ABSTRACT

Organisms are composed of organs and tissues that were formed and connected during development according to genetic programs and environmental factors. During the last two decades, molecular and genetic aspects have pushed integrated (system) approaches into the background. However, central integration between all structures and compartments of a body is mandatory for the functional stability of the organism. Thus, the general requirements for modern life science research are (1) to describe facts related to the whole body, and (2) to analyse underlying molecular and genetic mechanisms in a combined manner. Space Life Science Research offers the unique chance to keep thinking about Integrated Physiology alive due to the driving force of making spaceflight safe for men, and to develop not only physical but also pharmacological countermeasures in case of microgravity-induced disturbances of the integrated system, i.e., the organism. Thus, integrated physiology will benefit from molecular and genetic approaches, and vice versa.

INTRODUCTION

Organisms are composed of organs and tissues that were formed and connected during development to a unity according to genetic programs and environmental factors. Functional integration between all structures and compartments is mandatory for the functional stability of this unity. Integrated (system) physiology deals with this matter. In mathematics, integration is a method to calculate areas or volumes between defined margins by means of the addition of small elements. In Life Science, integration includes complex processes. Linear integration is rare; non-linear integration dominates. Cross-talking between elements modifies the total response. In sensory systems, two stimuli elicit a response that is - even from the statistical point of view - unequal to the sum of the responses that each stimulus induces. A classical example is lateral inhibition that was first detected in the visual system. Starting at the beginning of the 19th century, physiology became the dominating discipline in life science research. Technical developments favour physiological research and pushed anatomy aside. The fate of anatomy is now that of physiology.

THE EXPLOSION OF MOLECULAR BIOLOGY AND GENETICS

A search using PUBMED as databank revealed that the number of publications in system and integrated physiology is still increasing and even higher compared to most other disciplines. But after the 90-ties, the relative increase in publication frequency was surpassed by the modern life science disciplines such as genetics, molecular biology or biotechnology. The explosion of publication numbers makes clear that these so-called modern techniques will surpass integrated physiology also in absolute numbers in the near future. Genetics has already reached larger absolute numbers in the middle of the 80-ties (Figure 1).

One of the most prominent aspects in integrated physiology is nervous function and behaviour. Studies in these areas are still expressing the highest publication numbers, but also in these cases the relative increase became considerably lower since the early 90-ties compared to aspects of molecular biology and genetics such as gene expression, gene down regulation, cell cultures, etc. These facts have significant impact on the future of integrated physiology, and scientists have to be aware of the extreme competition and their own weak standing if they do not change their strategy in research.

Behavioural Physiology is a classical discipline in integrated physiology. It deals with the analysis of processes within the body only by visual or automated monitoring of animals that were either unaffected or manipulated by treatments such as modifications of the sensory input, sensory or motor deprivation, pharmacological treatments or lesions. In human research, the equivalent of this technique is Psychophysics. Behavioural physiology has a great history; its importance became obvious when three leading scientists in this field were awarded with the Nobel Prize in 1973: Karl von Frisch (Germany), Konrad Lorenz (Austria), and Nikolaas Tinbergen (The Netherlands). The importance of behavioural approaches is still obvious as demonstrated in the extreme large number of publications that exceeds each other technique (Figure 1, bottom plots). Although of basic importance and copied by thousands of scientists studies by means of behavioural analyses create mainly hypotheses; this approach could never find basic proofs of how behaviour is organized by physiological, genetic and molecular mechanisms. Modern molecular and genetic techniques such as early gene expression or down-regulation of genes present now the necessary tools to test hypotheses developed from classical systemic approaches, in particular with respect to development and adaptation.
Figure 1. Absolute number of publications (left) and relative number of publications with respect to 1990/1991 (right) for different fields of life science research, as well as for typical specific topics and methods for these fields. In this search, key words from the life science area were used. Counting was based on a PUBMED search between the years 1942 and 2005 with a resolution of 2 years lasting intervals. Note the significant higher relative increase of publications in molecular/genetic areas compared to physiology.

Early gene expression was originally used to study modifications within the brain during pathophysiological behaviour such as epileptic attacks in animal models (Popovici et al., 1990). But at that time, also intact animals were studied. One of these studies dealt with gene expression during the exploration behaviour of chicken in enriched visual environments. The results revealed a marked increase of c-fos expression in cerebellum and medial forebrain. Interestingly, the extent of this elevation was related to the duration of exploration: c-fos elevation occurred only if the 1-day or 2-day-old animals were allowed to experience the new environment for 30 min but not if they could explore it for 2 days (Anokhin et al., 1991). This study pointed to the importance of “novelty” during exploration of a new environment, and to loss of novelty significance during long-term exploration. These facts were already known to behavioural physiologists, but due to the molecular techniques they could now identify sites within the brain that were involved in the underlying adaptation causing the loss of novelty importance.

The introduction of knock-down techniques was a further major step that pushed molecular and genetic techniques in life science research. It became clear that “transcription of genes can be controlled by regulatory proteins that bind to sites on the DNA either nearby or at a considerable distance” (cited from Ptashne, 1986). Consequently, down-regulation of specific genes and rescue of affected systems by application of the respective RNA became a standard approach. Studies about the genetic basis of the early motor development in zebrafish are typical for this
experimental strategy. By means of knock-out techniques identification of specific genes responsible for the development of first motor response, the spontaneous coiling, and for the first inducible motor response, the induced escape was possible (Figure 2) (Cui et al., 2005). Interestingly, this study included the description of behavioural responses, i.e., a classic systemic approach.

THE COOPERATION BETWEEN PHYSIOLOGICAL AND MOLECULAR/GENETIC APPROACHES

The influence of modern molecular and genetic approaches is also becoming obvious in Space Life Science research. The incorporation of these aspects reveals the same dynamics in citation frequency as in ground based research albeit on a numerical lower level. The most dynamic development is seen in gene expression albeit the number of publications in space related physiology is still significantly higher than in those in genetics and molecular biology (Figure 3). For neurological and neurobiological problems, gene expression offers the unique chance to define sites in the nervous system that are affected by the weightlessness. However, the fundamental problem in space related studies is the low number of experiments and the diversity of animal species used for the experiments. In most cases, hypotheses about basic mechanisms of physiological adaptation to the space environment that include correlations between observations in integrated physiology on one side and molecular and genetic analyses on the other side have to rely on the comparative approach. It uses observations obtained from different species. The comparison between observations about gene expression in the vestibular nuclei of rats and both behavioural studies in fish and toads on the roll-induced vestibuloocular reflex (Sebastian et al., 2001; Horn, 2006) and psychophysical studies on postural sensation by astronauts (Clément et al. 2001) clearly demonstrate how physiology and molecular approaches and genetics benefit from the comparative approach to understand physiological mechanisms.

**Figure 2.** Molecular basis of motor development in zebrafish. - Left: Alternating slow contractions of the trunk (spontaneous coiling) is the earliest motor activity. In shocked (sho) gene embryos the frequency of coils/min is depressed with respect to the wild type (wt). The defect is caused by a mutation in the slc6a9 gene that encodes for the glycine transporter GlyT1. Function of the glycine transporter GlyT1 is required for normal spontaneous coiling behaviour because knock-down of GlyT1 function by E212 MO severely reduced the frequency of spontaneous coiling; injection of wild-type GlyT1 mRNA but not the mutant G81D mRNA rescues sho embryos. - Right: Mechanosensory stimulation of 24-28 hpf wild-type embryos elicits vigorous alternating contractions of the trunk (escape response). A sho embryo failed to respond to tactile stimulation. Knock-down of GlyT1 in the wild type phenocopied sho. Restoration of GlyT1 activity in sho embryos by means of wild-type mRNA rescued the mutant (modified from Cui et al., 2005).
It was shown that during a 16 days lasting space flight, a tonic depression of the number of Fos- and FRA-labelled cells developed in efferent (Balaban et al., 2002) (Figure 4A) but not in afferent vestibular nuclei (Pompeiano et al., 2002). It is likely that the depressed efferent influence causes an increased flow of activity from the peripheral vestibular structures indicating a sensitization of the vestibular system during exposure to microgravity. In fact, after a couple of days in microgravity, (1) the roll-induced vestibuloocular reflex in fish (Sebastian et al., 2001) (Fig. 4B) and toads (Horn 2006) was augmented for some developmental stages, and (2) the sensation of tilt by astronauts during artificial gravity stimulation was more pronounced for two days after a spaceflight compared to pre-flight observations (Clément et al., 2001) (Figure 4C). Although a number of physiological and morphological data such as increased activity of the vestibular nerve or increased number of synapses also correspond with a sensitization (for summary, cf. Horn, 2007), the observation of depressed efferent activity by molecular biological techniques can be considered as a step forward in understanding physiological adaptation in microgravity.

Much more convincing is to study aspects of physiological adaptation in one species only. However, this is so far rarely done. The studies in the early development of swimming in zebrafish are conclusive and indicate clearly the site of genes responsible for the early steps in motor activity; they can be considered as the best approach to be transferred to Space Life Science research. An attempt to bring molecular techniques and space flight experiments together has recently started. The aim is to analyse the fundamentals of microgravity induced modifications of the developing vestibular system in *Xenopus laevis*. First studies revealed that tadpoles in which the transcription factor XTcf of the wnt-pathway was knocked down by a morpholino injection in one of the two cells of the 2-cell stage embryo revealed similarities in their phenotype with those tadpoles that were exposed to microgravity; both treatment caused a significant depression of the rVOR (Figure 5) and similar abnormalities of swimming such as looping or spiralling (Horn et al., 2005).

**THE UNIQUE CHANCE FOR RESEARCH FOR A SAFE LIFE IN THE UNIVERSE**

If man plans to explore the universe and to send men on long-term missions, the focus exclusively on molecular biology and genetics as well as on radiation biology to study risks of this travel is a dangerous way. It neglects the fact that the stability of a body is maintained by integrative mechanisms within the body. In zebrafish, different organs and cell types show periods of maximum susceptibility to microgravity during developmental periods that coincide with specific developmental events (Shimada et al., 2005); however, in the adult fish they reveal stable coordination. Flight rats in LD conditions evidenced a pronounced phase delay in body temperature but not heart rate compared to controls (Fuller, 2006). The function of the vestibular system depends on the circulatory system, on feedback information from the motor system, on convergent processing of information originating in different sensory systems, on the endocrine system, etc. Recent research has demonstrated that the
Figure 4. Long-term modification of Fos- and FRA-labelled efferent vestibular neurons in rats during microgravity exposure (Neurolab mission 1998) that might be a basis of vestibular sensitization observed in behavioural experiments with fish (Oreochromis mossambicus; STS-84, 1997) and psychophysical experiments (astronauts from Neurolab, STS-90, 1998). - A. Differences \( \Delta \) in the number of labelled efferent vestibular neurons from flight and paired ground controls determined on flight days 2 and 14 (FD2 and FD14, respectively) and on the 1st and 13th day after landing (R+1 and R+13, respectively), and the histological demonstration for each one animal from the flight and ground group that shows the location of analysis. EVe, efferent vestibular nucleus; g7, genu of the facial nerve; mlf, medial longitudinal fasciculus; 6, abducens nucleus (modified from Balaban et al., 2002). - B. Augmentation of the roll-induced vestibuloocular reflex after a 9.5 days lasting exposure to microgravity (\( \mu g \)). Fish were rolled in 15°-steps around their longitudinal axis. The inset shows the eye posture during horizontal posture (left) and during a 90° lateral roll. n, number of fish (modified from Sebastian et al., 2001). - C. Increase of postural sensitization after a space flight. Astronauts had estimated their postural tilt during 1g-centrifugation before and after the space flight. The horizontal line defines the theoretical estimation. Astronauts underestimated the tilt before flight but overestimated it after landing for two days. Arrows indicate the effective forces during centrifugation (modified from Clément et al., 2001).

Can all this be analysed by the study of genes relevant for the circadian or vestibular system, exclusively? I have my doubts! Certainly, molecular biology and genetics will significantly push forward the development of effective countermeasures in case of pathological or stressing conditions. However, central integration between all structures and compartments of a body is mandatory for the functional stability of the organism. Thus, the general requirements for modern life science research are (1) to describe facts related to the whole body, and (2) to analyse underlying molecular and genetic mechanisms in a combined manner. Animal and human studies benefit from the co-operation between Integrated Physiology and Molecular/Genetic Sciences; scientific programs have to push forward cooperation instead of increasing competition.

CONCLUSION

Space Life Science Research deals with the matter to bring men in Space, let him live there and protect him against stressing conditions. They may be caused by radiation, isolation or other physiological stressors that might arise because of diverging modifications of physiological adaptation. Superimposed differences in the circadian rhythms will complicate adaptation to microgravity. Fundamental requirements for modern Life Science research are therefore (1) to describe facts related to the whole body, and (2) to analyse underlying molecular and genetic mechanisms in a combined manner. Space Life Science research, in particular, offers the unique chance to combine Gravitational Physiology and Molecular/Genetic Sciences as the driving force to make spaceflight safe for men, and to develop not only physical but also pharmacological countermeasures against microgravity-induced disturbances of the integrated organism. The holistic approach including molecular analyses and systems interplay is mandatory.
Figure 5. Depression of the roll induced vestibuloocular reflex in Xenopus laevis after a 10 days lasting microgravity exposure (German D2 Spacelab mission, 1993) (left) and by unilateral knock-down of the transcription factor XTcf of the wnt-pathway (right). Numbers below treatment indicate numbers of tadpoles. - Left. Comparison between the rVOR amplitude obtained from tadpoles exposed to microgravity (F0g), to 1g-centrifugation in orbit (F1g), and to Earth gravity (G1g). The rVOR amplitude is the maximal extent of eye movement during a complete 360° lateral roll. Each dot and circle indicates a tadpole (modified from Sebastian et al., 1996). - Right. The transcription factor XTcf was knocked down by a morpholino injection in one of the cells of the 2-cell stage embryo. Non, untreated embryos; control, injection of a non effective control morpholino; XTcf, injection of the transcription factor XTcf; ipsi and contra, eye on the injected side and opposite side, respectively; each tadpole is presented by a dot (contralateral eye) and a circle (ipsilateral eye). Experiments by the author in collaboration with D. Gradl, University of Karlsruhe, Karlsruhe, Germany, and N.A. El-Yamany, Helwan University, Helwan, Egypt.

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