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Salt Processing in Larval *Drosophila*: Choice, Feeding, and Learning Shift from Appetitive to Aversive in a Concentration-Dependent Way

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Abstract

Sodium and chloride need to be ingested and cannot be stored. Therefore, choice of habitat and diet as related to NaCl needs to be tightly regulated. We thus expect that the behavioral effects of salt are organized according to its concentration. Here, we comparatively "fingerprint" the reflex releasing (in choice and feeding experiments) versus the reinforcing effects of sodium chloride ("salt") in terms of their concentration dependencies, using larval *Drosophila*. Qualitatively, we find that the behavioral effects of salt in all 3 assays are similar: choice, feeding, and reinforcing effect all change from appetitive to aversive as concentration is increased. Quantitatively, however, the appetitive effects for choice and feeding share their optimum at around 0.02 M, whereas the dose–response curve for the reinforcing effect is shifted by more than one order of magnitude toward higher concentrations. Interestingly, a similar shift between these 2 kinds of behavioral effect is also found for sugars (Schipanski et al. 2008). Thus, for salt and for sugar, the sensory-to-motor system is more sensitive regarding immediate, reflexive behavior than regarding reinforcement. We speculate that this may partially be due to a dissociation of the sensory pathways signaling toward either reflexive behavior or internal reinforcement.

Key words: *Drosophila* larva, feeding, learning, taste, olfaction, sodium chloride

Introduction

This study provides a behavioral view of salt processing. We compare the dose–effect functions of sodium chloride regarding choice behavior, feeding, and learning in *Drosophila* larvae, an emerging experimental system to understand chemosensory function and its neurobiological bases (reviews by Gerber and Stocker 2007; Gerber et al. 2008) (Figure 1).

Sodium chloride (NaCl, ''salt'') is necessary for a multitude of physiological processes, not the least important being neuronal function. Both sodium and chloride need to be ingested and cannot be stored. Thus, both these elements need to be taken up, and choice of habitat and of diet as related to salt content needs to be a well-regulated process, balanced by excretion of surplus salt, if any. As therefore uptake of just the right amount of salt is required, one may expect the behavioral effects of salt being tightly regulated according to concentration. Indeed, the appetitive responses to low salt gradually turn into aversion as concentration is increased (adult: Arora et al. 1987; larvae: Miyakawa 1981; Liu et al. 2003). These opposing behavioral responses involve discrete molecular and cellular processes. (i) A member of the *pickpocket* (*ppk*) gene family (*ppk11*; the *ppk* gene family is homologous to the *epithelial* Na^+ *channel/degenerin* gene family [EnaC] in vertebrates: Lindemann 2001) is exclusively expressed in 3 pairs of gustatory sensory neurons of the larva. Expression of this gene is necessary for the appetitive behavioral responses to low salt but dispensable for the aversive responses to high salt (Liu et al. 2003). (ii) In adults, the so-called L1 neurons are activated by salt with low threshold (between 0.01 and 0.05 M), whereas the L2 neurons have their threshold at about one order of magnitude higher concentration (Ishimoto and Tanimura 2004). (iii) Also in adults, Marella et al. (2006; fig. 3) report that neurons likely expressing different members of the *Gr* gene family can be activated by salt with low threshold as well as by sugars (*Gr5a*) or by salt with high threshold as well as by bitter substances (*Gr66a*) (for further studies concerning *Gr* function also see Ueno et al. 2001; Wang et al. 2004; Marella et al. 2006; Dahanukar et al. 2007).

Given these dissociations between low- and high-threshold salt processing, we use the concentration dependencies of

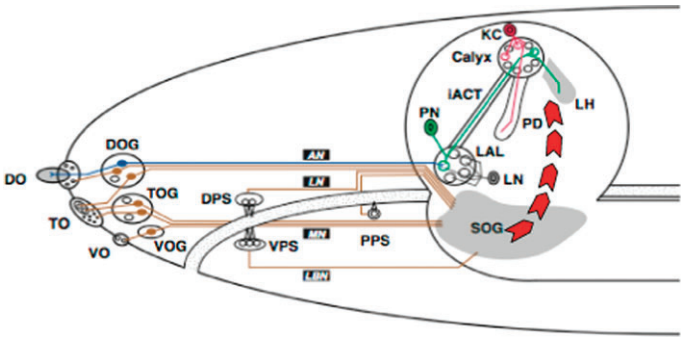

Figure 1 Chemosensory organs and pathways of larval *Drosophila*. Olfactory processing remains supraesophageal. Olfactory sensory neurons (blue) from the dorsal organ project toward the antennal lobe where they form synapses with both local interneurons and antennal lobe output elements, the projection neurons (green). These output neurons bifurcate: one branch directly innervates proposed premotor centers in the lateral horn, whereas the other branch forms a side loop via the mushroom bodies (red). Output from the mushroom bodies then presumably targets supraesophageal premotor centers as well. Taste processing (brown) bypasses the brain proper; rather, gustatory sensory neurons from the various external and internal taste organs project to the subesophageal ganglion. From there, motor centers in the ventral nerve cord and the mouthparts likely are innervated directly. With regard to odor–taste learning, modulatory interneurons are responsible to "short circuit" smell and taste: they receive input in the subesophageal ganglion and provide output toward the brain; the chevrons indicate this proposed pathway. Notably, separate kinds of modulatory interneuron seem to be responsible to carry appetitive (octopaminergic/tyraminergic neurons) and aversive (dopaminergic neurons) reinforcement (Schroll et al. 2006). Note that the actual connectivity toward the motor system is unknown; this, as the general layout of the chemosensory system, by and large corresponds to the situation in adult flies and insects in general. AN: antennal nerve, DO/DOG: dorsal organ/ganglion, DPS: dorsal pharyngeal sense organ, iACT: inner antennocerebral tract, KC: Kenyon cells, LAL: larval antennal lobe, LBN: labial nerve, LH: lateral horn, LN: local interneurons, LN: labral nerve, MN: maxillary nerve, PD: pedunculus, PN: projection neuron, PPS: posterior pharyngeal sense organ, SOG: subesophageal ganglion, TO/TOG: terminal organ/ganglion, VO/VOG: ventral organ/ganglion, VPS: ventral pharyngeal sense organ. Modified from Stocker (2006).

the salt effects as functional "fingerprints" to compare 2 kinds of behavioral function in larval *Drosophila* (see Schipanski et al. [2008] for a similar analysis regarding sugar processing):

- How does salt concentration affect reflexive behavior?
- How does salt concentration affect reinforcement function?

These 2 kinds of effect (i.e., reinforcing vs. reflex releasing) typically are dissociated in terms of the neuromodulators involved: for example, if honeybees are depleted of biogenic amines by injection of reserpine, compensatory injections of octopamine can restore the reinforcing effect of sugar but not its capacity to elicit ingestion reflexes (Menzel et al. 1999). Correspondingly, driving a single, identified octopaminergic neuron can substitute for the reinforcing effect of sugar but does not trigger ingestion reflexes (Hammer and Menzel 1995). In turn, dopamine injections can restore ingestion reflexes in reserpinized bees but not the reinforcing effect of sugar (Menzel et al. 1999) (see also de Araujo et al. 2008 concerning a dissociation of these functions in mice). Within this context, our study aims at parametrically dissociating the reflex releasing (in choice and feeding experiments) versus the reinforcing effects of NaCl in terms of their respective concentration dependencies.

Methods

We use third instar feeding stage larvae aged 5 days (±12 h) after egg laying. Flies of the Canton-S wild-type strain (Michels et al. 2005) are used which are kept in mass culture, maintained at 25 °C, 60–70% relative humidity and a 14/10 h light/dark cycle. Experiments are performed in red light under a fume hood at 20 °C –24 °C room temperature.

Choice behavior

Larvae are offered a choice between 2 substrates, one consisting of pure 1% agarose (electrophoresis grade; Roth, Karlsruhe, Germany) (PURE) and one of agarose with sodium chloride added at the indicated concentration (NaCl, purity 99.5%, Fluka/Sigma-Aldrich, Steinheim, Germany) (see inset of Figure 2).

Petri dishes of 90 mm inner diameter (Sarstedt, Nümbrecht, Germany) are equipped with a vertical barrier in the middle. These barriers are made from overhead transparencies and fixed to the rim of the plates with small stripes of tape. Parafilm is used to tighten the barrier. Then, the respective freshly boiled aqueous agarose solutions are poured into either side of the split petri dish to yield the desired combination of substrates on either side. Before the substances solidify, the barriers are gently torn out yielding a smooth yet sharp border between sides. After 20 min of cooling, plates are covered with their lids and left at room temperature overnight.

Thirty animals are placed to the middle of the plate. Then, animals are allowed to move about the plate for 15 min, until we determine the number of animals (#) located on either the sodium chloride side or the PURE side. Animals that dug into the agarose or crawled up the lids of the plates (approximately 5–15%) are not considered in data analysis. A preference index is calculated as

$$\mathrm{PREF} = (\#_{\mathrm{NaCl}} - \#_{\mathrm{PURE}})/\#_{\mathrm{TOTAL}} \quad (1)$$

Thus, positive values indicate attraction while negative values indicate repulsion.

Feeding behavior

To measure feeding, 30 larvae are placed on a petri dish filled with 1% agarose containing the chosen concentration of salt (see "Results") and 30% red food dye (RU9805; backfun.de,

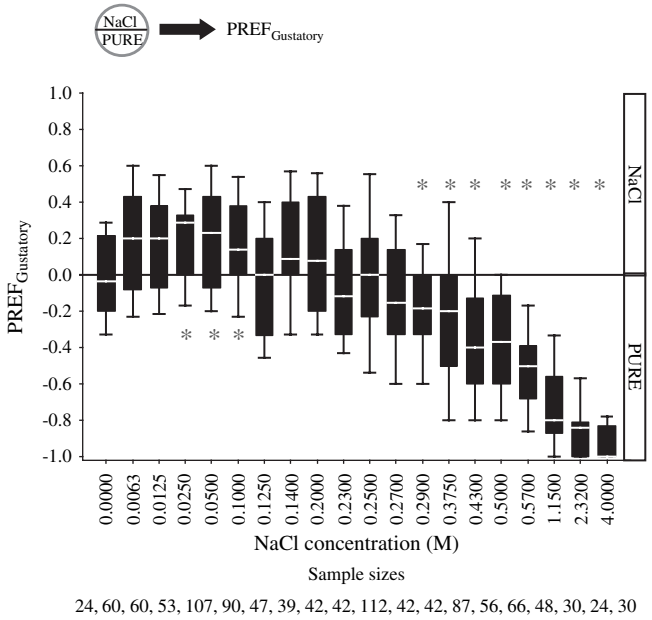

Figure 2 Choice. Preferences between plain agarose (PURE) versus various concentrations of salt; positive values indicate attraction and negative values repulsion. Behavior turns from appetitive to aversive as salt concentration is increased. *$P < .05/20$. Data are displayed as box plots, with the bold line indicating the median and box boundaries and whiskers the 25/75% and 10/90% quantiles, respectively.

Uhingen, Germany). On this substrate, the animals are allowed to feed for 15 min and then are washed in tap water and homogenized in 80 µl of distilled water. The homogenate is centrifuged (30 s, 13 200 rpm), and 50 µl of supernatant is loaded into each well of a 96-well plate (Hartenstein, Würzburg, Germany). Using a "Sunrise" spectrophotometer (Tecan AG, Männedorf, Switzerland), absorbance is measured at 500 nm. On each experimental day, we measure the absorbance of homogenate from animals that have been feeding on a plate containing no salt but only dyed agarose. We calculate a median absorbance from 3 to 15 such samples and take this value as baseline to be subtracted from all spectrophotometer readings on that experimental day; this subtraction then yields the feeding scores. Thus, if larvae feed as much in the presence of a given salt concentration as they do in its absence, feeding scores are zero; if they eat more or less than in the absence of salt, respectively, positive and negative feeding scores result. Per experimental day, 3–15 independent samples of 30 larvae each are measured per salt concentration.

Effect as reinforcer

For the learning experiments, larvae are offered a choice between a previously reinforced and a previously nonreinforced odor (see schematics in Figure 3A,C).

We use modified lids for the petri dishes with 15 concentrically arranged holes with 1-mm diameter to improve aeration. All petri dishes are homogeneous in that the complete dish either does or does not contain the reinforcer. Larvae receive either of 2 training regimens: either amyl acetate (AM, 99%; Merck, Hohenbrunn, Germany) is presented with reinforcement and 1-octanol (OCT, 99%; Fluka/Sigma-Aldrich) without reinforcement (AM+/OCT), whereas in the companion group the larvae are trained reciprocally (i.e., AM/OCT+). In half of the cases, we start with the trials involving AM, in the other half with the OCT-containing trials. In the test, we measure the distribution of the larvae between AM versus OCT. For the reinforced trials, we use petri dishes with sodium chloride added to the agarose at the indicated concentration; for the nonreinforced trials, we use petri dishes with only agarose.

Custom-made Teflon containers (diameter 5 mm) with perforated lids (7 concentrically arranged holes with 0.5 mm diameter each) are loaded with 10 µl of odorant (either AM diluted 1:50 in paraffin oil or OCT; Merck, Darmstadt, Germany) and placed onto the assay plate, which either does or does not contain the reinforcer. Thirty larvae are transferred to the assay plate and after 5 min are transferred to a fresh plate with the alternative odorant–substrate combination. This cycle is repeated 3 times. Then, animals are placed in the middle of an assay plate with AM on one side and OCT on the other. This test plate has no reinforcer added, unless noted otherwise.

After 3 min, we determine the number of animals on either side to calculate an odor preference [–1; 1] as the number of animals at the AM side ($\#_{AM}$) minus the ones at the OCT side ($\#_{OCT}$), divided by the total ($\#_{TOTAL}$):

$$\mathrm{PREF} = (\#_{AM} - \#_{OCT})/\#_{TOTAL} \qquad (2)$$

From alternately run, reciprocally trained groups we calculate a learning index [–1; 1]:

$$\mathrm{LI} = \left(\mathrm{PREF}_{AM+/OCT} - \mathrm{PREF}_{AM/OCT+}\right)/2 \qquad (3)$$

Thus, positive LIs indicate appetitive, negative values aversive memory.

Statistical analyzes

Nonparametric statistics (one-sample sign test, Kruskal–Wallis test, Mann–Whitney *U* test) are used throughout (*P* level .05). Where applicable, we divide this significance level by the number of single-group comparisons to maintain an experiment-wide error rate of 5% despite multiple comparisons (Bonferroni correction); if, for example, 20 single-group comparisons are performed (Experiment 1), we present *P* levels as $P < .05/20$ (i.e., .0025). Data are displayed as box plots, with the bold line indicating the median and box boundaries and whiskers the 25/75% and 10/90% quantiles, respectively. In all cases, sample sizes are presented within the figures only.

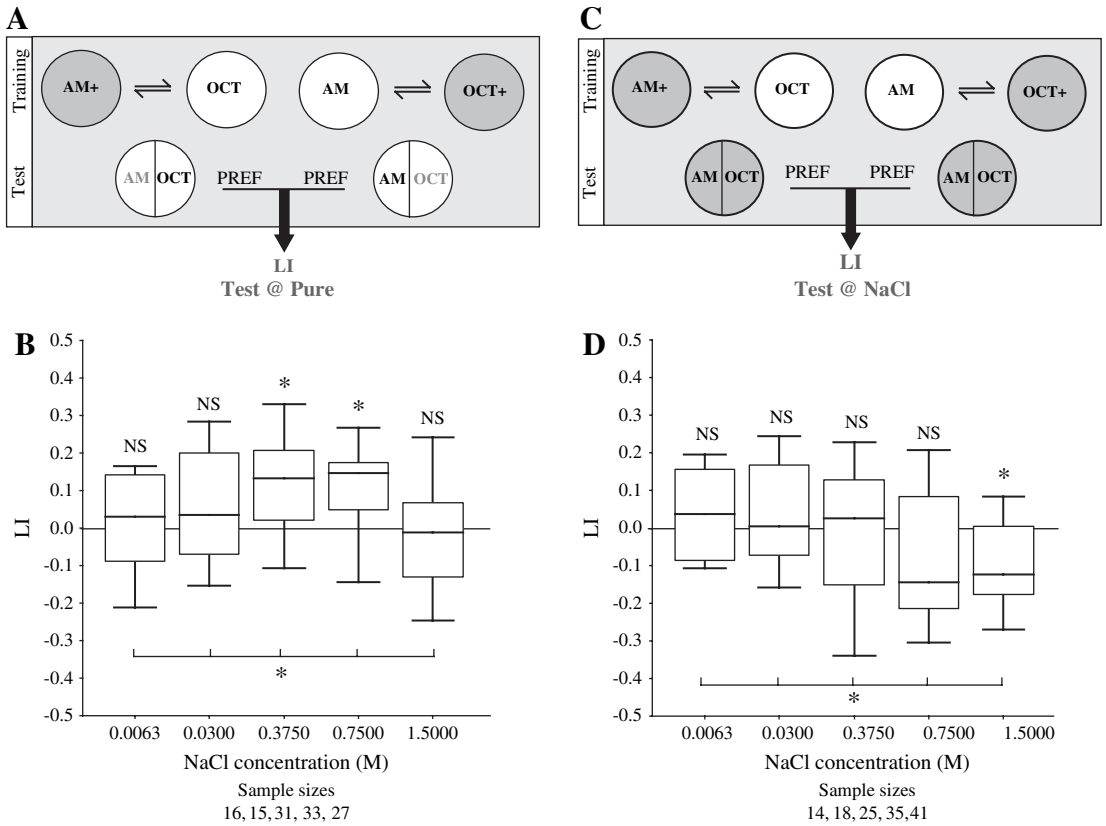

Figure 3 Reinforcement. **(A)** Schematic of the learning experiment. Larvae are trained with 2 odors (AM and OCT) and salt at the indicated concentration as reinforcer (+; indicated by dark gray shading). One group of larvae receives AM while crawling on a reinforcer-containing agarose plate, whereas OCT is presented in the absence of the reinforcer (AM+/OCT). Another group is trained reciprocally (AM/OCT+) (note that for half of the cases the sequence of trials is as indicated; for the other half, sequences are reversed: OCT/AM+ and OCT+/AM). Then, both groups are tested for their preference between AM and OCT. Associative learning shows by differences in preference scores between the groups trained AM+/OCT versus the reciprocally trained AM/OCT+ group. These differences are quantified by the learning index (LI). Positive LI values indicate appetitive learning, negative values aversive learning. **(B)** When testing is carried out in the absence of the reinforcer, low and high training concentrations of salt do not support positive learning scores, whereas intermediate concentrations do. **(C, D)** When testing is carried out in the presence of the reinforcer (indicated by the dark gray shading of the testing situation in C), learning scores are significantly negative only for the highest salt concentration. *$P < .05/5$. Other details as in the legend of Figure 2.

Results

Choice

Choice of NaCl is concentration-dependent when assayed in 20 experimental groups using concentrations between 0.0063 M and 4 M NaCl (Figure 2; Kruskal–Wallis test: $P < .05$, $H = 452.0$, degrees of freedom [df] = 19). Larvae are indifferent toward very low [0–0.0125 M] concentrations and show attractive responses to low concentrations [0.025–0.1 M]; as concentration is further increased, these responses gradually turn into aversion for high concentrations [0.29–4 M]; consequently, there is an intermediate concentration range at which appetitive and aversive properties cancel out [0.125–0.27 M] (all statements refer to one-sample sign tests and a P level of 0.05/20).

For convenience, in Figure 5A,B the results are plotted in terms of a normalized CHOICE score over concentration. Apparently, behavioral responses to NaCl are supported by 2 processes: an appetitive one at low concentrations (below 0.2 M) and an aversive component at high concentrations (above 0.2 M); both processes score even at intermediate (around 0.2 M) concentrations. Notably, the appetitive effect has its optimum at around 0.02 M NaCl.

Reinforcement

We next ask whether a similar concentration dependency is seen with respect to the effect of sodium chloride as a reinforcer. We had shown before that appetitive memories are behaviorally expressed only in the absence of the training reinforcer; arguably, this is because conditioned search behavior is expressed only if there is something to gain from searching, that is, if the sought-for situation is not already present (Gerber and Hendel 2006). Therefore, animals are trained with a given concentration of sodium chloride as reinforcer and then tested for their odor preference between the previously reinforced and the nonreinforced odor in the

absence of the reinforcer, that is, on petri dishes containing pure agarose (see schematic in Figure 3A). Clearly, the concentration of NaCl does influence test performance (Figure 3A, Kruskal–Wallis test: $H = 11.6$, df = 4, $P < .05$). Specifically, larvae do not show appetitive memory scores after training with either high (1.5 M) or low (0.03 M or less) concentrations; however, intermediate concentrations (0.375 and 0.75 M) do support appetitive memory (Figure 3A; all statements refer to one-sample sign tests at a P level of .05/5). Thus, the appetitive reinforcing effect of sodium chloride is concentration-dependent, with an optimum at intermediate concentrations, around 0.5 M NaCl.

In turn, we had shown before that aversive memories are behaviorally expressed only in the presence of the reinforcer; this conceivably is because conditioned escape behavior is expressed only if there is something to gain from that escape, that is, if the situation which the animals are in does indeed call for an escape (Gerber and Hendel 2006). Therefore, animals received the same kind of training as above but were tested on petri dishes containing the respective training reinforcer (see schematic in Figure 3B). Again, the concentration of NaCl obviously influences test performance (Figure 3B, Kruskal–Wallis test: $H = 13.9$, df = 4, $P < .05$). Larvae show aversive memory scores for 1.5 M sodium chloride but not for any lower concentration (Figure 3B; all statements refer to one-sample sign tests at a P level of .05/5); an apparent trend for aversive learning when using 0.75 M sodium chloride remains, due to the large scatter of the data, not significant (i.e., $P = .3$) despite a substantial sample size (i.e., $N = 35$). Thus, the aversive reinforcing effect of sodium chloride is concentration-dependent, being observable only for high concentrations.

For convenience, the results of both learning experiments are plotted as normalized LEARNING score over NaCl concentration in Figure 5. Apparently, the effect of NaCl as reinforcer turns from appetitive to aversive rather abruptly at and above 0.75 M; interestingly, the appetitive effect has its optimum at more than one order of magnitude higher NaCl concentrations as compared with the optimum for choice behavior.

Feeding

We finally ask which NaCl concentrations are ''appetizing'' (or ''disgusting'') using a photometer-quantified dye-feeding assay. The interesting question is whether such an ''appetizing'' effect would show for those concentrations of NaCl for which appetitive choice behavior is seen or for those concentrations which yield appetitive reinforcement.

When NaCl is added to the substrate, the amount eaten differs depending on NaCl concentration (Figure 4, Kruskal–Wallis test: $H = 70.72$, df = 4, $P < .05$). Given that larvae are continuous feeders (Carle 1969), increases in feeding are relatively difficult to detect; in our initial experiment, feeding scores for 0.03 M salt are not statistically significant when using the (rather conservative) Bonferroni correction (Figure 4A, one-sample sign test: $P > .05/5$). When repeating the experiment using this concentration, however, a small yet significantly positive feeding score can be substantiated (Figure 4B, one-sample sign test: $P < .05$).

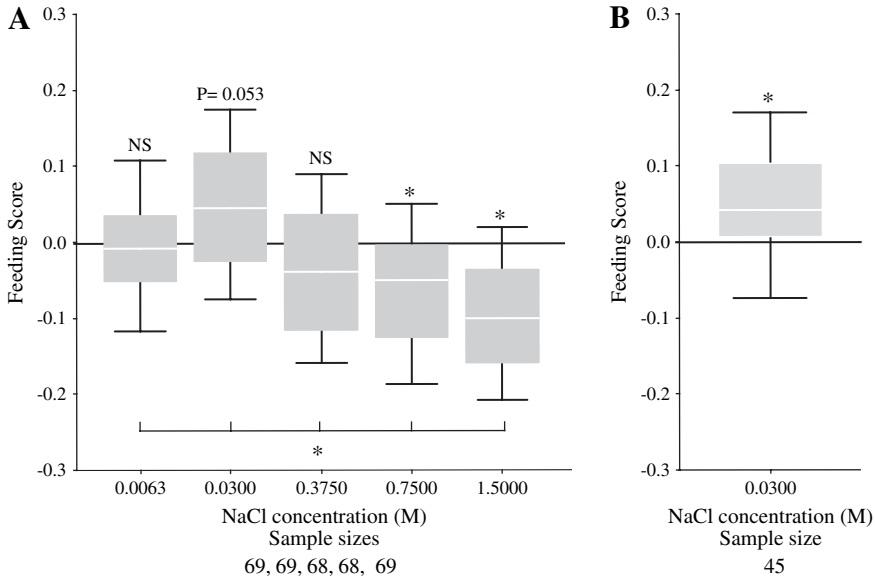

Figure 4 Feeding. Feeding of dyed substrate is assayed in the presence of various concentrations of salt and is quantified photometrically relative to a condition without salt in the substrate; positive values indicate upregulation and negative values downregulation of feeding. **(A)** High salt concentrations downregulate feeding, whereas low salt concentrations tend to upregulate feeding. *$P < .05/5$. **(B)** In a repetition of the experiment for 0.03 M salt, a slight upregulation of feeding can be statistically substantiated. *$P < .05$. Other details as in the legend of Figure 2.

In turn, larvae feed less at 0.75 and 1.5 M NaCl than when no NaCl is present (Figure 4A, one-sample sign tests: $P < .05/5$ for both 0.75 M and 1.5 M, respectively). Thus, feeding is slightly upregulated in the presence of low-concentration NaCl (0.03 M) and strongly downregulated in the presence of higher concentration NaCl (>0.75 M). Both processes score even at around 0.375 M NaCl (Figure 4A, one-sample sign test: $P > .05/5$).

When plotted in terms of a normalized FEEDING score across NaCl concentration (Figure 5), the concentration for which the "appetizing" effect of NaCl is seen fits the range of concentrations for which appetitive choice behavior is apparent but is shifted by about one order of magnitude towards lower concentrations relative to the appetitive learning effect.

Discussion

Qualitatively, the behavioral effects of sodium chloride are similar in all 3 cases tested: choice behavior, feeding behavior, and the reinforcing effect all change from appetitive to aversive as concentration is increased (Figure 5A,B).

The "titration point" of choice behavior as reported here (approximately 0.2 M; Figure 5A,B) is in line with data gathered 25 years ago reporting 0.1–0.2 M as the concentration of equally strong attraction and repulsion (Arora et al. 1987; Miyakawa 1981) as well as with recent data from Liu et al. (2003) who report such a draw at slightly above 0.2 M. Thus, the dose–effect curve for choice behavior of salt in larval *Drosophila* is remarkably reproducible.

Regarding feeding behavior, Hiroi et al. (2004) reported for adult flies that feeding is upregulated by salt at 0.1 M but is downregulated by 0.4 M salt, with the strongest "appetizing" effect between 0.05 and 0.1 M. This fits reasonably well with our results in the larva (Figure 5A) and suggests some functional conservation of salt processing between larva and adult. Based on the observation that most pharyngeal gustatory sensory neurons of the larva are retained into adulthood, such conserved function had already been proposed by Gendre et al. (2004).

Regarding a comparison of choice and feeding, we note that the concentration dependencies for both kinds of behavior match parametrically (Figure 5A): in both cases the effect changes from appetitive to aversive at around 0.2 M. Such shared dose–effect characteristics may suggest that both kinds of behavior rely on common input. Strikingly, the concentration where aversive effects start to unfold in both larva and adult and regarding both choice and feeding (approximately 0.2 M) fits with the electrophysiological threshold of the L2 neurons in adults which start to be activated between 0.1 and 0.4 M (Hiroi et al. 2004; Ishimoto and Tanimura 2004). This not only underscores the functional conservation between larva and adult as well as between the 2 kinds of reflexive behavior examined, but may also suggest a surprisingly straightforward relation between sensory physiology and reflexive behavior.

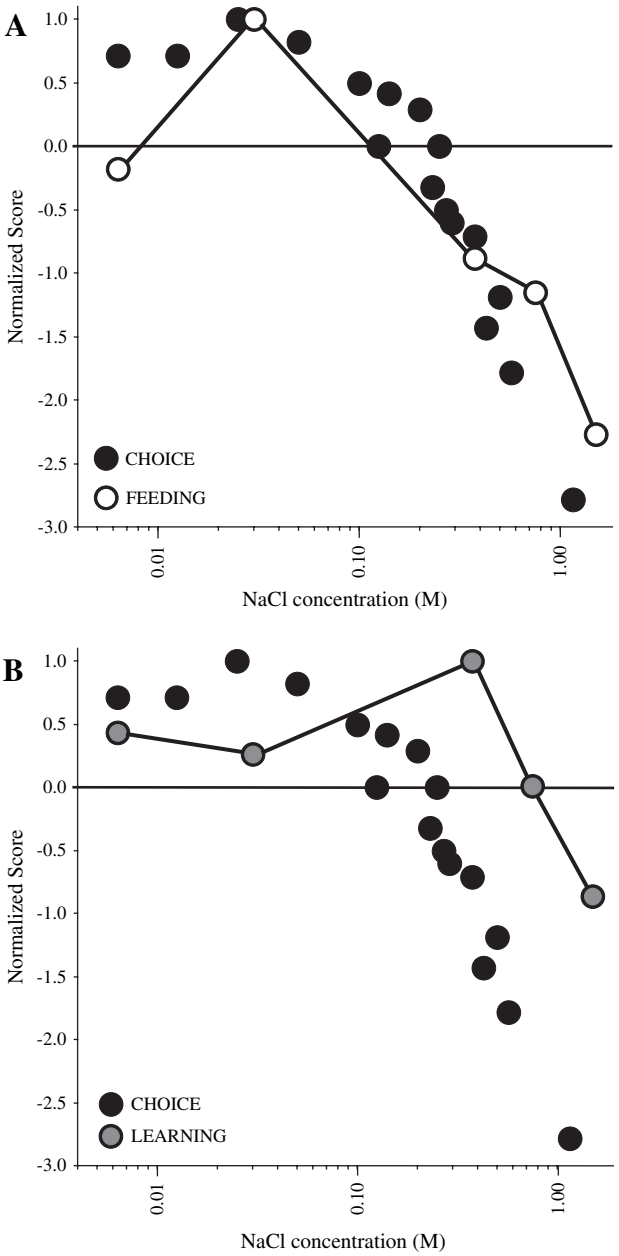

Figure 5 Summary. Semischematic illustration of the relation between choice, feeding, and learning. **(A)** We take the median of the salt preference values for each concentration (Figure 2) and express it relative to the highest score thus obtained; thus, the figure shows the maximum "CHOICE" score as "1." Then, we do accordingly for the median feeding values from Figure 4 and display them as "FEEDING" scores. The dose–effect characteristics between "CHOICE" and "FEEDING" appear similar. **(B)** To deal with the learning values in a similar way, we take the median learning index for a given training concentration as obtained when testing in the absence of the training reinforcer (Figure 3A) as well as the corresponding value for the learning index as obtained when testing in the presence of the training reinforcer (Figure 3B) and average these 2 values. Then, we do the same for all other concentrations and express the respective scores relative to the highest score thus obtained. These "LEARNING" scores then are plotted for comparison with the FEEDING scores. The dose–effect functions appear offset by at least one order of magnitude.

Reflexive behavior versus effect as reinforcer

To compare the dose–effect characteristics of the reflexive versus the reinforcing function of salt, we plot our data in a semischematic way (Figure 5). It is striking that the appetitive effects of salt for reflexive behavior, namely choice and feeding, share their optimum at around 0.02 M (Figure 5A), whereas the strongest effect of salt as appetitive reinforcer is seen for more than one order of magnitude higher concentrations (>0.2 M) (Figure 5B). In other words, the dose–response curve for the reinforcing effect is shifted by one order of magnitude toward higher concentrations. How can such a shift along the concentration axis come about?

One possibility may be that nongustatory processing, for example, via high-osmolarity sensors, selectively impinges upon the reflexive pathway to suppress appetitive tendencies for high salt concentrations. Given, however, that such sensors remain to be characterized in the larva, and given that this would leave the apparent ineffectiveness of relatively low salt concentrations as reinforcer unexplained, an alternative scenario may be warranted.

Suppose one and the same low-threshold salt sensor would be driving appetitive reflex behavior as well as appetitive reinforcement, and a high-threshold salt sensor would drive both aversive reflexes and aversive reinforcement. Could one, within such a scenario, yield the observed shift along the concentration axis? What if the connection of, for example, the low-threshold salt sensor toward reflex behavior would be tuned differently from its connection toward reinforcing neurons?

- A different gain of these connections would correspond to a multiplication step; such multiplication would yield altered amplitudes of attraction and repulsion but would leave the ''titration point'' between them unaffected. Thus, within such a scenario, the dose–response profile would not shift along the concentration axis.
- Introducing an additive effect also would not do so, as it would rather shift the dose–response profile along the ordinate toward higher or lower behavioral scores for a given concentration.
- Different signal-to-noise ratios would lead to different levels of scatter but would not qualitatively alter the dose–response profile.

Thus, as far as we can see, the assumption that both the reflexive and the reinforcing effects of salt draw upon common input pathways is incompatible with the observed shift of the dose–response curves along the concentration axis regarding these behavioral effects.

We therefore speculate that there may be 4 types of sensors: low-threshold salt sensors hooked up preferentially to appetitive reflex behavior, low-threshold salt sensors preferentially hooked up to appetitive reinforcement, and 2 types of high-threshold salt sensors, preferentially linked to aversive reflex behavior and aversive reinforcement, respectively. The heterogeneity of gustatory sense organs (Figure 1) and the complexity of the projection patterns of the gustatory sensory neurons in the subesophageal ganglion (Colomb et al. 2007) would seem permissive for such functional specialization; in particular, a division of labor between the external sense organs to support reflexive and of the internal sense organs to support the reinforcing effects of salt is conceivable (for a corresponding proposal with regard to mice see de Araujo et al. 2008). The observed shift in the behavioral dose–effect characteristics may then find its explanation either by the expression of differently tuned sets of salt sensors in these respective organs or by a 10-fold dilution of tastant by saliva upstream of the internal sense organs (for a more detailed discussion see Schipanski et al. in press).

To summarize, our study dissociates parametrically the reflex releasing (choice, feeding) from the reinforcing function of salt in terms of their respective dose–effect characteristics: the reinforcing effect is shifted by one order of magnitude toward higher concentrations (Figure 5). Interestingly, a similar shift between these 2 kinds of behavioral effect is also found for sugars (Schipanski et al. 2008), suggesting some degree of generality of such parametric dissociation. Thus, both in the case of salt and for sugar, the input pathways for gustatory behavior appear to be more sensitive than the ones supporting gustatory reinforcement.

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