EFFECTS OF ALTERED GRAVITY ON IDENTIFIED PEPTIDERGIC NEURONS OF THE CRICKET *ACHETA DOMESTICUS*.

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The development of organisms requires a permanent interplay between genes and environment. Identified neurons that are common in insects are excellent models to determine the specific contribution of these factors for the development of the nervous system. The aim of the study was to investigate effects of microgravity (µg) on identified peptidergic neurons in a cricket (*Acheta domesticus*) (1) if eggs were fertilized in space and (2) if the period of neuronal proliferation took place in µg. Proliferation is mainly completed after 50% of embryonic development. Thus, an 8 days lasting µg-exposure was sufficient to answer the question. The 10 days lasting Italian Soyuz taxi flight ENEIDE to ISS in April 2005 was used.

We chose neurons immunoreactive (ir) to allatostatin (AST), crustacean cardioactive peptide (CCAP), and perisulfakinin (PSK) because these neuropeptides are involved in developmental and neuronal processes (Homberg 1994). AST inhibits the synthesis of juvenile hormone; AST neurons are usually equipped with short axons; rarely do they extend to more than 4 segments (Fig. 1). CCAP has myotrope function with strong effect on heart activity; it probably contributes also to the regulation of molting. PSK is a myotrope neuropeptide; in addition, effects on the central nervous system were described. Due to its wide projections (Fig. 2), P-PC1 neurons probably exert a modulating effect on neuronal activity.

We considered these peptidergic neurons as model structures to study the sensitivity of the topological organization within the central nervous system to µg because it is very unlikely that gravity effects on the development of topological organization depends on specific neurotransmitters. This assumption might not be correct if the investigated neurons have mono- or polysynaptic input from gravity receptors such as the position sensitive interneuron PSI (cf. Sakaguchi and Murphey, 1983).

The experiment CRISP-2/ENEIDE was a follow-up study of the Neurolab experiment CRISP (Crickets in Space). Then post-flight observations on cricket larvae included neuroanatomical, neurophysiological, and behavioral studies of different developmental stages. All stages as well as embryos used in CRISP/Neurolab had a fully developed set of neurons at onset of the mission. The main observations were absence of significant behavioral modifications and anatomical changes of cerebral peptidergic neurons, but a significant depression of the sensitivity of the PSI (Horn, 2003; Horn et al., 2002).

A special experimental container was developed for inflight fertilization on ENEIDE. It consisted of an adult chamber and larval chambers, separated by egg collectors (Fig. 3). If females inseminated on ground had access to

Figure 1. Allatostatin A-PC2-ir neuron and its primary neurite within the right hemisphere from a space (middle) and ground (right) embryo. Post-flight fixation at 60-65% of embryonic development. The mature A-PC2-ir group consists of 6 neurons (left). At stage 60-65% embryos, only 4 out of the 6 neurons are visible in both, flight and ground embryos.

Figure 2. Basic projection pattern of a PSK PC1,2-ir neuron.

Figure 3. Cricket container for in-flight fertilization. On top the adult chamber (CC-AC), on bottom 2 larval chambers (CC-LC1,2); in-between 3 egg collectors (CC-EC1,2,3). They were filled with vermiculite; 2.5 days after launch, females had access to the vermiculite for 20 hrs to deposit eggs. Inset: individual egg collector with deposition area on top.

the vermiculite within the egg collectors, they deposited their eggs. During this process, the eggs were fertilized.

After termination of the 8 days lasting µg-exposure, the nervous system was taken from embryos or, after hatch-
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ing, from the 1st instars for immunocytochemical staining of neurons. The 1st fixation was done 6 hours after landing in Kazakhstan, a 2nd fixation 34 hours later in Moscow, and a 3rd fixation after hatching and testing of the geotaxis of the 1st instars. A total number of 112 embryos and 1st larvae from µg-fertilization were available, and 103 from on-ground fertilization.

The ENEIDE flight revealed that after in-µg fertilization, AST-ir-, PSK-ir- and CCAP-ir-neurons developed as after on-ground fertilization with respect to the location of somata and arborizations. This held not only for cerebral (Fig. 1), thoracic or abdominal (Figs. 4,5) neurons with short neurites, but also for PSK-ir neurons that project throughout the whole nervous system, with cell somata lying in the protocerebrum and arborizations within the cerebral, thoracic and abdominal ganglia (Figs. 2,6).

Figure 4. Somata of CCAP-ir neurons C-BM1 in the abdominal ganglia (AG) from 55% embryos (top and middle) and 1st instars (bottom). Left: Basic C-BM1-ir projection patterns.

Figure 5. Location of 2 types of CCAP-ir neuron in the terminal (last) ganglion of 1st instars. Neurons at the rostral corner of the ganglion: C-BM1+2-ir neuron. - Neurons at the median caudal location: C-TG neuron. Note the clear similarity of the arborization and the projection pattern of the flight and ground embryos. - Calibration 100 µm.

Thirty-three space embryos were reared up until hatching. They hatched about 1.5 days earlier than the ground reared embryos. The space larvae revealed no abnormal geotactic behaviour compared to ground larvae; however, the quantitative analysis concerning walking velocity and curvature of walking trajectories is not yet completed.

These studies demonstrate that developmental processes within the nervous system of insects are obviously under a dominant genetic control. This does not exclude an influence of epigenetic factors on other developmental processes in insects including the nervous, motor and sensory systems. The earlier hatching of the 1st larvae after inflight fertilization makes this hypothesis very likely.

Supported by DLR: grant 50WB0323 to Horn.

Figure 6. PSK PC1,2-ir neurons from two 1st larvae, after fertilization in µg (left) or on ground (right). Top: Somata of PC1,2-ir neurons in the brain. Middle: Projections of the neurons at the level of the 6th abdominal ganglion. Bottom: Projections of the neurons within the terminal ganglion (cf. Fig. 5a). Calibration 50 µm (top, middle), 100 µm (bottom).

REFERENCES


