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Is Feeding Behaviour in Crucian Carp Mediated by the Lateral Olfactory Tract?

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Abstract

Experiments were performed to investigate which bundle of the olfactory tract was essential for mediating feeding behaviour in crucian carp. Fish were divided in three groups: control fish, fish with only the lateral olfactory tracts (LOTs) intact and fish with the LOTs cut. The fish were maintained in physiological saline after surgery to preserve the remaining tracts and postoperative inspections revealed the functional status of the remaining tracts. With the injection of food odour into the aquaria the scores for various feeding behaviours—biting, snapping, mouth openings and vertical posture—were not significantly different between those of the control fish and the fish with the LOT intact. Those fish that had the LOT cut but the medial and lateral parts of the medial olfactory tract (mMOT, lMOT) intact had significantly lower feeding-related scores than the other two groups of fish. The results of the present study indicate that the LOT is necessary to maintain the full qualitative and quantitative extent of feeding behaviour in crucian carp.

Introduction

The olfactory system in fish mediates a number of behaviours that are essential for life processes: feeding, reproduction and alarm. In gadids, silurids and cyprinids, the olfactory tracts are well suited for experimental manipulations as both tracts are long and divided into distinct bundles (Sheldon, 1912). Each bundle connects to different areas of the brain and this organization is indicative of separate functional properties for each bundle, even though there are anatomical overlaps in projections seen in both carp, *Cyprinus carpio* (Ichikawa, 1975; von Bartheld *et al.*, 1984; Levine and Dethier, 1985), and cod, *Gadus morhua,* (Rooney *et al.*, 1992).

The functional implication of these projections has already been demonstrated by experiments involving electrical stimulation of discrete bundles of the olfactory tract in free swimming cod (Døving and Selset, 1980). Feeding behaviours were elicited by stimulation of the lateral olfactory tracts (LOTs), while spawning behaviour was induced by electrical stimulation of the lateral bundle of the medial olfactory tracts (lMOTs). Alarm-related behaviour, on the other hand, was seen in cod when electrical pulses were applied to the medial bundle of the medial olfactory tracts (mMOTs). Further evidence for the functional separation of olfactory tracts can be found in behavioural experiments with goldfish, *Carassius auratus*, which had been trained to discriminate amino acids and lost this ability after the

transection of their LOTs (von Rekowski and Zippel, 1993). This functional partitioning has also been demonstrated in goldfish as courtship behaviour is mediated by the medial olfactory tracts (MOTs) (Stacey and Kyle, 1983; Kyle *et al.*, 1987). Sex pheromones selectively elicited electrical activity in medial olfactory tracts of male goldfish (Sorensen *et al.*, 1991), whereas sperm release was induced by electrical stimulation of the medial olfactory tracts (Demski and Dulka, 1984). Alarm reaction in crucian carp *Carassius carassius*, also exhibit functional partitioning as this reaction is mediated by the mMOT (Hamdani *et al.*, 2000).

These experiments lay the foundation for the hypothesis that each bundle of the olfactory tract mediates a certain class of behaviour (Kotrschal, 2000). Some experiments, however, have provided conflicting evidence, as shown in the study by Stacey and Kyles on goldfish, which found that either the MOT or the LOT maintained intact levels of feeding response to food odours (Stacey and Kyles, 1983). As mentioned, this observation is in conflict with the general idea of a functional specificity of each olfactory tract bundle, and we therefore found it important to reinvestigate the functional specificity of the LOT as a pathway for various feeding behaviours. In the present study we present evidence that the LOT is necessary to maintain the full qualitative and quantitative extent of feeding behaviour in crucian carp.

Materials and methods

Crucian carp, *C. carassius* L., were caught in a small lake just outside the Oslo city boundry. They were transported to the aquarium facilities at the Department of Biology. The 12 crucian carp used for these experiments weighed between 15 and 22 g. All rearing and experimental procedures were conducted in accordance with the protocols described by the University of Oslo Animal Care Committee in order to respect the welfare of our experimental animals.

Experimental design

In view of the results from cod experiments indicating that the LOT mediates feeding behaviour (Døving and Selset, 1980) and the loss of discriminating behaviour seen by cutting the LOT in goldfish (von Rekowski and Zippel, 1993), we decided to study the feeding behaviours elicited by food odours in three groups of fish: controls, fish with only the LOT intact and fish with only the LOT cut. Three groups of fish were observed for a period of 2 min while injecting either physiological saline or food extract to the aquaria. Observations were made twice a day for 7 days starting 1 week after surgery.

Surgical operation

The fish were anaesthetized with benzocaine (45 mg/l), placed in a stand with running water through the mouth and over the gills, and operated on under a stereomicroscope. The skin just above the olfactory tracts was cut open and a portion of the dorsal cranium removed. The mesenchymeal tissue in the brain case was aspirated and the meninges around the olfactory tracts were removed with fine forceps. The olfactory tracts were clearly visible as three distinct bundles running from the olfactory bulb to the brain (Figure 1). The bundles were gently separated, taking care not to disrupt blood vessels. When cutting particular bundles of the olfactory tracts, care was taken to remove ~2 mm to prevent regeneration (von Rekowski and Zippel, 1993; Zippel *et al.*, 1993). We visually inspected the fibre bundles to control possible regeneration at the end of the experiments. The bundles of the olfactory tracts were cut symmetrically on both sides as follows:

- Two fish were sham operated, i.e. the brain case was opened and the meninges were removed to see the olfactory tracts, and two fish were anesthetized only. These four fish served as control.
- In four fish the LOT was left intact while the lMOT and mMOT were sectioned.
- In four fish the LOT was cut, while both the mMOT and the lMOT were left intact.

After sectioning the appropriate bundles, the cranial cavity was filled with a 2% solution of agar dissolved in physiological saline (g/l): NaCl (8.53), KCl (0.22),
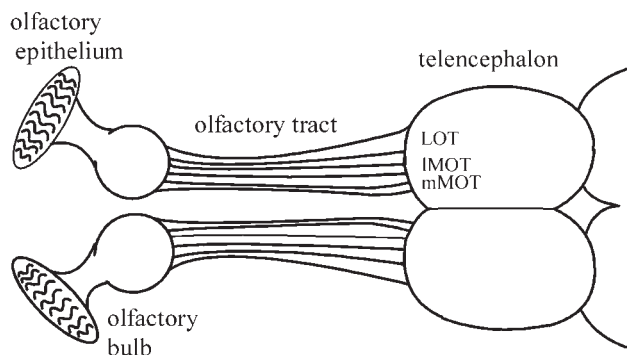

Figure 1 Schematic outline of the olfactory system and the forebrain in crucian carp illustrating the long olfactory tracts and their bundles. LOT, lateral olfactory tract; lMOT, lateral bundle of the medial olfactory tract; mMOT, medial bundle of the medial olfactory tract.

$MgSO_4 \cdot 7H_2O$ (0.25), $CaCl_2 \cdot 2H_2O$ (0.19). The fish recovered from the surgical operation in 25 l aquaria at 18°C with physiological saline as the aquatic medium. The aquaria bottoms were made of glass and not covered with gravel. Fish that had been subjected to corresponding types of treatments were grouped and placed in one of three aquaria. Fish were fed commercial salmon food pellets (EWOS 5A, Vextra) daily. Fish in all three aquaria swam to the place where pellets were introduced and started to snap at the food. During the week when observations of their behaviour were made, half of the physiological saline in the aquaria was replaced every day 3 h before trials.

Preparation of food extract

Salmon food pellets were suspended in physiological saline and mixed in a magnetic stirrer for 15 min. About 15% of the pellet dry weight was water soluble. The extract was filtered and injected into the aquaria at a concentration of 1.5 g dry weight of the water-soluble material per litre.

Analysis of behaviour

The different behaviour patterns of the fish were scored during 2 min periods when the fish were exposed to: (i) physiological saline or (ii) food extract. In total, 2×7 injections were made for each group of fish. Odorant stimuli were added to the aquaria via a polyethylene tube connected to a peristaltic pump with a flow rate of 5 ml/min for a duration of 2 min. When homogeneously distributed, the concentration of food odour in the aquarium would represent a 2500 times dilution of the introduced samples, i.e. 0.6 mg/l. The behaviour of the fish during the experiments was recorded by a video camera. Observations were made once every day for 7 days starting 1 week after the surgery. Total observation time for each group of fish was 28 min. The observer was not informed about the treatment of the different groups of fish.

The behaviours were scored from video recordings, which were repeated to follow the behaviour of each and every fish throughout the 2 min injection time. The following classes of

Table 1 Behaviour scores for the three groups of crucian carp exposed to physiological saline and food extract for a 2 min period

Behaviour	Control fish		LOT intact		LOT cut	
	Physiological saline	Food extract	Physiological saline	Food extract	Physiological saline	Food extract
Biting	12 ± 13.8	62.4 ± 23.4	15.3 ± 7.7	99.6 ± 63.8	1.0 ± 1.4	6.1 ± 9.2
Snapping	1.7 ± 2.9	4.9 ± 4.5	0.6 ± 1.1	5.3 ± 4.1	0	0
Mouth opening	4.0 ± 4.9	36.3 ± 18.6	5.9 ± 3.8	20.4 ± 14.2	3.1 ± 1.5	4.7 ± 4.6
Vertical posture	0.3 ± 0.5	3.1 ± 2.7	0	3.9 ± 3.0	0	0.1 ± 0.4
Distinct activity	0	12	0	21	0	0
Questionable activity	3	14	4	5	4	6
No response	25	2	24	2	24	22

feeding behaviours were scored: *biting* against the bottom, walls or congeners; *snapping*, a fast movement with the jaws at objects on the bottom or in mid-water or at the outlet of the plastic tube used for injecting substances; *mouth opening*, slow movements of the jaws in mid-water; *vertical posture*, where the fish were grasping objects on the bottom at an angle close to 90° to the bottom.

In addition to these behaviours, we saw other types of activity which could be interpreted as exploratory or search behaviour. Thus we observed the following behavioural parameters: activation, swimming downward, searching and location of the tube outlet. These behaviours were noted as present, questionable or not present for each of the seven sessions of the 2 min injection periods (Table 1).

The means and standard deviations are given for the biting, snapping, mouth openings and vertical posture behaviours (Table 1). To compare the different treatments, the scores for the four classes of behaviour patterns were pooled and two-tailed *t*-tests with different variance were made (Table 2).

Results

Behaviour during injection of physiological saline

Control and LOT-intact fish

The fish in these two aquaria swam slowly around in the middle or lower water layers, or they stood still for several minutes. These fish rarely snapped, or bit the walls or their congeners, and bottom food search with a vertical posture was not observed. During the 2 min period encompassing the injection of physiological saline into the tank, the scores related to feeding were low, and activation, swimming downward, searching and location of the tube outlet were seldom seen (Table 1).

LOT cut

The fish with the LOT cut and the mMOT and lMOT intact had initially darker skin coloration than fish of the other groups. The fish were quiet and stood still for hours in the same place. Biting or snapping happened very rarely. Food search behaviour was never seen. During the 2 min period of physiological saline injection the scores were low for all types of behaviour patterns (Table 1).

Behaviour during injection of food odour

Control and LOT-intact fish

The injections of food extract evoked typical food search behaviour both in control fish and fish with the LOT intact (Table 1). The first signs of an evoked reaction were mouth openings in mid-water, in which there were often 4–6 mouth openings in rapid sequence. The fish then dove towards the bottom of the tank and started to bite the bottom or walls of the aquarium. In several trials, the fish bit their congeners. The fish swam rapidly around in the aquarium and after 1 min gathered around the outlet of the injection tube, biting frequently. Several times during the 2 min injection period the fish adopted a vertical posture, snapping at the bottom. Occasionally, fish swam for short periods to the surface, only to return to the bottom, snapping at particles in the water.

One control fish did not show any signs of feeding behaviour and stood quietly near the surface. Post-mortem inspection of the brain revealed that the blood supply to the olfactory bulbs of this fish was impaired. This feature might explain the lower scores for the control fish than the fish with the LOT intact. The olfactory system in the other control fish was intact. The fish group with LOT intact had a normal blood supply to the olfactory bulbs and the interruption of the lMOT and mMOT could be confirmed.

LOT cut

The behaviour of the fish with the LOT cut was dramatically different from the other groups of fish. Injection of food extract did not induce any particular behaviour. The fish remained quiet and stood at the same place even if that

Table 2 Comparisions of the behavioural scores between the three different groups of crucian carp: the control fish, the fish with the LOT intact and those with the LOT cut

Stimuli	Fish group		Fish group	Probability (*t*-test)	Significance level
Physiological saline	Control fish	vs.	LOT intact	0.66325	NS
	Control fish	vs.	LOT cut	0.04046	*
	LOT intact	vs.	LOT cut	0.00519	**
Food extract	Control fish	vs.	LOT intact	0.61306	NS
	Control fish	vs.	LOT cut	0.00017	***
	LOT intact	vs.	LOT cut	0.00479	**
Physiological saline vs. food extract	Control fish			0.00045	***
	LOT intact			0.00966	**
	LOT cut			0.12982	NS

The stimuli were either physiological saline or food extract. The significance levels are: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, no significant difference.

happened to be close to the outlet of the injection tube. These fish did not show any signs of increased activity and snapping was never observed (Table 1). A slight increase in biting frequency was, however, observed in two of the seven trials. It should be noted that in these two cases the fish stayed close to the outlet of the injection tube.

Post-mortem inspection of the brain revealed that the blood supply to the olfactory bulbs of these fish was patent and that the lMOT and mMOT were intact. The LOTs that were cut had not regenerated.

Comparisons between the different treatments

Statistical analysis showed that there was no significant difference in feeding behaviour between the control and the LOT-intact fish (Table 2). This observation held true when injecting the physiological saline into the tank and when food extract was injected into the tank. On the other hand, those fish with the LOT cut had lower behavioural scores during the 2 min control period with the injection of physiological saline than the other two groups of fish. When injecting the food extract, the fish with the LOT cut had a highly significant lower score than the other two groups of fish. Comparing the 2 min periods of the injection of physiological saline and injection of food extract revealed significant differences for both the control fish and the fish with the LOT intact. However, there was no significant difference between these same treatments for the fish with the LOT cut, thus confirming the loss of sensitivity.

These results clearly show that the fish with the LOT cut displayed a drastically lower number of food search behaviours. Furthermore, the fish with only the LOT intact had the same behavioural scores as the control fish. In other words, the fish with the LOT cut had lost their ability to react to food odours.

Discussion

The results of the present study demonstrate striking differences in behaviour between the fish with LOT intact and those with LOT cut, suggesting that, in the absence of visual and tactile stimuli, the LOT is necessary to maintain the full qualitative and quantitative extent of feeding behaviour in crucian carp via the olfactory organ. This is not to say that all behaviour patterns that can be related to feeding are absent when the LOT is cut. Crucian carp with the LOT cut can respond to the presence of food pellets, and some reaction patterns could be observed in the absence of any discernible stimuli. It is also worth noting that food extract induced a slight increase in the frequency of biting among the fish with LOT cut; however, the behavioural patterns of mouth opening, snapping and vertical posture did not change upon injection of food extract. Biting is associated with feeding, but can also be part of aggressive and search behaviour. Feeding behaviour may also be evoked by the other chemsosensory systems (Kotrschal, 2000), which were intact in our experimental fish.

In two instances we saw feeding behaviour in the LOT-cut fish. These were observed when the fish happened to be close to the outlet of the injection tube; it seems possible that in these instances the concentration of food odour was sufficiently high to induce food search behaviour via the extraoral taste system. Taste buds are abundant (82–162 mm^{-2}) in the gular, forehead and operculum area in crucian carp (Gomahr *et al.*, 1992). It has been shown that anosmic channel catfish, *Ictalurus punctatus*, which have numerous external taste buds, do respond to food odours (Bardach *et al.*, 1967). Both electrophysiological and behavioural studies show that the external taste receptors are more sensitive than oral taste buds (Kanwal and Caprio, 1988; Kasumyan, 1999).

In behavioural trials the difference between thresholds for olfaction and gustation is high. The threshold concentrations for the most stimulatory taste substances are usually ~10^{-2}–10^{-4} M (Hidaka, 1982; Adams *et al.*, 1988; Jones, 1989; Lamb and Finger, 1995; Kasumyan and Morsy, 1996). The threshold concentrations for substances that induce food search behaviour in fish via the olfactory system

are least 2–5 orders of magnitude lower (Ellingsen and Døving, 1986; Kasumyan and Taufik, 1994). In natural waters the concentration of free amino acids that evoke food search behaviour are of the order of 10^{-6}–10^{-7} M (Poulet and Martin-Jezequel, 1983; Poulet et al., 1985; Williams and Poulet, 1986). These concentrations would evoke behavioural reaction via the olfactory but not the gustatory system.

Another interpretation of our results is that the fibres of the MOT can mediate feeding behaviours. Following crushing of the LOT, goldfish lost the ability to discriminate low (10^{-8} M) but not high (10^{-6} M) concentrations of stimuli (Zippel et al., 1993). Regeneration appeared after 2 weeks (von Rekowski and Zippel, 1993). This recovery time is so short that one can ask if the lesion included all fibres in the tract equally efficiently. The authors conclude that the LOT is responsible for the discriminatory ability, whereas similar concentrations of the rewarded and the concurrent stimuli (10^{-6} M) can also be discriminated via the MOT. Stacey and Kyle (Stacey and Kyle, 1983) stated that both the MOT and the LOT were needed to maintain feeding responses to food odours in goldfish. In the latter study, we speculate that post-operative recovery time may play a role. It is worth noting that in our experiments the period between surgical operation and behavioural trials was short and prevented the possibility of the olfactory nerve acquiring even limited amounts of patency or compensation by other sensory systems. This post-operative recovery period is an important consideration in fishes as research on the stellate sturgeon, *Acipenser stellatus*, demonstrated that they could begin to respond to food odour 3 months after bilateral cauterisation of the olfactory rosettes (Kasumyan and Devitsina, 1997). While the sturgeon's olfactory system was impaired however, the external gustatory system showed a peripheral proliferation and thus, to a certain degree, had compensated for the loss of olfactory sensitivity to food odours. We concluded that a period of 1 week is not enough for our experimental fish to recover an ability to respond to food stimuli by using the gustatory system.

A number of experiments using different techniques have yielded results that support the present findings. Electrophysiological studies of the nervous activity at the surface of the olfactory bulb of salmonids showed that the lateral part of the bulb responded to amino acids generally thought to be food odours, while the medial part respond to bile salts (Thommesen, 1978; Døving et al., 1980). These results have been confirmed by investigations into the bulbar responses in zebrafish (*Brachydanio rerio*) using voltage-sensitive dyes coupled with stimulation with different odorants (Friedrich and Korsching, 1997). Furthermore, amino acids evoked responses in the lateral part of the bulb, and fish pheromones (17α,20β-dihydroxy-4-pregnene-3-one-20-sulphate and prostaglandin $F_{2\alpha}$) and bile salts give responses in restrict regions of the medial olfactory bulb. Application of neural tracers to discrete regions of the olfactory bulb revealed that sensory neurons of a particular type are spread out in the olfactory epithelium (Morita and Finger, 1998). Neurons expressing specific putative olfactory receptors appear to be randomly distributed within the sensory epithelium (Ngai et al., 1993; Asano-Miyoshi et al., 2000). It is worth noting that there is a topographical projection from the olfactory bulb to the olfactory tract in both carp, *Cyprinus carpio* (Satou et al., 1979), and tench, *Tinca tinca* (Dubois-Dauphin et al., 1980), such that the majority of neurons that are situated in the medial part of the bulb projected to the medial tract and the majority of neurons in the lateral part of the bulb project to the lateral tract.

These results on the olfactory system in teleosts are supported by studies on the mammalian olfactory bulb, which have demonstrated that receptor neurons expressing a given receptor converge onto a small number of synaptic structures (glomeruli) in the olfactory bulb (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996). In future experiments on fish it will be important to describe which olfactory receptors are expressed in the different types of sensory neurons in the fish olfactory epithelium and what kind of behaviour patterns they mediate. An advance in this direction was recently published by Speca et al. (Speca et al., 1999) who showed that receptor 5.24 responded to basic amino acids. The implication of the present experiments is that sensory neurons that express receptors tuned to food odours terminate in a specific region of the olfactory bulb. The present study and that of Hamdani et al. (Hamdani et al., 2000) lay the basis for studies indicating which type of receptor neurons make synapses with neurons projecting to the MOT and the LOT (Hamdani et al., 2001).

Acknowledgements

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