Interneuronal Mechanism for Tinbergen’s Hierarchical Model of Behavioral Choice

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Summary

Recent studies of behavioral choice support the notion that the decision to carry out one behavior rather than another depends on the reconfiguration of shared interneuronal networks [1]. We investigated another decision-making strategy, derived from the classical ethological literature [2, 3], which proposes that behavioral choice depends on competition between autonomous networks. According to this model, behavioral choice depends on inhibitory interactions between incompatible hierarchically organized behaviors. We provide evidence for this by investigating the interneuronal mechanisms mediating behavioral choice between two autonomous circuits that underlie whole-body withdrawal [4, 5] and feeding [6] in the pond snail Lymnaea. Whole-body withdrawal is a defensive reflex that is initiated by tactile contact with predators. As predicted by the hierarchical model, tactile stimuli that evoke whole-body withdrawal responses also inhibit ongoing feeding in the presence of feeding stimuli. By recording neurons from the feeding and withdrawal networks, we found no direct synaptic connections between the interneuronal and motoneuronal elements that generate the two behaviors. Instead, we discovered that behavioral choice depends on the interaction between two unique types of interneurons with asymmetrical synaptic connectivity that allows withdrawal to override feeding. One type of interneuron, the Pleuro-Buccal (PIB), is an extrinsic modulatory neuron of the feeding network that completely inhibits feeding when excited by tactile contact while recording identified neurons from the feeding network. To understand how PIB might affect these alternative behaviors, we investigated the interactions of PIB with neurons of the withdrawal and feeding networks.

Stimulation of PIB activated whole-body withdrawal and simultaneously inhibited rhythmic feeding movements (example in Figure 2B; n = 6). A burst of spikes (Figure 2B) artificially evoked in PIB by current injection resulted in a single large contraction of the circumesophageal muscle, which is known to cause touch-induced whole-body withdrawal responses [4]. The same touch inhibited sucrose-driven rhythmic feeding movements of the buccal mass (feeding apparatus [10]). To understand how PIB might affect these alternative behaviors, we investigated the interactions of PIB with neurons of the withdrawal and feeding networks.

First, we asked how PIB drives withdrawal. We found that PIB is electrically coupled to motoneurons of the withdrawal-response network, and this plays a critical role in causing touch-induced withdrawal. A hyperpolarizing current pulse applied to PIB produced corresponding changes in membrane potentials of corecorded withdrawal motoneurons (Figure 2C1) that were located in several different ganglia of the CNS (Figure 2A). In the same preparation, application of lip touch caused a burst of spikes in PIB and motoneurons (Figure 2C2). Due to the extensive electrotonic connectivity of PIB with the withdrawal-response network, a current-induced burst of spikes in PIB depolarized the motoneurons and induced spiking (Figure 2C3) similar to that produced by touch. No other member of the withdrawal circuit was capable of eliciting withdrawal alone [5, 11]. It therefore seems reasonable to conclude that this behavioral response to touch results from a combination of distributed sensory input to all members of the withdrawal network [3] and the strong electrotonically mediated excitatory effects of PIB (Figure 2C3).

Next, we asked whether touch-induced burst responses in PIB are necessary for the touch-induced suppression of feeding in a sucrose-driven rhythm. Data supporting this necessity were obtained by recording PIB together with neurons of the feeding circuit, such as the B3 and B4 motoneurons (Figure 2A). By recording these motoneurons, we were able to monitor sucrose-driven “fictive feeding” activity, an in vitro correlate of behavioral feeding in the intact animal [10]. Motoneuronal bursts in response to sucrose were driven by synaptic inputs from the feeding central pattern generator (Figure 2D, expanded trace). PIB was normally silent (mean resting potential −75 ± 2.3 mV, n = 28), but experiments...
(n = 12) of the type shown in Figures 2D1 and 2D4 showed that PeD12 was strongly activated by touch. This was accompanied by a significant inhibition of the fictive feeding rhythm recorded in the B3 feeding motoneuron. An artificially induced burst of spikes in PeD12 had the same effect (Figure 2D2). There was no statistical difference in the inhibitory effect on feeding between these two methods of spike activation (Figure 2D3). To determine whether PeD12 is necessary for feeding inhibition, we compared the effects of touch with (Figure 2D5) and without (Figure 2D4) suppression of touch-induced PeD12 spikes. Statistical analysis showed that preventing PeD12 spikes by hyperpolarization removed the inhibition of fictive feeding by touch (Figure 2D6). We conclude that the touch-induced spiking of PeD12 is necessary for inhibition of feeding.

We compared the effects of touch on feeding in the semi-intact preparation in which feeding was monitored in vitro with the behavioral experiments using the same stimuli (Figure 1). There was no significant difference in the inhibitory effects of touch in the two types of experiments, justifying the use of the in vitro preparations for the neural analysis of the Tinnbergen choice mechanism (mean difference scores: behavioral, −3.1 ± 0.3, n = 16; in vitro, −2.5 ± 0.2, n = 17; Mann-Whitney test, U = 87, p = 0.07).

It was important to find out how PeD12 inhibited feeding because it was key to understanding how the two behavioral networks interacted. We found that there were no direct synaptic connections from PeD12 to neurons of the feeding network (Figure S2A). Instead, we showed that a PeD12 to PIB synaptic pathway mediated PeD12 inhibition of feeding, with PIB being the primary agent for feeding suppression. Evidence that PeD12 inhibited feeding via the PIB interneuron was obtained by corecording PeD12 and PIB and artificially manipulating their spike activity during a sucrose-driven rhythm (n = 3). Evoking a burst of spikes in PeD12 excited PIB, and this resulted in inhibition of feeding cycles recorded in the B3 motoneuron (Figure 3A1). Suppressing PIB activity by hyperpolarization prevented this inhibition (Figure 3A2), so PeD12 must have been acting via PIB. The ability of PIB alone to suppress feeding activity is shown in Figure 3A3, where a burst of spikes in PIB inhibited feeding in the absence of spike activity in PeD12.

These experiments suggest that PeD12 has an excitatory synaptic connection with PIB, and this was confirmed by showing that an artificially evoked burst of spikes in PeD12 drives an increase in the firing rate of PIB (Figure 3B1, left; n = 11). This connection was asymmetrical because there was no evidence of a corresponding synaptic connection from PIB to PeD12 (e.g., Figure 3B1, right) in the same preparation. More detailed experiments suggested that the PeD12-PIB synapse was chemically mediated and monosynaptic. Thus, calcium was required for transmission (n = 10) (Figure 3B2), and high concentrations of the divalent cations calcium and magnesium (Hi-Di saline), which blocked polysynaptic pathways [12, 13], did not block synaptic transmission (Figure 3B3, left; n = 8). When PIB spikes were suppressed by hyperpolarization in the same Hi-Di experiments, a slow depolarizing synaptic response was revealed (Figure 3B3, middle). Repeated triggering of single PeD12 spikes on a faster time base revealed the presence of short-latency 1:1 excitatory postsynaptic potentials (EPSPs) on PIB (Figure 3B3, right), also consistent with a monosynaptic connection. Dye-filling
Figure 2. The Interneuron PeD12 Plays a Key Role in Behavioral Choice by Activating Withdrawal and Inhibiting Feeding in Response to Touch

(A) The semi-intact head-brain preparation used for recording interneurons and motoneurons of the feeding and withdrawal-response networks. This preparation retains the sensory nerves that carry touch and chemical signals from the lips to the central motor circuits. Paired PeD12 and PlIB interneurons (light blue) are located in the pedal ganglia (PeG) and pleural ganglia (PlG), respectively. Feeding motoneurons, B3 and B4 (dark blue), are located in the buccal ganglia (BG). Motoneurons of the whole-body withdrawal network (yellow) are located in several CNS ganglia. The cerebral A cluster is the largest group (6–9 cells), with smaller numbers in the pedal G cluster (3–5 cells) and a single neuron (DLM) in the left parietal ganglion (LPaG). Other CNS ganglia are the right parietal ganglion (RPaG) and the visceral ganglion (VG).

(B) Responses to PeD12 stimulation recorded in the columellar muscle (CM) and the buccal mass (BM). The semi-intact preparation was used for these recordings, but for these experiments, the muscles involved in whole-body withdrawal (CM) and feeding ingestion (BM) were retained, and their contractions were recorded using a force transducer. Sucrose application drives rhythmic feeding movements in the BM until the evoking of a burst of spikes in PeD12 by current injection suppresses feeding despite the continued presence of sucrose. A single large contraction in the CM (*) is also caused by PeD12 stimulation (n = 6).

(C1–C3) Electrotonic coupling of PeD12 with motoneurons of the withdrawal-response network. Application of hyperpolarizing square current pulses to PeD12 causes similar but reduced responses in the three corecorded motoneurons (C1). Coupling coefficients recorded in the soma are 0.06 ± 0.01 (n = 5) between PeD12 and Parietal DLM motoneurons, 0.08 ± 0.1 (n = 5) between PeD12 and Pedal G cluster motoneurons, and 0.11 ± 0.02 (n = 12) between PeD12 and Cerebral A cluster motoneurons. Application of touch to the lips (C2) induces bursts of spikes in PeD12 and the three corecorded withdrawal-response motoneurons. A current-induced burst of spikes in PeD12 depolarizes the motoneurons and induces spiking in the motoneurons similar to that produced by touch (C3). All recordings shown in (C1)–(C3) are taken from the same preparation.

(D1–D6) Touch-induced spike activity in PeD12 with motoneurons of the withdrawal-response network. Application of hyperpolarizing square current pulses to PeD12 causes similar but reduced responses in the three corecorded motoneurons (C1). Coupling coefficients recorded in the soma are 0.06 ± 0.01 (n = 5) between PeD12 and Parietal DLM motoneurons, 0.08 ± 0.1 (n = 5) between PeD12 and Pedal G cluster motoneurons, and 0.11 ± 0.02 (n = 12) between PeD12 and Cerebral A cluster motoneurons. Application of touch to the lips (C2) induces bursts of spikes in PeD12 and the three corecorded withdrawal-response motoneurons. A current-induced burst of spikes in PeD12 depolarizes the motoneurons and induces spiking in the motoneurons similar to that produced by touch (C3). All recordings shown in (C1)–(C3) are taken from the same preparation.

(D1–D6) Touch-induced spike activity in PeD12 is both sufficient and necessary for inhibition of feeding. The expanded trace of a B3 fictive feeding burst shows the N1 (protraction), N2 (rasp), and N3 (swallow) phases of the feeding cycle (D1). The inhibition of feeding by touch (D1) is similar to that induced by artificial stimulation of PeD12 (D2), and there is no statistical difference in the two types of data (D3) (n = 6, mean difference scores: touch, −2.4 ± 0.2; PeD12 depolarization, −2.0 ± 0.3; Wilcoxon signed-rank test, W = 8, p = 0.2). Hyperpolarizing PeD12 to suppress spiking (D5) during touch prevents the inhibition of feeding by touch (D4), producing a statistically significant reduction in the difference score (D6) (n = 9, mean difference scores: touch, −2.2 ± 0.2; PeD12 hyperpolarization, −0.9 ± 0.2; Wilcoxon signed-rank test, W = 36, p = 0.014).

In this figure and in the following figures, horizontal bars indicate that either a depolarizing (black) or a hyperpolarizing (gray) square current pulse has been applied. Difference scores in this and other figures are calculated by subtracting the number of feeding bursts in the 20 s before touch from the number of bursts in the 20 s after touch. Error bars show ±SEM.
experiments revealed the sites of potential synaptic contacts between the two neurons (Figure S1). The arborization of PeD12 (Figure S1A1, red) and PIB (Figure S1A1, green) indicated two areas where the neurites intertwined. These were potential sites of the synaptic interactions. One of these areas was close to the cell body of the PeD12 cell (Figure S1A2), and the other was close to the PIB cell body (Figure S1A3). Together, these experiments provide evidence for a monosynaptic chemical pathway between PeD12 and PIB (Figure S3B4).

To validate the role of the PeD12-PIB synaptic pathway in behavioral choice, we had to show that PIB inhibits a sucrose-driven feeding rhythm. Although interneuron PIB inhibits feeding behavior [2], little is known about its sensory inputs [1-4], and in particular, about whether its response to strong tactile inputs is sufficient to suppress feeding rhythms. PIB fired tonically during sucrose application, but its baseline activity was insufficient to inhibit fictive feeding. A single touch stimulus produced a maintained depolarization of PIB and an increase in tonic firing (Figure 3C1). These touch-induced increases in PIB tonic firing rate resulted in an inhibition of the fictive feeding rhythm (Figures 3C1 and 3B1; n = 11). Similar inhibition was produced by an artificially evoked burst of spikes in PIB (Figure 3C2), indicating that increased firing in PIB was sufficient to suppress sucrose-induced feeding. A statistical comparison of the effects of touch versus the depolarization of PIB found that there was no difference in the inhibition of the fictive feeding responses produced by the two types of stimulation (Figure 3C3). The necessity for the touch-induced increase in firing of PIB for feeding inhibition was tested. PIB was hyperpolarized, and the effects of touch on fictive feeding were compared with (Figure 3D2) and without (Figure 3D1) hyperpolarization. There was a significantly smaller difference score in the hyperpolarized state (Figure 3D3). These results show that the increase in tonic firing in PIB induced by touch is both sufficient and necessary for the inhibition of feeding.

Finally, we showed that there were no synaptic connections between PIB and motoneurons of the withdrawal-response network (Figure S2B); therefore, PIB has no role in the control of whole-body withdrawal responses.

Discussion

Our results conform to the competitive model for behavioral selection originating in the ethological literature [2, 3] and provide an interneuronal mechanism for it. We propose that behavioral choice in response to conflicting sensory inputs depends on inhibitory synaptic interactions between autonomous networks that control incompatible behaviors. Extensive electrophysiological and anatomical investigations ([4-6]; Figure S2) show that the feeding and whole-body withdrawal circuits operate as autonomous units, consistent with the Tinbergen model. This type of inhibitory interaction between autonomous networks was suggested to occur in the mollusk Pleurobranchaea to explain the “dominance” of feeding over withdrawal [15, 16]. More recent studies [17, 18] have described the mechanism that mediates another type of competitive behavioral interaction in the same animal. Here, the dominance of swimming over feeding was shown to involve the asymmetrical synaptic inhibition of the feeding central pattern generator (CPG) circuit by a CPG interneuron from the swim circuit [17]. This differs from our example, where interneurons extrinsic to the feeding network are involved (Figure 4). The switch from feeding to defensive withdrawal is mediated by two identified interneurons with asymmetrical synaptic connectivity that allows withdrawal to always over-ride feeding. One of these interneurons (PIB) completely inhibits the feeding rhythm when it is excited by the second of the two neurons (PeD12). Crucially, PeD12 also plays an important role in driving the whole-body withdrawal behavior, and it is responsive to strong tactile stimuli that evoke the defensive behavior. This pivotal neuron therefore has a dual function: in response to a strong aversive stimulus, it simultaneously activates the withdrawal motor circuit, acting as an extrinsic modulatory interneuron, and monosynaptically excites the PIB, which enhances tonic inhibition to the feeding motor circuit to suppress feeding. Thus, this simple asymmetric circuit joins the two motor networks and underlies the dominance of defensive withdrawal. By activating PeD12, the animal can simultaneously shut down grazing and initiate whole-body defensive withdrawal.

Defensive withdrawal of the whole animal is known to be at the top of the behavioral choice hierarchy in Lymnaea [19], so it would be expected that the inhibitory connection is asymmetrical to achieve this dominance. This differs from the original Tinbergen model, where reciprocal inhibition was proposed to prevent two behaviors being coexpressed. The reciprocal inhibition model is more likely to occur when inhibitory interactions between two autonomous behaviors require a more flexible relationship [20]. With high-value defensive behaviors, it is imperative that they override all other behaviors to prevent predation so that asymmetrical inhibitory interactions are present rather than reciprocal inhibition. In our experiments, we used starved animals to increase their responsiveness to food, but, despite this manipulation, food-driven feeding rhythms were still inhibited by touch. Tinbergen [2] considered that behavioral state would be an important determinant of behavioral selection, but our data suggest that aversive sensory stimulation triggering life-preserving behavioral responses overrides the effects of behavioral state.

Our Lymnaea example of behavioral choice is fundamentally different to other systems where the alternative behaviors share elements of one another’s circuits. Here, behavioral choice depends on overlapping combinations of interneurons that are active during different behaviors. In the leech (Hirudo), for example, the selection of one of four different behaviors (swimming, shortening, crawling, or bending) depends on a unique combination of firing in the same interneurons [21].

A key feature of all those systems that involve network configuration is that similar groups of muscles and motoneurons are used in various combinations, so the units of motor control are not unique to a particular behavior. The Tinbergen model occurs when the elements of motor control are autonomous, and selection depends on hierarchically based control mechanisms, where behaviors are selected by inhibition of less-valued behaviors.

In conclusion, two distinctly different models have been proposed to explain how switching between behaviors is achieved at the level of neuronal networks. The selection of different combinations of active neurons in shared wider networks determines which of a limited subset of behaviors is expressed where there is significant overlap in the neuronal machinery and muscles controlling more than one behavior. The inhibition of one circuit by another determines the behavioral outcome when the choice is made between behaviors controlled by dedicated nonoverlapping networks. Our example is an interesting case of the second model. Moreover, it provides insight into the cellular and synaptic details of the way inhibition mediates behavioral choice. For example, our
Figure 3. Monosynaptic Connection between PeD12 and PlB Mediates the Touch-Induced Inhibition of Feeding, and PlB is Both Sufficient and Necessary for Inhibition of a Sucrose-Driven Feeding Rhythm

(A1–A3) PeD12 inhibition of sucrose-driven fictive feeding is due to the excitation of the PlB interneuron (A1). A current-evoked burst of spikes in PeD12 increases tonic firing in the PlB interneuron and suppresses rhythmic bursting in the B3 motoneuron. The inhibition of the feeding pattern by PeD12 is

(legend continued on next page)
results suggest that there is an important role for the modulation of tonic inhibition in explaining the hierarchical coupling between behavioral responses to aversive and rewarding sensory stimuli. We therefore suggest that the regulation of tonic inhibition by interneurons constitutes a common mechanism that is central to adaptive behavioral switching in other systems [22, 23].

Experimental Procedures

Experimental Animals

Animals from a laboratory-bred stock of *Lymnaea stagnalis* were used in the experiment. Details of their maintenance are described in the Supplemental Experimental Procedures.

Behavior

Animals were starved for 2 days before the experiments. Sucrose-driven feeding activity was initiated by perfusion of 0.02 mM sucrose. Von Frey hairs (4 g) were used to induce whole-body withdrawal. The procedure was video recorded and analyzed using ImageJ software. Feeding scores were calculated by subtracting the number of feeding cycles in the 20 s after the touch from the number of cycles in the 20 s before.

Preparations

Experiments were performed on semi-intact preparations containing the entire CNS and attached lips and tentacles (Figure 2A) [8, 24–26]. A modified semi-intact preparation, containing the main feeding muscle (buccal mass) and the columellar muscle, responsible for the whole-body withdrawal was also used to measure contractions induced by neuronal stimulation. A detailed description of preparations, stimulation and recording protocols, explanation of choice of neurons recorded, and data analysis methods are described in the Supplemental Experimental Procedures.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and two figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.07.044.

Author Contributions

Z.P. and M.C. carried out the electrophysiological experiments with the assistance of Z.L. M.C. carried out the behavioral experiments. S.N. did the confocal microscopy. G.K. and M.O. were involved in planning the experiments, discussing the results, and writing the manuscript. P.R.B. and I.K. each played a major role in the design of the experiments, analysis of the data, and production of the manuscript.

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References

1. Kristan, W.B. (2008). Neuronal decision-making circuits. Curr. Biol. 18, R928–R932.

2. Tinbergen, N. (1951). The Study of Instinct (New York: Oxford University Press).

3. McCleery, R. (1983). Interactions between activities. In Animal Behaviour, P.J.B. Slater and T.R. Halliday, eds. (Oxford: Blackwell), pp. 132–165.
4. Ferguson, G.P., and Benjamin, P.R. (1991). The whole-body withdrawal response of Lymnaea stagnalis. II. activation of central motoneurones and muscles by sensory input. J. Exp. Biol. 158, 97–116.
5. Ferguson, G.P., and Benjamin, P.R. (1991). The whole-body withdrawal response of Lymnaea stagnalis. I. identification of central motoneurones and muscles. J. Exp. Biol. 158, 63–95.
6. Benjamin, P.R. (2012). Distributed network organization underlying feeding behavior in the mollusk Lymnaea. Neural Syst. Circuits 2, 4.
7. Kemenes, I., Straub, V.A., Nikitin, E.S., Staras, K., O’Shea, M., Kemenes, G., and Benjamin, P.R. (2006). Role of delayed nonsynaptic neuronal plasticity in long-term associative memory. Curr. Biol. 16, 1269–1279.
8. Mara, V., O’Shea, M., Benjamin, P.R., and Kemenes, I. (2013). Susceptibility of memory consolidation during lapses in recall. Nat. Commun. 4, 1578.
9. Alania, M., Sakharov, D.A., and Elliott, C.J. (2004). Multilevel inhibition of feeding by a peptidergic pleural interneuron in the mollusc Lymnaea stagnalis. J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. 190, 379–390.
10. Rose, R.M., and Benjamin, P.R. (1979). The relationship of the central motor pattern to the feeding cycle of Lymnaea stagnalis. J. Exp. Biol. 80, 137–163.
11. Syed, N.I., and Winlow, W. (1991). Coordination of locomotor and cardiorespiratory networks of Lymnaea stagnalis by a pair of identified interneurones. J. Exp. Biol. 158, 37–62.
12. Getting, P.A. (1981). Mechanisms of pattern generation underlying swimming in Tritonia. I. neuronal network formed by monosynaptic connections. J. Neurophysiol. 46, 65–79.
13. Elliott, C.J., and Benjamin, P.R. (1989). Esophageal mechanoreceptors in the feeding system of the pond snail, Lymnaea stagnalis. J. Neurophysiol. 61, 727–736.
14. Alania, M., Dyakonova, V., and Sakharov, D.A. (2004). Hyperpolarization by glucose of feeding-related neurons in snail. Acta Biol. Hung. 55, 195–200.
15. Kovac, M.P., and Davis, W.J. (1977). Behavioral choice: neural mechanisms in Pleurobranchaea. Science 198, 632–634.
16. Kovac, M.P., and Davis, W.J. (1980). Neural mechanism underlying behavioral choice in Pleurobranchaea. J. Neurophysiol. 43, 469–487.
17. Jing, J., and Gillette, R. (1995). Neuronal elements that mediate escape swimming and suppress feeding behavior in the predatory sea slug Pleurobranchaea. J. Neurophysiol. 74, 1900–1910.
18. Jing, J., and Gillette, R. (2000). Escape swim network interneurons have diverse roles in behavioral switching and putative arousal in Pleurobranchaea. J. Neurophysiol. 83, 1346–1355.
19. Winlow, W., Moroz, L.L., and Syed, N.I. (1992). Mechanisms of behavioural selection in Lymnaea stagnalis. In Neurobiology of Motor Programme Selection, J. Kien, C.R. McCrohan, and W. Winlow, eds. (Oxford: Pergamon Press), pp. 55–72.
20. Anderson, D.J. (2012). Optogenetics, sex, and violence in the brain: implications for psychiatry. Biol. Psychiatry 71, 1081–1089.
21. Kristan, W.B., and Gillette, R. (2007). Behavioural choice. In Invertebrate Neurobiology, G. North and R.J. Greenspan, eds. (Cold Spring Harbor: Cold Spring Harbor Laboratory Press), pp. 533–553.
22. Grillner, S., Hellgren, J., Ménard, A., Saitoh, K., and Wikström, M.A. (2005). Mechanisms for selection of basic motor programs—roles for the striatum and pallidum. Trends Neurosci. 28, 364–370.
23. Benjamin, P.R., Staras, K., and Kemenes, G. (2010). What roles do tonic inhibition and disinhibition play in the control of motor programs? Front. Behav. Neurosci. 4, 30.
24. Kemenes, G., Staras, K., and Benjamin, P.R. (1997). In vitro appetitive classical conditioning of the feeding response in the pond snail Lymnaea stagnalis. J. Neurophysiol. 78, 2351–2362.
25. Staras, K., Kemenes, G., and Benjamin, P.R. (1999). Cellular traces of behavioral classical conditioning can be recorded at several specific sites in a simple nervous system. J. Neurosci. 19, 347–357.
26. Kemenes, I., Kemenes, G., Andrew, R.J., Benjamin, P.R., and O’Shea, M. (2002). Critical time-window for NO-cGMP-dependent long-term memory formation after one-trial appetitive conditioning. J. Neurosci. 22, 1414–1425.