Werner M. Graf: **Research Interests**

How do we move? - An interdisciplinary approach to study movement execution and perception

Movements constitute and integral part of our daily life, either for locomotor activities, orientation in physical space, avoidance of obstacles or moving objects. At the same time, initiation, cessation, as well as perception and detection of these movements, whether actively created, or passively experienced, are important components of the generation of our overall behavioral repertoire. Unequivocal interpretation of self-motion by the nervous system requires converging multisensory information that takes into account rotational and translational displacements of eyes, head and body in three-dimensional space. It also necessitates a comparison of congruent and conflicting input originating from different sensors. Basic reflex functions, such as compensatory eye movements following head- or body movements, for instance, largely rely on complementary vestibular, visual and neck-proprioceptive inputs. For higher cortical processing, as well, a single sensory quality by itself can no longer be of perceptual importance, since every displacement of the head in space will stimulate labyrinthine receptors detecting either rotational (semicircular canals) or linear (otoliths) accelerations in addition to the visual, auditory and somatosensory inputs. Furthermore, multisensory input can be used to clarify ambivalent monosensory information.

Dr. Graf's scientific activities are focused on the perception of movement, and the sensory-motor transformations in context of differentiation of active versus passive movements, as well as motor learning and developmental aspects. A crucial sensory system for the detection of movement, whose existence we are unaware of under normal circumstances, is our sense of balance, i.e., the labyrinth, or the vestibular system. We become acutely aware of this sense only, when it malfunctions, resulting e.g. in vertigo, motion sickness (sea sickness, space motion sickness in astronauts, etc.). The differentiation between active and passive movements is not trivial, and considerable research effort has been devoted to it since the problem was formulated by von Holst and Mittelstaedt in the 1950s. However, groundbreaking experiments have not been conducted until only a few years ago using comparisons about neuronal discharge in actively versus passively moving animals.

The overall goal of specific experiments planned for the next 10-15 years is to describe the neuronal structure-function relationship of spatially and temporally coordinated eye-, head- and body movements in context of the perception of self-motion, including some specific developmental and motor learning aspects.

Methods and Models:

In order to achieve these goals, a wide range of neurobiological approaches are being employed, often in combination, in Dr. Graf's laboratories and in collaboration with other investigators. These approaches include:

- <u>neuroethology:</u> analysis of behavior in normal and lesioned animals in conjunction with video-, film-, x-ray cinematography and three-dimensional movement analysis.
- <u>neurophysioloy:</u> electrical central and peripheral stimulation, natural visual and vestibular stimulation, field potential analysis, single cell extra-and intracellular recordings, eye movement registration with the magnetic search coil, quantitative EEG and intracranial recording and stimulation in humans.
- <u>neuroanatomy:</u> extra- and intracellular horseradish peroxidase application, 2-deoxyglucose and thymidine autoradiography, immunocytochemistry, transneuronal labeling with a virus tracer.
- <u>neuro-imaging:</u> i.e., fMRI.
- <u>molecular biology:</u> PCR, gene screening.
- <u>modeling</u> of the functional properties of sensory-motor systems (matrix analysis, tensor analysis).

We are using non-human primates as experimental animals because of the training aspects and the similarity with human neuroanatomy. Furthermore, comparison with human brain imaging data can now be established, complementing electrophysiological and neuroanatomical findings obtained in experimental primates. The flatfish model will allow us to determine specific genes, environmental and hormonal factors, and the cell lineages involved in embryonic plasticity, and particular learning mechanisms.

Research Projects:

Three major axes of research are envisioned that use a multidisciplinary approach to address the various problems of movement perception:

<u>1) Neuronal Correlates of Movement Perception</u>
Neuronal correlates of movement detection and perception of visual and somatosensory qualities have been demonstrated in many areas of the so-called "dorsal visual stream areas" (MT, MST, LIP, VIP, etc.). The role and importance of vestibular signals for these functions have only recently been shown in recordings from VIP (ventral intraparietal area) conducted in Dr. Graf's laboratory. Area VIP of macaque monkeys is located in the fundus of the intraparietal sulcus. It bridges the gap between the "dorsal visual processing stream" (e.g. LIP, lateral intraparietal area) and the somatosensory and premotor system (MIP). Area VIP is thus considered to play an important role in the analysis of self-motion and multimodal representation of the three-dimensional movement space. Our recordings revealed directionally selective responses for vestibular stimulation, and to visual as well as tactile stimulation.

The experiments planned for the near-future will investigate the role of vestibular signals during active versus passive semi-constrained head movements (recordings in MT/MST, VIP, and the vestibular cortices: 2v and PIVC). Since cortial neurons are presumed to be involved in spatial orientation and perception of movement, we find a wide array of responses, even a change of directional selectivity. Our working hypothesis is that intraparietal sulcus neurons are involved in the decision making process of differentiating between active and passive movements. By contrast, reflex movement-related vestibular activity in brain stem premotor circuits are largely *suppressed* during active movements (Current Research Project: Neuronal correlates of movement perception using non-human primates and behavioral tasks in tandem with recordings in the parietal cortex).

Long-term plans in that regard involve the development of a paradigm, which we have termed "the running monkey paradigm". This paradigm will allow to provide information about sensory activity during actual active locomotion of bipedally walking non-human primates. Thus, we will have an animal model at hand for direct comparison with human physiology and eventually pathophysiology, opening completely new avenues for basic research, therapy and patient rehabilitation. For instance, vestibular stimulation can alleviate the neurological syndrome of spatial hemineglect. This approach will open a direct link to applied research.

The experiments in monkeys will be compared with data from human subjects. To that end, the active-passive movement differentiation will be explored by fMRI. Furthermore, correlates for perceptive functions in humans will be obtained using quantitative EMG, and depth electrode placements (available in pre-surgery epilepsy candidates). Current investigations involving human subjects target prevention of falls and improvement of car driving skills in the elderly (using a wide range of psychophysical, clinical and simulator approaches with normal elderly and patients).

2) Neuronal Circuits Underlying Perceptive and Motor Functions

We have begun to visualize entire context-related neuronal circuits with the retrograde transneuronal tracing using rabies virus. In experiments in guinea pigs and primates we were able to show the entire neuronal circuitry involved in horizontal eye movement control. The results signal an ever increasing complexity of afferent pathways and control circuits converging onto the different layers of the investigated networks and onto the final motoneuronal pathway. In addition to the well-known motor pathways, circuitry involved in navigation (hippocampus), movement perception (cortical areas), motivation, etc., were revealed as well. Recent intracortical injections of the tracer revealed the afferent pathways to VIP/MIP and MT/MST regions (Current Research Project: Neuronal circuits underlying perceptive and motor functions using non-human primates and the technology of transneuronal tracing with rabies virus to map specific neuronal pathways).The full potential of this powerful method to determine entire functional neuronal networks involved in specific behaviors will be accomplished by perfecting central injection techniques, and the development of intracellular injections. Applying this technology to neuronal development will certainly lead to new insights in that dynamic field of research. In future applications, we are envisioning to employ this technique to demonstrate the missing and/or altered circuits and neuronal elements in genetically modified animals, such as *knock-out* mutants, in order to clarify the pathophysiology of related syndromes and behaviors.

3) Adaptation and Learning

In this context, we make use of a particular animal model offered by Nature: the flatfish. Flatfish are a natural paradigm for studying adaptive changes of compensatory eye movements (the vestibulo-ocular reflex, VOR). During metamorphosis, flatfish tilt 90 degrees to one side or the other to become bottom-adapted adult animals. In this position, the labyrinths are rotated 90 degrees relative to their premetamorphic orientation in space. Structurally, this arrangement requires a neuronal pathway from the horizontal semicircular canals to muscles that move the eye vertically. The morphological substrate subserving adaptation of the VOR in post-metamorphic (adult) flatfish was obtained with a number of morphological and electrophysiological methods. This system allows the study of a closely defined developmental plasticity process. Single cell morphology of vestibular neurons revealed a unique innervation pattern linking horizontal vestibular neurons to vertical eye muscles. We have furthermore discovered an interesting asymmetry in vestibular nuclei innervation in these animals based on swimming behavior after hemilabyrinthectomy. In future experiments of the ontogenetic development of eye movement circuits in flatfish, we will determine the cell lineages of the development of this particular vestibulo-oculomotor connectivity using immunohistochemistry (GABA, glycine), in situ hybridization (GAP-43), thymidine autoradiography and molecular biology methodology (PCR cloning). Furthermore, the ontogenetic development of specific behaviors, e.g., eye movements, will be determined. The flatfish is an unprecedented model in vertebrate hierarchy to understand mechanisms of adaptation to a changing life situation.

Overall, the combined results of these research projects will provide new insights into the perceptive and motor production aspects of self-movement, as well as in the inherent adaptive potential. The employment of ambitious and complex paradigms will require considerable short-term investment, but yield invaluable long-term results for both basic research as well as for practical application in everyday life and patient rehabilitation. For instance, deficits in motion perception account for a high percentage of accidents in the elderly, either in vehicle crashes, falls in the house, or other living situations. The laboratory is currently involved in developing training programs to alleviate these problems in the elderly (Current Research Project: Prevention of falls and improvement of car driving skills in the elderly using a wide range of psychophysical, clinical and simulator approaches with normal elderly and patients), while exploring in parallel in the non-human primate model the possible pathophysiological mechanisms and potential rehabilitation avenues with neurophysiological and neuropharmacological methods.