as osmundalactone and 5-hydroxy-2-hexen-4-olide, respectively, by infra-red and proton magnetic resonance spectral analysis, and by mass spectral analysis of their acetates (Fig. 2). Although these compounds have been artificially prepared from osmundalin, a glucoside of osmundalactone (Hollenbeak et al., 1974), this is the first report on them as natural products.

REFERENCES

Olfactory Neural Pathway and Sexual Pheromone Responses in the Deutocerebrum of the Male Silkworm Moth, Bombyx mori (Lepidoptera: Bombycidae)

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It is well known that male silkworm moths display a dramatic mating behavior when the antennal olfactory receptor cells receive even a very few molecules of the sexual pheromone, Bombykol, emitted from a female (Butenandt et al., 1959). These receptor cells of the male antennae respond only to the Bombykol extracted from the female lure gland (Schneider, 1969, 1970) and their receptive mechanism is relatively well known (Kassling, 1971). However, very few studies have been done on the function of the neurons in the deutocerebrum and olfactory higher centers of the silkworm moth, Bombyx mori (Kanazaki and Shibuya, 1981).

This study was undertaken to investigate the olfactory neuronal pathway in the deutocerebrum and the responses of the secondary neurons to Bombykol in the male silkworm moth.

Many silkworm cocoons purchased from a local farmer were kept in a refrigerator at about 5°C. In each series of the experiment, about 100 of the pupae emerged at room temperature and became adult moths in two weeks. Male and female moths were kept in separate rooms.

For morphological studies, scales, cuticles and tracheal membranes of the head of a male moth were carefully removed under a binocular microscope with the brain being isolated. The preparation was fixed with a Susa fixative for 12 hr. After dehydration and cleaning with butanol, three-dimensional preparations 14 μm in thickness were made in horizontal, transverse and sagittal directions of the brain. The sections were impregnated with silver (Bodian's method modified according to Ootsuka, 1962) and observed with a light microscope.

To record electrical responses of the deutocerebrum neurons to Bombykol, the male moth was immobilized by putting the neck into a thin slit of a fixed chamber made of acrylic plastic. Using a binocular microscope the brain was carefully exposed by removing the scales and cuticles of the head. Muscles in the head were then cut and removed to prevent any movement of the brain. A glass microelectrode filled with a 15% potassium ferricyanide solution was used. The resistance of the microelectrode was 100–150 M ohms. Impulse responses led with a microelectrode were observed with an oscilloscope (Nihon-Kohden VC-9) through a preamplifier (MEZ-8201) and recorded with a data recorder (TEAC R-60).

The lure glands were isolated from female moths about 7 hr after emergence and Bombykol extracted from them with n-hexan. A gas chromatograph determined that 1 ml of the solution contained about

1 μg of Bombykol ($10^9$). The standard curve was determined with cetyl alcohol, which has the same carbon number (C16) as Bombykol. The Bombykol (concentration: $10^{-10}$-$10^{-5}$) was used as the odorant. The odorant (Bombykol; 0.2 ml) was put onto a small filter paper ($1 \times 1$ cm) and this was then placed in a glass cartridge. The odorant was transmitted to the antenna by a constant flow of pure air (1.5 ml/sec). Air in the shield room was exhausted during the experiment through a duct with a ventilator.

The antennal nerves of the silkworm moth, as in most insects, morphologically terminate in the ipsilateral deutocerebrum. In serial sections of the histological preparations it was observed that most axonal fibers of the nerves connected with the secondary neurons in the glomeruli of the deutocerebrum. In the male silkworm moth, the deutocerebrum had an egg-shaped macrogglomerulus in the dorsal portion and many small egg-shaped microglomeruli in the ventral portion. The size of the macrogglomerulus was about $150 \times 200 \mu m$ and the microglomerulus $30 \times 40 \mu m$. Usually one macrogglomerulus and 50 to 60 microglomeruli were observed in each deutocerebrum of the male moth. However, no macrogglomeruli were found.

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Fig. 1. Sagittal sections of the deutocerebrum of the male (A) and female (B) silkworm moths. Thickness of sections is about 14 μm. Dorsal side is upward and anterior to the left. AN: antennal nerve, MAGL: macrogglomerulus, Arrows indicate microglomeruli. Scale: 50 μm.

A. PHASIC TYPE

B. TONIC TYPE

Fig. 2. Two types of response of the medial cell group (MCG) neurons to Bombykol in the deutocerebrum of a male moth. A: phasic type, B: tonic type. Horizontal lines under each record indicate stimulus (concentration: $10^9$). Calibration: 10 mV, Time 1 sec.
in histological preparations of the deutocerebrum of 28 female moths (Fig. 1, A and B).

There were two groups of secondary neuron somata, in the deutocerebrum of the male silkworm moth, the lateral cell group (LCG) and the medial cell group (MCG). It was usually observed that dendrites of the LCG neurons were sent processes into the microglomeruli and those of the MCG neurons into the macroglomerulus. The diameters of the neuron somata in both groups were 15 to 20 μm. Small axonal bundles from the deutocerebrum ran to the ipsilateral protocerebrum as the tractus olfactorio-globularis (TOG). The TOG ran near the β-lobe and the central body and diverged into two two branches in the dorsal portion of the central body, one branch apparently reaching the calyx of the mushroom body and the other the lateral protocerebral lobe. The histological structure of the deutocerebrum of the silkworm moth is very similar in many ways to those of the Saturniidae moths studied by BOECKH and BOECKH (1979).

MCG neurons responded well to various concentrations of Bombykol. Spontaneous impulses of the single neurons showed a higher frequency than the receptor cells (0 to 2 Hz) (SCHNEIDER, 1970). Two types of responses to Bombykol in the MCG single neurons, namely the phasic and tonic types, are shown in Fig. 2. Impulse frequency of both types of responses increased to about 26/sec in the phasic type and to about 30/sec in the tonic type (concentration: 10−6), increasing with an increase in concentration. However, the LCG neurons seldom responded to Bombykol.

It is considered that the two types of responses in the MCG neurons are probably important as neuro-

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ERRATUM

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"Tocusta migratoria L." in the title should read "Locusta migratoria L."