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Olfactory input increases visual sensitivity in zebrafish: a possible function for the terminal nerve and dopaminergic interplexiform cells

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Summary

Centrifugal innervation of the neural retina has been documented in many species. In zebrafish *Danio rerio*, the only so-far described centrifugal pathway originates from terminal nerve (TN) cell bodies that are located in the olfactory bulb. Most of the TN axons terminate in the forebrain and midbrain, but some project *via* the optic nerve to the neural retina, where they synapse onto dopaminergic interplexiform cells (DA-IPCs). While the anatomical pathway between the olfactory and visual organs has been described, it is unknown if and how olfactory signals influence visual system functions. We demonstrate here that olfactory input is involved in the modulation of visual sensitivity in zebrafish. As determined by a behavioral assay and by electroretinographic (ERG) recording, zebrafish visual sensitivity was increased upon presentation of amino acids as olfactory stimuli. This effect, however, was observed only in the early morning hours when zebrafish are least sensitive to light. The effect of olfactory input on vision was eliminated after lesion of the olfactory bulbs or after the destruction of DA-IPCs. Intraocular injections of a dopamine D_2 but not a D_1 receptor antagonist blocked the effect of olfactory input on visual sensitivity. Although we cannot exclude the involvement of other anatomical pathways, our data suggest that the TN and DA-IPCs are the prime candidates for olfactory modulation of visual sensitivity.

Key words: centrifugal pathway, terminal nerve, dopamine, olfactory stimulation, retina, visual sensitivity, zebrafish, *Danio rerio*.

Introduction

Centrifugal innervation of the neural retina has been described in many species, including horseshoe crabs (Barlow et al., 1980; Battelle, 1991; Battelle et al., 1982, 2000), reptiles (Halpern et al., 1976), fish (Arey, 1916; Springer, 1983; Munz et al., 1982), birds (Cowan, 1970; Uchiyama, 1989), rats (Itaya, 1980), cats (Brooke et al., 1965) and monkeys (Gastinger et al., 1999). In fish, different brain areas such as the olfactory bulb, the telecephalic area, the preoptic area, the dorsomedial optic nucleus, the lateral pretectum or the optic tectum may project axons to the neural retina (Vanegas and Ito, 1983; Lima and Urbina, 1998). The centrifugal pathway that has drawn the widest attention originates in TN cell bodies that are located in the olfactory bulb (Fig. 1; see also Arey, 1916; Fujita et al., 1985; Pinelli et al., 2000). This olfactoretinal centrifugal pathway is found in all fish species described thus far. The terminal nerve (TN) receives synaptic input from the olfactory bulb as well as some other brain areas, many of which seem to be involved in processing sensory information of other modalities such as visual and somatosensory information (Yamamoto and Ito, 2000). The TN contains two classes of neuropeptides, molluscan cardioexcitatory tetrapeptide (FMRFamide) and gonadotropin hormone-releasing hormone-like peptide (GnRH) (Stell et al., 1984). While most of the TN axons project to the forebrain and midbrain, some enter the retina where they synapse with dopaminergic interplexiform cells (DA-IPCs; Zucker and Dowling, 1987).

In fish retinas, the DA-IPC is the only cell type that synthesizes dopamine (Witkovsky and Dearry, 1992). DA-IPCs synapse with virtually all other retinal cell types (Dowling and Ehinger, 1978; Yazulla and Zucker, 1988; Yazulla and Studholme, 1997). Dopamine functions as a major neuromodulator in the retina (Dowling, 1986; Negishi et al., 1990). One of the important effects of dopamine is to modulate glutamate receptor sensitivity (Knapp and Dowling, 1987; Knapp et al., 1990). In the outer retina, dopamine regulates retinomotor movements (Dearry and Burnside, 1986). It also plays a role in rod–cone or horizontal cell coupling *via* the modulation of gap junctions (Krizaj et al., 1998). In the inner retina, dopamine modulates potassium currents of ON bipolar cells (Fan and Yazulla, 2001) and the spike frequency of ganglion cells (Vaquero et al., 2001).

So far, the function of the olfactory bulb-retina connection remains enigmatic. It has been speculated that the TN transmits olfactory information to other brain areas. Since FMRFamide and GnRH are the major transmitters, it has been hypothesized that the TN plays a role in the physiological mechanisms involved in sexual behavior (Demski and Northcutt, 1983; Schreibman and Margolis, 1987; Oelschlager et al., 1998).

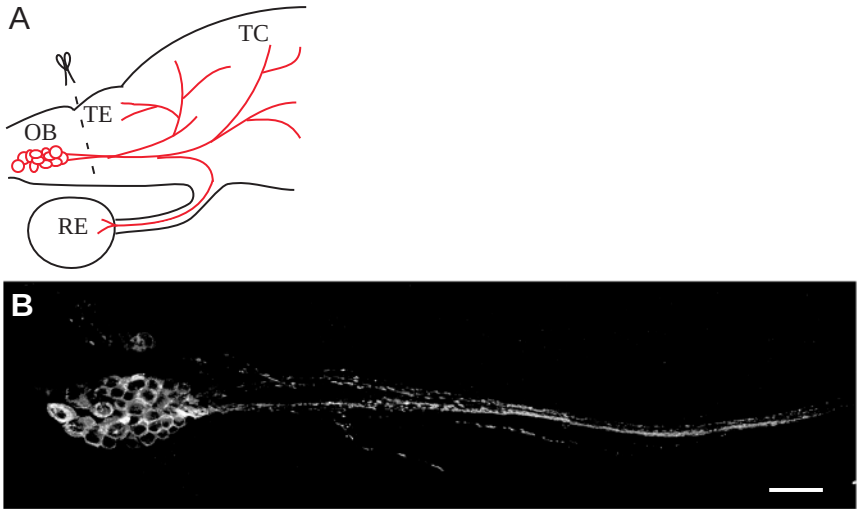

Fig. 1. Olfactoretinal centrifugal pathway in zebrafish. (A) Schematic representation of the location and projections of the terminal nerve (TN). Red circles and red lines label TN cell bodies and their axons, respectively. The broken line drawn between OB and TE indicates the location of the olfactory bulb lesion. OB, olfactory bulb; TE, telencephalon; TC, tectum opticum; RE, retina. (B) A confocal image of a whole-mount preparation of the olfactory bulb showing TN cell bodies and their axons (labeled with an antibody against FMRFamide). Anterior is to the left. Scale bar, approximately 35 μm for B.

Recent studies have suggested a role of TN input in visual function. *Via* a dopamine mechanism, for example, FMRFamide and GnRH alter the size of the receptive fields of horizontal cells (Umino and Dowling, 1991). FMRFamide and GnRH also affect spike activity of retinal ganglion cells (Walker and Stell, 1986). Weiss and Meyer (1988) have demonstrated that olfactory stimuli modulate the amplitude of the b-wave in the electroretinogram (ERG), suggesting that FMRFamide and GnRH may have a role in the regulation of bipolar cell activity.

In the present study, we have evaluated the effect of olfactory stimulation on visual sensitivity in zebrafish. Our data suggest that there is a functional connection between the olfactory organ and the neural retina. It seems likely that both the TN and DA-IPCs are anatomical candidates that play a role in the modulation of visual sensitivity by olfactory stimulation.

Materials and methods

Animals and maintenance

Zebrafish *Danio rerio* Hamilton were maintained in our fish facility as described (Westerfield, 1995) under a 14 h:10 h light:dark cycle (room fluorescent light, 06.00–20.00 h). Zebrafish were kept in recirculating tank water (reverse osmosis water with Instant Ocean Salt added, 0.8 g l^{-1}, pH 7.0) heated to 28°C. They were fed twice per day, at 09.00 h and at 13.00 h, with freshly hatched brine shrimp and dried plankton, respectively. Zebrafish used in this study were aged 4–12 months.

Behavioral assay

Zebrafish visual sensitivity was assessed using a behavioral assay based on the visually mediated escape response when they encounter a threatening object (Li and Dowling, 1997; see also Fig. 2). The behavioral test apparatus consisted of a transparent circular container surrounded by a rotating drum. A black segment was marked on white paper that covered the inside of the drum and served as a threatening stimulus. The drum was illuminated from above with a halogen lamp (maximum intensity, 475 μW cm^{-2}), and was turned at 10 rotations min^{-1} by a motor. The fish were viewed on a monitor attached to an infrared video camera.

Normally zebrafish swim in circles along the wall of a circular container. However, when challenged by a black segment revolving around the container, the fish show an escape response, i.e. they rapidly reverse the direction of swimming. To evaluate behavioral visual sensitivity, we measured the threshold of the light intensity required for the fish to react to the black segment with an escape response. The

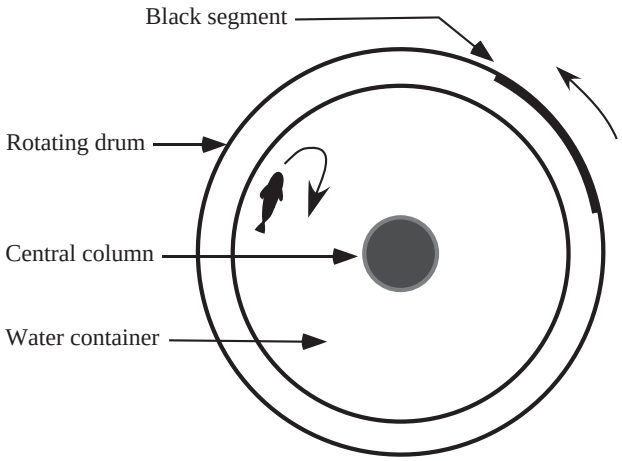

Fig. 2. Diagram of the behavioral test apparatus. The transparent water container is placed in the center of a drum that rotates clockwise or counterclockwise. A black segment printed on white paper attached on the inside of the drum serves as a visual stimulus. When the black segment comes into view, the fish reacts with an 'escape' response, i.e. by reversing its swimming direction. The column in the center of the container prevents the fish from swimming across the center. Above the apparatus is suspended a light-source, the intensity of which is controlled by neutral density filters. An infrared video camera was used to observe the behavior of the fish at low light levels.

testing light was always started at a dim light level. If no escape responses were observed (within three encounters between the fish and the rotating black segment), the light intensity was increased by removing neutral density filters (in steps of 0.5 log unit) until the fish showed the escape response.

Olfactory stimulation

Amino acids are basic odorants for zebrafish. Analyzing activation patterns of the glomuruli and their cross-adaptation using electro-olfactography led to the proposal to divide the amino acids as olfactory stimuli into four groups: short-chained neutral, long-chained neutral, basic, and acidic (Friedrich and Korsching, 1997, 1998; Caprio and Byrd, 1984; Michel and Lubomudrov, 1995; Zippel et al., 1993, 1997). In the present study, we examined the effect of representatives of each of the four groups: L-alanine, L-methionine, L-arginine and L-aspartic acid, on zebrafish visual sensitivity. The effect of amino acids on visual sensitivity was determined by comparing the threshold light intensities before and after amino acid administration at which an escape response occurs.

Amino acids were dissolved in regular tank water, and the pH of the solution was adjusted to 7.0. Following pre-odor visual threshold measurements, 1.0 ml of odor solution was slowly injected into 200 ml water of the experimental container. The final concentrations of each amino acid were 0, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} mol l^{-1}. Post-odor threshold measurements were made within 30 s after amino acid administration. The experimenter was not aware of the concentration of the amino acids used in each experiment. Data obtained before and after each amino acid or sham stimulation were compared by a paired Student's *t*-test.

Electroretinographic recording

Procedures for electroretinographic (ERG) recordings were similar to those described (Brockerhoff et al., 1995; Li and Dowling, 1997). The zebrafish was anesthetized with 4% 3-aminobenzoic methylesther and immobilized with 10% gallamine triethiodide. The zebrafish was placed on its ventral side at a 45° slanting angle on a wet sponge and most of its body was covered with a wet paper towel. A slow stream of fish water was directed *via* a spout into the mouth to keep the fish oxygenized. A beam of halogen light (maximum light intensity 670 μW cm^{-2}) was directed by a mirror system to the eye of the fish. The light intensity was controlled by neutral density filters.

ERGs were recorded after 30 min of dark adaptation. First, we determined the lowest light intensity that evoked a b-wave of at least 10 μV. The fish received five light stimuli of 10 μs with an inter-stimulus interval of 5 s. The light intensity was initially set at a dim level, and was increased in 0.5 log steps until a 10 μV b-wave was recorded. Following this pre-odor visual threshold measurement, the fish was allowed to dark-adapt for about 2 min, then a solution of 50 μl of either tank water or methionine dissolved in tank water was released over the nasal cavity contralateral to the recorded eye. Within 10 s after olfactory stimulation, the threshold light intensity to evoke a 10 μV b-wave was again determined. This experiment was carried out twice during the day, in the early (06.00–09.00 h) and late morning (09.00–12.00 h).

Bulbectomy

Zebrafish were anesthetized with 4% 3-aminobenzoic methylester. Under a dissecting microscope, the olfactory bulbs were severed by a microblade inserted approximately 1 mm caudal of the nasal cavities. The blade was lowered at a slanting angle of approximately 45° in the caudal direction, and was pulled along a trench of approximately 1 mm on each side of the midline in the lateral direction. In all cases the olfactory bulbs were severed, and sometimes a part of the telencephalon was ablated as well. The fish were allowed to recover for 5–7 days before threshold measurements were made. No obvious differences in swimming behavior were observed between control and experimental animals.

Administration of drugs

DA-IPCs were destroyed by coinjections of 6-hydroxydopamine (6-OHDA) and pargyline (Lin and Yazulla, 1994; Li and Dowling, 2000b). Approximately 2 μl of a 1:1 mixture solution (5 μg ml^{-1}) was injected into the vitreous of each eye. The injection was repeated the next day. Visual threshold measurements were performed after 2 weeks of 6-OHDA injections when the DA-IPCs were completely or nearly completely depleted. SCH23390 (a dopamine D_1 receptor antagonist) and sulpiride (a D_2 receptor antagonist) were dissolved in phosphate-buffered saline (PBS) and PBS/HCl, respectively, and were further diluted in PBS. The final concentrations of SCH23390 and sulpiride were estimated to be approximately 100 μmol l^{-1}. All chemicals were obtained from Sigma (St Louis, MO, USA).

Immunostaining

Specimens were fixed in 4% paraformaldehyde in PBS. Both the TN cell bodies and TN axons were stained with a polyclonal antibody against FMRFamide (Chemicon, CA, USA). DA-IPCs were stained with an antibody against tyrosine hydroxylase (Chemicon, CA, USA). Rhodamine- and FITC-conjugated secondary antibodies (Sigma) were used to visualize FMRFamide and tyrosine hydroxylase antibodies, respectively.

Results

Olfactory stimulation with amino acids increases behavioral visual sensitivity

We measured the light thresholds required to evoke escape responses before and after amino acid stimulation. Among the amino acids tested, arginine produced the most robust effect, followed by methionine, alanine and aspartic acid (Fig. 3). Arginine increased visual sensitivity at every tested concentration: 10^{-6} mol l^{-1} ($t_{(16)}$=–2.22, P<0.05), 10^{-5} mol l^{-1} ($t_{(17)}$=–2.38, P<0.05], 10^{-4} mol l^{-1} ($t_{(17)}$=–3.43, P<0.005] and

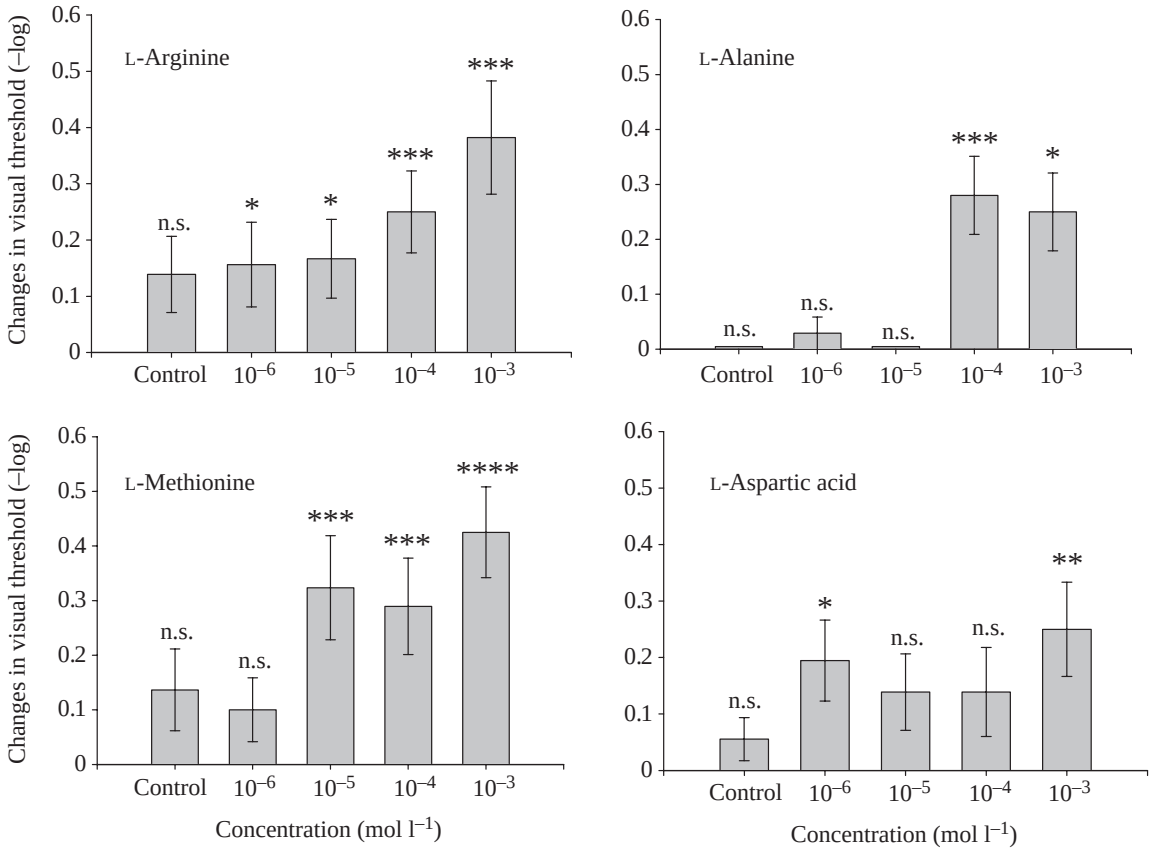

Fig. 3. Changes of behaviorally assessed visual sensitivity in zebrafish induced by different concentrations of amino acids. Values are means ± S.E.M.; n.s., not significant; *P<0.05; **P<0.01; *** P<0.005; ****P<0.001.

10^{-3} mol l^{-1} ($t_{(16)}$=–3.79, P<0.005). Methionine increased visual sensitivity for the three highest concentrations: 10^{-5} mol l^{-1} ($t_{(16)}$=–3.40, P<0.005), 10^{-4} mol l^{-1} ($t_{(18)}$=–3.28, P<0.005) and 10^{-3} mol l^{-1} ($t_{(19)}$=–5.10, P<0.0001). Alanine elicited an increase in visual sensitivity at a concentration of 10^{-4} mol l^{-1} ($t_{(16)}$=–3.41, P<0.005) and 10^{-3} mol l^{-1} ($t_{(18)}$= –2.25, P<0.05). Application of aspartic acid at the highest dose (10^{-3} mol l^{-1}) increased visual sensitivity ($t_{(17)}$=–3.0, P<0.01). It is not clear whether the decrease in the light threshold at the low dose (10^{-6} mol l^{-1}) was due to a bimodal distribution ($t_{(17)}$=–2.72, P=0.015). Adding regular tank water without amino acids did not change the behavioral visual threshold.

The above-described experiments were carried out in the early morning when zebrafish are least sensitive to light, due to a circadian effect (Li and Dowling, 1998). In a separate experiment, we examined whether olfactory stimulation increases visual sensitivity when the zebrafish are most sensitive to light, i.e. in the late afternoon. In the late afternoon hours, application of amino acids (methionine, 10^{-3} mol l^{-1}) produced no effect on behavioral visual sensitivity ($t_{(16)}$= –2.06, P>0.05; not shown). This suggests that olfactory stimulation by amino acids could not increase the absolute visual sensitivity level when it was already at its peak, probably due to a ceiling effect.

In our behavioral test, the criterion used to score a visual threshold was the escape response, elicited by the rotating black segment (Li and Dowling, 1998). We have, of course, to be aware that zebrafish show some spontaneous turning behavior, which cannot readily be discerned from visually mediated escape responses. To test if the increase in visual sensitivity was confounded by an increase in spontaneous turning behavior, we recorded the number of turns before and after amino acid administration in the absence of visual cues. This experiment was performed in the dark in order to exclude any reaction by the fish to other unspecified visual stimuli, in the absence of the black segment. In the dark, the number of changes in swimming direction before and after amino acid administration was similar ($t_{(23)}$=1.23, P>0.2; not shown).

Olfactory stimulation increases ERG sensitivity in the early morning

To investigate further the effect of olfactory stimulation on retinal sensitivity, we recorded full-field ERGs in dark-adapted zebrafish. ERG has been widely used in the evaluation of outer retinal sensitivity (Dowling, 1987). We compared light intensities required to evoke threshold (10 μV) b-waves before and after olfactory amino acid stimulation. In the early morning, application of amino acids (methionine 10^{-3} mol l^{-1})

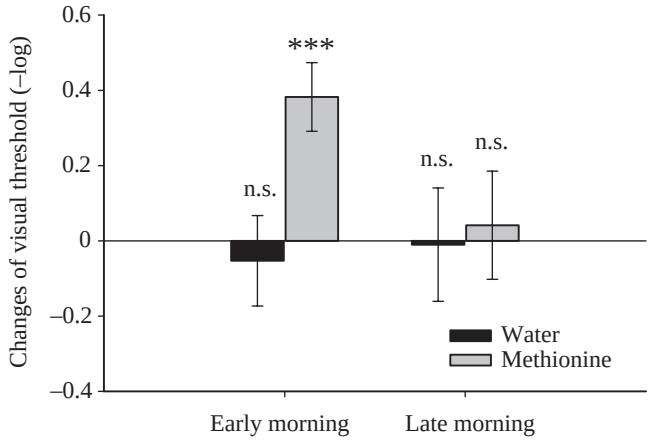

Fig. 4. Effect of olfactory stimulation with methionine (10^{-3} mol l^{-1}) on the ERG b-wave sensitivity. Changes of the b-wave threshold after application of either methionine or regular tank water were determined at early morning, 06.00–19.00 h and late morning, 09.00–12.00 h. Values are means ± S.E.M.; n.s., not significant; ***P<0.001.

decreased the b-wave threshold by approximately 0.4 log units ($t_{(34)}$=–4.19, P<0.001) (Fig. 4). The effect of methionine on ERG sensitivity, however, was seen only in the experiments carried out in the early morning, when the visual sensitivity of zebrafish is at its lowest level (Li and Dowling, 1998). When tested at a later time (09.00–12.00 h), methionine did not have any effect on the light threshold for the b-wave (Fig. 4).

Bulbectomy eliminates the modulation of visual sensitivity by amino acids

Fish possess at least two classes of chemoreceptors, olfactory receptors located in the epithelium in the nasal cavity and taste receptors located in the oral cavity, on the gills, barbells or fins (Hara, 1992; Marui and Caprio, 1992). To distinguish if the effect of amino acid stimulation on vision is mediated by olfactory or gustatory pathways, we measured visual sensitivity in zebrafish after bulbectomy. Bulbectomy resulted in a strong reduction of FMRFamide immunostaining in the forebrain, midbrain and retina (Fig. 5). This suggests that the TN is the main supplier for FMRFamide in those areas (see also Pinelli et al., 2000). While methionine increased visual sensitivity in control fish, it was not effective in bulbectomized fish (Fig. 6; Table 1). This strongly suggests that the effect of amino acids on visual sensitivity is indeed mediated by the olfactory system.

Table 1. *Number of subjects, number (percentage) of responders, and visual thresholds before and after application of methionine (10^{-3} mol l^{-1}) for control and manipulated zebrafish*

			log(Threshold intensity)	
Groups	Total tested	Responders (%)	Before methionine	After methionine
Control	25	19 (76)	–4.7±0.16	–5.3±0.19
OB lesion	12	0 (0)	–4.8±0.3	–4.7±0.21
6-OHDA	23	3 (13)	–3.1±0.15	–3.1±0.16
SCH23390	6	5 (83)	–4.7±0.11	–5.3±0.21
Sulpiride	6	1 (17)	–4.8±0.17	–5.0±0.18

Values are means ± S.E.M.
OB, olfactory bulb.
For details of manipulations, see text.

Dopamine mediates olfactory signals in the retina

In the retina, the target cells of the TN are DA-IPCs (Zucker and Dowling, 1987). We examined the effect of dopamine depletion on the modulation of visual sensitivity by olfactory stimulation. DA-IPCs were destroyed by intraocular injections of 6-OHDA. Depletion of dopamine in the retina resulted in elevation of the absolute visual threshold. This may be due to blockage of rod signaling transmission to the inner plexiform

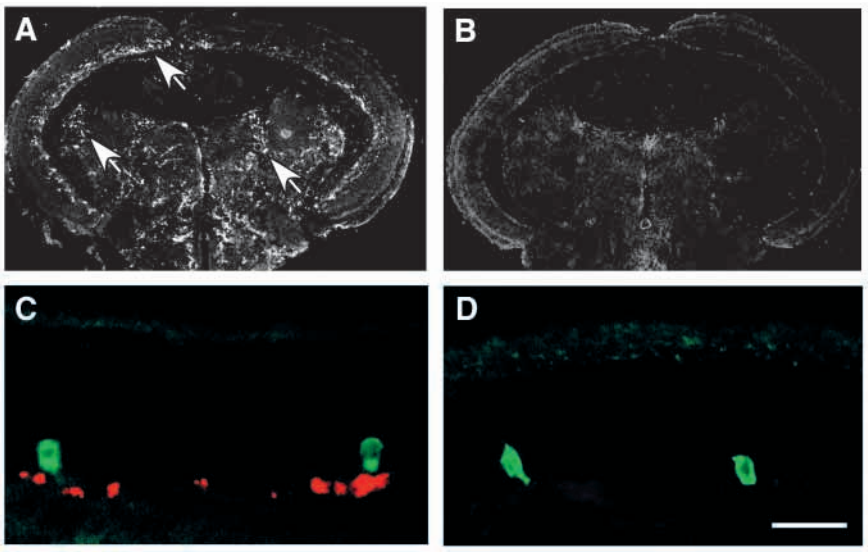

Fig. 5. Immunostaining of midbrain and retinal sections of control (A,C) and bulbectomized (B,D) zebrafish. (A,B) FMRFamide staining was seen in the midbrain of control (A, arrows) but not bulbectomized fish (B). (C,D) Double-labeled retinal sections of control (C) and bulbectomized (D) zebrafish with antibodies against FMRFamide and tyrosine hydroxylase identifying terminal nerve (TN) axons (red) and dopaminergic interplexiform cells (DA-IPCs) (green), respectively. Note the absence of FMRFamide staining in bulbectomized zebrafish. Scale bar, approximately 350 μm (A,B), 35 μm (C,D).

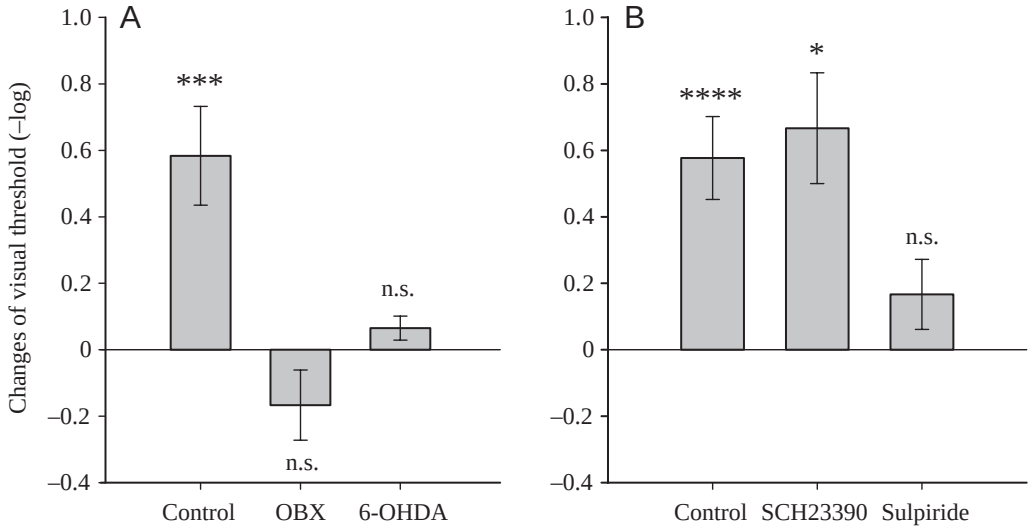

Fig. 6. Behavioral visual threshold changes in response to olfactory stimulation with methionine (10^{-3} mol l^{-1}). (A) Visual threshold changes in control, bulbectomized (OBX) and 6-OHDA-treated zebrafish. (B) Visual threshold changes in control, dopamine D1 (SCH23390) and D2 (sulpiride) receptor antagonist-injected zebrafish. Values are means ± S.E.M.; n.s., not significant; ***P<0.005; ****P<0.001.

layer (see also Li and Dowling, 2000b). In 6-OHDA-treated animals, the effect of olfactory stimulation on vision was no longer evident. Following methionine application the absolute visual threshold of 6-OHDA-treated animals remained virtually unchanged from the visual threshold measured before amino acid administration ($t_{(22)}$=−1.82, P>0.08) (Fig. 6; Table 1).

To investigate further the dopaminergic mechanism underlying the olfactory-visual interaction, we measured visual sensitivity following olfactory stimulation using zebrafish in which either dopamine D_1 or D_2 receptor activity was blocked (using the dopamine D_1 antagonist SCH23390 or the D_2 antagonist sulpiride). Following methionine stimulation, the visual sensitivity of SCH23390-injected fish increased significantly ($t_{(5)}$=−4.0, P<0.05), similar to control fish sham-injected with PBS. However, no obvious threshold changes were observed in sulpiride-injected fish ($t_{(5)}$=−1.58, P>0.1) (Fig. 6; Table 1), which suggests that in the retina, olfactory signals are mediated by dopamine *via* its D_2 receptors.

Discussion

Olfactory input modulates zebrafish visual sensitivity

We demonstrated that amino acids added to the swimming water increased behavioral visual sensitivity in zebrafish. Bulbectomy eliminated this effect, suggesting that amino acids affect vision *via* the olfactory, not the gustatory system. The effect of olfactory input on vision is dose-dependent. In response to high concentrations (i.e. 10^{-4} or 10^{-3} mol l^{-1}) of amino acids, the behavioral visual sensitivity increased by about 0.5 log units. Lower concentrations (10^{-5} mol l^{-1} and lower) produced little or no changes.

The fact that amino acids decrease visual thresholds only at higher doses might seem problematic. We know from both electro-olfactogram and imaging studies that they stimulate the olfactory nerve at much lower doses, i.e. from 10^{-9} mol l^{-1} (Hara, 1992; Michel and Lubomudrov, 1995; Zippel et al., 1993, 1997; Friedrich and Korsching, 1997). Valentincic et al. (2000) have also shown that bullhead catfish can be trained to search for food when exposed to amino acids in concentrations as low as 10^{-7} mol l^{-1}. The fundamental difference is that we determined the amino acid concentration that affects visual sensitivity, not the lowest concentration that can be detected by the olfactory system.

The ERG data confirmed that olfactory stimulation increases visual sensitivity. The b-wave threshold was decreased after application of methionine. Similarly, Weiss and Meyer (1988) reported that in fish the b-wave amplitude was increased after presenting food extracts as olfactory stimuli. It is likely that the increase of ERG sensitivity after olfactory stimulation is due to changes in the activity of the retinal bipolar cells. We know that the b-wave in dark-adapted fish mainly arises from activity of ON bipolar cells and of glial Müller cells, which both are characterized by a potassium flux (Stockton and Slaughter, 1989; Miller and Dowling, 1970). These potassium currents are modulated by dopamine (Fan and Yazulla, 2001). The only source of dopamine in the fish retina is the DA-IPC (Witkovsky and Dearry, 1992). DA-IPCs receive input from the TN (Stell et al., 1984; Zucker and Dowling, 1987), which in turn receives input from the olfactory bulbs (Yamamoto and Ito, 2000). Thus, the TN projection to the DA-IPC seems to provide an ideal pathway for the dopaminergic modulation of the bipolar cells after olfactory stimulation, and thus to modulate ERG threshold.

Using a psychophysical assay, Davis et al. (1988) found that bilateral ablation of the olfactory bulb and telencephalon had no effect on the response threshold to a conditioned stimulus, i.e. a spot of red light on a dark background. They concluded, therefore, that the TN had no role in the regulation of visual sensitivity. It is noteworthy that they did not offer olfactory stimulants, so their conclusion only applies to spontaneous TN activity. In a previous study, Li and Dowling (2000a) examined the effect of bulbectomy on visual sensitivity in zebrafish. They found that following short dark adaptation the visual threshold of bulbectomized fish was similar to that in

control fish. However, following prolonged dark adaptation their visual threshold started to fluctuate and was sometimes more than 3 log units higher than before. In control fish, on the other hand, prolonged dark adaptation had no such effect. It is not clear why these studies (Davis et al., 1988; Li and Dowling, 2000a) elicited different results. There may be several reasons, e.g. the characteristics of the stimulus (Davis and colleagues used red light, Li and Dowling white light), the duration of the dark adaptation, or the time of day when the experiment was performed. As we demonstrated here, the effect of olfactory stimulation on vision was observed only in the early morning when the circadian visual sensitivity is low. When repeated in the late morning or late afternoon, the effect was diminished.

Olfactory modulation of visual sensitivity is mediated by a dopaminergic mechanism

Dopamine is an important neural modulator in the retina (Dowling, 1986; Witkovsky and Dearry, 1992). Dopamine release is under the control of light (Weiler et al., 1997; Kirsch and Wagner, 1989) and a circadian clock (Doyle et al., 2002; Ribelayga et al., 2002). Although many questions remain about the mechanism of retinal dopamine functions, one of the main pictures emerging is that dopamine plays a role in the modulation of neural circuitry during light and dark adaptation (Dowling, 1986; Witkovsky and Dearry, 1992). This seems to be true for short-term reactions to ambient illumination changes, as well as for long-term adaptation during the circadian cycle.

TN input may play a crucial role in short-term and/or long-term light/dark adaptation. Umino and Dowling (1991) have shown that both GnRH and FMRFamide have effects on horizontal cells by either simulating or antagonizing, respectively, the effects of dopamine. Retinal ganglion cells increased their spontaneous activity in the dark after administration of both GnRH and FRMRamide (Stell et al., 1984). Interestingly, retinal GnRH content depends on diurnal and seasonal factors and on the state of light/dark-adaptation (Ball et al., 1989). We found that olfactory stimulation increased visual sensitivity only in the early morning, not in the late afternoon when visual sensitivity and retinal dopamine release are already high (Li and Dowling, 1998; Ribelayga et al., 2002). This shift of visual sensitivity from an early morning state to a late afternoon state could be blocked by a dopamine D_2 antagonist. Manglapus et al. (1999) found, using ERG measurements, that in Japanese quail retinas dopamine D_2 agonist administered at night simulated the daytime state of the retina with respect to b-wave amplitude and rod–cone dominance. A D_2 antagonist administered during the day, on the other hand, shifted the retina functionally to a state of night time. It is possible that changes of b-wave threshold, as seen in the present study and by Manglapus et al. (1999), or changes of b-wave amplitude as seen by Weiss and Meyer (1988), are due to dopaminergic modulation of outer retinal first-order elements, such as photoreceptor cells, that possess D_2 receptors (Stella and Thoreson, 2000).

The increase of absolute visual sensitivity in response to olfactory stimulation also suggests that dopamine is needed for the modulation of rod signaling transmission. It has been demonstrated that dopamine plays a role in the regulation of rod signaling transmission in the inner retina. In zebrafish with the DA-IPCs destroyed, rod signaling transmission to the inner plexiform layer was blocked (Li and Dowling, 2000b), possibly due to desensitization of dopamine receptors located on the retinal bipolar cells. Fan and Yazulla (2001) demonstrated that in 6-OHDA treated goldfish, the modulatory effect of dopamine on outward potassium current of bipolar cells is diminished. Similarly, in *night blindness b* mutants, which are characterized by degeneration of TN fibres and DA-IPCs (Li and Dowling 2000a), the effect of dopamine on the outward potassium current is lost (Yu and Li, 2003).

TN is the prime candidate for olfactory modulation of visual sensitivity

We propose that the olfactoretinal branch of the TN is involved in transmitting olfactory signals to the retina. Although this hypothesis awaits conclusive proof, we present here four lines of evidence in favor of it. (1) Although other centrifugal pathways have been described in different fish species, the TN is the only direct anatomical pathway between olfactory bulb and retina thus far identified (Stell et al., 1984). (2) Depletion of dopamine or intraocular injections of a dopamine D_2 antagonist prevents the modulation of visual sensitivity by olfaction. So far the only centrifugal pathway described in zebrafish that synapses onto the DA-IPCs is the TN (Li and Dowling, 2000a). (3) FMRFamide and GnRH alter the activity of both outer and inner retinal neurons (Walker and Stell, 1986; Umino and Dowling, 1991). Except for the TN, no other brain areas have been reported to send fibers to the retina that contain FMRFamide or GnRH. (4) The visual defect displayed by olfactory bulbectomized or dopamine-depleted fish mimic to some extent the visual defect of a mutant zebrafish, *night blindness b*, in which the olfactoretinal centrifugal pathway is disrupted. The characteristics of *night blindness b* mutants are a reduced number of DA-IPCs, fewer FMRFamide fibers in the retina and, in the behavioral assay, intermittent decreases in visual sensitivity after prolonged dark adaptation (Li and Dowling, 2000a).

Although olfactory bulb neurons synapse onto TN cell bodies (Yamamoto and Ito, 2000), so far no obvious changes in the electrophysiological characteristics of the TN have been recorded as a result of chemosensory stimulation (Fujita et al., 1991). Recently, Folgueira et al. (2002) have shown in trout that the nucleus subglomerulosis is a link between the visual and chemosensory systems. Interestingly, this nucleus projects to the optic tectum. It is known that the optic tectum is involved in the visual perception of objects and has a role in visually mediated escape responses (Springer et al., 1977; Herrero et al., 1998). Although chemosensory stimuli could modulate visual functions at the level of the optic tectum, we can exclude this explanation for our findings, because destruction of the retinal DA-IPCs and intraocular application of the D_2

antagonist sulpiride eliminate the effect of amino acids on visual function. This demonstrates that the olfacto-visual transduction described here is located in the retina. Furthermore, visual sensitivity is commonly assumed to be a function of the retina. A lesion of the optic tectum can eliminate the escape response (Springer et al., 1977); however, this will be an all-or-none effect, i.e. it will not influence the visual threshold under which an escape response can be elicited. Finally, the nucleus subglomerulosis receives gustatory, not olfactory information (Folgueira et al., 2002).

Conclusion

The zebrafish has recently become a genetic model for visual and olfactory physiology (Baier, 2000), behavioral and developmental neurobiology (Fetcho and O'Malley, 1997; Gahtan and O'Malley, 2001), and circadian biology (Cahill, 2002). In the present study, we provide *in vivo* evidence for functional olfactory-retinal sensory integration. We present several lines of evidence that the TN and DA-IPCs are the anatomical components for olfactoretinal centrifugal modulation.

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