Research Results in Pharmacology

Research Results in Pharmacology 8(4): 185–196
UDC 615.212
DOI 10.3897/rrpharmacology.8.91390

Research Article

Classical analgesic drugs modulate nociceptive-like escape behavior in *Drosophila melanogaster* larvae

Thamyris Santos-Silva[1], Caio Fábio Baeta Lopes[1], Jennifer Diniz Soares Guimarães[2], Felipe Berti Valer[3], Gustavo Campos Silva e Kuhn[4], Thiago Roberto Lima Romero[1], Lígia Araújo Naves[2], Igor Dimitri Gama Duarte[1]

1 *Department of Pharmacology, Institute of Biological Science, Federal University of Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil*

2 *Department of Physiology and Biophysics, Institute of Biological Science, Federal University of Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil*

3 *Department of Cellular and Molecular Biology, Ribeirão Preto Medical School, University of São Paulo, São Paulo, SP, 14049-900, Brazil*

4 *Department of Genetics, Ecology and Evolution, Institute of Biological Science, Federal University of Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil*

Corresponding author: Igor Dimitri Gama Duarte (dimitri@icb.ufmg.br)

Academic editor: Oleg Gudyrev ♦ **Received** 9 August 2022 ♦ **Accepted** 5 December 2022 ♦ **Published** 22 December 2022

Citation: Santos-Silva T, Lopes CFB, Guimarães JDS, Valer FB, Kuhn GCS, Romero TRL, Naves LA, Duarte IDG (2022) Classical analgesic drugs modulate nociceptive-like escape behavior in *Drosophila melanogaster* larvae. Research Results in Pharmacology 8(4): 185–196. https://doi.org/10.3897/rrpharmacology.8.91390

Abstract

Introduction: Nociceptive stimulus triggers escape responses in *Drosophila melanogaster* larvae, characterized by 360° rolling behavior along its own body axis. Therefore, it is possible to study analgesic drugs based on this stereotypical nociceptive-like escape behavior. Here, we aimed to develop an analgesic predictive validity test of thermal nociception through *D. melanogaster* larvae.

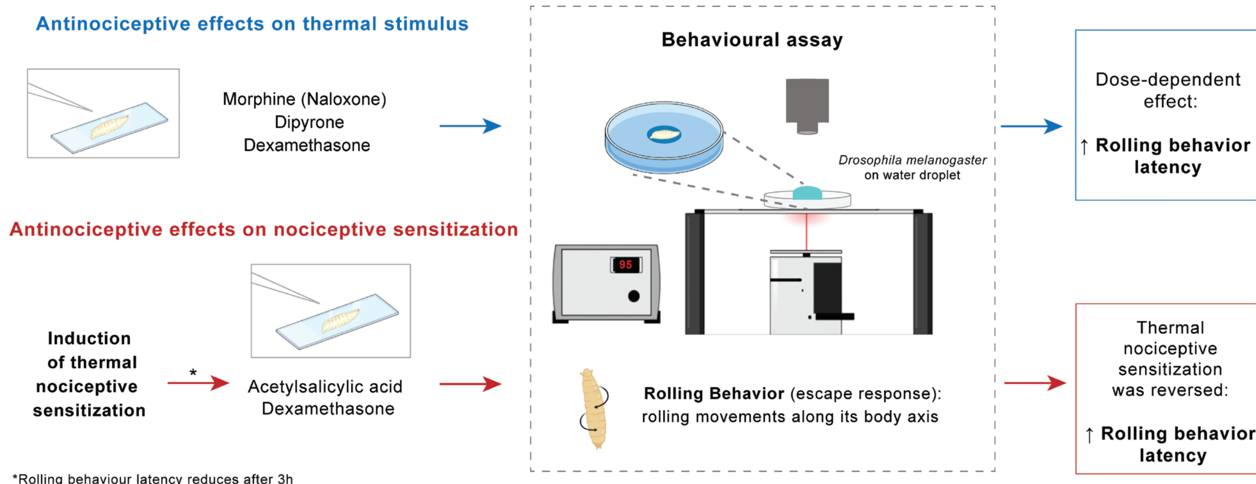
Materials and methods: We evaluated the effect of classical analgesics (morphine, dipyrone, acetylsalicylic acid (ASA) and dexamethasone (DXM)) in the rolling behavior latency of *D. melanogaster* larvae exposed to thermal-acute noxious stimulus and nociceptive sensitization paradigm. Drugs were injected into hemocoel (100 nL) before nociceptive measurement.

Results and discussion: Rolling behavior latency was increased by morphine (2, 4, 8 and 16 ng) in dose-dependent manner. Naloxone (4 ng) fully reversed maximum effect of morphine. Dipyrone (32, 64 and 128 ng) and DXM (8 and 16 ng) elicited dose-dependent antinociceptive effects. Exposure of larvae to 97% of maximal infrared intensity induced nociceptive sensitization, i.e., latency changed from 12 to 7.5 seconds. ASA (25, 50 and 100 ng) and DXM (4, 8 and 16 ng) were administered 150 min after nociceptive sensitization and displayed reverse sensitization in rapid onset (30 min after injection). DXM (16 ng), injected prior to nociceptive sensitization, displayed a delay in the onset of action (150 min after injection). Locomotor behaviors were not affected by analgesic substances.

Conclusion: Our findings open perspectives for evaluation and discovery of antinociceptive drugs using *D. melanogaster* larvae model.

Copyright *Santos-Silva T et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC-BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Graphical abstract



Keywords

antinociception, corkscrew, noxious stimuli, thermal nociception.

Introduction

The ethical principles and guidelines for scientific experimentation comprise methods that reduce and/or replace the use of animals, especially those subjected to painful conditions (Díaz et al. 2020; Fontana et al. 2021). Attempting to minimize the use of mammalian vertebrates in research, "low-order" animals, such as non-mammalian vertebrates and invertebrates, have been adopted for partial replacement in various aspects of neuroscience (Moulin et al. 2021). For example, to elucidate genetic and cellular processes of diseases or to initial drug screening (Lee and Min 2019; Papanikolopoulou et al. 2019; Millet-boureima and Selber-hnatiw 2021).

The study of pain in animals is based on their nociceptive behavior, namely the capacity to respond to potentially damaging stimuli (Abboud et al. 2021). The ability to detect and respond to noxious stimuli is present throughout the Metazoan kingdom, including in vertebrates such as *Drosophila melanogaster*, known as the fruit fly (Williams et al. 2019). *D. melanogaster* has been used for over a century in biological research (Kimble and Nüsslein-Volhard 2022), as a model organism, in drug discovery, genetic research and molecular pathways, among other studies (Pandey and Nichols 2011; Verheyen 2022). This species also has anatomical and physiological components that trigger responses to avoid potentially harmful stimuli (Im and Galko 2012; Lopez-Bellido et al. 2019; Dason et al. 2020; Lopez-Bellido and Galko 2020).

Tracey et al. (2003) described a paradigm for investigation of nociception in third instar larvae of *D. melanogaster*. A hot noxious stimulus evoked escape response mediated by nociceptive neurons (Burgos et al. 2018; Gu et al. 2022). The escape response consists first of body curvature, in which the head and tail move simultaneously. Then, larvae present rolling movements along their axis, like a corkscrew (Oswald et al. 2011), which occurs in variable numbers and most often with a 360° rotation. Although the escape behavior can be induced experimentally, it occurs in their natural habitat after attacks by *Leptopilina* parasitic wasps (Tokusumi et al. 2017). Female wasps lay their eggs inside *Drosophila* larvae, which defend themselves by moving the anterior and posterior region and/or exhibit the rolling behavior response triggered by somatosensory activation (Robertson et al. 2013).

Sensory neurons in Drosophila larvae are present within the epidermis and extend through the body wall (Orgogozo and Grueber 2005). Class IV multidendritic neurons (mdIV) are polymodal nociceptors, which encode and transduce different noxious stimuli (Smith and Lewin 2009; Im and Galko 2012). mdIV are present in both ventral and dorsal regions of larvae and have a complex branching pattern (Grueber et al. 2002). These neurons are essential to nociceptive behavior, and, when blocked, they significantly impair thermal and mechanical nociception behavior, whereas their optogenetic activation is enough to trigger the stereotyped rolling response (Hwang et al. 2007; Dason et al. 2020). Nociceptive rolling behavior is also initiated at lower-threshold temperatures and at higher frequencies when the temperature increased rapidly (Luo et al. 2017a).

D. melanogaster larvae have a relatively simple nervous system and exhibit sensory response to high intensity mechanical and thermal stimuli. The robust escape behavior upon nociceptive thermal stimulus is well defined,

allowing the determination of the larvae thermal nociception, as well as the replacement of mammalians in pain research. To validate *D. melanogaster* larvae as a model to detect potential analgesic substances upon nociceptive stimulus and pain-induced sensitization, we assessed the effects of opioid, nonsteroidal and steroidal substances in infrared noxious stimulus. This methodology would contribute to the progress in the nociceptive biology field and the reduction of mammals in animal experimentation.

Materials and methods

Animals

Third instar larvae of *D. melanogaster* (wild-type, WT) were provided by the Evolutionary Cytogenetic Laboratory of Department of General Biology – Institute of Biological Science, Federal University of Minas Gerais (Brazil). The fly strain used was derived from WT *D. melanogaster* specimens collected in nature and kept for successive crossings that ensure genetic homogeneity. All animals were descendant from a single female fly. *D. melanogaster* larvae were grown at 24±1 °C in a natural light/dark cycle of 12 hours and maintained in glass vials containing standard corn meal food media mixture (51% cornmeal, 14% agar, 14% yeast, 11% soy flour, 9% sugar, and 1% methylparaben) prepared in de-ionized water.

Drugs and chemicals

Artificial hemolymph contained (mM): NaCl (117.5), KCl (20), $CaCl_2$ (2), $MgCl_2 \cdot 6H_2O$ (8.5), $NaHCO_3$ (10.2), NaH_2PO_4 (4.3), HEPES (8.6), L-glutamine (10), and glucose (20) (Bijelik et al. 2005). Morphine (Merck, Germany), Naloxone (Sigma, USA), Dipyrone (Sigma, USA) and Dexamethasone (Aché, Brazil) were dissolved or diluted in artificial hemolymph. Acetylsalicylic acid (Sigma, USA) was dissolved in artificial hemolymph with DMSO 0.002%.

Hemocoel injections

Each *D. melanogaster* larva was immobilized on a microscope slide with a double-sided tape and placed under an electrophysiology micromanipulator (Molecular Device, HL-U pipette holder; Siskiyou, MC1000e-J) coupled to 40× objective. A 200 µL tip, pulled out to a fine tip over a low flame, was used to transfer 100 nL of drug solution to a glass micropipette (tip diameter 3 µm). This glass micropipette was coupled to the micromanipulator and used to insert the tip into the hemocoel, between A4–A5 larvae segments. Positive air pressure, supplied by a 5 ml syringe connected to polyethylene tubing in the back of the glass micropipette, was used to dispense solutions into the hemocoel. All solutions were injected to final volume of 100 nL. Immediately after the injection, larva was carefully removed from double-sided tape with water and a brush. This procedure was adapted from Bijelic et al. (2005).

Rolling behavior assay

Each *D. melanogaster* larva was placed in water droplet (20 µL) on a petri dish. The petri dish was positioned on glass surface of a Hargreaves apparatus (Ugo Basile, Italy). Infrared radiation source (infrared light with 8V voltage and 50W power) was perpendicularly positioned under the glass, towards the water droplet. Rolling Behavior Latency, time (seconds, s) from incidence until escape motion (360° rolling along body axis), was defined as the nociceptive measurement and visualized by binocular loupe (16× magnification, Zeis). The upper cutoff was set to 32 s, as when this value was reached, the infrared light was switched off automatically. The rolling behavior assay was conducted blind to drug and dose injection.

Infrared radiation intensity

To determine the best working infrared radiation intensity, 20 larvae per group were exposed under different relative intensities of infrared radiation lamp (85, 90, 95 and 99% of apparatus maximum power). 95% was chosen for other procedures, in which the rolling behavior latency was observed in 11.7±1.3 s.

Measurement of water droplet temperature

Water droplet temperature (20 µL) was measured for 95% infrared intensity. Measurements were made by thermocouple connected to a computer every 5 s, for 30 s. It was recorded and analyzed by Clampex 10.5 and Clampfit 10.5 software, respectively. The following second order polynomial function was used to determinate water droplet temperature (y) after rolling behavior latency at 95% infrared intensity (x):

$$y = 25.36 + 0.4721x - 0.008134x^2$$

Effects of analgesic substances on infrared nociception

Basal rolling behavior latency was initially recorded (n = 7 per group). Morphine (2, 4, 8 and 16 ng), dipyrone (32, 64 and 128 ng) and dexamethasone (DXM; 4, 8 and 16 ng) were evaluated at 0, 5, 10, 15, 20 minutes (min) after hemocoel injection (a.i.h) until 30, 60 or 120 min. The involvement of opioid receptors was assessed by concomitant administration of 16 ng morphine and naloxone (1, 2 or 4 ng) after injection. Doses were established from those used in mice, considering proportions of body weights. Artificial hemolymph was used as negative control for all the experiments.

Effects of analgesic substances on locomotor behavior

After hemocoel injection of vehicle, morphine (16 ng), dipyrone (128 ng) or dexamethasone (16 ng), each *D. melanogaster* larva (n = 10 per group) was gently placed on a Petri dish with 1% agar over a graph paper with 0.1 cm².

Locomotor behaviors were registered for 2 minutes in the peak of antinociceptive action (1 min after morphine and dipyrone injection and 150 min after for dexamethasone). Drosophila locomotion patterns were categorized as striding (when larva moves linearly covering a significant distance) or non-striding (when larva turns its head sideways and bends its body without moving long distances) (Lahiri et al. 2011; Aleman-Meza et al. 2015). The results were reported as the total striding locomotor distance (cm^2) and the total number of non-striding movements.

Induction of thermal nociceptive sensitization

After basal rolling behavior latency measurement, each larva was exposed to 95, 97 or 99% infrared intensity during 32 s. The SHAM group consists of animals placed on the apparatus for 32 s without exposure to infrared stimulus, whereas the NAÏVE group was not previously exposed to the apparatus. Rolling behavior latency was evaluated at 95% infrared intensity, each 30 min up to 300 min (5 hours). 97% infrared intensity was selected to induce infrared nociceptive sensitization.

Effects of ASA and DXM on infrared-induced nociceptive sensitization

Basal rolling behavior latency was initially measured. Artificial hemolymph was used as control for all treatments. ASA (25, 50 and 100 ng) or DXM (4, 8 or 16 ng) were administered 150 min after infrared-induced nociceptive sensitization. Alternatively, DXM (16 ng) was administered before the nociceptive sensitization protocol. Rolling behavior latency was measured each 10 min for 60 min.

Statistical analysis

D. melanogaster larvae were randomly distributed between experimental and control groups. Rolling behavior latency was represented as mean±standard error of the mean (SEM) (n = 7 per group). Maximum possible effect (MPE) was defined by the following equation, adapted from (Le Bars et al. 2001):

$$MPE = \frac{RL_t - RL}{C_0 - RL} \times 100$$

where, RL_t is rolling behavior latency after treatment; RL is rolling behavior latency of the control group; C_o is cut-off time (32 s).

Data were presented as the mean ± the standard error of the mean (SEM). All the data were subjected to tests to verify the homogeneity of variances (Bartlett's test) and if they followed a normal distribution (Shapiro–Wilk test). A two-way analysis of variance (ANOVA) repeated measures with Bonferroni's *post-hoc* test was performed to compare treatments at different time points. One-way ANOVA followed by the *post hoc* Bonferroni test was performed to compare the differences among treatments in a determined time point. All statistical analyses were performed using a GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, California, USA). Values of $p < 0.05$ were considered statistically significant.

Results and discussion

Choice of infrared radiation intensity

Rolling behavior latencies for 85% and 90% were 12.6±3.0 s and 12.3±2.7 s, respectively. 95% displayed mean rolling behavior latency in 11.7±1.3 s. The lowest rolling behavior latency was observed for 99% (9.8±2.6 s) (Fig. 1A). 95% was chosen to evaluate potential antinociceptive substances.

Water droplet temperature of rolling behavior

The water droplet average temperature that evoked rolling behavior latency was 29.8 °C (Fig. 1B).

Morphine antinociceptive effect in *D. melanogaster* larvae

Morphine (2, 4, 8 and 16 ng) affected rolling behavior latency in a dose-dependent manner (treatment: $F_{(4, 270)} = 213.64$; time: $F_{(9, 270)} = 274.56$; interaction: $F_{(36, 270)} = 38.27$, $p < 0.0001$) (Fig. 2A). The highest dose (16 ng) produced 91% MPE at 0 min, i.e., rolling behavior latency reached 30.2±0.6 s after hemocoel injection. Thresholds reduced gradually but did not return to the baseline even 120 min after hemocoel injection. The 57% MPE was observed immediately after the administration of 8 ng of morphine (23.3±0.5 s). This effect decreased with time and lasted for 90 min. Morphine (4 ng) evoked rolling behavior latency in 19.6±0.3 s (39% MPE), whereas morphine (2 ng) – in 15.3±0.5 s (18% MPE). The antinociceptive effect of those doses lasted 20 and 10 min, respectively. Naloxone was able to reverse morphine antinociceptive effect in dose-dependent manner ($F_{5, 36} = 305.9$, $p < 0.0001$). Naloxone 4 ng completely reversed morphine effect (11.9±0.4 s), while doses of 2 and 1 ng exhibited partial reversal (16.8±0.9 s and 23.0±1.8 s, respectively). Naloxone (4 ng) administered alone had no effect in comparison to vehicle group (Fig. 2B).

Dipyrone acts as an antinociceptive substance in *D. melanogaster* larvae

Hemocoel injections of dipyrone (32, 64 and 128 ng) elicited dose-dependent antinociception (rolling behavior latencies were 16.4±0.4 s, 21.3±1.5 s and 26.2±0.9 s, respectively; treatment: $F_{(3, 168)}=19.93$; time: $F_{(7, 168)}=110.08$; interaction: $F_{(21, 168)}=21.84$; $p < 0.0001$) (Fig. 3). All doses produced the peak effect immediately after administration, in which MPE increased according to the dose: 23, 47 and 71%, respectively. Dipyrone effect lasted for 10 min for the highest dose and 5 min for other doses.

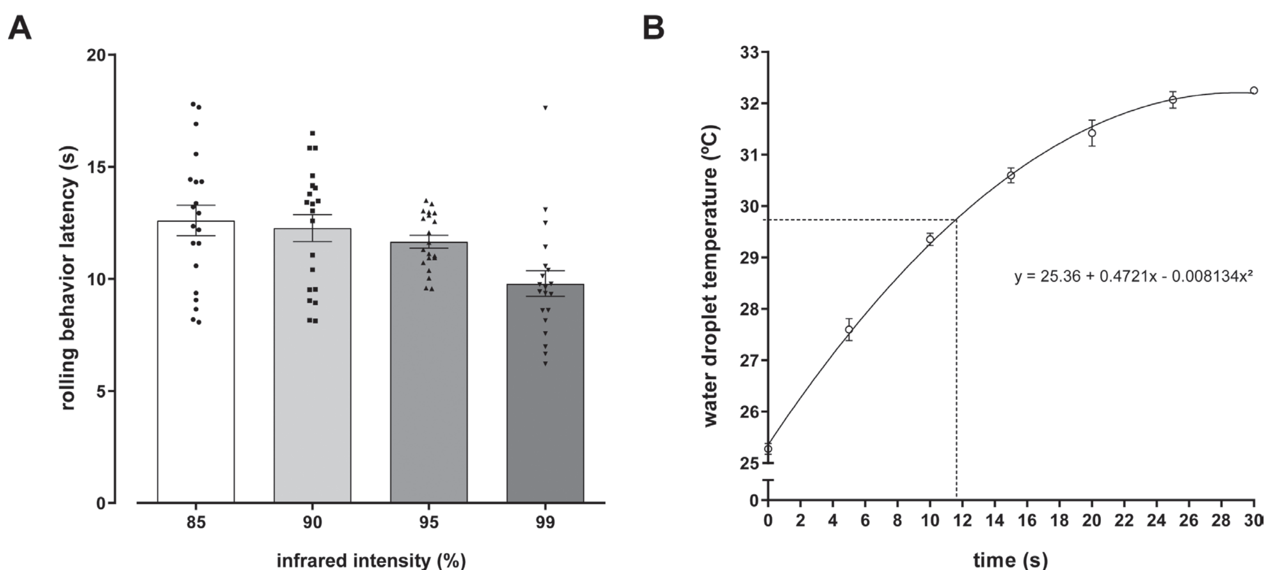


Figure 1. Infrared radiation intensity and time/temperature curve. **(A)** Larvae were exposed to different relative intensities of infrared radiation (85, 90, 95 and 99% of apparatus maximum potency). The traced line represents the rolling behavior latency mean±SEM of 20 larvae per group. 85%=12.6±3.0 s; 90%=12.3±2.7 s; 95%=11.7±1.3 s; 99%=9.8±2.6 s. Each symbol represents rolling behavior latency of a single larvae. **(B)** The water droplet (20 µL) was placed on a petri dish and submitted to infrared radiation at 95% maximum potency. Each point represents mean±SEM for 4 measurements determined with a thermocouple, 5 s each. Dotted plot represents the relationship between rolling behavior latency (11.7 s) and temperature (29.8 °C), determined by a second order polynomial regression curve.

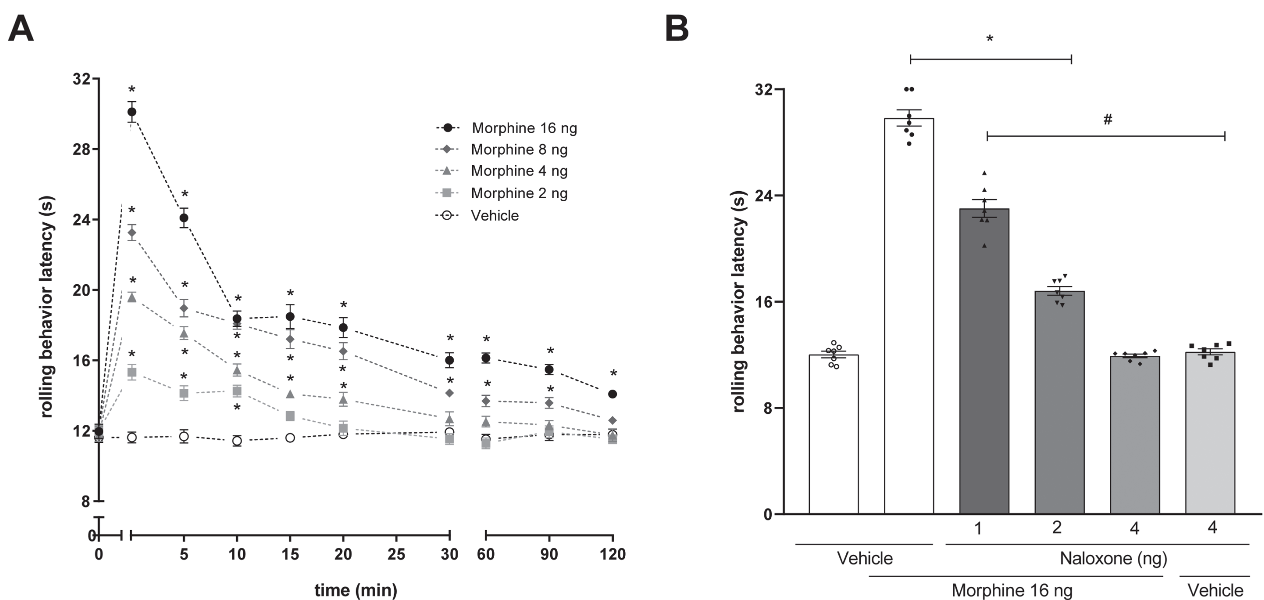


Figure 2. Antinociception induced by morphine in *D. melanogaster* larvae. **(A)** Morphine (2, 4, 8 and 16 ng) was injected into hemocoel. Each line represents mean±SEM of 7 animals per group. $^{*}p < 0.05$, significant difference compared with vehicle group (Two-way ANOVA repeated measures and Bonferroni test). **(B)** The non-selective antagonist, naloxone (1, 2 and 4 ng) was administered concomitantly with morphine (16 ng). Each bar represents mean±SEM of 7 animals per group. $^{*}p < 0.05$ significant difference compared with vehicle group; $^{\#}p < 0.05$ indicated a significant difference compared to morphine (16 ng) (One-way ANOVA and Bonferroni test). Vehicle = artificial hemolymph.

Infrared-induced nociceptive sensitization

Exposing larvae for 32 s to 95% infrared intensity did not induce nociceptive sensitization during the time observed (5 hours after induction). 97% intensity sensitization peaked 180 min after induction. At this time, rolling behavior latency was reduced from 12.1±0.5 s to 7.5±0.4 s. Similarly, the group exposed to 99% intensity reduced rolling behavior latency from 11.8±0.4 s to 7.3±0.4 s; however, sensitization peaked after 120 min (treatment: $F_{(4, 406)} = 13.43$; time: $F_{(14, 406)} = 5.52$; interaction: $F_{(56, 406)} = 8.51$; $p < 0.0001$) (Fig. 4). Rolling behavior

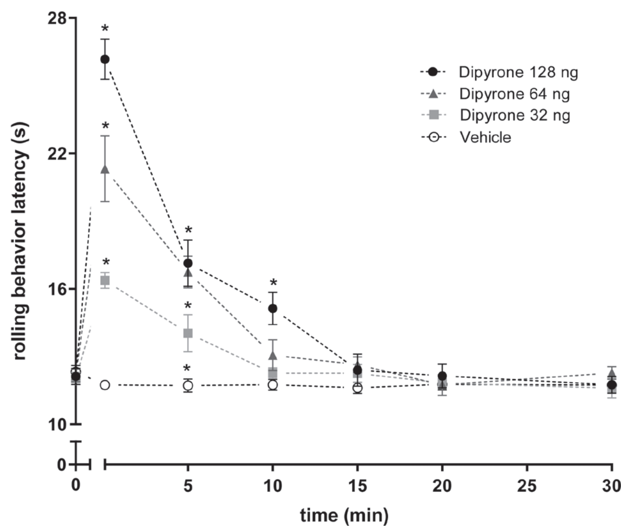


Figure 3. Dipyrone induced antinociception in *D. melanogaster* larvae. Dipyrone (32, 64 and 128 ng) was injected into hemocoel. Each line represents mean±SEM of 7 animals per group. $^{*}p < 0.05$ indicated significant difference compared with vehicle group (Two-way ANOVA repeated measures and Bonferroni test). Vehicle = artificial hemolymph.

latencies were significantly different 30 min before sensitization peak when compared to SHAM rolling behavior latency and returned to the baseline 60 min after sensitization peak (97 and 99%). There were no statistical differences between SHAM and NAÏVE groups, i.e., exposure to the apparatus did not interfere with the observed responses. 97% infrared intensity was selected to induce nociceptive sensitization in the following protocols.

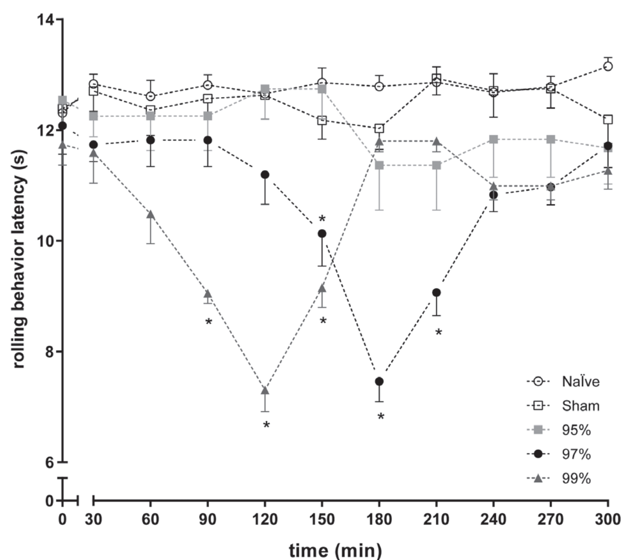


Figure 4. Infrared-induced nociceptive sensitization in *D. melanogaster* larvae. Larvae were exposed for 32 s to 95, 97 or 99% maximal infrared intensity. Each line represents mean±SEM for 7 animals per group. $^{*}p < 0.05$ significant difference compared with SHAM rolling behavior latency (Two-way ANOVA repeated measures and Bonferroni test). NAÏVE group was not previously exposed to the apparatus. SHAM group was placed on apparatus during the same time interval without exposure to infrared radiation. Vehicle = artificial hemolymph.

ASA reversed infrared-induced nociceptive sensitization

ASA (25, 50 and 100 ng), injected 150 min after infrared-induced nociceptive sensitization, produced significant and dose-dependent increment in rolling behavior latency (treatment: $F_{(4, 210)} = 204.86$; time: $F_{(7, 210)} = 118.30$; interaction: $F_{(28, 210)} = 15.22$; $p < 0.0001$) (Fig. 5A). ASA (25 and 50 ng) partially reversed infrared-induced nociceptive sensitization. These effects occurred for 40 min, with peaks at 30 and 20 min after hemocoel injection, respectively. 10 min after hemocoel injection, ASA (100 ng) completely reversed nociceptive sensitization. This effect was significant over time and peaked 20 min after hemocoel injection. ASA (100 ng) was also evaluated without induction of nociceptive sensitization. Rolling behavior latency assessed for this group was not different when compared to negative control group (Fig. 5B).

Short-term and long-term effects of DXM in infrared-induced nociceptive sensitization

DXM (4, 8 and 16 ng), was injected 150 min after infrared-induced nociceptive sensitization, produced a dose-dependent profile (treatment: $F_{(4, 300)} = 107.92$; time: $F_{(10, 300)} = 77.57$; interaction: $F_{(40, 300)} = 11.96$; $p < 0.0001$) (Fig. 6A). Nociceptive sensitization was completely reversed by DXM (8 and 16 ng). Both effects persisted for 50 min, with peaks 30 min after hemocoel injection. Dexamethasone (4 ng) produced a significant antinociceptive effect upon sensitization from 10 to 60 min after hemocoel injection, peaking at 20 min after hemocoel injection. The rapid onset and shorter duration of the effect indicates a short-term effect of DXM, which is defined as acute antinociceptive action of DXM. Regarding the long-term effect, DXM (16 ng) reversed rolling behavior latency 150 min after hemocoel injection. and lasted for 60 min (treatment: $F_{(1, 120)} = 283.68$; time: $F_{(10, 120)} = 34.15$; interaction: $F_{(10, 120)} = 26.77$; $p < 0.0001$) (Fig. 6B). DXM (8 and 16 ng) (without previous nociceptive sensitization) elicited antinociceptive effects 20 and 15 min after hemocoel injection, respectively. In both groups, peak effect was also observed 30 min a.i.h (treatment: $F_{(4, 270)} = 2.91$; $F_{(7, 210)} = 118.30$; $p < 0.0001$; interaction: $F_{(36, 270)} = 6.00$; $p < 0.0378$) and was no longer detected 60 min after hemocoel injection. DXM (4 ng) did not show significant effect when compared to vehicle group (Fig. 6C).

Locomotor behaviors of D. melanogaster larvae were not affected by analgesic substances

The striding and non-striding locomotor behaviors of *D. melanogaster* larvae were not significantly different in the peak of antinociceptive action compared to vehicle group (Fig. 7).

It is hard to estimate, even roughly, the number of animals used in research worldwide. Currently, only a few countries collect, analyze and publish this data, making it difficult to precisely quantify animal usage, but this

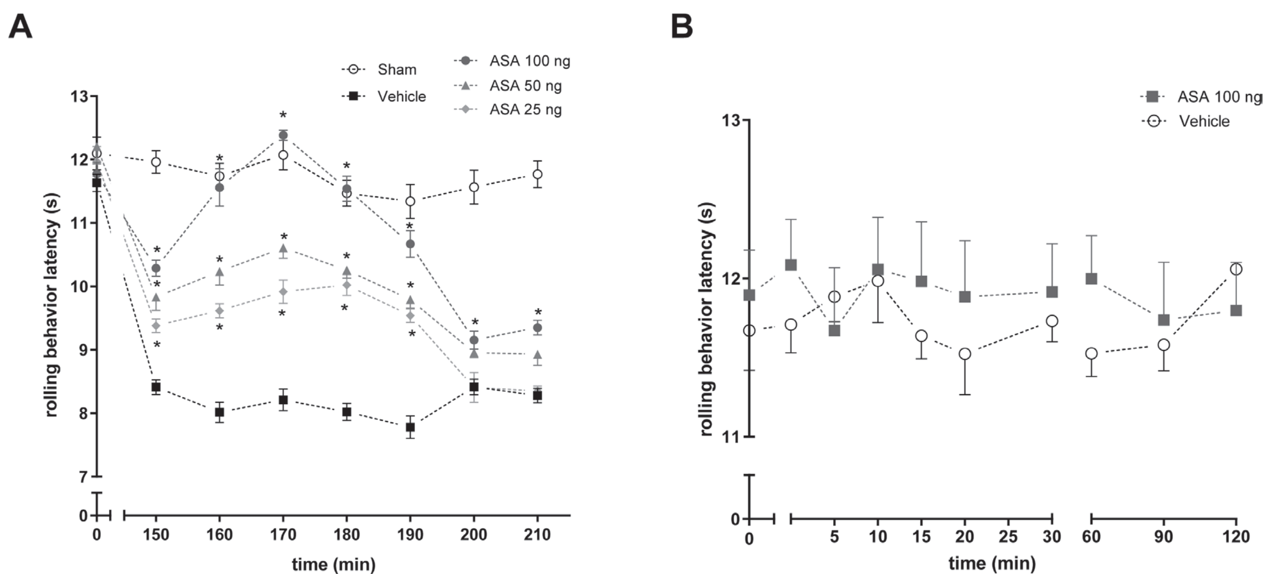


Figure 5. ASA reversed infrared-induced nociceptive sensitization. **(A)** ASA (25, 50 and 100 ng) was injected into hemocoel 150 min after induced nociceptive sensitization. Each line represents mean±SEM for 7 animals per group. $^{*}p < 0.05$; significant difference compared with vehicle group (Two-way ANOVA repeated measures and Bonferroni test). Nociceptive sensitization: larvae were exposed to 97% infrared intensity during 32 seconds. SHAM group was placed on the apparatus for the same time interval without exposure to radiation. **(B)** ASA (100 ng) was injected into hemocoel. Each line represents mean±SEM of 7 animals per group. Vehicle = artificial hemolymph and DMSO 0.002%.

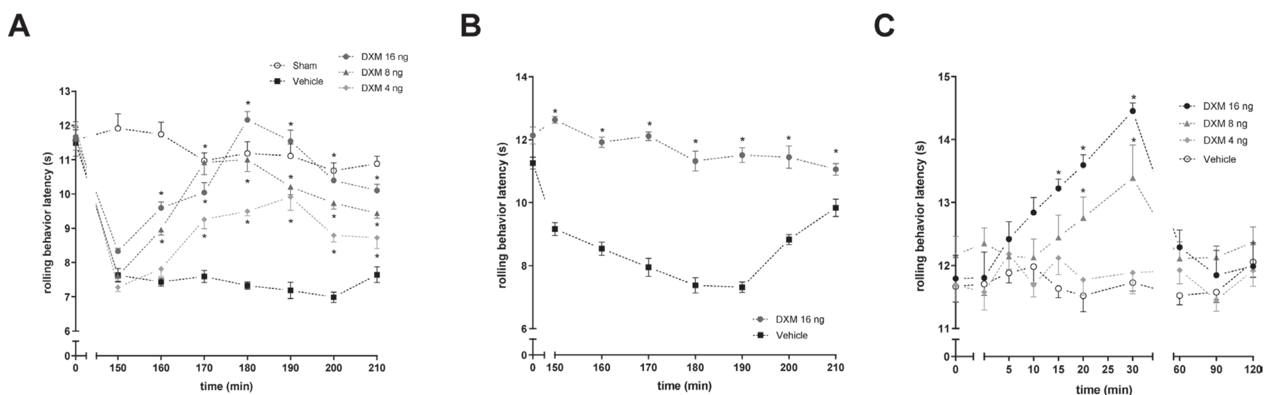


Figure 6. Short-term and long-term effects of DXM in *D. melanogaster* larvae. **(A)** DXM (4, 8 and 16 ng) was injected into hemocoel 150 min after induced nociceptive sensitization. **(B)** DMX (16 ng) was injected into hemocoel prior induced nociceptive sensitization. **(C)** DXM (4, 8 and 16 ng) was injected into hemocoel. Each line represents mean±SEM of 7 animals per group (Two-way ANOVA repeated measures and Bonferroni test). $^{*}p < 0.05$ indicated significant difference compared with vehicle group. Nociceptive sensitization: larvae were exposed to 97% infrared intensity for 32 seconds. SHAM group was placed on the apparatus for the same time interval without exposure to radiation time. Each line represents mean±SEM of 7 animals per group. Vehicle = artificial hemolymph.

number is large, reaching up to 190 million higher animals killed per year (Taylor and Alvarez 2019). There is great effort aimed at reducing this number, and the requirement of an ethics committee approval for publishable research conducted on animals reflects this struggle. Despite this, new approaches are needed and the use of invertebrates for research purposes is gaining attention, especially those well characterized, such as *Drosophila melanogaster*.

Here we show that morphine, dipyrone and DXM, well-known analgesic substances, are capable of eliciting antinociceptive effects on thermal nociception in *D. melanogaster* larvae. In addition, infrared-induced nociceptive sensitization was achievable in the scope of the proposed model. Such sensitization was shown to be reversed by ASA and DXM, in which DXM produced short and long-term effects.

Several studies demonstrated nociceptive behavioral responses of *D. melanogaster* larvae upon mechanical stimulus (Hwang et al. 2007; Jang et al. 2019, 2022) and local or global thermal stimulation (Oswald et al. 2011; Chattopadhyay et al. 2012; Yoshino et al. 2017). The present study aimed at induced thermal nociception using an infrared lamp was based on the classical Hargreaves method for murines (Hargreaves et al. 1988). The 95% apparatus maximal infrared power was chosen since the rolling behavior response variation allows the detection and visualization

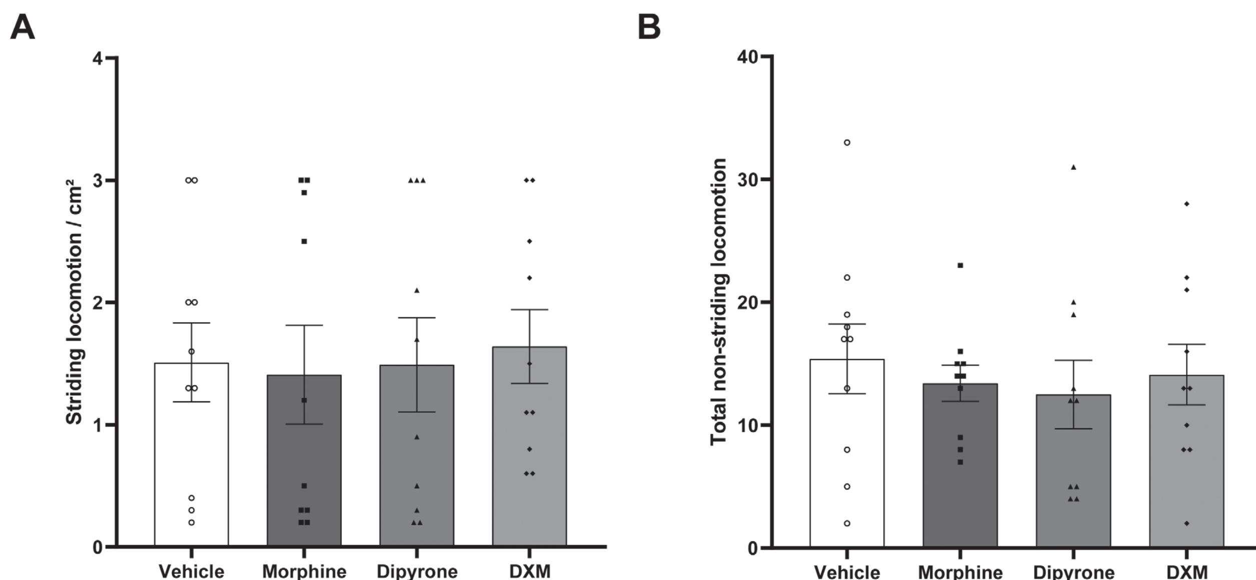


Figure 7. Effects of analgesic substances on locomotor behaviors. Morphine (16 ng), dipyrone (128 ng) and DXM (16 ng) did not affected the striding (locomotor distance, cm²) and non-striding behavior (total number of movements) when compared to vehicle group. Locomotor behaviors were evaluated in the peak of action of each drug (morphine and dipyrone: t = 1 min post-injection; DXM: t = 30 min post-injection). Data represents mean±SEM of 10 animals per group. Vehicle = artificial hemolymph.

of analgesic effects. In contrast, the dispersed rolling behavior latency at 85 and 90% could impair the detection of antinociceptive effect since the cut-off time of apparatus is 32 s. At 99% intensity stereotypic behaviors appeared, such as whipping and tremors, followed by rolling behavior. Those behaviors are typically elicited after rolling behavior (Oswald et al. 2011; Chattopadhyay et al. 2012), therefore, it could damage larvae tissue exposed to 99%.

Radiation from the infrared lamp is absorbed mainly by water molecules, causing agitation, hence, heating (Hardy et al. 1951). This kinetics can be measured by temperature. To assess the water droplet temperature at the rolling behavior latency, we measured the related heating kinetics. Using a second order polynomial fitting to the obtained curve, we estimated that the water droplet temperature was 29.8 °C at mean rolling behavior latency. Considering that infrared beam directed to water droplet plus larva system has a high potency and homogenously irradiates the entire system, it is expected that the surrounding water will heat more slowly than the larval tissues. Indeed, water specific heat (1.0 cal.g^{-1} °C^{-1}) is 25% higher than the specific heat of larval insect, namely 0.8 cal.g^{-1} °C^{-1} (Shinozaki 1957), resulting in larval temperatures being higher than those measured in pure water droplet. As an example, and considering that the amount of heat transferred is proportional to specific heat, a water droplet at 30 °C would indicate a larva at 38 °C, a temperature consistent with activation of peripheral nociceptors was observed in literature (Tracey et al. 2003; Oswald et al. 2011; Chattopadhyay et al. 2012). Moreover, nociceptive rolling behavior is also initiated at lower-threshold temperatures and at higher frequencies when the temperature increased rapidly (Luo et al. 2017b).

Here, analgesic agents were injected into *D. melanogaster* larvae hemocoel, a cavity filled with hemolymph, which bathes the organs with oxygen and nutrients and maintains larvae osmolarity (Bijelic et al. 2005). Hemocoel injection provides a bolus of drugs, which are expected to be evenly distributed throughout larval tissues in quick manner due to its small size. It is also known that all drugs used cross the blood brain barrier in mammals, rendering please check as capable of permeating neuronal tissues in *D. melanogaster* larvae (Schirmeier and Klämbt 2015). Considering the complexity of this fluid, artificial hemolymph was used as a drug vehicle and administered in all control groups. As expected, artificial hemolymph has no antinociceptive effects. To rule out any mechanical impairment of larval rolling behavior related to hemocoel injection, rolling behavior latency of groups treated only with vehicle was compared to those of sham animals. No significant difference was found in latency for rolling behavior between those groups.

The first analgesic investigated with the proposed model was morphine, an opioid commonly used to treat pain (Devereaux et al. 2018). The presence of opioid peptide, enkephalin, has been demonstrated in the nervous system of *D. melanogaster* (Pagés et al. 1983). Accordingly, opioid drugs have been shown to bind stereo-specifically and with high affinity to neuronal tissue of the fruit fly *D. melanogaster* (Santoro et al. 1990). Although no *bona fide* opioid receptor has been identified in flies using bioinformatics (Birgül et al. 1999), four receptors with structural similarities to mammalian somatostatin, galanin and opioid receptors, as well different neurotransmitters were found from all stages of *D. melanogaster* (Hewes and Taghert 2001; Estacio-Gómez et al. 2020; Nässel and Zandawala 2020; Dvořáček and Kodrík 2021). We showed that morphine was able to increase rolling behavior latency in a dose-dependent manner. The possible role of opioid-like receptors in morphine antinociception is suggestive from our results, since naloxone, a non-selective opioid receptor, fully reversed morphine effects.

Dipyrone is a well-known and effective analgesic and antipyretic, commonly used in human health care and veterinary practice (Jasiecka et al. 2014). In *D. melanogaster* larvae, dipyrone also had dose-dependent antinociceptive effect. This result opens perspectives aiming to elucidate the still controversial mechanism of action, since *D. melanogaster* can be manipulated genetically and used in conjunction with other pharmacological tools.

We also investigated whether nociceptive sensitization could be induced by infrared radiation, detected by infrared stimulus and reversed by specific pharmacological agents. Nociceptive sensitization is characterized by the facilitation and increase in nociceptor responses, triggered by a decreased threshold to mechanical, chemical or thermal tissue injuries (Woolf 2018). Here, we induced nociceptive sensitization in *D. melanogaster* larvae through acute exposure to distinct intensities (95, 97 and 99% maximal potency of the apparatus) of infrared radiation, during 32 s. Sensitization peaked after three and two hours (97% and 99%, respectively), characterized by a reduction in baseline rolling behavior latencies.

Several inflammatory mediators, such as prostaglandins (PGs), are essential during inflammatory processes and consequently play central roles in nociceptive sensitization (Gold and Gebhart 2010; Jang et al. 2020; Oikawa et al. 2022). PGs are derived from arachidonic acid and synthesized by cyclooxygenases (COX), which are irreversibly inhibited by ASA, a non-steroidal anti-inflammatory drug (Roth and Majerus 1975). Pagés et al. (1986) determined the existence of COX-like activity in *D. melanogaster*. A possible *Cox* homolog is *Pxt* (peroxinctin-like) with conserved COX-like catalytic residues (Tootle and Spradling 2008; Scarpati et al. 2019). To investigate whether nociceptive sensitization could be related to an inflammatory response, increasing doses of ASA were administered into sensitized larvae. Dose-dependent antinociceptive effect was detected. However, the ASA effect is attenuated over time, i.e., inhibition of sensitization does not persist. Since the drug irreversibly inhibits COX, we posit that other inflammatory mediators could be present, but further studies are needed.

DXM is a synthetic analogue of glucocorticosteroids that acts mainly via steroid receptors (Smoak and Cidlowski 2004). During inflammation, the most important factor blocked by DXM is the nuclear factor kappa B (NF-κB), with consequent decrease in the levels of COX and TNF. DXM also regulates inflammatory resolution genes, such as annexin 1, which requires time to the onset of action (Smoak and Cidlowski 2004; Jiang et al. 2015). In mammals, non-genomic mechanisms have also been described, characterized by their rapid action onset, hence defined as short-term effects (Panettieri et al. 2019). Several orphan steroid receptors were identified in Drosophila, as well as two annexins, involved in cell differentiation and insect development (Johnston et al. 1990; Carney and Bender 2000). Moreover, the Estrogen Related Receptor (*Err*) gene was identified as the closest genetic vertebrate glucocorticoid receptor homolog in *D. melanogaster* (Bartolo et al. 2020). Here, DXM reversed dose-dependent sensitization approximately 20 min after injection; then, we categorized such an action as a short-term effect. The same doses increased rolling behavior latency without prior exposure to nociceptive sensitization. We also identified possible long-term genomic effects of DXM.

Systemic injections of analgesic drugs could impact nociception and sensory neurons specifically or affect motor neurons and central neurons in the nociceptive circuit. To demonstrate that the antinociceptive effects by analgesics are sensory/nociceptive rather than locomotory/motor, we evaluated the striding and non-striding movements of larvae injected with vehicle, morphine, dipyrone or DXM (drugs that produced antinociception without infrared-induced nociceptive sensitization). As expected, the results presented here are due to antinociceptive effects and not due to inhibition of normal locomotion and motor coordination.

Over the past decades, studies in pain-suppressed behavior along with pain-evoked behaviors have been performed to improve the translation of putative analgesic drugs from animals to humans. Although neither *D. melanogaster* nociceptive models nor pain-suppressed behaviors have been described yet, it has been shown that many proteins play an important role in nociception and other behaviors. For example, the TRPA channel protein (painless) is required for thermal and mechanical nociception and for an abnormal fly male–male courtship behavior (Wang et al. 2011). The loss of G-Protein-Coupled Neuropeptide Y-Like Receptor leads to abnormal larval sugar-averse behavior as well as a delay in the thermal aversive response (Xu et al. 2010). Here, we evaluated the use of acute noxious thermal stimulus on larvae and the suppression of the avoidant behavior they evoke by classical analgesics. It is known that larvae exposed to long global heating of their bodies, in a model similar the one applied here, display a seizure-like behavior that is followed by paralysis (Chattopadhyay et al. 2012). Most of the larvae exposed to that paradigm end up becoming non-viable pupas, an occurrence linked to the cited pain-evoked behavior. It would be interesting to test pupal viability of treated vs. sham larvae, to address how pain suppression could impact pupation.

Conclusion

In sum, the characterization of behavioral escape responses, modulation of nociception and antinociceptive effects of distinct classes of drugs suggest that *D. melanogaster* could be used in pain pharmacological research and nociceptive biology. The easy genetic manipulation and availability of usable pharmacological tools appear as an alternative in the drug discovery process. In addition, *Drosophila* approaches to physiological and pharmacological pain will reduce nociceptive experimentation in mammals, as one of the animal experimentation ethical principles.

Funding

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES (Grant Number 1652082). All authors contributed and have approved the final manuscript. The manuscript was revised by a British-born text reviewer, in compliance with the journal standards.

Conflict of interest

The authors declare no conflict of interest.

References

- Abboud C, Duveau A, Bouali-benazzouz R, Massé K, Mattar J (2021) Animal models of pain: diversity and benefits. Journal of Neuroscience Methods 348: 108997. https://doi.org/10.1016/j.jneumeth.2020.108997 [PubMed]
- Aleman-Meza B, Jung SK, Zhong W (2015) An automated system for quantitative analysis of *Drosophila* larval locomotion. BMC Developmental Biology 15: 1–12. https://doi.org/10.1186/s12861-015-0062-0 [PubMed] [PMC]
- Bartolo G, Gonzalez LO, Alameh S, Valencia CA, Martchenko Shilman M (2020) Identification of glucocorticoid receptor in *Drosophila melanogaster*. BMC Microbiology 20(1): 1–12. https://doi.org/10.1186/s12866-020-01848-x [PubMed] [PMC]
- Bijelic G, Kim NR, O'Donnell MJ (2005) Effects of dietary or injected organic cations on larval *Drosophila melanogaster*: Mortality and elimination of tetraethylammonium from the hemolymph. Archives of Insect Biochemistry and Physiology 60: 93–103. https://doi.org/10.1002/arch.20085
- Birgül N, Weise C, KreienKamp HJ, Richter D (1999) Reverse physiology in *Drosophila*: identification of a novel allatostatin-like neuropeptide and its cognate receptor structurally related to the mammalian somatostatin/galanin/opioid receptor family. The Embo Journal 18(21): 5892–5900. https://doi.org/10.1093/emboj/18.21.5892 [PubMed] [PMC]
- Burgos A, Honjo K, Ohyama T, Qian CS, Shin GJ, Gohl DM, Silies M, Tracey WD, Zlatic M, Cardona A, Grueber WB (2018) Nociceptive interneurons control modular motor pathways to promote escape behavior in *Drosophila*. eLife 7: e26016. https://doi.org/10.7554/eLife.26016 [PubMed] [PMC]
- Carney GE, Bender M (2000) The *Drosophila* ecdysone receptor (EcR) Gene is required maternally for normal oogenesis. Genetics 154: 1203–1211. https://doi.org/10.1093/genetics/154.3.1203
- Chattopadhyay A, Gilstrap A V., Galko MJ (2012) Local and global methods of assessing thermal nociception in drosophila larvae. Journal of Visualized Experiments 63: 3837. https://doi.org/10.3791/3837 [PubMed] [PMC]
- Dason JS, Cheung A, Anreiter I, Montemurri VA, Allen AM (2020) *Drosophila melanogaster* foraging regulates a nociceptive-like escape behavior through a developmentally plastic sensory circuit. PNAS 117: 23286–23291. https://doi.org/10.1073/pnas.1820840116 [PubMed] [PMC]
- Devereaux AL, Mercer SL, Cunningham CW (2018) DARK classics in chemical neuroscience: morphine. ACS Chemical Neuroscience 9: 2395–2407. https://doi.org/10.1021/acschemneuro.8b00150 [PubMed]
- Díaz L, Zambrano E, Flores ME, Contreras M, Crispín JC, Alemán G, Bravo C, Armenta A, Valdés VJ, Tovar A, Gamba G, Barrios-Payán J, Bobadilla NA (2020) Ethical considerations in animal research: The principle of 3R's. Revista de Ivestigacion Clinica 73(4): 199–209. https://doi.org/10.24875/RIC.20000380 [PubMed]
- Dvořáček J, Kodrík D (2021) *Drosophila* reward system – A summary of current knowledge. Neuroscience and Biobehavioral Reviews 123: 301–319. https://doi.org/10.1016/j.neubiorev.2020.12.032 [PubMed]
- Estacio-Gómez A, Hassan A, Walmsley E, Le LW, Southall TD (2020) Dynamic neurotransmitter specific transcription factor expression profiles during *Drosophila* development. Biology Open 9(5): bio052928. https://doi.org/10.1242/bio.052928 [PubMed] [PMC]
- Fontana F, Figueiredo P, Martins JP, Santos HA (2021) Requirements for animal experiments: problems and challenges. Small 17(15): 1–8. https://doi.org/10.1002/smll.202004182 [PubMed]
- Gold MS, Gebhart GF (2010) Nociceptor sensitization in pain pathogenesis. Nature Medicine 16(11): 1248–1257. https://doi.org/10.1038/nm.2235 [PubMed] [PMC]
- Grueber WB, Jan LY, Jan YN (2002) Tiling of the *Drosophila* epidermis by multidendritic sensory neurons. Development (Cambridge, England) 129(12): 2867–2878. https://doi.org/10.1083/jcb.140.1.143 [PubMed]
- Gu P, Wang F, Shang Y, Liu J, Gong J, Xie W, Han J, Xiang Y (2022) Nociception and hypersensitivity involve distinct neurons and molecular transducers in *Drosophila*. Proceedings of the National Academy of Sciences of the United States of America 119: 1–11. https://doi.org/10.1073/pnas.2113645119 [PubMed] [PMC]
- Hardy JD, Goodell H, Wolff HG (1951) The influence of skin temperature upon the pain threshold as evoked by thermal radiation. Science 114(2954): 591–601. https://doi.org/10.1126/science.114.2954.149 [PubMed]
- Hargreaves K, Dubner R, Brown F, Flores C, Joris J (1988) A new and sensitive method for measuring thermal nociception. Pain 32: 77–88. https://doi.org/10.1016/0304-3959(88)90026-7 [PubMed]
- Hewes RS, Taghert PH (2001) Neuropeptides and neuropeptide receptors in the *Drosophila melanogaster* genome. Genome Biology 11(6): 1126–1142. https://doi.org/10.1101/gr.169901 [PubMed] [PMC]
- Hwang RY, Zhong L, Xu Y, Johnson T, Zhang F, Tracey WD (2007) Nociceptive neurons protect *Drosophila* larvae from parasitoid wasps. 17(24): 2105–2116. https://doi.org/10.1016/j.cub.2007.11.029 [PubMed] [PMC]
- Im SH, Galko MJ (2012) Pokes, sunburn, and hot sauce: drosophila as an emerging model for the biology of nociception. Developmental Dynamics 241(1): 16–26. https://doi.org/10.1002/dvdy.22737 [PubMed] [PMC]
- Jang W, Lim JY, Kang S, Kim M, Hwang SW, Kim C (2022) *Drosophila* ppk19 encodes a proton-gated and mechanosensitive ion channel. Scientific Reports 12(1): 18346. https://doi.org/10.1038/s41598-022-23236-3 [PubMed] [PMC]
- Jang W, Lee S, Choi SI, Chae HS, Han J, Jo H, Hwang SW, Park CS, Kim C (2019) Impairment of proprioceptive movement and mechanical nociception in *Drosophila melanogaster* larvae lacking Ppk30, a *Drosophila* member of the Degenerin/Epithelial Sodium

Channel family. Genes, Brain and Behavior 18(5): 1–8. https://doi.org/10.1111/gbb.12545 [PubMed]

- Jang Y, Kim M, Hwang SW (2020) Molecular mechanisms underlying the actions of arachidonic acid-derived prostaglandins on peripheral nociception. Journal of Neuroinflammation 17(1): 1–27. https://doi.org/10.1186/s12974-020-1703-1 [PubMed] [PMC]
- Jasiecka A, Maślanka T, Jaroszewski JJ (2014) Pharmacological characteristics of metamizole. Polish Journal of Veterinary Sciences 17(1): 207–214. https://doi.org/10.2478/pjvs-2014-0030 [PubMed]
- Jiang C, Liu L, Li Z, Buttgereit F (2015) The novel strategy of glucocorticoid drug development via targeting nongenomic mechanisms. Steroids 102: 27–31. https://doi.org/10.1016/j.steroids.2015.06.015 [PubMed]
- Johnston PA, Q TCS, Reynoldsli A (1990) Two novel annexins from drosophila. Cloning, characterization, and differential expression in development. The Journal of Biological Chemistry 265(19): 11382–11388. https://doi.org/10.1016/S0021-9258(19)38604-1 [PubMed]
- Kimble J, Nüsslein-volhard C (2022) The great small organisms of developmental genetics: *Caenorhabditis elegans* and *Drosophila melanogaster*. Developmental Biology 485: 93–122. https://doi.org/10.1016/j.ydbio.2022.02.013 [PubMed] [PMC]
- Lahiri S, Shen K, Klein M, Tang A, Kane E, Gershow M, Garrity P, Samuel ADT (2011) Two alternating motor programs drive navigation in *Drosophila* larva. PLoS ONE 6(8): e23180. https://doi.org/10.1371/journal.pone.0023180 [PubMed] [PMC]
- Lee S, Min K (2019) Translational medicine of aging *Drosophila melanogaster* as a model system in the study of pharmacological interventions in aging. Translational Medicine of Aging 3: 98–103. https://doi.org/10.1016/j.tma.2019.09.004
- Lopez-Bellido R, Galko MJ (2020) An improved assay and tools for measuring mechanical nociception in drosophila larvae. Journal of Visualized Experiments 164: e61911. https://doi.org/10.3791/61911 [PubMed] [PMC]
- Lopez-Bellido R, Himmel NJ, Gutstein HB, Cox DN, Galko MJ, Galko MJ (2019) An assay for chemical nociception in *Drosophila* larvae. Philosophical Transactions of the Royal Society B: Biological Sciences 374(1785): 20190282. https://doi.org/10.1098/rstb.2019.0282 [PubMed] [PMC]
- Luo J, Shen WL, Montell C (2017a) TRPA1 mediates sensation of the rate of temperature change in *Drosophila* larvae. Nature Neuroscience 20(1): 34–41. https://doi.org/10.1038/nn.4416 [PubMed] [PMC]
- Luo J, Shen WL, Montell C (2017b) TRPA1 mediates sensation of the rate of temperature change in *Drosophila* larvae. Nature Neuroscience 20(1): 34–41. https://doi.org/10.1038/nn.4416 [PubMed] [PMC]
- Millet-boureima C, Selber-hnatiw S (2021) Drug discovery and chemical probing in *Drosophila*. Genome 64(2): 147–159. https://doi.org/10.1139/gen-2020-0037 [PubMed]
- Moulin TC, Covill LE, Itskov PM, Williams MJ, Schiöth HB (2021) Rodent and fly models in behavioral neuroscience: An evaluation of methodological advances, comparative research, and future perspectives. Neuroscience and Biobehavioral Reviews 120: 1–12. https://doi.org/10.1016/j.neubiorev.2020.11.014 [PubMed]
- Nässel DR, Zandawala M (2020) Hormonal axes in *Drosophila*: regulation of hormone release and multiplicity of actions. Cell and Tissue Research 382(2): 233–266. https://doi.org/10.1007/s00441-020-03264-z [PubMed] [PMC]
- Oikawa I, Kondo S, Hashimoto K, Kashiwabara A, Tanimoto H, Furukubo-Tokunaga K, Honjo K (2022) A descending inhibitory mechanism of nociception mediated by an evolutionarily conserved neuropeptide system in *Drosophila*. bioRxiv preprint: 2003–2005. https://doi.org/10.1101/2022.03.08.483420
- Orgogozo V, Grueber WB (2005) FlyPNS, a database of the *Drosophila* embryonic and larval peripheral nervous system. BMC Developmental Biology 5: 2–7. https://doi.org/10.1186/1471-213X-5-4 [PubMed] [PMC]
- Oswald M, Rymarczyk B, Chatters A, Sweeney ST (2011) A novel thermosensitive escape behavior in drosophila larvae. Fly 5(4): 304–306. https://doi.org/10.4161/fly.5.4.17810 [PubMed] [PMC]
- Pagés M, Jiménez F, Ferrús A, Peralta E, Ramírez G, Gelpí E (1983) Enkephalin-like immunoreactivity in *Drosophila melanogaster*. Neuropeptides 4(1): 87–98. https://doi.org/10.1016/0143-4179(83)90012-4 [PubMed]
- Pagés M, Roselló J, Casa J, Gelpí E, Gualde N, Rigaud M (1986) Cyclooxygenase and lipoxygenase-like activity in *Drosophila melanogaster*. Prostaglandins 32(5): 729–740. https://doi.org/10.1016/0090-6980(86)90195-4 [PubMed]
- Pandey UB, Nichols CD (2011) Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. Drug Delivery 63(2): 411–436. https://doi.org/10.1124/pr.110.003293 [PubMed] [PMC]
- Panettieri RA, Schaafsma D, Amrani Y, Koziol-White C, Ostrom R, Tliba O (2019) Non-genomic effects of glucocorticoids: an updated view. Trends in Pharmacological Sciences 40(1): 38–49. https://doi.org/10.1016/j.tips.2018.11.002 [PubMed] [PMC]
- Papanikolopoulou K, Mudher A, Skoulakis E (2019) An assessment of the translational relevance of *Drosophila* in drug discovery. Expert Opinion on Drug Discovery 14(3): 303–313. https://doi.org/10.1080/17460441.2019.1569624 [PubMed]
- Robertson JL, Tsubouchi A, Tracey WD (2013) Larval defense against attack from parasitoid wasps requires nociceptive neurons. PLoS ONE 8(10): e78704. https://doi.org/10.1371/journal.pone.0078704 [PubMed] [PMC]
- Roth GJ, Majerus PW (1975) The mechanism of the effect of aspirin on human platelets. I. Acetylation of a particulate fraction protein. Journal of Clinical Investigation 56(3): 624–632. https://doi.org/10.1172/JCI108132 [PubMed] [PMC]
- Santoro C, Hall LM, Zukin RS (1990) Characterization of two classes of opioid binding sites in drosophila melanogaster head membranes. Journal of Neurochemistry 54(1): 164–170. https://doi.org/10.1111/j.1471-4159.1990.tb13297.x [PubMed]
- Scarpati M, Qi Y, Govind S, Singh S (2019) A combined computational strategy of sequence and structural analysis predicts the existence of a functional eicosanoid pathway in *Drosophila melanogaster*. PLoS ONE 14(2): e0211897. https://doi.org/10.1371/journal.pone.0211897 [PubMed] [PMC]
- Schirmeier S, Klämbt C (2015) The *Drosophila* blood-brain barrier as interface between neurons and hemolymph. Mechanisms of Development 138(1): 50–55. https://doi.org/10.1016/j.mod.2015.06.002 [PubMed]
- Shinozaki J (1957) The specific heat of insects. Journal of The Faculty of Science Hokkaido University 13: 470–474. https://doi.org/http://hdl.handle.net/2115/2727
- Smith ESJ, Lewin GR (2009) Nociceptors: a phylogenetic view. Journal of Aomparative Physiology A 195(12): 1089–1106. https://doi.org/10.1007/s00359-009-0482-z [PubMed] [PMC]
- Smoak KA, Cidlowski JA (2004) Mechanisms of glucocorticoid receptor signaling during inflammation. Mechanisms of Ageing

and Development 125(10–11): 697–706. https://doi.org/10.1016/j.mad.2004.06.010 [PubMed]

- Taylor K, Alvarez LR (2019) An estimate of the number of animals used for scientific purposes worldwide in 2015. ATLA Alternatives to Laboratory Animals 47: 196–213. https://doi.org/10.1177/0261192919899853 [PubMed]
- Tokusumi Y, Tokusumi T, Schulz RA (2017) The nociception genes painless and Piezo are required for the cellular immune response of *Drosophila* larvae to wasp parasitization. Biochemical and Biophysical Research Communications 486(4): 893–897. https://doi.org/10.1016/j.bbrc.2017.03.116 [PubMed]
- Tootle TL, Spradling AC (2008) *Drosophila* Pxt: a cyclooxygenase-like facilitator of follicle maturation. Development 135(5): 839–847. https://doi.org/10.1242/dev.017590 [PubMed] [PMC]
- Tracey WD, Wilson RI, Laurent G, Benzer S (2003) Painless, a *Drosophila* gene essential for nociception. Cell 113(2): 261–273. https://doi.org/10.1016/S0092-8674(03)00272-1 [PubMed]
- Verheyen EM (2022) The power of *Drosophila* in modeling human disease mechanisms. Disease Models & Mechanisms 15(3): 2020–2022. https://doi.org/10.1242/dmm.049549 [PubMed] [PMC]
- Wang K, Guo Y, Wang F, Wang Z (2011) *Drosophila* TRPA channel painless inhibits male-male courtship behavior through modulating olfactory sensation. PLoS ONE 6(11): e25890. https://doi.org/10.1371/journal.pone.0025890 [PubMed] [PMC]
- Williams CJA, James LE, Bertelsen MF, Wang T (2019) Analgesia for non-mammalian vertebrates. Current Opinion in Psychology 11: 75–84. https://doi.org/10.1016/j.cophys.2019.07.001
- Woolf CJ (2018) Pain amplification—A perspective on the how, why, when, and where of central sensitization. Journal of Applied Biobehavioral Research 23: 1–9. https://doi.org/10.1111/jabr.12124
- Xu J, Li M, Shen P (2010) A G-protein-coupled neuropeptide Y-like receptor suppresses behavioral and sensory response to multiple stressful stimuli in *Drosophila*. Journal of Neuroscience 30: 2504–2512. https://doi.org/10.1523/JNEUROSCI.3262-09.2010 [PubMed] [PMC]
- Yoshino J, Morikawa RK, Hasegawa E, Emoto K (2017) Neural circuitry that evokes escape behavior upon activation of nociceptive sensory neurons in drosophila larvae report neural circuitry that evokes escape behavior upon activation of nociceptive sensory neurons in drosophila larvae. Current Biology 27(16): 2499–2504.e3. https://doi.org/10.1016/j.cub.2017.06.068 [PubMed]

Author contributions

- **Thamyris Santos-Silva**, MSc. Department of Pharmacology, Institute of Biological Science, e-mail: thamyrisantos@gmail.com, ORCID ID https://orcid.org/0000-0001-5129-5695. The author was engaged in investigation, designing methodology, and writing the original draft.

- **Caio Fábio Baeta Lopes**, MSc. Department of Pharmacology, Institute of Biological Science, e-mail: caioblopes@yahoo.com, ORCID ID https://orcid.org/0000-0002-0080-2308. The author was engaged in investigation and designing methodology.

- **Jennifer Diniz Soares Gumarães**, MSc. Department of Physiology and Biophysics, Institute of Biological Science, e-mail: jenniferdiniz.guimaraes@hotmail.com, ORCID ID https://orcid.org/0000-0002-7787-4553. The author was engaged in investigation and designing methodology.

- **Felipe Berti Valer**, PhD. Department of Cellular and Molecular Biology, Ribeirão Preto Medical School, e-mail: fbvaler@gmail.com, ORCID ID https://orcid.org/0000-0001-6286-3811. The author was engaged in investigation and designing methodology.

- **Gustavo Campos Silva Kuhn**, PhD, Professor. Department of Genetics, Ecology and Evolution, Institute of Biological Science, e-mail: guto.cs.kuhn@gmail.com, ORCID ID https://orcid.org/0000-0002-6280-7964. The author was engaged in design methodology.

- **Thiago Roberto Lima Romero**, PhD, Professor. Department of Pharmacology, Institute of Biological Science, e-mail: thiromero@gmail.com, ORCID ID https://orcid.org/0000-0001-9723-0395. The author was engaged in investigation and equipment and reagent resources.

- **Lígia Araújo Naves**, PhD, Professor. Department of Physiology and Biophysics, Institute of Biological Science, e-mail: lanavesk@gmail.com, ORCID ID https://orcid.org/0000-0002-2668-4098. The author was engaged in designing methodology of research.

- **Igor Dimitri Gama Duarte**, PhD, Professor. Department of Pharmacology, Institute of Biological Science, e-mail: dimitri@icb.ufmg.br, ORCID ID https://orcid.org/0000-0003-3891-5225. The author was engaged in investigation, equipment and reagent resources, formal analysis, conceptualization, reviewing and editing the draft, and supervision of the research.