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BRAIN-PITUITARY AXIS DEVELOPMENT IN THE CEBAS MINIMODULE

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I. Introduction

The CEBAS minimodule system is a man-made aquatic ecological system that incorporates animals, plants, snails, and microorganisms. It has been proposed that CEBAS will lead to a multigenerational experimental facility for utilization in a space station as well as for the development of an aquatic CELSS to produce animal and plant biomass for human nutrition. In this context, research on the reproductive biology of the organisms within the system should receive the highest priority. Thus, the goals of our proposal were to provide information on space-flight-induced changes in the brain-pituitary axis and in the organs that receive information from the environment in the vertebrate selected for the CEBAS Minimodule program, the freshwater teleost *Xiphophorus helleri* (the swordtail). We studied the development of the brain-pituitary axis in neonates, immature and mature swordtails using histology, cytology, immunohistochemistry, morphometry, and *in situ* histochemistry to evaluate the synthesis, storage, and release of neurotransmitters, neuroregulatory peptides, neurohormones, and pituitary hormones as well as the structure of the organs and cells that produce, store, or are the target organs for these substances.

We flew experiments in the CEBAS-minimodule on two shuttle missions, STS-89 and STS-90. In both flights four gravid females and about 200 juvenile (7 days old) swordtails (*Xiphophorus helleri*) constituted the aquatic vertebrates to be studied, in addition to the plants and snails that were studied by other team members. In a sample sharing agreement developed with Dr. Volker Bluem, organizer of the CEBAS research program, we received a small number of the juveniles and shared the brains of two adult females.

II. Animals Studied

Animals Received from Flights: Four females and 65 juvenile swordtails returned from STS-89; we received 15 juveniles and one adult female brain. In addition, seven living specimens were taken back to our aquatic facility at Brooklyn College to monitor their post flight growth and development. Four females and 25 juveniles returned from STS-90; we received 2 juveniles (head only) and one adult female brain.

III. Antisera Used in Study

CASI

Antisera used (all with ABC Elite kits):

- a) rabbit anti-(porcine) neuropeptide Y (NPY). From Peninsula Co.; 1:200
- b) rabbit anti-(porcine) dynorphin. From Peninsula; 1:200
- c) rabbit anti-(human) androgen receptor. From Affinity Bioreagents; 1:300
- d) rabbit anti-(synthetic) neurotensin. From Incstar; 1:400
- e) rabbit anti-(porcine) galanin (GAL). From Chemicon International; 1:400
- f) rabbit anti-(synthetic) FMRF-amide. From Incstar; 1:400

IV. Observations

A. Ground Based Studies

The following results were obtained from intensive investigations of the brain-pituitary-gonad axis of ground based animals. These data provided the basis of comparison for the study of the lab module and flight animals of STS-89 and STS-90.

1. Androgen Receptors

- localized in the ventral nucleus lateralis tuberis (NLT) of males and female brains. This immunoreactivity was seen in short, curved, beaded strands that appeared to be along cell membranes or perhaps, axons. No nuclear or cytoplasmic was found in this region, to date.
- localized in the neurohypophysis (NH) and pars intermedia (PI) of the pituitary gland. In the NH the nature of the immune response was granular and scattered while in the PI it appeared to be located within the cytoplasm of cells, often on the nuclear membrane. Few scattered granules also were seen in the ventral caudal pars distalis (CPD). These granules appeared to be located either in the extracellular areas of the CPD.

2. Galanin

- the immune response was found to be much more intense in males than in the two females studied. We must determine if this is true for most females.
- immunoreactive (ir) cell bodies were seen in the lateral, ventral NLT with a great deal of ir-material in the more dorsal regions. This ir-material was so dense and profuse that it was difficult to discern whether it formed tracts, but the overall orientation of this material would seem to indicate that it was in tracts either leading to or away from the ir-cell bodies in the ventral region of this brain nuclei.
- beaded tracts were also found in the preoptic area, particular in the nucleus preopticus (NPO) and the nucleus preopticus periventricularis (NPP).
- immunoreactivity extended caudally into the hindbrain region and anterior portions of the spinal cord.
- in the pituitary gland, ir-material was found as granules in the NH and medial CPD.

3. FMRF-amide

- appears to be ubiquitous in the brain, with short, scattered, beaded tracts in most brain regions, including the telencephalon, NPO, NPP, NLT, and to regions of the hindbrain.
- ir-cell bodies found in the nucleus olfactoryretinalis (NOR) and in the NLT.
- the orientation of ir-tracts appears to indicate that neuronal fibers extend in an arc from the pre-optic areas (NOR, NPO, NPP), to the pituitary regions of the brain (NLT).

- in the pituitary gland, ir-material was seen in the NH and in the CPD. This immunoreactivity appeared to be localized around pituitary cells.

4. Neurotensin

- ir-cell bodies were seen in the lateral sections of the NLT.
- beaded tracts were found in the NLT and NPP but their orientation is not yet clear.
- in the pituitary gland, ir-material was seen in the NH

5. Neuropeptide Y

- ir-cell bodies were found in the NOR of mature females, but not of males, as of now. The processes of these cells appear to extend in an upward arc towards the pituitary region.
- in males and females, ir-material was seen in the NPO, NLT, ventral tegmentum (VT) and the hindbrain. With the exception of the VT, material was seen as scattered beaded tracts. In the VT, two - three ir-cell bodies were found in addition to the tracts.
- in the pituitary gland, ir-material was found in the NH, CPD and the PI. In the CPD and PI, the immunoreactivity was within the cytoplasm of pituitary cells.

6. Dynorphin

- to date, we have seen ir-dynorphin only within cells of the PI.

B. Data From STS-89 & 90

Analysis of data STS-89 and STS-90 support the hypothesis that microgravity will alter, albeit very subtly, the functioning of the neuroendocrine system. The neonate swordtails that we have examined to date have shown altered patterns of spatial distribution of two of the neuropeptides that we studied (FMRF-amide and galanin). Observations are presented in tabular form (below) for purposes of brevity.

Flight Module Animals

The distribution of NPY, NT, AR, and DYN was essentially identical in the STS animals and the controls. However, we found subtle changes in the distribution of ir-FMRF-amide and profound changes in the distribution of GAL. The distribution of FMRF-amide in the brains of the flight animals appeared sparse when compared to the laboratory module controls. That is, there were less than the normal number of tracts containing ir-FMRF-amide in the flight animals.

GAL, which has never been localized in the NOR of any laboratory or ground experiment animal, appeared intensely immunoreactive in this nucleus in all flight animals.

Postflight Surviving Animals

Neonatal animals that were part on the STS-89 mission were returned to the laboratory. Two females that flew in the shuttle and 5 ground control animals, fish that were 7 days old at the time of the flight, did very well in our aquatic center. They were monitored for growth from the time of their arrival by determining their weight and standard length biweekly. There was no significant difference in growth rate and total length reached when flight animals and lab module animals were compared. Additionally, both groups displayed similar ages at which they reached sexual maturity and when they delivered their first of several broods. One flight animal died on 9/15/00, the other flight fish died on 2/25/01. Thus both lived to what we consider to be the average life span for this species. It is interesting to note that as of today 8/01, none of the lab module fish have died. We have no explanation for this observation.

SUMMARY TABLE (STS-89, STS-90)

	FLIGHT MODULE	LAB MODULE
GALANIN	ir-GAL in NOR increased ir-tracts pituitary; no difference	absent no difference pituitary; no difference
FMRF-amide	less ir-tracts pituitary; no difference	normal (more than flight) pituitary; no difference
NPY		No difference
DYNORPHIN		No difference
NEURONTENSIN		No difference
ANDROGEN RECEPTORS		No difference
GROWTH		No difference
AGE AT MATURITY		No difference
TIME OF FIRST BROODS		No difference
TIME OF DEATH	died before lab module animals	

IV. Publications, Presentations, and Other Accomplishments:

- Bottalico, A.G., Magliulo-Cepriano, L., and Schreibman, M.P. :Evidence of an androgen specific steroid receptor in the brain of a fish (Genus *Xiphophorus*) (Abstract). Soc. Neurosci., 24:1383 (1998).
- Flynn, K.M., Miller, S.A., Schreibman, M.P. Effects of MK-801 on puberty in the platyfish (Abstract).@ Soc. Neurosci., 23: 2055 (1997).
- Flynn, K.M., Miller, S.A., Schreibman, M.P. Sexually dimorphic effects of NMDA inhibition on brain-pituitary-gonad (BPG) axis histology in the platyfish (Abstract).Soc. Neurosci., 24:1383 (1998).
- Flynn, K.M., Schreibman, M.P., and Magliulo-Cepriano, L. Developmental changes in NMDA receptor expression the platyfish brain. Brain Res., 771, 142-146 (1997).
- Magliulo-Cepriano, L., Schreibman, M.P., and Bluem, V. Brain-pituitary axis development in the CEBAS minimodule (Abstract) Soc. Neuroscience, 23:2050 (1997).
- Magliulo-Cepriano, L., Schreibman, M.P., and Bluem, V. Investigations into the development of the brain-pituitary axis in hypogravity (Abstract). Amer. Zool., 37:156 (1998).
- Schreibman, M.P., Magliulo-Cepriano, L., Bluem, V., and Paris, F. Preliminary report of brain-pituitary axis development in the CEBAS-minimodule: shuttle flights STS-89 and STS-90 (Abstract). Gravitational and Space Biol. Bull., 12:53 (1998).
- Paris, F., Bluem, V., Schreibman, M.P., and Magliulo-Cepriano, L. Localization of GnRH in the brain and pituitary of swordtails flown in the CEBAS minimodule. I. Appl. Physiol.(2000)
- Schreibman, M.P., Magliulo-Cepriano, L., Bluem, V. Localization of neuropeptides in the

brain and pituitary gland of space-flown swordtails. Submitted to Appl. Physiol. (2001).


Additional Note:

The interest of the general public in our experiments and in the space program, in general, was, and is, incredible and beyond our expectations. Our participation in the two shuttle missions was given thorough coverage on the first page of the Metro section of the New York Times and in several other New York newspapers and magazines. We were interviewed on several radio talk programs and featured on the Discovery Channel. Many presentations on the CEBAS program have been made by the investigators at universities, high and junior high schools and at social, political and religious organizations, frequently with the title, "Fish For Launch".

There have been no inventions.

Final property value is over \$5,000.

Submitted by,

A handwritten signature in black ink, appearing to read "Martin P. Schreibman". The signature is written in a cursive style with a long horizontal flourish at the end.

Martin P. Schreibman