. Connectomes can help you find potential convergence points, but we don't know the strength of connections or if they are actually active. However, a connectome can only show the different anatomical

connections between neurons, but it does not tell us if a certain neuron is active. This is where techniques like calcium imaging and the manipulation of neurons come into play. Future research should combine detailed whole-brain imaging with targeted manipulation during naturalistic multisensory behaviour and also cameras from multiple angles to help capture everything.

In Winding et al the connectome was for only one *Drosophila*, and I think is a snapshot in time of its developmental stage; for future improvement, scientists can compare reconstructions on a large scale from different larvae to see which connections are universally shared between them.

We also cannot forget the crucial role timing plays, which is not yet explored; for instance, when 2 cues come at the same time they may be considered different to when 2 cues come at slightly different times, which can lead to them not getting combined. The temporal timing windows in which the larval circuits can combine information could help in understanding how the nervous system can distinguish between different events.

The studies so far did not take into consideration the possibility of specific internal states affecting how sensory modalities are integrated; for instance, a *Drosophila* might be hungry, and that could significantly influence how it weighs and values different sensory signals. Neuromodulators may be helpful for this limitation but their specific impact on the multisensory pathways are still not well understood. Furthermore, it is not evident how learning, developmental stage, and internal state can modify multisensory integration. For instance, Hunger can change the relative weighting and priority of sensory cues without necessarily changing the underlying anatomical connections.

Most functional experiments are done in a laboratory setting to explore specific favorable combinations of stimuli. Many studies used artificial neuronal activation methods like optogenetics or thermogenetics {Ohyama, T. et al., 2015; Gepner, R. et al., 2015}. These can be useful for testing neurons but may not produce the same response as a neuron responding to a natural stimuli. Furthermore, most of the studies tested no more than 2 modalities, which can provide a closed, limited view by disregarding essential pieces in the puzzle, in comparison to if they compared more modalities together {Ohyama, T. et al., 2015; Gepner, R. et al., 2015; Pan, G. et al., 2023}.

Natural environments differ significantly as they provide a multitude of sensory signals that are sent at a range of intensities and rates. Most studies in the laboratory setting focus on interaction between two modalities, but how 3, 4, or more modalities all interact at the same time has so far been challenging to address in the laboratory. Therefore, a question arises: Are the same circuit computations performed when larvae actually face a complex natural threat? I, therefore, think that future experiments need to be conducted in more complex naturalistic conditions where other factors like wind and temperature, for example, can also play a role or as an alternative, they could try to simulate natural environments and provide more complex, naturalistic stimuli. For example, one could image larval neurons responding to real predators.

Another crucial point for future research is to take into account how an animal's motion influences how sensory stimuli are integrated. So imaging neuronal activity in freely behaving animals, as they are

responding to naturalistic stimuli would be helpful. This would make it much more similar to the natural environment in comparison to just presenting simple stimuli to fixed animals.

CONCLUSION

This paper's main aim was to review the current understanding on how the brain integrates information from different sensory modalities to then guide behaviour in tractable Drosophila larvae as a model system. Drosophila is a very useful and appropriate model system, not just because it has multisensory neurons but the genetic flexibility and accessibility it provides and the availability of its entire synaptic resolution connectome, all help researchers shift from just observing to actually studying the pathways of these multisensory neurons.

This review of literature in Drosophila larvae supports the conclusion that multisensory integration is not performed by one specialised neuron or just at one late level of processing; rather, a multi-level multimodal integration occurs, where the sensory signals meet at many points throughout the circuit. The multilevel convergence architecture helps explain why combined signals together can yield a higher chance of an action than either signal alone. In Ohyama et al.(2015), the larval escape circuit demonstrates the best example of this multilevel organisation, showing that nociceptive and mechanosensory information converge in the Basin neurons early and then meet again in pathways that lead towards the command-like Goro neurons and also other neurons that can control different parts of the escape response. Furthermore, we saw how the escape response wasn't controlled by solely one command neuron but instead was arranged into many motor modules. Therefore, it is evident that the larva's response arises from the activity of a much wider complex network that connects the sensory evidence to the most appropriate output action. We saw how multisensory integration was used to produce and achieve various outcomes; for example, Pan et al. showed how weak nociceptive signals can be cancelled out through gentle mechanical input. However, Gepner et al showed how the brain integrates olfactory and visual information to determine if a larva turns as it navigates. Winding et al. (2023) highlighted how extensively branched and recurrent the connections are throughout the larva, which further supports this.

In summary, this paper supports a perspective of multisensory integration where it's not only a late step done in an isolated part of the brain; instead, it is looked at with the perspective of being multilevel and recurrent. All this evidence supports the idea that multisensory integration is not just one more late processing step in an isolated part of the brain. In this model, sensory signals can interact near the receptor terminals, meet in first-order interneurons, then they could meet again in downstream sensorimotor pathways and go to influence command-like and motor output circuits. MSI is now multi-level, multi-modal and a highly recurrent process that takes combinations of input via the senses and turns them into behaviour. Ohyama et al. (2015) provide an example of synergistic, multilevel integration during escape. Burgos et al. (2018) demonstrate how nociceptive pathways can activate specific motor elements to perform the escape response. Gepner et al. (2015) show that visual and olfactory signals are combined to guide navigation decisions, and Pan et al. (2023) show that MSI can suppress weak signals through presynaptic gating, not just always enhancing behaviour. Winding et al.

(2023) put all these individual pieces together within a brain-wide network; in this case is the synaptic-resolution connectome of an entire brain, showing the comprehensive set of anatomical interactions between all sensory modalities.

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