ANATOMICAL SUBSTRATE FOR SPATIAL REPRESENTATION OF LOCATION OF ODOR IN AN INSECT BRAIN

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In mammals and insects, olfactory receptor neurons with the same odorant receptor are randomly distributed in the olfactory sense organs, but they send axons that converge onto discrete glomeruli in the first-order center, creating an odotopic map depending on quality of an odor rather than its location. However, to date, information about whether or how position of odor is mapped in each glomerulus has been lacking. Olfactory receptor cells in the antennae of the cockroach *Periplaneta americana* send their axons down either the two parallel nerves of almost equal thickness, depending on whether they are located on the anterior or posterior half of the flagellum. For the first step to elucidate neural substrate underlying processing of location of odors, projection fields of sensory axons in the two sensory nerves were investigated with the use of anterograde dye injection. The results suggested that olfactory receptor cells in either of the two nerves projected to all glomeruli but the rate of amount of axon terminals from the two nerves was different between glomeruli. This projection-bias seen in glomeruli may be the basis for a spatial representation of location of odor.

RAPID RESPONSES OF ZEBRAFISH MELANOPHORES TO BACKGROUND CHANGES AFTER TRAINING WITH CYCLICAL CHANGES OF WHITE AND BLACK BACKGROUNDS

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To examine the mechanism of fish colour change, we developed a new procedure for analysing the behaviour of melanophores in zebrafish responding to background changes. We used adult zebrafish, the mutant *leopard*, which has lines of spots consisting of melanophores overlying iridophores. Twelve zebrafish were set against a white or a black background under controlled illumination and kept for about 40 days during which the background was changed alternately every two days. The area of the melanophores relative to that of the iridophore-spots was analyzed as the Melanophore Index (MI). After 5 cycles (20 days) of background changes the MI of the dorsal melanophores, whose mean MI was 0.17 before the training session, increased to 0.45-0.92 on the black background, while it decreased to 0.01-0.05 on the white background. In these fish, a rapid MI decrease was observed within 10 seconds of the background change from black to white. In contrast, the MI increased more slowly after the background change from white to black. The results indicate that the zebrafish melanophores respond to background changes and their behaviour can be modulated by learning.

EFFECTS OF STRESS ON THE BLIND SIDE PIGMENTATION IN THE JAPANESE FLOUNDER

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Effects of stress on the Blind Side Pigmentation(staining)in the Japanese flounder *Paralichthys Olivaceus* were examined. That is, we conducted the following experiments. In the Japanese flounder derive from an aquatic farm, a negative relationship between the incidence of pigmented areas in the blind side and the concentrations of plasma cortisol was found. In cortisol-treated fish, Blind Side Pigmentation was inhibited. No relationship between the pigmentation rate and plasma cortisol concentration was observed. In RU486(Mifepristone)-treated fish, the pigmentation was promoted. These results suggest that stress responsiveness(especially responsiveness of interrenal gland) was depressed in the pigmented fish.

MORPHOLOGICAL COLOR CHANGES AND APOPTOSIS IN MELANOPHORES OF LARVAL MEDAKA, *ORYZIAS LATIPES*

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Many teleost fish can adapt their body color to a background color by changing the morphology and density of their skin pigment cells. The density of skin pigment cells in many teleost fish decreases during longterm adaptation to a white background. We previously reported that apoptosis is responsible for the decrease in melanophores and that a sympathetic neurotransmitter, norepinephrine, stimulates pigment-aggregation response of melanophores and prolonged response induces their apoptosis in medaka, *Oryzias latipes*. It is known that fish larvae can adapt to a background color immediately after hatching. In the present study, we compared melanophore apoptosis in larvae (stage 37-42) with that in adult medaka during long-term adaptation to a white background. Melanophore density became significant 3 days after hatching between black- and white-adapted medaka. But, melanophore did not decrease by apoptosis in these larvae, and NE did not induce apparent apoptosis in cultured melanophores derived from embryo cells. These results suggested that the regulation system of apoptosis is different between larvae and adult fish.

ROLES OF RH GENE FAMILY IN AMMONIA SECRETION FROM THE GILL OF TELEOST FISH

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Ammonia excretion from the gill in teleost fish is essential for nitrogen elimination and acid-base regulation, but the molecular bases are still largely unknown. Recently, it has been suggested that members of Rh family mediate ammonia transport in the mammals. The Rh family is originated from Rhesus-associated glycoprotein (RhAG) that is known as one of major blood group antigens on surface of red blood cells. Among the Rh genes, RhBG and RhCG are expressed in the kidney and possibly involved in ammonia metabolism. These findings lead us to hypothesize that the Rh genes would work for ammonia excretion in the fish gill. Here, we identified four RhAG, one RhBG and two RhCG candidates in fugu genome database and successfully isolated all of the cDNA fragments from *Takifugu rubripes* by RT-PCR. Analyses by Northern blotting and *in situ* hybridization revealed that, among the fugu Rh genes, fRhBG and fRhCG2 were specifically expressed on the secondary lamella in the gill, suggesting that they are involved in ammonia excretion from the gill. Currently, we are analyzing subcellular localization of the products and the activity to transport ammonia.

MEDULLARY NUCLEUS REGULATING ACTIVITY OF THE GLOSSOPHARYNGEAL-VAGAL MOTOR COMPLEX, A MAIN MOTOR NUCLEUS CONTROLLING THE DRINKING BEHAVIOR

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The drinking behavior of the seawater eel is controlled by the glossopharyngeal-vagal motor complex (GVC) in the medulla. The GVC innervates cholinergically the upper esophageal sphincter muscle (UES), which consists of striated muscle fibers. When the GVC nuerons are activated, the UES is constricted, thus drinking stops. To start drinking, the GVC must be inhibited by some factor from upstream circuits. For this purpose, catecholamines may play a role, because adrenaline, noradrenaline and dopamine inhibit the GVC activity (Ito et al., 2004). Morphorogically, tyrosine hydroxylase immunoreactive neurons have been demonstrated in the vagal lobe (LX), the commissural nucleus of Cajal (NCC), the area postrema (AP) and the reticular formation (RF) of the medulla. The present study aims to isolate the upstream nucleus which inhibits the GVC activity catecholaminergically. When the LX was stimulated electrically, the neuronal activity of the GVC was altered, inhibited or activated. Relationships between the electrical stimuli and the GVC responses were examined.

CONTRACTION OF THE UPPER ESOPHAGEAL SPHINCTER MUSCLE IN THE SEAWATER EEL: NEURAL AND HORMONAL CONTROL

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Tha upper esophageal sphincter muscle (UES) plays critical role in drinking water. The eel UES innervated cholinergically by the glossopharyngeal-vagal motor complex (GVC) in the medulla. Among ten branches of the vagal nerves from the medulla, only one branch (called X5) was effective to constrict the UES with optimal frequency of 20 Hz. The presence of the optimal frequency was explained by the intracellular calcium concentrations. The eel UES was also influenced by vasotocin and isotocin. Vasotocin constricts the UES and isotocin relaxes it. By separating muscle cells from nerve endings pharmacologically, mode of action of these peptides was examined.

MULTIPLE NATRIURETIC PEPTIDES IDENTIFIED IN THE MOST PRIMITIVE EXTANT RAY-FINNED FISH CREATE A DEEPER UNDERSTANDING OF THEIR EVOLUTION

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The natriuretic peptide (NP) family is composed of ANP, BNP, VNP and four CNPs (CNP-1 through 4) in teleost fish.However, only CNP-3 exists in elasmobranchs. In order to trace the process of diversification in ray-finned fish, identification of NPs in the controstean fish, sturgeon, resulted in identification of ANP, BNP, VNP and CNP-2. However, it is not known when CNP is diversified into 4 types. Thus, bichir, Polypterus endlicheri, was used in this study, as it is believed to be the most primitive extant ray-finned fish and diverged earlier than sturgeon in the bony-fish lineage. We cloned cDNAs encoding ANP, BNP., VNP and three CNPs (CNP-1, 3, and 4) from the heart and brain. Thus it is obvious that four CNPs already exist in the bichir prior to the independent genome duplication that occurred in the teleost lineage. This is the first observation that CNP-3, ANP, BNP, and VNP co-exist in a single fish species including teleost fish, thereby confirming that three cardiac peptide genes, ANP, BNP and VNP, are produced by tandem duplication from the CNP-3 gene.