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The different effects of structurally-related sulfakinins on *Drosophila melanogaster* odor preference and locomotion suggest involvement of distinct mechanisms

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Abstract

Sulfakinins are myoactive peptides and antifeedant factors. Naturally-occurring drosulfakinin I (DSK I; FDDYGHMRFNH₂) and drosulfakinin II (DSK II; GGDDQFDDYGHMRFNH₂) contain sulfated or nonsulfated tyrosine. We discovered sDSK II and nsDSK II influenced *D. melanogaster* larval odor preference. However, sDSK I, nsDSK I, MRFNH₂, and saline did not influence odor preference. We discovered sDSK I and nsDSK I influenced larval locomotion. However, sDSK II, nsDSK II, MRFNH₂, and saline did not influence locomotion. Our novel data suggest distinct mechanisms underlie the effects of DSK I and DSK II peptides on odor preference and locomotion, parameters important to many facets of animal survival.

Keywords

Cholecystokinin; Drosulfakinin; Feeding; Odorants; Olfactory

1. Introduction

The ability to recognize and respond to odors in the environment plays crucial roles in diverse behaviors which are critical to animal survival, from avoiding a stealth predator to locating luscious food or a desirable mate. Over the past several years there has been considerable progress made in olfaction research [14, 19, 25, 39]. However, much remains to be learned at the cellular and molecular levels about how cues are received and processed, and responses are formulated and performed. The task of feeding involves locating food and digestion which requires the coordination of numerous physiological functions including smell, locomotion, and gut motility. Peptides act as messengers and regulators of these activities and are often pleiotropic.

The environment is full of olfactory cues which impact a broad spectrum of animal behaviors throughout development, from the immature larva to the mature adult. The *Drosophila melanogaster* larva is often used to investigate animal response to olfactory cues

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because of its relatively simple olfactory structures yet complex behaviors [13, 15, 28]. Additionally, homology between the fruit fly and vertebrate genomes further suggests what is learned can be translated to other organisms in order to delineate the roles of peptides as messengers and modulators of crucial behaviors.

The first sulfakinin identified was leucosulfakinin (LSK; EQFEDY(SO₃H)GHMRFNH₂); it was isolated from a cockroach brain extract based on its myotropic activity on gut [29]. Subsequently, other myoactive sulfakinin peptides were identified based on structural similarity to LSK [7, 8, 11, 12, 17, 18, 22, 26, 30, 31, 35, 37, 38, 40]. The first sulfakinin gene identified, *D. melanogaster* sulfakinin (Dsk), encodes two structurally-related peptides, drosulfakinin I (DSK I; FDDYGHMRFNH₂) and drosulfakinin II (DSK II; GGDDQFDDYGHMRFNH₂) [34]. Like their vertebrate homologues, the cholecystokinin (CCK) peptides, naturally-occurring sulfakinins contain a sulfated or nonsulfated tyrosyl residue [1, 3, 5, 17, 23, 31, 32, 35, 36]. The sulfated and nonsulfated drosulfakinin I and drosulfakinin II peptides are designated sDSK I and nsDSK I and sDSK II and nsDSK II, respectively.

In addition to their myoactive effects, sulfakinins elicit a decrease in food intake [9, 24, 27, 41]. Cholecystokinin, discovered based on its myoactive property, is also a satiety factor [42]. Putative G-protein coupled receptors (GPCRs) were identified by searching the *D. melanogaster* genome database and two sulfakinin receptors, DSK-R1 and DSK-R2, were found by homology to the cholecystokinin receptors, CCKR1 and CCKR2 [4, 10, 16]. The tissue distributions and binding requirements of the sulfakinin receptor are not yet delineated; the tissue distributions and binding requirements of the cholecystokinin receptors differ from one another. Distinct neuronal pathways underlie the effects of CCKR1 and CCKR2 agonists in olfactory recognition [21].

Drosulfakinin immunoreactive material localizes to the *D. melanogaster* medial protocerebrum in several cells from which complex arborizations originate and project to impinge on regions of the brain associated with olfaction [33]. However, to our knowledge no publication reports the analysis of sulfakinins on odor response. The myotropic effects of sulfakinins on gut, the role of sulfakinins in antifeedant behavior, and the importance of movement in many critical biological activities including foraging, led us to investigate the role of drosulfakinin peptides in odor preference and locomotion. Our results together with published reports are consistent with the conclusion that the structurally-related DSK peptides act to influence odor preference and locomotion through distinct mechanisms.

2. Materials and methods

2.1 Animals

D. melanogaster Oregon R wild type strain flies were raised at 22° C under a 12hr:12hr light:dark cycle. The animals were maintained on standard cornmeal molasses media. Third instar feeding larvae were collected, rinsed with deionized water, and blotted dry on a laboratory wipe immediately before use in an assay.

2.2 Chemicals

The sulfated and nonsulfated DSK I and DSK II peptides, and MRFNH₂, a C-terminal sulfakinin truncation analog, were synthesized by standard Fmoc protocol with a Protein Technologies Symphony synthesizer and purified by reversed phase HPLC. The structures of the peptides were confirmed by amino acid analysis and mass spectrometry. The odorants used were reagent grade butyl alcohol (BuOH; Sigma-Aldrich, St. Louis, MO) and SequanalTM grade ethyl acetate (Ac; Pierce Chemical Co., Rockford, IL). The agarose used was electrophoresis grade (Schwarz Mann Biotech, Cleveland, OH).

2.3 Assays

The odor preference assay was based on established protocols described in the literature [13], however, there were some important changes. We conducted the assay in disposable polystyrene Petri dishes (Fisher Scientific, Pittsburgh, PA); a dish was circular with a 100 mm diameter x 15 mm depth. Each dish lid contained 5 holes, 1 hole in the center and 2 holes each spaced 2 cm from the center on a vertical axis or on a horizontal axis; the holes were made with a 21 gauge needle. A dish containing approximately 8 ml of 1% agarose was used in the assay and referred to as a “plate”. The plates were made fresh the day of the experiment and allowed to solidify and cool for at least 20 minutes prior to being used. Odorant reservoirs were made from the lids of 0.5 ml flat-top microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA). The reservoirs were placed along the midline of each plate, at the extreme left and right, directly across from each other on the horizontal axis. Immediately prior to the experiment, the reservoirs were filled with either 95µl of an odorant or deionized water (control). The plate was placed on top of a sheet of paper which contained a grid of vertical lines and horizontal lines separated by 0.5 cm. The area of the paper on which the plate was placed was divided into 3 sections by 2 vertical lines each drawn 1.5 cm from the center vertical axis of the dish; the sections defined odor preference zones. The central section was 3 cm wide and did not contain an odorant reservoir, thus, it was referred to as the neutral zone. The remaining sections, the left odorant zone and the right odorant zone, each contained an odorant reservoir and were identified in the data by the content of the reservoir. The zone in which an odorant was placed did not significantly affect odor preference.

To begin the olfactory preference assay an individual larva was injected with 40 nl of DSK peptide, truncation analog, or saline (5mM HEPES, 128 mM NaCl, 36 mM sucrose, 4 mM MgCl₂, 2 mM KCl, and 1.8 mM CaCl₂, pH 7.1). The final concentration of peptide or analog in the animal was 1 µM calculated based on the volume and concentration of the solution injected, and the estimated volume of hemolymph calculated based on animal length and diameter. The injection site was in the second anterior segment, lateral to the midline. After injection, a larva was placed in the center of an olfactory assay plate. Typically, four larvae were individually injected in a series after which each larva was placed on a separate plate and all larvae were monitored simultaneously by an individual who performed the experiment without knowledge of the injectant; peptide, analog or saline. No statistical difference was observed among the four animals compared to conducting the assay with one animal. The time from injection to placement on a plate was recorded and, on average, the total time for injection of four larvae and placement on the plates was 1 to 1½ minutes. The path of each larva was traced on the lid of the plate over a period of 5 minutes noting intervals of every 20 seconds with tick marks.

The locomotion assay used a similar protocol to the olfactory preference assay, however, a larger circular dish, 150 mm diameter x 15 mm depth (Falcon, Becton Dickinson, Franklin Lakes, NJ), was used to allow the animals more space in which to move. The assay recorded larval movement in the absence of odorants. Locomotion plates contained approximately 12 ml of 1% agarose and were placed on 0.5 cm grid paper described above. Lids were placed on the locomotion plates during the assay; unlike the odor preference assay, the lids used on locomotion assay plates contained no holes.

In order to perform the locomotion assay an individual larva was injected with 40 nl of DSK peptide, truncation analog, or saline. The final concentration of peptide or analog in the animal was 1 µM calculated based on the volume and concentration of the solution injected and the estimated volume of hemolymph. The injection site was the same as for the olfactory preference assay, in the second anterior segment, lateral to midline. After injection, a larva was placed in the center of a locomotion assay plate. Typically, four larvae were

individually injected after which each larva was placed on a separate plate and monitored simultaneously by an individual who performed the experiment without knowledge of the injectant. No statistical difference was observed among the four animals. The time from injection to placement on a plate was recorded and, on average, the total time for injection of four larvae and placement on the plates was 1 to 1½ minutes. The path of each larva was traced on the lid of the plate over a period of 5 minutes.

All injections were performed with a pipette made from borosilicate non-filament 1.2 mm glass (World Precision Instruments (WPI), Sarasota, FL) pulled using a Flaming/Brown Micropipette Puller Model 9-87 (Sutter Instrument, Novato, CA). The tip of each pipette was ground on sandpaper to a tapered point measuring 5-7 µm. A pipette was filled with a solution and placed in a micromanipulator (WPI); delivery of a measured volume was done using a calibrated micrometer (WPI).

2.4 Data analysis

In the olfactory assay the number of 20-second tick marks in the path was counted for each larva, individually, for each of the 3 zones, the left odorant zone, the neutral zone, and the right odorant zone of each plate. A preference index (PREF) was calculated for each animal by subtracting the number of 20-second tick marks in the right zone from the number of 20-second tick marks in the left zone and dividing that by the total number of tick marks. Thus, a preference for the odorant in the left zone is indicated by a positive PREF index, and a preference for the odorant in the right zone is indicated by a negative PREF index. A PREF index of 1 indicates the larva preferred the odorant in reservoir in the left zone; a PREF index of -1 indicates the larva preferred the odorant in the reservoir in the right zone. Averaged PREF indices are reported; error bars represent standard error. Statistical analysis was done using ANOVA; p values = 0.02 were considered to be significant. In the locomotion assay the number of 0.5 cm grid lines a larva crossed in 5 minutes was determined. Averaged numbers of lines crossed are reported; error bars represent standard error. Statistical analysis was done using ANOVA; p values < 0.001 were considered to be significant.

3. Results

In order to investigate whether DSK peptides played a role in olfactory preference we first looked at the effect of saline as a control to establish our assay. The effect of saline on odor preference in the presence of BuOH was that larvae preferred an odorant (left zone) rather than no odorant (central zone) or water (right zone) (Fig. 1A). Overall, the number of larvae with an odor preference for BuOH (n = 42) was larger than the number of animals (n = 18) which preferred the other two zones combined, no odorant (n = 14) and water (n = 4). Sixty larvae were subjected to this odor preference paradigm in the experiment reported in Fig. 1A; repetition of the assay resulted in the same outcome. The total number of animals analyzed in the two experiments was 122.

Similarly, the effect of saline on odor preference in the presence of Ac was that larvae preferred an odorant rather than no odorant or water (Fig. 1B). Overall, the number of larvae with an odor preference for Ac (n = 38) was larger than the number of animals (n = 22) which preferred the other two zones combined, no odorant (n = 18) and water (n = 4). Sixty larvae were subjected to this odor preference paradigm (Fig. 1B); repetition of this assay resulted in the same outcome. The total number of animals analyzed in the two experiments was 120.

When presented with a choice between BuOH and Ac, the effect of saline on odor preference was that larvae preferred Ac (Fig. 1C). Overall, the number of larvae with a

preference for an odorant ($n = 32$), Ac ($n = 27$) or BuOH ($n = 5$), was larger than the number of animals which preferred the neutral zone ($n = 28$), the only zone to contain no odorant reservoir. Sixty larvae were subjected to this odor preference paradigm (Fig. 1C); repetition of this experiment resulted in the same outcome. The total number of animals analyzed was 171.

As a further control for the effect of exogenously applied peptide, we tested the effect of MRFNH₂ on odor preference. The effect of MRFNH₂ in the presence of BuOH was that larvae preferred an odorant rather than water with a PREF index of 0.50 ± 0.05 (Fig. 2A). The total number of larvae analyzed in this experiment was 111. In comparison, the effect of saline on odor preference in the presence of BuOH was that larvae preferred BuOH to water with a PREF index of 0.67 ± 0.03 (Fig. 2A). The total number of larvae analyzed in this experiment was 62.

The effect of MRFNH₂ on odor preference in the presence of Ac was that larvae preferred an odorant rather than water with a PREF index of 0.24 ± 0.04 (Fig. 2B). The total number of larvae analyzed in this experiment was 112. In comparison, the effect of saline on odor preference in the presence of Ac was that larvae preferred Ac to water with a PREF index of 0.31 ± 0.05 (Fig. 2B). The total of animals analyzed in this experiment was 60.

When presented with a choice between BuOH and Ac, the effect of MRFNH₂ on odor preference was that larvae preferred Ac rather than BuOH with a PREF index of 0.30 ± 0.07 (Fig. 2C). The total number of larvae analyzed in this experiment was 111. In comparison, the effect of saline on odor preference was that larvae preferred Ac to BuOH with a PREF index of 0.31 ± 0.05 (Fig. 2C). The total number of animals analyzed in this experiment was 111.

We tested the effects of all four DSK peptides (sDSK I, nsDSK I, sDSK II, nsDSK II) on odor preference. The effects of sulfated and nonsulfated DSK I and DSK II peptides on odor preference in the presence of BuOH was that larvae preferred an odorant rather than water with PREF indices of 0.60 ± 0.04 (sDSK I), 0.75 ± 0.03 (nsDSK I), 0.47 ± 0.05 (sDSK II), and 0.30 ± 0.08 (nsDSK II) (Fig. 3A). The total numbers of larvae analyzed in this experiment were 96 (sDSK I), 62 (nsDSK I), 122 (sDSK II), and 60 (nsDSK II). In comparison, the effect of saline on odor preference in the presence of BuOH was that larvae preferred an odorant to water with a PREF index of 0.67 ± 0.03 (Fig. 3A). The effects of sDSK II and nsDSK II were statistically significant compared to saline, p values 0.004 and 0.00004, respectively; the effects of sDSK I and nsDSK I were not statistically significant, p values 0.2 and 0.7, respectively.

The effects of sulfated and nonsulfated DSK I and DSK II peptides on odor preference in the presence of Ac was that larvae preferred an odorant rather than water with PREF indices of 0.34 ± 0.04 (sDSK I), 0.31 ± 0.05 (nsDSK I), 0.20 ± 0.04 (sDSK II), and 0.20 ± 0.05 (nsDSK II) (Fig. 3B). The total numbers of larvae analyzed in this experiment were 97 (sDSK I), 62 (nsDSK I), 122 (sDSK II), and 62 (nsDSK II). In comparison, the effect of saline on odor preference in the presence of Ac was that larvae preferred an odorant to water with a PREF index of 0.31 ± 0.05 (Fig. 3B). None of these effects were statistically different compared to saline; p values were 0.5 (sDSK I), 0.9 (nsDSK I), 0.2 (sDSK II), and 0.2 (nsDSK II).

When presented with a choice between BuOH and Ac, the effect of sulfated and nonsulfated DSK I and DSK II peptides on odor preference was that larvae preferred Ac rather than BuOH with PREF indices of 0.16 ± 0.06 (sDSK I), 0.29 ± 0.04 (nsDSK I), 0.07 ± 0.04 (sDSK II), and 0.15 ± 0.05 (nsDSK II) (Fig. 3C). The total numbers of larvae analyzed in this experiment were 60 (sDSK I), 109 (nsDSK I), 122 (sDSK II), and 61 (nsDSK II). In

comparison, the effect of saline on odor preference was that larvae preferred Ac to BuOH with a PREF index of 0.31 ± 0.05 (Fig 3C). The effects of sDSK II, and nsDSK II were statistically different compared to saline, p values 0.00005 and 0.02, respectively; the effects of sDSK I and nsDSK I were not statistically significant, p value 0.03 and 0.8, respectively.

To investigate the role of DSK peptides in movement, we determined the effect of saline, MRFNH₂, and the four DSK peptides on larval locomotion. The average number of lines crossed in 5 minutes by larvae was 51 ± 4 (saline), 50 ± 2 (MRFNH₂), 32 ± 2 (sDSK I), 31 ± 2 (nsDSK I), 42 ± 2 (sDSK II), and 49 ± 3 (nsDSK II) (Fig. 4). The numbers of larvae analyzed were 29 (saline), 40 (MRFNH₂), 40 (sDSK I), 40 (nsDSK I), 37 (sDSK II), and 40 (nsDSK II).

The effects of sDSK I and nsDSK I were statistically different from saline, p values 0.000002 and 0.000004, respectively; the effects of sDSK II and nsDSK II were not significant, p values 0.01 and 0.6, respectively.

Discussion

Peptides are widely distributed in the central nervous system and gastrointestinal tract where they are involved in numerous roles including messengers and modulators of animal behavior. Sulfakinins affect feeding and gut motility, and are similar in structure to vertebrate CCK peptides which act in olfactory recognition, learning and memory, feeding, and gut motility. The myotropic effects of sulfakinins on gut, the role of sulfakinins in antifeedant behavior, and the presence of DSK immunoreactive material in regions of the brain involved in olfaction led us to investigate the role of drosulfakinin peptides in odor preference. We chose the *D. melanogaster* larva because it is an established model for sensory studies.

We found saline did not affect odor preference of larvae when presented with a choice between odorant (Ac or BuOH) and water, consistent with previous studies performed on *D. melanogaster*. The overall preference for Ac over BuOH when given the choice may be due to the greater volatility of acetate. The number of animals in the neutral zone may have been due to the volatility of the odorants causing the area to be an odor-rich environment like a food source. A C-terminal truncation DSK analog, MRFNH₂, served as a peptide control to demonstrate activity was not ubiquitous to RFNH₂-containing peptides but was specific to the sulfakinin structure(s).

The ability of animals to recognize odors in the environment is crucial to many diverse behaviors including finding a food source. Peptides are known to play key roles in feeding behavior; peptides may also be pleiotropic. Sulfated DSK II and nonsulfated DSK II significantly affected larval preference for BuOH in the BuOH/water paradigm and Ac in the Ac/BuOH paradigm. However, sDSK I and nsDSK I did not significantly affect larval odor preference for any paradigm. We believe this is the first report of the influence of a sulfakinin in odor preference.

The ability of animals to move is critical for foraging, an act in which odor cues are involved. Movement was important in responding to the odor preference assay we employed. Thus, we also examined the effect of the four DSK peptides on whole animal locomotion. The effects of sDSK I and nsDSK I were statistically different from saline. However, sDSK II and nsDSK II did not significantly affect larval locomotion. We believe this is the first report of the influence of a sulfakinin in locomotion.

The difference in the influence of DSK II and DSK I peptides suggests distinct mechanisms are involved in the effects of these structurally-related peptides. The mechanisms by which

sulfakinins affect physiological parameters are not yet known. Two GPCRs, DSK-R1 and DSK-R2, with homology to cholecystokinin receptors, CCKR1 and CCKR2 [10], were identified from the *D. melanogaster* genome database [4, 16]. The binding requirements of one sulfakinin receptor candidate, DSK-R1, were examined using sulfated and nonsulfated forms of a DSK I analog, Leu⁷-DSK I (FDDYGHLRFNH₂) [20]. Binding the analogs to *in vitro* expressed protein led the authors to conclude only a sulfated DSK I activated DSK-R1; they report the nonsulfated DSK I analog binds the expressed receptor protein about 3000-fold less. Unfortunately, no data are provided to demonstrate that the Leu⁷-substituted DSK I analogs accurately reflect the binding of the naturally-occurring sulfakinin peptides, nor do binding data reflect biological activities. The report does not investigate DSK-R2 binding requirements, nor does it examine sulfated or nonsulfated DSK II ligand-receptor binding.

Our data indicate there was a structure requirement for the effects of DSK peptides on odor preference and locomotion activities. We observed distinct effects of the peptides even though the sulfakinins are highly similar in structure. The C-terminal structures of sulfated and nonsulfated DSK I and DSK II peptides are -FDDY(SO₃H)GHMRFNH₂ and -FDDYGHMRFNH₂, respectively. The N-terminal structures are different, DSK II is a 5 amino acid N-terminal extension (GGDDQ-) of DSK I. Our data suggest the N terminal extensions are important in ligand-receptor binding. In addition, our data demonstrate that both forms of the sulfakinins, the sulfated and nonsulfated peptide of a given sulfakinin, DSK II or DSK I, are functional in odor preference or locomotion, respectively. This information provides further insight into the mechanisms involved in the influence of sulfakinins on biological parameters.

Based on the potential for two DSK receptors and mechanisms involved in the physiology of other neuropeptides, in particular CCK, models which explain the data can be developed. Sulfakinins are structurally and functionally similar to vertebrate CCK peptides which act through two GPCRs designated CCK1R and CCK2R [10]. CCK1R shows high specificity for sulfated CCK; CCK2R has the same affinity for both sulfated and nonsulfated CCK. Selective binding and/or spatial expression of DSK receptors may help to explain the influence of the structurally-related DSK peptides on odor preference and locomotion. Our data are consistent with the ability of both sDSK II and nsDSK II to bind and act through a specific DSK-RX₁ to influence odor preference; X₁ may be 1 or 2 [4, 16]. In this model, both sulfated and nonsulfated DSK I would bind and act through another distinct DSK-RX₂, X₁ ≠ X₂, different from the receptor to which DSK II peptides bind, to influence locomotion.

Inhibitory local neurons (LNs) in the insect antennal lobe are involved in regulation of olfactory processing [6]. Interestingly, these neurons have been shown to be peptidergic and contain FMRFNH₂ immunoreactive material in moth [2]. The decrease in odorant preference in response to exogenous DSK peptides is consistent with the hypothesis that peptidergic LNs are involved in inhibition of olfactory processing. This study is important as the first confirmation through behavioral testing of a possible role for sulfakinin peptides in olfactory preference.

Understanding how information is received and processed from olfactory cues is crucial to delineate feeding behavior. Our data that DSK II peptides are involved in odor preference and DSK I peptides are involved in locomotion are novel. Additionally, the finding that both sulfated and nonsulfated sulfakinins are effective provides additional information for the investigation of the mechanisms which underlie these conserved peptides. The influences on odor preference and locomotion and the distribution of DSK peptides in the brain, suggest sulfakinins, like cholecystokinins, may play a role in feeding-related behavior(s).

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Fig 1A.

The effect of saline on odor preference; BuOH odorant. The number of animals (y-axis) and the zone in which they spent a plurality of time (x axis). The left zone reservoir contained butanol (BuOH), the central or neutral zone contained no reservoir (unlabeled), and the right zone reservoir contained water (H2O). The number of animals analyzed was $n = 60$.

Fig 1B.

The effect of saline on odor preference; Ac odorant. The number of animals (y-axis) and the zone in which they spent a plurality of time (x-axis). The left zone reservoir contained acetate (Ac), the central or neutral zone contained no reservoir (unlabeled), and the right zone reservoir contained water (H₂O). The number of animals analyzed was n = 60.

Fig 1C.

The effect of saline on odor preference; Ac and BuOH odorants. The number of animals (y-axis) and the zone in which they spent a plurality of time (x-axis). The left zone reservoir contained acetate (Ac), the central or neutral zone contained no reservoir (unlabeled), and the right zone reservoir contained butanol (BuOH). The number of animals analyzed was $n = 60$.

Fig 2A.

The effects of saline and MRFNH₂ on odor preference; BuOH odorant. PREF (y axis) equals (# animals in left zone - # animals in right zone)/(total # of animals). The left zone reservoir contained butanol (BuOH) and the right zone reservoir contained water (H₂O); x axis. The number of animals analyzed was n = 120 (saline) and n = 113 (MRFNH₂).

Fig 2B.

The effects of saline and MRFNH₂ on odor preference; Ac odorant. PREF (y axis) equals (# animals in left zone - # animals in right zone)/(total # of animals). The left zone reservoir contained acetate (Ac) and the right zone reservoir contained water (H₂O); x axis. The number of animals analyzed was n = 120 (saline) and n = 112 (MRFNH₂).

Fig 2C.

The effects of saline and MRFNH₂ on odor preference; Ac and BuOH odorants. PREF (y axis) equals (# animals in left zone - # animals in right zone)/(total # of animals). The left zone reservoir contained acetate (Ac) and the right zone reservoir contained butanol (BuOH); x axis. The number of animals analyzed were n = 123 (saline) and n = 111 (MRFNH₂).

Fig 3A.

The effects of saline and sulfakinins on odor preference; BuOH odorant. A positive PREF index (y axis) indicates animals preferred the odor in the left reservoir butanol (BuOH) compared to the right reservoir water (H₂O) in response to the injectant; x axis. Data represent 110 larvae assayed for each injectant. Error bars represent % error. An asterisk (*) denotes sulfakinin odor preference data statistically significant compared to saline (p 0.02).

Fig 3B.

The effects of saline and sulfakinins on odor preference; Ac odorant. A positive PREF index (y axis) indicates animals preferred the odor in the left reservoir acetate (Ac) compared to the right reservoir water (H₂O), in response to the injectant; x axis. Data represent 100 larvae assayed for each injectant. Error bars represent % error. No sulfakinin odor preference data were statistically significant compared to saline ($p = 0.02$).

Fig 3C.

The effects of saline and sulfakinins on odor preference; Ac and BuOH odorants. A positive PREF indicates a systematic preference for the left odorant (Ac); negative PREF indicates a systematic preference for the right odorant (BuOH). Data represent 110 larvae assayed for each injectant. Error bars represent % error. An asterisk (*) denotes significant compared to saline ($p < 0.02$).

Fig 4.

The effects of saline and sulfakinins on locomotion. Results displayed as number of lines crossed in 5 minutes. Data represent 29 larvae assayed for each injectant. Asterisks represent effects statistically different from saline with p values < 0.001. Error bars represent standard error.