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FINAL TECHNICAL REPORT

BASIC GRAVITATIONAL REFLEXES IN THE LARVAL FROG

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Description of Research

Little is known about how vertebrates are able to sense gravity and how they process this information to generate appropriate motor responses. This investigation was designed to determine how a primitive vertebrate, the bullfrog tadpole, is able to sense and process gravitational stimuli. Because of the phylogenetic similarities of the vestibular systems in all vertebrates, the understanding of the gravitational reflexes in this relatively simple vertebrate should elucidate a skeletal framework on a elementary level, upon which the more elaborate reflexes of higher vertebrates may be constructed.

The tadpole manifests a powerful counter-rolling response of the eyes to static tilt of the head. In addition, the fact that amphibians are cold blooded means that their cellular metabolism is much lower than that of mammals. Consequently, the entire head can be maintained *in vitro* with the brain, sensory structures, and eye muscles exposed. The strong gravitational reflexes are still evident under these conditions and persist for several days. Such conditions permit detailed and reproducible electrophysiological and anatomical investigations of this reflexive behavior.

The purpose of this study was to understand how the nervous system of the larval amphibian processes gravitational information. This study involved predominantly electrophysiological investigations of the isolated, alert (forebrain removed) bullfrog tadpole head. The focus of these experiments is threefold: 1) to understand from whole extraocular nerve recordings the signals sent to the eye following static gravitational tilt of the head; 2) to localize neuronal centers responsible for generating these signals through reversible pharmacological ablation of these centers; 3) to record intracellularly from neurons within these centers in order to determine the single neuron's role in the overall processing of the center. This study has provided information on the mechanisms by which a primitive vertebrate processes gravitational reflexes.

Accomplishments

Quantification of the behavior. A software event detection technique, which has been developed over the last fifteen years, has been finalized and published. This technique allows for analysis of spontaneously-occurring voltage deflections over time. Electrophysiological recordings digitized (at 50 KHz) over many minutes can be rapidly and automatically analyzed. The publication illustrates this technique for spontaneously occurring synaptic potentials recorded intracellularly from VIIIth nerve afferents that are consequent from convergent hair cell innervation of the afferents. This technique allows for tens of thousands of EPSPs to be analyzed during this period. With respect to the experiments in this study that involve whole nerve recordings from the trochlear and medial rectus nerves, this same technique can be applied to detect and quantify the extracellularly recorded nerve action potentials. Since these whole nerve recordings are more stable than the intracellular recordings of EPSPs, digitized periods are often over an hour in duration, allowing for continuous quantitation of hundreds of thousands of spike potentials that occur during the recording period. Since the amplitude (as well as other parameters) of each spike potential is quantified, whole nerve recorded spike potentials can be parceled according to size, and their size can then be related to the time of occurrence during a tilt stimulus.

Responses to static tilt of the head. Trochlear motoneurons increase their frequency of firing with nose up tilts and decrease it with nose down tilts. Motoneurons innervating the medial rectus muscle respond reciprocally--i.e. they increase their activity with nose down tilts and decrease their activity with nose up tilts. For both the trochlear and medial rectus motoneurons, the larger sized units are phasic and adapt more rapidly than the smaller sized units, which appear to have a larger tonic component and which adapt less rapidly. Typically larger units fire only during a movement (relating them more to velocity), while smaller units appear to be modulated both by the table movement as well as by the absolute position of the table (relating them both to table velocity and position). During the period of this grant, the principal investigator constructed a servo-controlled tilting system for his electrophysiological setup. This system allowed for precisely controlled movements of the table of a range of ± 10 degrees and at velocities between 0.023 and 2 degrees per second. Clearly compensatory trochlear responses were seen even at the lowest velocities and involved principally the smallest amplitude units, suggested a tilt position signal is communicated from the inner ear (probably the utricle) through the central nervous system to the trochlear motoneurons.

Pharmacological studies. Iontophoresis of the kynurenic acid, an antagonist of the excitatory transmitter glutamate, into the extraocular motor nuclei reversibly abolishes spike activity in the extraocular motor nerves. Intracellular recordings from oculomotor neurons show complex mixtures of EPSPs and IPSPs following electrical stimulation of the vestibular nerves and afferent brainstem pathways to this nucleus. Bath application of kynurenic acid reversibly blocks over 95 % of the EPSPs. Both these findings are consistent with the hypothesis that the principal excitatory transmitter to the oculomotor nucleus is glutamate or a related compound.

Preliminary investigations in the turtle. The advantage of investigating static gravitation responses in the tadpole is that the head can be maintained viably in vitro for a number of hours after decapitation. The disadvantage is that tadpoles and frogs do not possess both types of hair cells found in mammals, but only have type II hair cells. Consequently, interpretations of results from tadpoles cannot easily be extended to mammals and birds, because of this increased complexity. Homeothermic organisms such as birds and mammals have higher metabolic demands than poikilothermic vertebrates such as frogs, and consequently isolation of relatively intact portions of the nervous system or inner ear do not survive in vitro. Turtles, however, have both types of hair cells as is found in mammals and birds and also are poikilothermic, so portions of the inner ear and nervous system (and possibly the whole head) can be maintained viably in vitro. It is thus of interest to compare the tilt sensitivity of vestibular and extraocular motoneurons in the turtle to that in the tadpole to attempt to discover the additional role(s) type I hair cells have in producing compensatory behavior to static tilt. Preliminary investigations at the end of this grant period have focused upon investigations of the inner ear of the turtle and have attempted to compare the basic aspects of inner ear organization in the turtle with that known in the frog and tadpole. These investigations indicate that the turtle inner ear can be maintained in vitro with a similar viability as is found in the frog, and that the hair cell transmitter is likely to be the same in both these animals. In addition, ultrastructural investigation of the types of synaptic contact found between hair cells, afferents, and efferents is quite similar to that found in the other amniotes (i.e. birds and mammals). This research has promising directions with respect to understanding the comparative aspects of the static vestibular reflexes.

Significance of the Accomplishments

In general, the above accomplishments provide a step toward the understanding of how these primitive vertebrates sense gravitational stimuli and how they process these stimuli and integrate them into reflex control.

The development of the quantification technique is an important step in the ability to investigate how gravity can influence eye movements. Moreover, this technique is generalizable to any spontaneously occurring events that can be represented as voltage deflections over time, indicating that it has a potential use other than its strict application in this study.

The similarity of the responses from the trochlear versus medial rectus motoneurons (although their receptive field properties are different) suggests that different inner ear endorgan signals converge upon these motoneurons, but that the quality of the processing of these signals is similar. The contribution of individual endorgans and specific vestibular nuclear complex neurons in channeling the static and dynamic signals remains to be determined.

The finding that glutamate is the main excitatory transmitter to the oculomotor nucleus indicates that glutamate is the principal excitatory transmitter of the entire vestibulo-ocular reflex. Glutamate (or a related compound) is the hair cell transmitter, it is the transmitter of VIIIth nerve afferents, it is the transmitter of excitatory commissural vestibular neurons, and it is the transmitter of vestibulo-oculomotor neurons.

These findings provide basic information as to the organization of gravitationo-ocular reflexes in a relatively simple lower vertebrate. These studies can provide a basic framework for understanding how these reflexes are organized and elaborated upon in the more complex vestibulo-ocular behaviors exhibited by higher vertebrates.

The preliminary investigations in the turtle are a new promising route towards understanding the different contributions of type I versus type II hair cells in generating compensatory reflex behavior, and should they continue, would suggest how the neuronal organization responsible for mediating this behavior is tailored to suit the individual animals and their repertoire of behaviors.

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