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Report

Flies Cope with Uncontrollable Stress by Learned Helplessness

Zhenghong Yang,[1,2] Franco Bertolucci,[2,3] Reinhard Wolf,[1] and Martin Heisenberg[1,*]
[1]Rudolf-Virchow-Center, Joseph-Schneider-Strasse 2, 97080 Würzburg, Germany

Summary

In a wide range of animals, uncontrollable stressful events can induce a condition called "learned helplessness." In mammals it is associated with low general activity, poor learning, disorders of sleep and feeding, ulcers, and reduced immune status, as well as with increased serotonin in parts of the brain. It is considered an animal model of depression in humans [1–4]. Here we investigate learned helplessness in *Drosophila*, showing that this behavioral state consists of a cognitive and a modulatory, possibly mood-like, component. A fly, getting heated as soon as it stops walking, reliably resumes walking to escape the heat. If, in contrast, the fly is not in control of the heat, it learns that its behavior has no effect and quits responding. In this state, the fly walks slowly and takes longer and more frequent rests, as if it were "depressed." This downregulation of walking behavior is more pronounced in females than in males. Learned helplessness in *Drosophila* is an example of how, in a certain situation, behavior is organized according to its expected consequences.

Results

Learned helplessness was first studied in dogs [1] and later in many other species, notably rat, mouse, fish, cockroach, and slug. In a typical experimental design, two groups of animals ("master" and "yoked") are exposed to electric shocks. Master animals can terminate the shock by their behavior. Yoked animals receive the same sequence and amount of electric shocks but have no influence on their onset and duration. Subsequently, learning performance of the two groups is compared in the same or a different experimental setting. In contrast to the master animals, those of the yoked group do not try to escape the electric shocks at this stage and are generally inactive.

In order to deal with a stressful or dangerous event, an animal needs in its behavioral repertoire a suitable module or strategy (e.g., avoidance, escape). Often, however, the situation does not immediately reveal what the right behavior might be. In this case, the animal may just try out something. It generates behavior and searches for changes in the occurrence of the event that coincide with its own activity. Once it finds a correlation, it labels the respective behavioral module "effective" for further use with similar events (operant learning). Consistently effective behaviors may gradually turn into responses and, on an evolutionary timescale into sensory-motor reflexes. Some events, however, are persistently uncontrollable. For this important condition, animals have developed a special kind of memory called "learned uncontrollability."

Learned uncontrollability must be a basic behavioral property. It has even been described for highly reduced animal preparations: a single cockroach leg connected to the corresponding isolated thoracic ganglion can be operantly trained to remain lifted [5, 6]. If such a preparation is subjected to uncontrollable electric shocks before the conditioning, acquisition takes much longer than in naive preparations or in pretrained ones that have received exactly the same sequence of electric shocks [5]. Apparently, learned uncontrollability can be closely linked to the behavior in question and stored in the vicinity of the pattern generator for this behavior. (We call learned uncontrollability a "cognitive state" because it represents implicit knowledge about the relation between the animal and its environment.)

We show here that learned helplessness in *Drosophila* is more than learned uncontrollability. In addition to the cognitive part, it has a modulatory, motivational component that shows in the low behavioral activity of the animal in this state. Learned uncontrollability, the cognitive part, has briefly been described before [7].

We have developed a new learning paradigm called no-idleness learning. A fly in a small chamber [8] (Figure 1A) receives heat if it stops walking for more than 1 s (idle allowance). This is long enough to be taken as a moment of rest and short enough to make it easy for the fly to note the coincidence between the stop and the onset of heat. With an idle allowance of 2 s, the fly needs significantly longer to learn the relation (see Figure S1 available online; two-sample t test; $p < 0.001$). The fly can instantly terminate the heat by resuming locomotion (master fly; Figure 1B). A second fly (yoked; Figure 1C) receives the same sequence of heat and no heat as its master but has no control of onset and duration. After 10 min of conditioning for master and yoked flies, locomotion is measured without heat for 30 s (Figure 2).

Resting master flies hit by the heat resume walking about 1 s after the onset (escape latency), i.e., after about 2 s of rest (idle event = idle allowance + escape latency; for definition of "idle event," see Supplemental Information). In most cases, responses are fast enough not to let the heat reach its peak value of 37°C. Over the 10 min training period, the mean escape latency gradually increases to 1.7 s (Figure 2A). Flies may adapt to the mean temperature and therefore tolerate a longer rise. Escape latencies have a very small variance throughout the conditioning period, showing the high reliability of the response.

Due to their escape responses, master flies have shorter idle events than the control flies (Figure 2A) and hence would get less overall rest during the 10 min. Yet they actually get even more (Figure 2C). They overcompensate by increasing the frequency of idle events (Figure 2B). Maybe short stops provide less rest than longer ones. Alternatively, heat pulses increase the demand for rest.

For yoked flies, the pretest phase (P) without heat pulses does not differ from that of master flies. In the training phase also, the heat pulses do not differ between yoked and master

[2]These authors contributed equally to this work
[3]Present address: Seckenheimerhauptstrasse 197a, 68239 Mannheim, Germany
*Correspondence: heisenberg@biozentrum.uni-wuerzburg.de



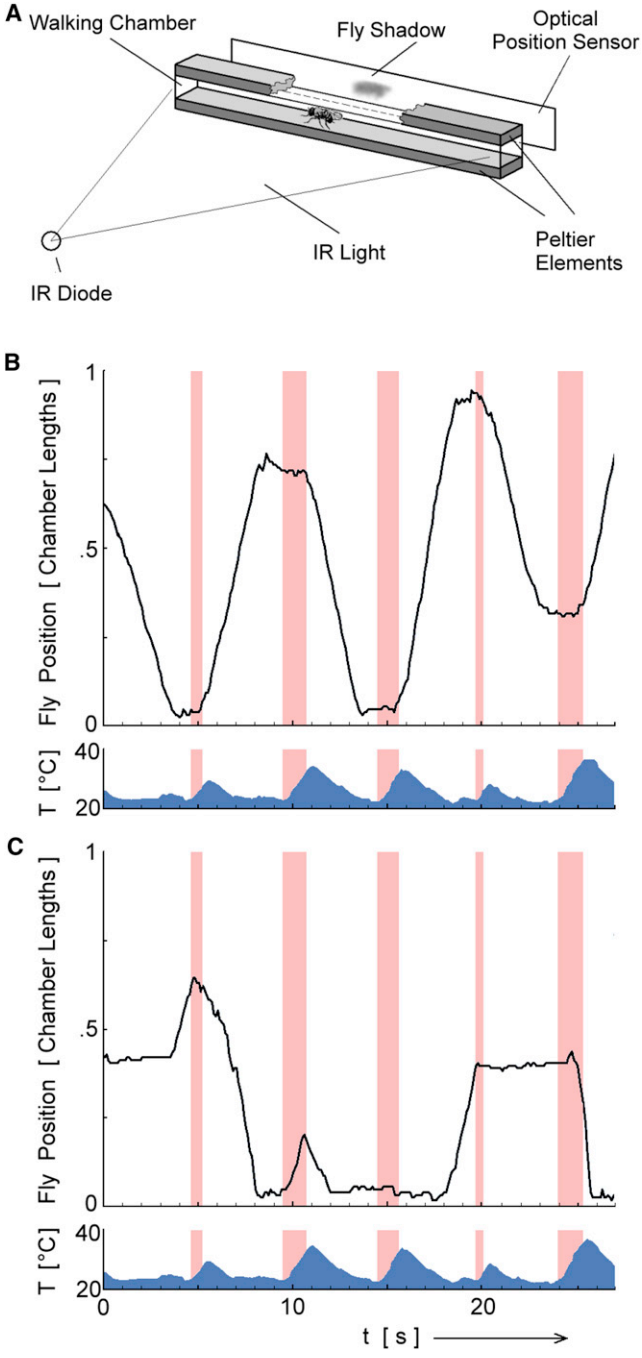


Figure 1. Walking Behavior in Heat Box

(A) Schematic of heat box. Single flies can walk back and forth in darkness. Their position is continuously recorded, and their temperature can be switched between 24°C and 37°C (heat shock).

(B and C) Time traces (black curves) of fly position in chamber. Vertical pink bands: time periods of heat "ON." In the lower boxes, the upper margin of the blue area indicates the actual temperature T(t). Master flies are punished by heat shock if they rest for more than 1 s (B). Resuming locomotion turns off the heat instantly. Yoked flies, if hit by the heat during walking, may turn around and walk in the opposite direction (first and second encounter) (C).

flies, but for the yoked flies, the pulses come and go independently of their own behavior. In rare cases, yoked flies happen to be hit by a heat pulse after about 1 s of idleness. If this is the first encounter with heat, the experience of these flies is, at this moment, still indistinguishable from that of master flies. They start walking in order to escape the heat. In Figure 3A, we compare the mean response latencies for yoked flies with those of master flies, considering all those cases in which the yoked fly has generated an idle allowance of 1 s or longer at the onset of the heat pulse. For the different master and yoked pairs, these paired events occur at different times in the conditioning period, and different numbers of nonmatching events are interspersed (not shown in Figure 3A). As the data show, the yoked flies quickly learn that they have no influence on the duration of the heat pulses and quit responding (long escape latencies of yoked flies in Figure 3A). Already in the first event, which on average is preceded by five events that did not meet the criteria for the yoked flies, the mean escape latency in the yoked flies is significantly longer than in the master flies (Mann-Whitney U test, $p < 0.05$). Control experiments show that in master flies, a longer idle allowance does not lead to a longer response latency (Figure S1), except in the first minute of training. We conclude that the elevated response latency in yoked flies after the first few paired events must be due to the lack of correlation between the flies' actions and the changes in temperature. In other words, a few uncorrelated events are enough to substantially loosen the stimulus-response contingency in escape behavior.

If heat arrives while a yoked fly is walking, the fly often turns around immediately (first heat encounter in Figure 1C). It is tempting to assume that the fly interprets the increasing temperature during forward walking as a spatial gradient. As the heat may continue to rise after the turnaround, the fly occasionally even resumes the previous direction. Turnarounds to increasing heat during walking are more frequent at the beginning of the training phase than later (Figure 3B; see Figure S3 for detection of turnarounds). Flies seem to learn that rising heat during walking does not reflect spatial gradients and suppress turnaround responses. Idle events of yoked flies resemble, in their mean duration and large variance, those in control flies (Figure 2A; two-sample t tests; $p > 0.05$) but are significantly more frequent (Figure 2B).

In all three groups—master, yoked, and controls—walking activity decreases over the 10 min conditioning period (Figure 2C). Controls are tested at 27°C to keep the mean temperature during training similar to that of master and yoked flies (24°C–37°C). At 24°C the decrease is very similar to that at 27°C, as shown in a separate experiment (Figure S2).

Interestingly, yoked flies in the experiment of Figure 2 reduce their activity distinctly more (59%) than master flies (44%). Note that the mean amount of heat in the conditioning phase is exactly the same for master and yoked flies. Slight differences of the individual heat boxes, as shown in Figures 1B and 1C, are compensated by having the same number of master and yoked flies tested in each heat box. The different walking activity cannot be attributed to the escape responses of the master flies because this effect is overcompensated by the frequency of stops, as pointed out above (Figure 2C). It can only be attributed to a larger demand for rest from the yoked flies.

Once the conditioning period is over, the frequency of idle events of the master and yoked flies drops to the level in the controls (Figure 2B), suggesting that the increase during conditioning is largely responsive. However, the difference in activity between master and yoked flies persists. Yoked flies still are distinctly less active than master flies (Figure 4A; ANOVA post hoc; ***$p < 0.001$). Interestingly, yoked flies also

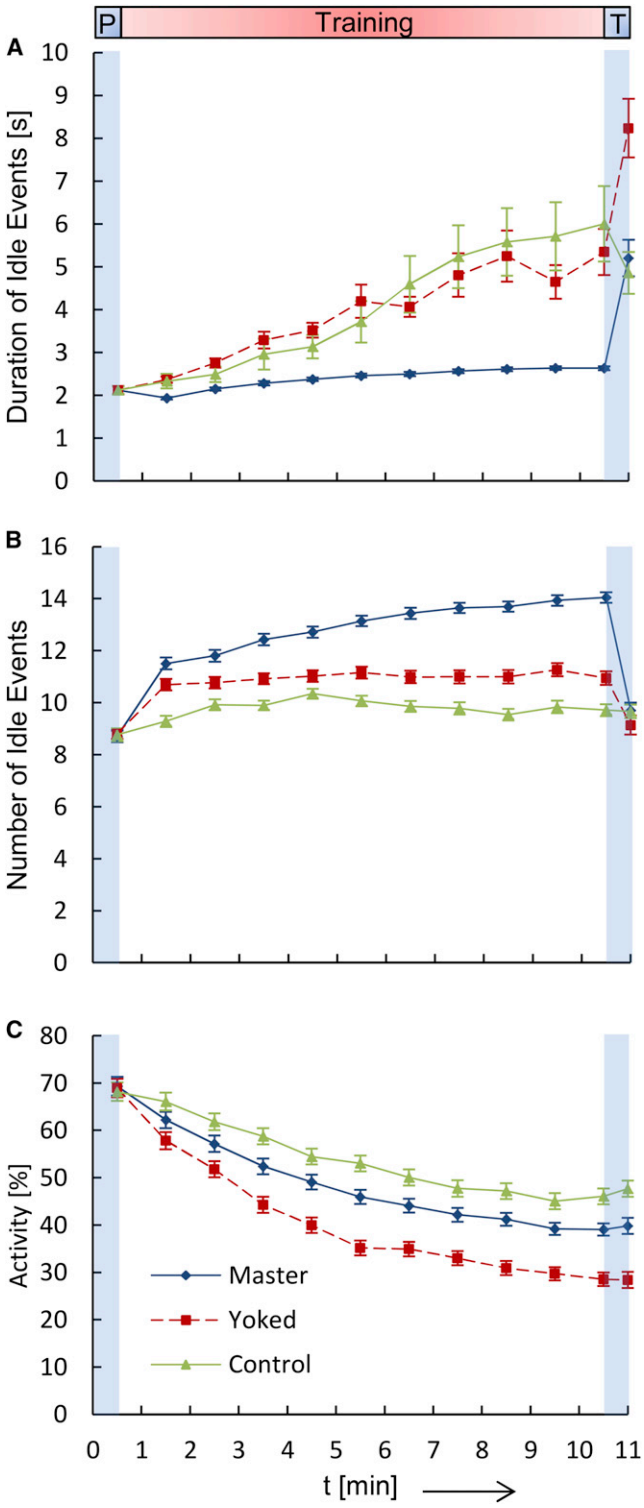


Figure 2. Walking Activity and Rest Periods of Master, Yoked, and Control Flies in the Heat Box

Data points refer to 0.5 or 1 min measuring periods and are shown at the end of the respective periods. Top: experimental time course; P: pretest without heat pulses; conditioning: master and yoked pairs receive the same heat pulses, and control flies get no heat pulses; T: test, no heat.

(A) Mean durations of rest periods > 1 s (rest periods = idle events; flies are scored as idle as long as their position has changed by less than 0.86 mm. (For further definition see Supplemental Explanation of Terms.) Throughout training, the durations of idle events do not differ significantly between yoked and control flies (paired t tests).Values are the means of the mean durations of idle events of the individual flies.

(B) Mean number of idle events per min.

(C) Activity (% time spent walking; activity = 1 − ∑idle events). Only female data are shown. n = 180 for master and yoked pairs and controls. Error bars in this and the following figures represent SEMs. (If not visible, error bars are smaller than symbols.)

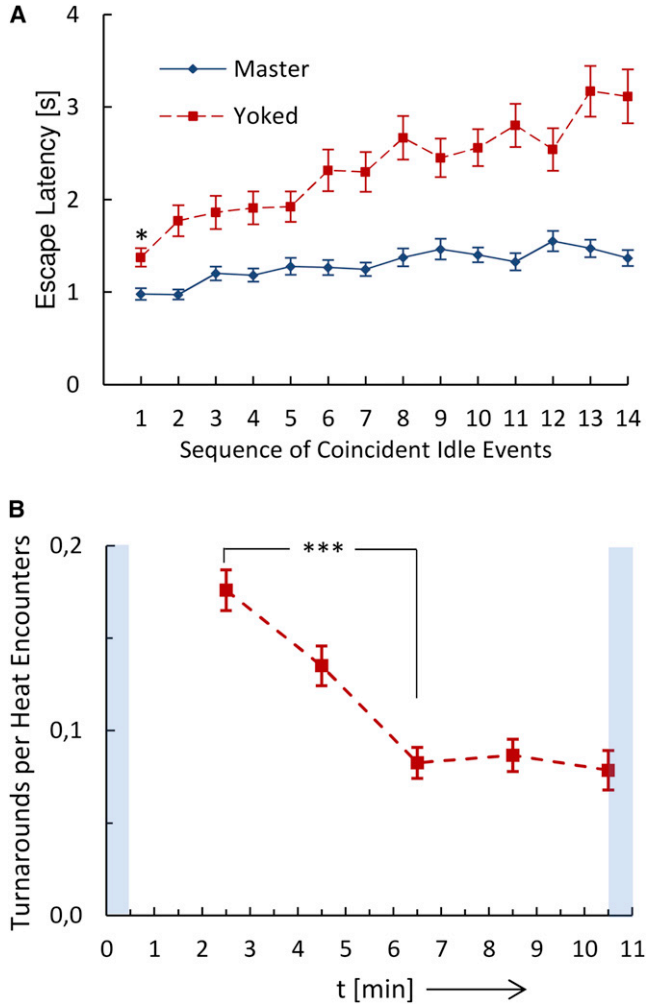


Figure 3. Innate Responses Are Suppressed if They Have No Effect

(A) Escape latencies of master and yoked flies for the first 14 coincident events. Events are coincident if the yoked fly is idle for at least 1 s when hit by the heat pulse. Escape latencies are significantly different between master and yoked flies. At the first coincident event, this difference (Mann-Whitney U test; $p < 0.05$) may be in part due to the variable idle period prior to the heat pulse in yoked flies (see Figure S1). The same data set is used as in Figure 2. n = 180 for all events.

(B) Turnaround responses of yoked females during heat encounters. A heat encounter is scored if the fly has been walking for 1 s when heat is switched on. A turnaround has to occur within 2 s after heat onset to be scored. n = 190 females (including the 180 flies of Figures 2 and 3A). To facilitate statistical evaluation, we pooled data for 2 min periods. See Figure S4 for a definition of "turnarounds."

walk more slowly than master flies in this phase (Figure 4B). This low walking speed is quite remarkable, considering that it lies even below the range of slow-walking mutant flies [9]. It is the previous experience of uncontrollability that has left the yoked flies in this state.

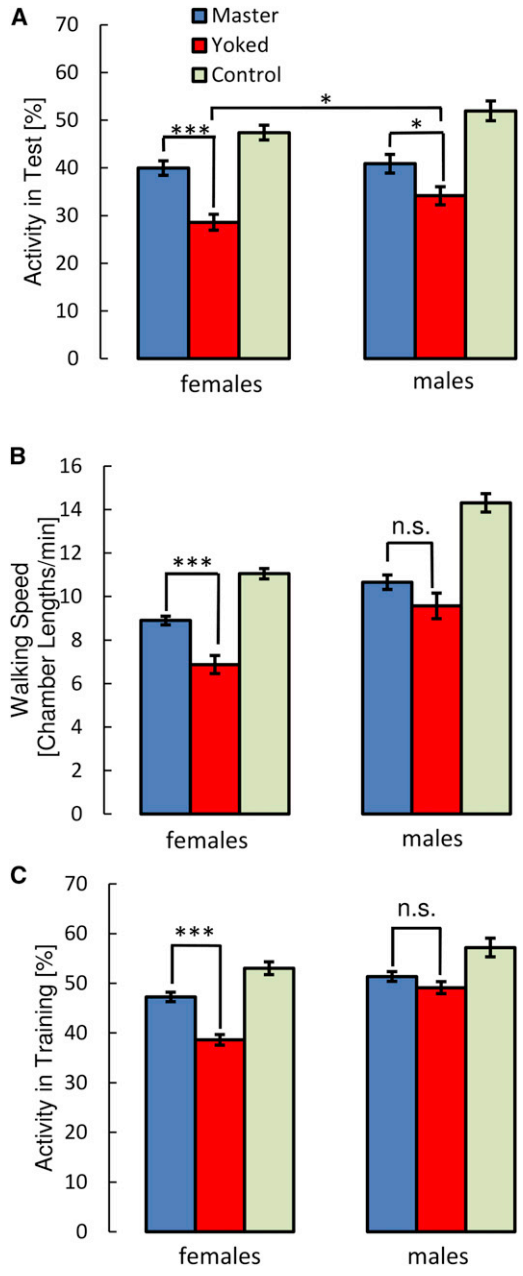


Figure 4. Females Are More Affected by Learned Helplessness Than Males

Comparison of walking activity and walking speed of master (blue), yoked (red), and control (green) flies.

(A) Walking activity during 30 s test after training (no heat pulses). Activity is defined as in Figure 2C. For master flies, no difference is found between females and males, whereas activity is significantly lower in yoked females than in yoked males (one-way ANOVA; post hoc analysis; $p < 0.05$).

(B) Mean walking speed (distance walked during time of walking) for test after training is different for master and yoked females ($p < 0.001$), but not males ($p > 0.05$; ANOVA post hoc).

(C) Mean walking activity during training (for time course in females, see Figure 2C). There was no significant difference between master and yoked males (ANOVA post hoc; $p > 0.05$). Female master and yoked pairs: n = 180; male master and yoked pairs: n = 143; control females: n = 180; control males: n = 126). ***$p < 0.001$; *$p < 0.05$; n.s., $p > 0.05$.

Because learned helplessness in mammals and the occurrence of depression in humans both show gender differences, we scored females and males separately. Data so far have been from females. The walking behavior of males in the heat box is quite similar. For instance, during the conditioning phase in both genders, master and yoked flies have the same mean walking speed (Figure S4). With respect to learned helplessness, however, the data reveal interesting differences (Figure 4). In the final test after training in the yoked group, females are significantly less active than males, although no female/male difference is found in the master group (Figure 4A; $p < 0.05$ between yoked flies; no significance [n.s.] between master flies; ANOVA post hoc). A similar gender difference is observed for walking speed in the test. Whereas in yoked females the suppression is substantial, it is not significant in males (Figure 4B; ANOVA post hoc). Finally, yoked males hardly reduce walking activity at all over the 10 min training period compared to master males (Figure 4C).

Discussion

Most studies on learned helplessness measure learned uncontrollability, and motivational effects are just mentioned [4]. Learned uncontrollability is a special kind of operant learning. Trying to control external stimuli by behavioral actions and strategies is costly in time and metabolic energy. Persistent uncontrollability should therefore eventually lead to a suppression of these futile attempts. In the heat box, the suppression is directly observed for escape and turnaround responses (Figures 3A and 3B). Whether the flies try other behaviors in addition is not known. Both responses are innate, because the flies have not experienced any heat pulses or other external stressors before the experiment to have learned them. The suppression of the escape responses in females can be sufficiently complete to impair subsequent place learning in the heat box [10].

What makes learned helplessness in *Drosophila* particularly interesting is that the suppression of innate responses is accompanied by a reduction of walking activity and walking speed. The cognitive state of operant memory and the motivational state of passivity can be separately and quantitatively documented. Both components had contributed to the term "learned helplessness" in mammals.

Earlier studies have reported an "immunization effect" in learned helplessness [11]. If the animal first experiences the stressor as controllable and only later as uncontrollable, it does not give up trying as readily as with only the second experience. It will be interesting to find out whether the cognitive and the motivational components show the immunization effect to the same extent. Also, how closely the motivational component in flies resembles that in mammals further research must tell. Learned helplessness may have developed early in the evolution of animal behavior, well before the separation of the vertebrates and arthropods. Already at that time it was the major task of the brain to organize the animal's behavior.

Evidently, the behavior described here is not a disease or trauma. The fly copes with a stressful situation for which it has no ready-made answer in its behavioral repertoire (see also [4]). The suppression of escape and turnaround responses as well as the reduction of walking speed and activity are the fly's adaptations to 10 min of being trapped in a small, dark hole and receiving dangerous heat pulses. The fly cannot do anything about the heat pulses. It can only try to optimize the balance between enduring and not missing a chance for escape. This balance may be different for males and females.

Experimental Procedures

Apparatus

The fly is confined to a small chamber (heat box [8]; inner size: 27.5 × 4 × 2 mm; Figure 1A) in complete darkness. The position of the fly's infrared (IR) shadow is recorded at a resolution of 0.2 mm using a linear optical sensor and an IR-LED. The fly is considered "idle" (not active) as long as the position signal has changed by less than 0.86 mm. Flies not active throughout the experiment are excluded from evaluation. Temperature in the chamber is controlled by two Peltier elements, above and below the fly, that are switched between 24°C and 37°C (punishment). Heat is switched on and off synchronously in two chambers by the behavior of the fly in one of the two chambers (master fly). Heat is switched on if the master fly rests for more than 1 s (idle allowance); it is switched off as soon as the fly resumes walking.

Animals

Canton-S flies of the Würzburg stock collection are grown on standard cornmeal fly food at 25°C and 60% relative humidity in a 14:10 hr light:dark cycle. They are 3–6 days old and have been kept as mixed groups on fresh food vials for 48 hr before the experiment. Our fly maintenance was regularly supervised by the government agency FB-Verbraucherschutz, Veterinärwesen und Lebensmittelüberwachung, Würzburg.

Statistical Treatment

All measurements in Figures 2, 3, and 4 are given as means and SEMs of n flies. Independent groups of data are compared by Mann-Whitney U test, two-sample t tests, or one-way ANOVA (post hoc analysis), where applicable. Statistical significance is shown as $*p < 0.05$, $**p < 0.01$, or $***p < 0.001$.

Supplemental Information

Supplemental Information includes four figures, Supplemental Explanation of Terms, Author Contributions, and Author Information and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.03.054.

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References

1. Seligman, M.E., and Maier, S.F. (1967). Failure to escape traumatic shock. J. Exp. Psychol. *74*, 1–9.
2. Maier, S.F., and Watkins, L.R. (2005). Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. Neurosci. Biobehav. Rev. *29*, 829–841.
3. Willner, P. (1986). Validation criteria for animal models of human mental disorders: learned helplessness as a paradigm case. Prog. Neuropsychopharmacol. Biol. Psychiatry *10*, 677–690.
4. Eisenstein, E.M., and Carlson, A.D. (1997). A comparative approach to the behavior called "learned helplessness." Behav. Brain Res. *86*, 149–160.
5. Horridge, G.A. (1962). Learning of leg position by the ventral nerve cord in headless insects. Proc. R. Soc. Lond. B Biol. Sci. *157*, 38–52.
6. Eisenstein, E.M., and Cohen, M.J. (1965). Learning in an isolated prothoracic insect ganglion. Anim. Behav. *13*, 104–108.
7. Brown, G.E., Mitchell, A.L., Peercy, A.M., and Robertson, C.L. (1996). Learned helplessness in *Drosophila melanogaster?* Psychol. Rep. *78*, 962–963.
8. Wustmann, G., Rein, K., Wolf, R., and Heisenberg, M. (1996). A new paradigm for operant conditioning of *Drosophila melanogaster*. J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. *179*, 429–436.
9. Wosnitza, A., Bockemühl, T., Dübbert, M., Scholz, H., and Büschges, A. (2013). Inter-leg coordination in the control of walking speed in *Drosophila*. J. Exp. Biol. *216*, 480–491.
10. Bertolucci, F. (2008). Operant and classical learning in *Drosophila melanogaster*: the *ignorant* gene (*ign*). Dissertation, Würzburg University, Würzburg, Germany.
11. Seligman, M.E.P., Rosellini, R.A., and Kozak, M.J. (1975). Learned helplessness in the rat: time course, immunization, and reversibility. J. Comp. Physiol. Psychol. *88*, 542–547.