### ROLE OF THE EXTRAOCULAR PHOTORESPONSIVE NEURONS IN THE RESPIRATORY BEHAVIOR OF THE MARINE MOLLUSC, *ONCHIDIUM*

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Extraocular photoresponsive neurons, Ip-2/Ip-1 in the *Onchidium* ganglion respond to light with a hyperpolarizing receptor potential. The electrical coupling of Ip-2/Ip-1 cells showed that each of beating or bursting discharges is transmitted synchronously along both the axonal branchings. The Ip-2/Ip-1 cells functioning as interneurons were also motoneurons innervating the pneumostome with pulmonary sac. We also suggested that their hyperpolarizing photoresponses play a role in depressing the synaptic transmission and in diminishing more the following respiratory behavior. On the other hand, *Onchidium* are intertidal and amphibian molluscs. Thus, at low tide animals open the pneumostome to begin an aerial respiration. The present study suggests that Ip-2/Ip-1 cells are possible candidates in generating the above aerial respiration behavior.

### THE SMALL-TYPE KENYON CELLS ARE ACTIVE IN THE BRAIN OF DANCER HONEYBEES

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The honeybee can transmit the information regarding the location of food source to their nest mates using the dance communication. With the aim to identify brain regions involved in the dance communication, we previously developed an experimental system to identify active regions in the honeybee brain, by detecting the expression of a novel immediate early gene, *kakusei*, as a marker for neural excitation. In the present study, we examined the expression patterns of *kakusei* in the brains of nurse bees, dancers or followers. With quantitative analysis, larger number of signals was detected in the dancer brain, especially in the small-type Kenyon cells in the mushroom bodies. These results strongly suggest that the activity of small-type Kenyon cells is elevated during dance behavior. Although the role of the small-type Kenyon cells in neural processing is still obscure, they are projected by antennal and optic lobes, and constitute a complex neural circuit with the extensive recurrent inputs. Thus, the small-type Kenyon cells may participate in the higher neural processing which is required for construction and representation of the dancing behavior.

### DEVELOPMENT OF A NOVEL SMALL TELEMETRY SYSTEM FOR ACQUISITION AND STIMULATION USING ELECTROMAGNETIC INDUCTION

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In order to know neural mechanisms from sensory reception to behavior in animals under free conditions, we developed a small telemetry system for acquisition of physiological signals and electrical stimulation. The telemeter weighs only 0.5 g, which is light enough to load on a large-size insect. We supplied power for the telemeter by electromagnetic induction externally, therefore, the telemeter has no batteries and is suitable for long-term recording and stimulation. For evaluation of the system, we stimulated the ventral nerve cord of a quiescent hawkmoth (Agirus convolvuli) and acquired induced muscle potentials that associated with wing flapping. We are now applying this system to freely moving insects (silkmoths, hawkmoths and crickets) for developing this system as a useful technique in neuroscience. Supported by PROBRAIN.

### IDENTIFICATION AND LOCALIZATION OF VISUAL PIGMENT OPSIN mRNAs IN THE EYE OF THE PALE GRASS BLUE *ZIZEERIA MAHA*

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We initiated a molecular biological analysis in order to elucidate the distribution of spectral photoreceptors in the eye of a flower visiting Pale grass blue, *Zizeeria maha*. We identified three mRNAs each encoding visual pigment opsin of UV- (ZmUV), blue- (ZmB) or long wavelength- (ZmL) absorbing type by RT-PCR. We localized these mRNAs in the retinal tissue by in situ hybridization. The labeling pattern with the probe specifically hybridize to the ZmUV mRNA (UV probe) yielded three types of ommatidia: type I with a single labeled cell, type II with two labeled cells, and type III with no labeled cells. This pattern was also clear in the labeling with the B probe, but in a compensatory manner. The L probe labeled 6 photoreceptors in all types of ommatidia. The labeling pattern is found to be basically same in other flower visiting insects including butterflies and bees. Some of the ommatidia of *Zizeera* bear deep red pigmentation around the rhabdom, which seems to function as red color filter for the ommatidia. The spectral sensitivity of ZmL-containing photoreceptors may thus be polymorphic because of the filtering effect of the red pigment.

### DEVELOPMENT OF ADULT COMPOUND EYE IN THE BUTTERFLY PAPILIO XUTHUS

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The compound eye of the swallowtail butterfly, *Papilio xuthus*, is composed of three types of ommatidia each containing different set of spectral photoreceptors. How are the different ommatidia formed in the pupa? Here we first studied the structural change of the eye during the pupal stage, which lasted for 10 days, and divided the process into three phases. In the phase I (day 1-3), the cells form clusters corresponding to the ommatidia, but cell types could not be identified. In the phaseII(day 4-6), precursor cells of photoreceptors, pigment cells, and lens could be identified histologically. The ommatidia rapidly elongate during the phaseIII(day 7-10). We next performed RT-PCR to detect five mRNA of visual pigment opsins (PxUV, PxB, PxL1-3) in the template polyA RNAs extracted from the pupal eyes of different days after pupation. PxUV and B were detected already in the 1 day pupae, but PxL1, 2, and 3 were detected day 6, 2, and 4, respectively. We identified the photoreceptor precursors that express these opsin mRNAs by histological in situ hybridization, and found that three ommatidial types could be recognized on day 6, and then the ommatidia elongate to mature.

### POLARIZATION DISCRIMINATION IN FORAGING PAPILIO BUTTERFLIES

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Compound eye photoreceptors of many insects are polarization-sensitive. Kelber reported that Papilio butterflies see difference in polarization-angle as difference in color when laying eggs. We tested whether this holds also for foraging Papilio or not. We trained Papilio to feed on sucrose solution in front of an unpolarized (UP) red light. The butterflies were then subjected to three unrewarded dual-choice tests. In test 1, we presented two UP red lights, one with different intensities: the butterflies always chose one with stronger intensity. In test 2, we presented a UP red and a horizontally or vertically polarized red light, and we changed the intensity of the UP red. The butterflies changed their selection depending on the intensity of the UP red. In test 3, we presented UP red, whose intensity was changed, with an UP blue or green light. Here the butterflies always chose the training color. Judging from these results, we concluded that feeding Papilio butterfly recognize the difference in polarization-angle as the difference in intensity, but not as color difference.

### DIFFERENT STAGES OF MEMORY FORMATION IN FEMALE CRICKET FIGHTING

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Isolated female crickets engage into agonistic interactions, which follow a very stereotyped behaviour pattern and deal the establishment of a dominant-subordinate relationship. After an initial encounter, subordinate individuals form a short-term memory of loss, which lasts for about 15-30 minutes. If subordinate individuals were faced with the same opponent for a second time within this interval, they predominantly responded with avoidance. Within one hour after the initial fight individuals fully recovered their aggressive motivation. However, two hours after the first encounter we observed a sudden relapse into avoidance behaviour. Using various different tools, we investigated the mechanisms underlying these different stages of memory formation.

### EFFECTS OF DOPAMINERGIC AGENTS ON IMPULSIVITY AND INTRACEREBRAL MONOAMINES IN THE DOMESTIC CHICK

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Fertile chicken eggs were treated *in ovo* prior to incubation with catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA). The hatched chicks were quantified their spontaneous motor activities on days 3-14 posthatch and their impulsivities on days 6-10 posthatch. The contents of intracerebral monoamines were quantitatively estimated by immunohistochemical and HPLC analyses. Furthermore, some of chicks were injected intraperitoneally with dopaminergic precursor L-dihydroxyphenylalanine (L-DOPA) on days 9-10 posthatch, and the effects were examined. The spontaneous motor activity and impulsivity were significantly enhanced in 6-OHDA-treated chicks as compared with controls. The contents of intracerebral dopamine (DA) were significantly lesser in the 6-OHDA-treated animals than those in controls at just before hatching (Day 18 embryo). The enhanced impulsivities in 6-OHDA-treated chicks were suppressed after L-DOPA treatments and their intracerebral DA contents were approximately the same level as those of 6-OHDA-untreated controls. These results suggested that intracerebral dopamine was involved in the inhibitory control of impulsivity in domestic chick.