EFFECTS OF AGING ON EXCITATORY JUNCTIONAL POTENTIALS RECORDED FROM CRICKET FLIGHT MUSCLES

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Excitatory junctional potentials (EJPs) and miniature excitatory juncitonal potentials (MEJPs) were recorded intracellularly from cricket (*Gryllus bimaculatus*) flight muscles 112a (hindwing depressor) and 119 (hindwing elevator) on appropriate days after imaginal moult. Cricket flight muscles are considered to be suitable for investigating functional changes with aging, since they are known to start to degenerate during the first 2–3 days after imaginal moult. In muscle 112a, which showed a remarkable decrease in muscle mass with aging, the EJP and the MEJP amplitude, and the MEJP frequency decreased during the first 2–3 days after imaginal moult and then increased gradually. However, inserting a microelectrode into the muscle fiber became difficult after the sixth day following the imaginal moult. In muscle 119, the EJP and MEJP amplitudes and the MEJP frequency increased gradually by the fifteenth day after imaginal moult. It is suggested that the fall in EJP amplitude in muscle 112a after the second day following imaginary moult is caused by a decrease in quantal content and the following gradual rise is due to an increase in quantal size.

CHARACTERIZATION OF SQUID VOLTAGE-DEPENDENT CALCIUM CHANNEL α1 AND β SUBUNIT

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Multiple classes of voltage-dependent calcium channel (VDCC) α1 subunit have been molecularly identified and are often termed L-, N-, P/Q-, R- and T-type calcium channels on the basis of their electrophysiological and pharmacological properties. Auxilial β subunits may also contribute to functional diversity of VDCC. To characterize a squid VDCC, we cloned VDCC α1 subunit ($LoCa_v1$) and β subunit (Loβ1) cDNA from the squid (*Loligo bleekeri*) optic lobe and performed functional expression experiments in *Xenopus* oocytes. Whole cell currents were measured by two electrodes voltage clamp method. $LoCa_v1$ current coexpressed with Loβ1 was high voltage activated and lasted for long during activation and not inhibited by any drugs and toxins. Loβ1 reduced inactivation of mammalian $Ca_v2.3$ current similar to mammalian β 2a subunit. Immunohistochemical examinations revealed punctate immunoreactive structures in neuropil area in the optic lobe, which suggests that $LoCa_v1$ may be localized at presynaptic terminal.

LOCALIZATION OF IONOTROPIC GLUTAMATE RECEPTOR SUBTYPES IN THE OPTIC TECTUM OF RAINBOW TROUT *Oncorhynchus mykiss*

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The distribution of glutamate receptor subtypes was examined in the optic tectum of rainbow trout *Oncorhynchus mykiss*. Glutamate is a putative neurotransmitter for the visual transduction in fish. First, we examined Ca^{2+} responses to glutamate in the tectum by a Ca^{2+} imaging method. The application of ionotropic glutamate receptor agonists (NMDA and AMPA) increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in a part of the cell body layer, stratum periventriculare (SPV). We named this layer "SPV I". On the other hand, a metabotropic glutamate receptor agonist, *t*-ACPD, did not induce a Ca^{2+} elevation in the tectum. Second, we examined the subtypes of ionotropic glutamate receptor in the tectum by *in situ* hybridization and immunohistochemistry. The mRNA of a NMDA receptor subunit was found in SPV I, whereas the immunoreactive signals distributed in the stratum fibrosum et griseum superficiale (SFGS) and the stratum griseum centrale (SGC). The SFGS and SGC contain the dendrites of neurons in SPV I. These results suggest that ionotropic glutamate receptors are involved in the visual information processing in salmonid.

Action spectrum of foraging behavior in the Japanese yellow swallowtail butterfly, *Papilio xuthus*.

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We established an experimental protocol to evaluate learning and discrimination of monochromatic light by the Japanese yellow swallowtail butterfly, *Papilio xuthus*. By using the protocol we measured the action spectrum of foraging behavior of *Papilio*. First, we trained butterflies to feed on a certain monochromatic light projected from below to a transparent screen inserted in the floor. After confirming that they learned the wavelength, typically after 10 days of training, we tested the butterflies one by one. We presented training wavelengths for each individual at different intensities, and recorded whether the butterflies perform foraging behavior under free-flight as well as under tethered conditions. The lowest intensity required to elicit foraging behavior was recorded as the sensitivity threshold. The sensitivity of visit in free-flight condition peaks at 420 nm, whereas the sensitivity of proboscis extension in tethered condition peaks at 360, 500 and 600nm. This difference suggests that the visit and the proboscis extension are controlled by independent mechanisms at least in part.

Coexpression of three long-wavelength absorbing visual pigments in proximal photoreceptors in the retina of *Papilio xuthus*

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The compound eyes of *Papilio xuthus* contain five classes of spectral receptors, peaking at the UV, violet, blue, green, and red wavelength regions, and another type of receptors with abnormally broad sensitivity spectrum (A-cell). We previously cloned five different opsin cDNAs, UV, B, L1, L2 and L3, and localized their mRNAs in the retina by *in situ* hybridization.

We thus found that the ommatidia can be divided into three types in terms of the combination of the expressed opsin mRNAs. It appeared that some photoreceptors express more than two opsin mRNAs. To improve the reliability of the labeling we here created a new probe to detect the mRNA of a green opsin, L1, and obtained increased labeling density in *in situ* hybridization. In contrast to our previous probe corresponding to the 200 bases of C-terminus non-coding region, the new one is approximately 1600 bases long, containing both the whole open reading frame and the non-coding region. We found that the mRNAs of L1 (green), L2 (green) and L3 (red) are coexpressed in the A cells suggesting that these three opsins broaden the sensitivity spectrum of A-cells. Interestingly, the coexpression seems to hold only in males.

Minimum visual angle required for color discrimination in the Japanese yellow swallowtail butterfly, *Papilio xuthus*

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We established a Y-maze experimental set-up to quantify the color vision properties in the butterfly *Papilio*. First we trained *Papilio* to feed on nectar on either blue, green, yellow, or red disk presented vertically. We then subjected the butterflies to select one of the disks each presented vertically at the end of the two arms of the Y-maze. We put a disk of training color in one arm and a gray disk whose *Papilio*-subjective brightness was identical to the color disk in another arm. We changed the size of disks so as to test the visual angles of 5.7, 3.4, 2.3, 1.7, 1.1, and 0.7 degree. A butterfly was allowed to enter one of the arms for 6 times per a test. When the butterfly extended the proboscis towards the disk of trained color, we counted the visit as a positive. It turned out that blue, yellow, and red disks were clearly discriminated from gray when the visual angle was larger than 1.7 deg. However, for green discrimination the angle was 2.7 deg. The results suggest that the information processing is color dependent. Interestingly, the visual angle similarly measured for honeybees is 5.0 degree, implying that *Papilio* has improved visual acuity in this respect.