RESEARCH ARTICLE

Immediate and punitive impact of mechanosensory disturbance on olfactory behaviour of larval Drosophila

Timo Saumweber1,2,§, Carmen Cano3,*, Juliane Klessen2, Katharina Eichler3,‡, Markus Fendt4,5 and Bertram Gerber2,3,5,6,§

ABSTRACT

The ability to respond to and to learn about mechanosensory disturbance is widespread among animals. Using Drosophila larvae, we describe how the frequency of mechanosensory disturbance (‘buzz’) affects three aspects of behaviour: free locomotion, innate olfactory preference, and potency as a punishment. We report that (i) during 2–3 seconds after buzz onset the larvae slowed down and then turned, arguably to escape this situation; this was seen for buzz frequencies of 10, 100, and 1000 Hz, (ii) innate olfactory preference was reduced when tested in the presence of the buzz; this effect was strongest for the 100 Hz frequency, (iii) after odour-buzz associative training, we observed escape from the buzz-associated odour; this effect was apparent for 10 and 100, but not for 1000 Hz. We discuss the multiple behavioural effects of mechanosensation and stress that the immediate effects on locomotion and the impact as punishment differ in their frequency-dependence. Similar dissociations between immediate, reflexive behavioural effects and reinforcement potency were previously reported for sweet, salty and bitter tastants. It should be interesting to see how these features map onto the organization of sensory, ascending pathways.

KEY WORDS: Drosophila, Learning, Memory, Olfaction, Punishment, Mechanosensation

INTRODUCTION

Drosophila larvae have but 10,000 neurons, yet display a relatively rich behavioural repertoire (Vogelstein et al., 2014; for reviews, see Cobb, 1999; Diegelmann et al., 2013; Gerber and Stocker, 2007; Schleyer et al., 2013): they are not only able to feed, smell and taste, to sense visual, tactile and noxious stimuli, temperature and vibration, but also use these kinds of sensory information for learning. Larvae form associative memories between an odour and rewards such as fructose (Scherer et al., 2008), whereas high salt concentrations or bitter substances as well as electric shocks can be used as punishment (Aceves-Pina and Quinn, 1979; El-Keredy et al., 2012; Niewalda et al., 2008). We focus on the behavioural impact of mechanical disturbance (‘buzzes’). In particular our experiments concern (i) the impact of buzzes on locomotion and on (ii) innate olfactory behaviour, as well as (iii) their potency as punishment (Eschbach et al., 2011).

Locomotion in larval Drosophila is studied mostly in Petri dish arenas covered with an agarose substrate. Their behaviour consists of runs, accomplished by peristaltic waves of muscular contraction that propagate from back to front (e.g. Gomez-Marin and Louis, 2014). Runs feature low-amplitude side movements (<20 degrees/s) of the first 1–3 segments, called head weathervaning. Weathervaning can support slightly curved runs and does not entail a break of the peristaltic wave (Gomez-Marin and Louis, 2014). Peristaltic runs can be interrupted to accommodate reorientation events. Upon such an interruption the larvae typically show more pronounced side movements of their head (~60 degrees/s). Dependent on when the peristaltic wave is re-initiated during these movements, the body is pushed forward in this new orientation. The mechanosensory chordotonal organs and the brain hemispheres are apparently dispensable for these locomotor patterns, arguing they are produced by circuitry in the ventral nerve cord; however, brain and mechanosensory input are required for the integration of these locomotor patterns into adaptive, biologically meaningful behaviour (Bermi et al., 2012; Caldwell et al., 2003; Ohyama et al., 2013; Wu et al., 2011).

Interestingly in the current context, the presentation of a buzz can both interrupt peristaltic running (Eschbach et al., 2011; Ohyama et al., 2013; Zhang et al., 2013) and serve as punishment in an associative olfactory learning experiment (Eschbach et al., 2011) (both these effects may under natural conditions help larvae to avoid predatory wasps (Zhang et al., 2013)). In these experiments an odour A is presented with a buzz, but another odour X is presented without a buzz. After such training, the preference between both odours is tipped to the disadvantage of the previously punished odour (Fig. 1). In accordance with what has been found for other types of aversive olfactory learning in the larva (Apostolopoulou et al., 2014a; Apostolopoulou et al., 2014b; El-Keredy et al., 2012; Gerber and Hendel, 2006; Niewalda et al., 2008; Schleyer et al., 2011; Schroll et al., 2006; Selcho et al., 2009), such learned behaviour can best be understood as an escape strategy. Consider that you will not run out of a movie theatre upon seeing the emergency exit sign, but only when there is an emergency to escape from. Likewise, the
larvae do not show conditioned escape during the test unless the punishment is present and escape indeed is warranted (for buzz as punishment (Eschbach et al., 2011)). In other words, the smell of the previously punished odour does not itself trigger escape, but gives direction to an escape which is otherwise triggered – namely by the buzz.

The current study aims to further our understanding of the behavioural impact of buzz-mechanosensation in larval Drosophila. We parametrically describe the potency of buzzes of various frequency (10, 100, 1000 Hz) as punishment, as well as their impact on free locomotion and olfactory preference behaviour.

**RESULTS**

**Buzz as punishment**

*Drosophila* larvae were trained such that one odour, namely either n-amyl acetate or 1-octanol (AM, OCT), was associated with a buzz as punishment (–). Then, the larvae were offered a choice between AM and OCT (Fig. 1A,B). Preference scores as displayed in Fig. 1C (left) were defined such that positive scores indicate a choice of AM while negative scores indicate a choice of OCT. We used ‘standard’ 0.2 s-duration buzzes at a frequency of 100 Hz (Eschbach et al., 2011). Preference scores were shifted towards OCT after AM–/OCT training as compared to AM/OCT–training (Fig. 1C, left). Correspondingly, the associative performance index, which measures the difference in preference, was significantly negative (Fig. 1C, right). Increasing buzz duration by a factor of ten, i.e. from 0.2 s to 2 s, did not increase this associative effect (Fig. 1D; for the underlying preference scores, see supplementary material Fig. S1), suggesting that the punitive effect of the buzz might be largely exerted by its onset (Zhang et al., 2013).

Next, we asked whether the frequency of buzz punishment has an influence on associative scores (Fig. 2B; supplementary material Fig. S2B). Buzzes of 10 Hz and 100 Hz support significantly negative associative performance indices, whereas 1000 Hz buzzes did not. A relatively low frequency of 10 Hz supported the same level of associative effect as the standard 100 Hz buzz, while the scores using 1000 Hz buzzes were less relative to the 100 Hz buzz.

We were surprised to observe that the 1000 Hz buzz did not support a punitive effect. As mentioned in the Introduction, both for bad-taste and for the buzz as punishment, learned behaviour is part of an escape process and is expressed only in the presence of the punishment. Therefore the lack of associative effect of a 1000 Hz buzz may either be because no odour-buzz memory is established, or because the 1000 Hz buzz during testing does not allow the behavioural expression of an otherwise intact odour-buzz memory. Given that the standard buzz of 100 Hz was effective as punishment (middle plot in Fig. 2B), we trained larvae with such a standard 100 Hz buzz, but tested them in the presence of a 1000 Hz buzz. It turned out that associative scores were intact (Fig. 2C; supplementary material Fig. S2C). This argues that a 1000 Hz buzz is permissive for learned escape. In turn, as the standard 100 Hz buzz was also permissive for learned escape (middle plot in Fig. 2B), we trained larvae with a 1000 Hz buzz, but tested them in the presence of the standard 100 Hz buzz. In such an experiment, associative scores were zero...
In turn, 1000 Hz buzzes cannot function as punishment (D) and ns indicate P values. Buzzes differing in frequency between training and test. Odour-buzz memory, if established using a 100 Hz buzz, can be behaviourally expressed at 1000 Hz. Presenting the buzz during the test is required, because conditioned avoidance is not behaviourally expressed if there is no ‘reason’ to escape (see Introduction for rationale). (B) Associative performance indices when using buzzes at the indicated frequencies. Associative performance is observed for 10 Hz and 100 Hz, but not for 1000 Hz buzzes. * and ns refer to P < 0.05/3 and P > 0.05/3 (OSS-tests), respectively. # refers to P < 0.05/2 (MWU-test, P < 0.05/2; U = 327.0). □ refers to P < 0.05 (KW-test: P = 0.05; H = 7.71; df = 2). From left to right, sample size is N = 32, 38, 28. (C,D) Associative performance indices when using buzzes differing in frequency between training and test. Odour-buzz memory, if established using a 100 Hz buzz, can be behaviourally expressed at 1000 Hz (C). In turn, 1000 Hz buzzes cannot function as punishment (D). * and ns indicate P < 0.05 and P > 0.05, respectively (OSS-tests) (N = 50, 43).

Fig. 2. Buzz as punishment: frequency-dependence. (A) Sketch of the experimental design using buzz frequencies of 10 Hz, 100 Hz and 1000 Hz. Presenting the buzz during the test is required, because conditioned avoidance is not behaviourally expressed if there is no ‘reason’ to escape (see Introduction for rationale). (B) Associative performance indices when using buzzes at the indicated frequencies. Associative performance is observed for 10 Hz and 100 Hz, but not for 1000 Hz buzzes. * and ns refer to P < 0.05/3 and P > 0.05/3 (OSS-tests), respectively. # refers to P < 0.05/2 (MWU-test, P < 0.05/2; U = 327.0). □ refers to P < 0.05 (KW-test: P = 0.05; H = 7.71; df = 2). From left to right, sample size is N = 32, 38, 28. (C,D) Associative performance indices when using buzzes differing in frequency between training and test. Odour-buzz memory, if established using a 100 Hz buzz, can be behaviourally expressed at 1000 Hz (C). In turn, 1000 Hz buzzes cannot function as punishment (D). * and ns indicate P < 0.05 and P > 0.05, respectively (OSS-tests) (N = 50, 43).

Buzz as modulator of innate olfactory behaviour
We offered the larvae a choice between an odour side (either AM or OCT) versus a blank side of a Petri dish and recorded their preference – and did so either in the presence or in the absence of a buzz (Fig. 3A,C). Given that it takes 3–5 min for the larvae to distribute themselves between both sides of the Petri dish (Fig. 3B), we chose to focus on the data at 5 min. This was done for either 10, 100, or 1000 Hz buzzes. In the presence of 10 Hz and 1000 Hz buzzes the larvae behaved the same as larvae tested without a buzz; to our surprise, however, in the presence of 100 Hz buzzes innate odour preference was strongly decreased, for both the odours employed (Fig. 3D).

We conclude that 100 Hz buzzes, but not 10 or 1000 Hz buzzes, strongly modulate innate olfactory behaviour – while, as mentioned above, associative aspects of olfactory processing remain unaffected in the presence of a 100 Hz buzz.

Buzz as modulator of locomotion
We monitored locomotion of individual larvae and quantified their innate behaviour with respect to buzzes of 10, 100, or 1000 Hz. We present speed and turning propensity upon the very first (Fig. 4), the 10th (supplementary material Fig. S3) and the 60th buzz within a 5 min period (supplementary material Fig. S4). We normalized data to the 2 s before the onset of the respective buzz as baseline. In keeping with Eschbach et al. (Eschbach et al., 2011), the larvae ‘startled’, that is they briefly slowed down and then turned in response to a 100 Hz buzz (Fig. 4B3,C3). The speed dropped below baseline at second 2, yet returned to baseline while turning was still in progress, until at second 3 to 4 after the buzz the new direction was assumed. The same qualitative pattern of results was found for 10 Hz and 1000 Hz buzzes (Fig. 4B2,C2,B4,C4). These results were surprisingly stable over dozens of repetitions of the buzz (for the 10th and 60th buzz, see supplementary material Fig. S3, Fig. S4).

We conclude that innate behaviour towards buzzes is a rather repetition-stable behaviour consisting of sequential slowing-down and turning, and that this behaviour does not depend on the frequency of the buzzes, at least not across 10, 100, and 1000 Hz.

DISCUSSION
We demonstrate that mechanical disturbances (buzzes) impact immediate behaviour and are effective as punishment – and that buzzes of different frequency differ in impact across the types of behaviour assayed: 10 Hz buzzes function as punishment, do not modulate innate olfactory behaviour, and induce startle. Buzzes of 100 Hz also serve as punishment, do reduce innate olfactory preference and elicit startle. Lastly, 1000 Hz buzzes cannot serve as punishment, do not modulate innate chemotaxis, and do make larvae startle. How can these differences in frequency-dependence be understood?

For sugars, salt, and quinine, mismatches have been reported between the dose-effect functions of immediate and reinforcing effects (El-Keredy et al., 2012; Niewalda et al., 2008; Russell et al., 2011; Schipanski et al., 2008). For example, figure 5 in El-Keredy et al. found that the suppressing effect of quinine on feeding is shifted by about one order of magnitude towards higher concentrations as compared to the punishing effect of quinine.
The authors suggested that different sensory neurons differing in dose-effect function and differentially hooked up to feeding behaviour versus reinforcement signalling are responsible for these effects. This was confirmed by Apostolopoulou et al.: ablating Gr33a-Gal4 positive gustatory sensory neurons reduces feeding-suppression by quinine, but leaves punishment processing unaffected (Apostolopoulou et al., 2014b).

A buzz interrupts peristaltic running and induces a brief hunch, followed by large-amplitude sideways movements of the head and ensuing peristaltic runs into a new direction (Bharadwaj et al., 2013; Ohyama et al., 2013; Wu et al., 2011; Zhang et al., 2013) (Fig. 4). This sequential pattern of behaviour is reminiscent of startle in mammals (supplementary material Fig. S5): upon a sudden and intense visual, tactile or acoustic stimulus, mammals interrupt current behaviour, close their eyes, flatten their ears, bend their spine and limbs and stiffen their neck (these behaviours are typically measured as ‘startle’). In a second phase, the eyes are widely opened, the ears pricked, and, while the spine and body parts remain bent, the head is rotated sideways (Landis and Hunt, 1939; Strauss, 1929; Gerber et al., 2014; Koch, 1999; Yeomans and Frankland, 1995). As in larvae, these behaviours seem to initially protect the subject, followed by attempts at threat localization, reorientation, and preparation for a fight or flight decision.

Regarding the neurogenetics of sensing mild mechanical disturbance like buzzes, the precise targeting of chordotonal neurons within the central nervous system is required (Wu et al.,...
Two filter papers were fixed to the Petri dish lid, both loaded with 10 μl of the same odour (e.g. AM) and the lid was put on the Petri dish. Then, 50 larvae were collected from their rearing vials, washed and transferred to the Petri dish. For punishment, 60 disturbances were applied at a frequency of 100 Hz (‘buzzes’ in the following), each lasting 0.2 s and presented evenly spaced in time for 5 min. The larvae were then transferred to a fresh Petri dish and OCT was presented, without the buzz (AM–/OCT). This cycle was repeated two more times (in half of the cases larvae were punished during the 1st, 3rd and 5th trial, while otherwise they were punished in the 2nd, 4th and 6th trial).

For testing, larvae were transferred to a Petri dish equipped with AM on one side and OCT on the other. After 5 min, we counted the number of larvae in the middle (0.5 cm wide stripe), on the AM side and on the OCT side. A preference index is calculated as:

\[
\text{PI} = \frac{(\text{AM} - \text{OCT})}{\text{TOTAL}}
\]

Likewise, a preference index \(\text{PI}_2\) was determined for larvae of the reciprocally trained group (AM/OCT–). The performance index \(\text{PI}_2\) was defined as the difference in preference between the reciprocally trained groups:

\[
\text{PI}_2 = \frac{(\text{AM} - \text{OCT})}{2}
\]

Positive scores thus indicate appetitive memory, while negative scores indicate aversive memory, that is a punitive effect of the buzz. Testing was performed in the presence of the buzz (see Introduction for rationale).

**Buzz as modulator of olfactory preference**

Two 7-mm² filter papers were fixed to the Petri dish lid, one of which was loaded with odour (10 μl of either AM or OCT) while the other one was left blank. A group of 50 larvae was collected and transferred to the middle of an agarose-filled Petri dish. The Petri dish was then placed into the assay box described above. After 1, 3 and 5 min we determined the number of larvae on either the odour side or the blank side or the middle stripe, allowing us to calculate a preference score as:

\[
\text{PREF} = \frac{(\text{ODOUR} - \text{BLANK})}{\text{TOTAL}}
\]

This experiment was performed either as described, or in the presence of the buzz.

**Buzz as modulator of locomotion**

We determined two key parameters of the behaviour towards the buzz, namely changes in speed and changes in turning propensity. Single larvae were observed for 5 min, moving over an agarose-filled Petri dish. During this time, buzzes of 0.2 s duration were presented, evenly spaced in time, and data were recorded for offline analyses. For the first buzz as well as for the 10th and the 60th buzz, we determined speed (mm/s; 1 voxel = 0.33 mm) and turning propensity (°/s) (for details, see Eschbach et al., 2011; Eschbach, 2011). Baseline speed and turning propensity were determined for the 2 s before the buzz; data were then scored for the 1st, 2nd, 3rd and 4th second after onset of the buzz. Data are presented normalized to baseline: negative scores thus indicate slowing down and turning less, respectively, while positive scores indicate speeding up and turning more.

All three experiments were performed using buzzes at frequencies of 100 Hz, as in Eschbach et al. (Eschbach et al., 2011), as well as buzzes of one order of magnitude lower and higher frequency (10 Hz, 1000 Hz). All experiments comply with applicable law and regulations.

**Statistics**

Statistical analyses were non-parametric throughout and performed with Statistica on a PC (Statsoft 7.0, Tulsa, USA). To compare across multiple groups, we used Kruskal–Wallis tests (KW); Mann–Whitney U-tests (MWU) were used for pairwise comparisons. To test for differences from chance level we used One-Sample Sign-tests (OSS). In cases of multiple comparisons, we applied a Bonferroni correction by dividing 0.05 by the number of comparisons.
number of comparisons made (presented as P<0.05/3 in cases of e.g. three comparisons); this ensures a within-experiment error rate below 5%. Results of statistical analyses are presented in the figure legends. Data are presented as box-whisker plots (middle line: median; box: lower and upper quartile; whiskers: 90th and 10th percentile).

List of abbreviations
AM: n-amy acetate; OCT: octanol; TRP: transient receptor potential; NOMPC: No mechanoreceptor potential C; NANC: nanchung; IAV: inactive.

Acknowledgements
We thank Claire Eschbach and Hannah Haberkern for introducing us to the paradigm, Silvia Petter, Holger Reim and Roswitha Jungnickel for technical assistance, and Rupert Glassow, Bert Klagger, Christian König, BirgitMichels, Michael Schleyer for discussion and comments. We are particularly grateful to Klaus Schildberger for his extended, generous support and hospitality in his Department.

Competing interests
The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Author contributions
Developed the concept and designed the experiments: T.S., C.C., B.G. Performed the experiments: T.S., C.C., J.K., K.E., M.F. Analysed the data: T.S., C.C. Prepared and edited the manuscript before submission: T.S., M.F., B.G.

Funding
This study received institutional support from the Leibniz Institut für Neurobiologie (LIN) Magdeburg, the Wissenschaftsgemeinschaft Gottfried Wilhelm Leibniz (WGL), and the Universities of Leipzig and Magdeburg. This study was supported by grants from the Bundesministerium für Bildung und Forschung (BMBF Bernstein Network Insect inspired robotics to B.G.), the Deutsche Forschungsgemeinschaft (CRC 779 to B.G. as well as M.F.), and the European Commission (FP7-ICT project Miniature Insect Model for Active Learning [MINIMAL] to B.G.).

References
Aceves-Piña, E. O. and Quinn, W. G. (1979). Learning in normal and mutant Drosophila larvae. Science 206, 93-96.
Apostolopoulou, A. A., Hersperger, F., Mazija, L., Widmann, A., Wüst, A. and Thum, A. S. (2014a). Composition of agarose substrate affects behavioral output of Drosophila larvae. Front. Behav. Neurosci. 8, 11.
Apostolopoulou, A. A., Mazija, L., Wüst, A. and Thum, A. S. (2014b). The neuronal and molecular basis of quinine-dependent bitter taste signaling in Drosophila larvae. Front. Behav. Neurosci. 8, 6.
Bemi, J., Pulver, S. R., Griffith, L. C. and Bate, M. (2012). Autonomous circuitry for substrate exploration in freely moving Drosophila larvae. Curr. Biol. 22, 1861-1870.
Bharadwaj, R., Roy, M., Ohyama, T., Sivan-Loukianova, E., Delannoy, M., Lloyd, T. E., Zlatic, M., Eberl, D. F. and Kolodkin, A. L. (2013). Cbl-associated protein regulates assembly and function of two tension-sensing structures in Drosophila. Development 140, 627-638.
Calder, J. C., Miller, M. M., Wing, S., Soll, D. R. and Eberl, D. F. (2003). Dynamic analysis of larval locomotion in Drosophila chordotonal organ mutants. Proc. Natl. Acad. Sci. USA 100, 16053-16058.
Cardona, A., Saalfeld, S., Preibisch, S., Schmid, B., Cheng, A., Pulokas, J., Tomancak, P. and Hartenstein, V. (2010). An integrated micro- and macroarchitectural analysis of the Drosophila brain by computer-assisted serial section electron microscopy. PLoS Biol. 8, e1000502.
Cardona, A., Saalfeld, S., Schindelin, J., Arganda-Carreras, I., Preibisch, S., Longair, M., Tomancak, P., Hartenstein, V. and Douglas, R. J. (2012). TrakEM2 software for neural circuit reconstruction. PLoS ONE 7, e38011.
Cobb, M. (1999). What and how maggots smell? Biol. Rev. Camb. Philos. Soc. 74, 425-459.
Diegelmann, S., Klagges, B., Michels, B., Schleyer, M. and Gerber, B. (2013). Maggot learning and Synapsin function. J. Exp. Biol. 216, 939-951.
El-Kedery, A., Schleyer, M., König, C., Eikm, A. and Gerber, B. (2012). Behavioural analyses of quinine processing in choice, feeding and learning of larval Drosophila. PLoS ONE 7, e40525.
Eschbach, C. (2011). Classical and operant learning in the larvae of Drosophila melanogaster. PhD thesis, Graduate School of Life Sciences, Julius-Maximilians-Universität, Würzburg, Germany.
Eschbach, C., Cano, C., Haberkern, H., Schraut, K., Guan, C., Triphan, T. and Gerber, B. (2011). Associative learning between odorants and mechanosensory punishment in larval Drosophila. J. Exp. Biol. 214, 3987-3995.
Fushiki, A., Kohsaka, H. and Nose, A. (2013). Role of sensory experience in functional development of Drosophila motor circuits. PLoS ONE 8, e62199.
Gerber, B. and Hendel, T. (2006). Outcome expectations drive learned behaviour in larval Drosophila. Proc. Biol. Sci. 273, 2965-2968.
Gerber, B. and Stocker, R. F. (2007). The Drosophila larva as a model for studying chemosensation and chemosensory learning: a review. Chem. Senses 32, 65-89.
Gerber, B., Yarali, A., Diegelmann, S., Wotjak, C. T., Paulli, P. and Fendt, M. (2014). Pain-relief learning in flies, rats, and man: basic research and applied perspectives. Learn. Mem. 21, 232-252.
Gomez-Marin, A. and Louis, M. (2014). Multilevel control of run orientation in Drosophila larval chemotaxis. Front. Behav. Neurosci. 8, 38.
Koch, M. (1999). The neurobiology of startle. Prog. Neurobiol. 59, 107-128.
Landis, C. and Hunt, W. A. (1939). The Startle Pattern. New York: Farar and Rinehart.
Niewalda, T., Singhal, N., Fia, A., Saumweber, T., Wegener, S. and Gerber, B. (2008). Salt processing in larval Drosophila: choice, feeding, and learning shift from appetitive to aversive in a concentration-dependent way. Chem. Senses 33, 685-692.
Ohyama, T., Jovanic, T., Denisov, G., Dang, T. C., Hoffmann, D., Kerr, R. A. and Zlatic, M. (2013). High-throughput analysis of stimulus-evoked behaviors in Drosophila larva reveals multiple modality-specific escape strategies. PLoS ONE 8, e71706.
Russell, C., Wessnitzer, J., Young, J. M., Armstrong, J. D. and Webb, B. (2011). Dietary salt levels affect salt preference and learning in larval Drosophila. PLoS ONE 6, e20100.
Scherer, S., Stocker, R. F. and Gerber, B. (2003). Offactory learning in individuals assayed Drosophila larvae. Learn. Mem. 10, 217-225.
Schipsansi, A., Yarali, A., Niewalda, T. and Gerber, B. (2008). Behavioral analyses of sugar processing in choice, feeding, and learning in larval Drosophila. Chem. Senses 33, 563-573.
Schleyer, M., Saumweber, T., Nahrendorf, W., Fischer, B., von Alpen, D., Pauls, D., Thum, A. and Gerber, B. (2011). A behavior-based circuit model of how outcome expectations organize learned behavior in larval Drosophila. Learn. Mem. 18, 639-653.
Schleyer, M., Diegelmann, S., Michels, B., Saumweber, T. and Gerber, B. (2013). ‘Decision making’ in larval Drosophila. In Invertebrate Learning and Memory (ed. R. Menzel and P. R. Benjamini), pp. 41-55. London: Elsevier Ltd.
Schroll, C., Riemenesperger, T., Bucher, D., Ehrmer, J., Völker, T., Erbgrüth, B., Gerber, B., Hendel, T., Nagel, G., Buchner, E. et al. (2006). Light-induced activation of distinct modulatory neurons triggers appetitive or aversive learning in Drosophila larvae. Curr. Biol. 16, 1741-1747.
Selcho, M., Pauls, D., Han, K. A., Stocker, R. F. and Thum, A. S. (2009). The role of dopamine in Drosophila larval classical olfactory conditioning. PLoS ONE 4, e5897.
Strauss, H. (1929). Das Zusammenschrecken. J. Psychiatr. Psychol. 39, 111-231.
Vogelstein, J. T., Park, Y., Ohyama, T., Kerr, R. A., Truman, J. W., Priebe, C. E. and Zlatic, M. (2014). Discovery of brainwide neural-behavioral maps via multiscalpe unsupervised structure learning. Science 344, 388-392.
Wu, Z., Sweeney, L. B., Ayoob, J. C., Chak, K., Andreone, B. J., Ohyama, T., Kerr, R., Luo, L., Zlatic, M. and Kolodkin, A. L. (2011). A combinatorial semaphorin code instructs the initial steps of sensory circuit assembly in the Drosophila CNS. Neuron 70, 281-298.
Yeomans, J. S. and Frankland, P. W. (1995). The acoustic startle reflex: neurons and connections. Brain Res. Brain Res. Rev. 21, 301-314.
Zhang, W., Yan, Z., Jan, L. Y. and Jan, Y. N. S. M. (2013). Sound response mediated by the TRP channels NOMPC, NANCHUNG, and INACTIVE in chordotonal organs of Drosophila larvae. Proc. Natl. Acad. Sci. USA 110, 13612-13617.