Sensorimotor integration underlying prey-catching behavior of the frog

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Ph.D. thesis

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1. Introduction

The organization and function of the central nervous system of amphibians achieved a high developmental level during the evolution. Frogs represent a prominent group of amphibians because in the point of behaviour they adapt highly to their lifestyle. Several morphological and physiological information are available in literature related to their unique behavioural reactions but we still have to answer numerous questions to understand these complex systems. The maintence of equilibrium and the proper timing of extremly rapid, goal-directed movements have a remarkable importance during prey-catching and feeding behavior of the frogs coordinated by a complex sensorimotor system. Cerebellum, which has several afferent and efferent connections, plays a central role in this intricate system. The cerebellum is divided into three main parts in frog. The middle area is the corpus cerebelli; the two lateral parts are called as lobus auricularis, which function as the vestibulocerebellum. The reciprocal connection between the cerebellum and the vestibular system has a great importance in the maintence of equilibrium of the body during locomotion. Vestibular receptors, the three semicircular canals (anterior, posterior and horizontal) and the three otolith organs (saccule, utricule and lagena) are located in the special regions of the membranous labyrinth. The activity of the cerebellum is also influenced by the somatosensory inputs originating from the proprioceptors of the skin, muscles and joints, which are crucial in the coordination of the rapid, goal-directed movements. The pray-catching behaviour is evoked by a visual stimulus that is conveyed into the optic tectum by the optic nerve. According to the previous physiological experiments it is supposed that a stereotyped motor pattern is programmed into a complex neuronal network of central nervous system, called as central pattern generator (CPG). The CPG receives several direct and indirect sesory and motor inputs from different parts of the peripheral and central nervous system (optic tectum, cerebellum, vestibular nuclear complex, motor nuclei) that determine the pattern of behaviour. It is assumed that the information deriving from central pattern generator reach the motor nuclei through the last order premotor interneurons. The hypoglossal nucleus is one of the most important efferent components of the pray-catching behaviour innervating the protractor and retractor muscles of the tongue. The hypoglossal nucleus is divided into three subunits. The motoneurons of the dorsomedial subnucleus supply the protractor muscles (geniohyoid, genioglossus) and contribute to the innervation of the hyoglossal and intrinsic muscles of

the tongue. The ventrolateral subnucleus supplies the retractor muscles (sternohyoid) and plays a role in the innervation of the geniohyoid and intrinsic muscles. The intermedier subnucleus innervates the omohyoid, the geniohyoid and intrinsic tongue muscles. It has been suggested that the cerebellum, the brainstem and their afferent and efferent connections have a great importance during the coordination of extremly rapid, goal-directed movements underlying prey-catching and feeding behavior. In order to understand the function of this complex neuronal network it is necessary to be acquainted with the morphological background on the connections of individual elements.

2. Aims of this study

The aim of our study is to examine two of the most important components of the sensorimotor system underlying the control of prey-catching and feeding behavior in the frog.

2. 1. Organization of the dye-coupled cerebellar granule cells labeled from afferent vestibular and dorsal root fibers

In anurans, the spinal prorioceptive and vestibular afferent fibers reach the cerebellum directly and terminate as mossy fibers on the dendrites of granule cells. The integration of these primary afferent inputs plays an important role in the regulation of activity of the cerebellum and in the modulation of motor processes underlying prey-catching behavior. In our previous experiments, it has been demonstrated that the afferent vestibular and somatosensory inputs are transmitted through both chemical and electrical synapses to cerebellar granule cells. At the light microscopic level, the dye-coupling, which is a transneuronal transport of low-molecular weight tracer from one neuron to another, is considered as a sensitive indicator of the electrical transmission. In our earlier work, we have described an organotopical distribution of labyrinthine end-organs in the vestibular nuclei and in the other parts of the brainstem of the frog. **In the first part of our study we have focused on the dye-coupled cerebellar connections of the nerves of individual labyrinthine organs and of dorsal root fibers of limb-innervating segments of the spinal cord in order to examine a possible somatotopic organization.**

2. 2. Organization of last-order premotor interneurons related to the protraction and retraction of the tongue

Prey-catching behaviour of the frog is elicited by a moving visual stimulus. The majority of inputs is recived by the contralateral optic tectum and the tectal output is conveyed to the motor nuclei of the brainstem and spinal cord via the descending tectobulbar and tectospinal pathways. An important efferent component of the pray-catching behaviour is the hypoglossal nucleus responsible for the activation of various muscles that control the protraction and retraction of the tongue. Relatively little

information is available about neuronal structures, which are involved in the transmission of tectal output to hypoglossal motoneurons. The findings that direct tectal fiber terminals cannot be shown on hypoglossal motoneurons suggest the presence of intercalated last-order premotor interneurons. No morphological data are available about premotor interneurons of hypoglossal nucleus of lower vertebrates. Since the motoneurons of the hypoglossal nucleus display a musculotopic organization we can assume matching organization in the spatial distribution of their last-order premotor interneurons as well. **The aim of the second part of our study is to analyze the organization and morphology of the last-order premotor interneurons related to the protractor and retractor muscles of the tongue.**

3. Materials and methods

3. 1. Animals used in experiments

The experiments were performed on 97 common water frogs, *Rana esculenta*, in accordance with state regulations and with the approval of the University Animal Care Committee.

3. 2. Organization of the dye-coupled cerebellar granule cells labeled from afferent vestibular and dorsal root fibers

3. 2. 1. Dye-coupled neuronal tracing technique

Our previous experiments demonstrated that the primary afferent vestibular inputs are transmitted through chemical and electrical synapses (gap junctions) to their second order neurons in the cerebellum, which are the granule cells. Applying low-molecular-weight tracer, neurobiotin, to afferent vestibular and dorsal root fibers of the frog, neurobiotin was conveyed via axonal transport to their central terminals and through the gap junctions into the perikarya and dendrites of cerebellar granular cells. Consequently, this neuronal tracing technique is suitable to the labeling of primary afferent fibers, their terminals and the related secondary neurons. The specificity of dye-coupled labeling of cerebellar granule cells was tested in our previous work in which the glycyrrhetinic acid, the inhibitor of gap junctional coupling was injected into the cerebellum or applied directly to afferent fibers prior to the neurobiotin labeling. In all cases the dye-coupled neurons almost completely disappeared from the cerebellum.

3. 2. 2. Application of neurobiotin to the nerves of vestibular sense organs and the spinal dorsal root fibers

The frogs were deeply anesthetized with 0.1 % MS 222 (tricaine methane-sulfonate; Sigma).

In one group of animals (n = 55) the individual branches of the vestibular nerve were exposed from a ventral approach following incision of mucosa lining the roof of oral cavity and the opening of the bony otic capsule. Individual branches of the vestibular nerve were

cut near their receptors and placed on a small piece of parafilm. Neurobiotin crystals (Vector) were applied to the proximal stump of the nerve and covered with a mixture of silicon oil and grease in order to prevent the leakage of the tracer.

In the other group of animals (n = 30) the appropriate vertebral arches were removed and either the dorsal root of second, or eighth, or ninth spinal segments were exposed. The neurobiotin labeling was performed similarly to that of the vestibular branches.

In one animal only one nerve was labeled.

In the third group of animals (n = 2) the brain containing the cerebellum was removed and fixed in 10 % formalin, embedded in paraffin and either serial cross sections or horizontal sections of 30 µm were made. After deparaffinization and hydration sections were stained with Cresyl Violet for 10 min, dehydrated and coverslipped.

3. 2. 3. Visualization and mapping of dye-coupled granule cells

After surgical preparation the animals were kept in refrigerator for 3-6 days, and they were reanesthetized with MS 222 and perfused transcardially with isotonic saline and subsequently with a solution of 2 % paraformaldehyde and 1.25 % glutaraldehyde in 0.1 % phosphate buffer; pH 7.4. The brain including the cerebellum was removed and fixed by immersion in the same fixative for overnight. The specimens were washed in 0.1 M phosphate buffer, followed by 10 % and 20 % sucrose dissolved in the same buffer solution. 60 µm serial horizontal or cross sections were made with a freezing microtome. The sections were washed for 10 minutes in 0.1 M PB and PBS and incubated for 1 hour in PBS containing 0.001 % extravidin (Sigma). The sections were then washed for 10 minutes in PBS, 0.1 M PB and 0.05 M TRIS buffer (pH 8) and incubated in 0.05 M TRIS buffer containing 0.075 % diaminobenzidine (Sigma), 0.6 % nickel – ammonium sulfate and 0.015 % hydrogen peroxide until the reaction product was visualized as a black precipitate. Finally, sections were washed in TRIS buffer, mounted on gelatin-coated slides, dried overnight and coverslipped.

The contour of the cerebellum and the position of dye-coupled granule cells were drawn from representative sections using Neurolucida equipment (MicroBrightField, Inc., Colchester, VT, USA) and granule cells were superimposed on the corresponding Nissl-stained sections. Because the thickness of Nissl-stained sections was half of the neurobiotin labeled specimens, every second of the original 32 sections in rostrocaudal and

18 in ventrodorsal direction was used for mapping. The specimens were photographed by using a Nikon microscope.

3. 3. Organization of last-order premotor interneurons related to the protraction and retraction of the tongue

3. 3. 1. Retrograde labeling of the last-order premotor interneurons

The experiments were carried out on 10 frogs. Under MS 222 anaesthesia (0.01 %, tricaine methane-sulfonate; Sigma, St. Louis, MO, USA) the caudal portion of the occipital bone and the roof of fourth ventricle were removed. A glass micropipette with a tip diameter of 10-20 µm was filled with 10 % biotinylated dextran amine (BDA, 10 kD, Molecular Probes) dissolved in 0.1 M phosphate buffer, pH 7.4. The tracer was injected in case of five animals into the caudal part of the dorsomedial subnucleus and in case of five animals into the caudal part of the ventrolateral subunit of the hypoglossal nucleus. It is known that the BDA is taken up by axon terminals, dendrites and perikarya and transported both anterograde and retrograde directions. The dorsomedial subnucleus contains the hypoglossal motoneurons of the protractor muscles; the ventrolateral subnucleus supplies the retractor muscles of the tongue. The tracer was injected by iontophoresis, using positive direct current of 5 µA with pulse duration of 7 sec followed by 3 sec intervals for a period of 8-10 min. The place of injection was determined by the coordinates of Kemali and Braitenberg (1969).

3. 3. 2. Visualization and mapping of last-order premotor interneurons

After a survival period of 5 days, the animals were reanaesthetized and transcardially perfused with isotonic saline for 2-3 min, followed by a fixative containing 2.5 % glutaraldehyde, 0.5 % paraformaldehyde and 0.2 % picric acid in 0.1 M phosphate buffer, pH 7.4. The brainstem was excised and fixed by immersion in the same fixative for overnight. They were washed in 0.1 M phosphate buffer followed by 10 % and 20 % sucrose dissolved in the same buffer solution. Blocks of tissue were cut with a vibratome at a thickness of 60 µm. For visualizing BDA, sections were treated with avidin-biotin complex and then with a nickel-enhanced DAB chromogen reaction. The localization of BDA labeled cells was drawn from consecutive sections with the aid of Neurolucida

(MicroBrightField, Inc., Colchester, VT, USA). Photographs were taken with a Nikon microscope.

4. Results and discussion

Applying neuronal tracing techniques we have examined two of the most important components of the sensorimotor system underlying the control of prey-catching and feeding behavior in the frog. We have mapped the dye-coupled cerebellar connections of the primary afferent fibers related to the individual vestibular receptor end-organs and the dorsal root fibers of the limb-innervating segments of the spinal cord, and we have demonstrated the organization and morphology of the last-order premotor interneurons related to the protractor and retractor muscles of the tongue.

4. 1. Organization of the dye-coupled cerebellar granule cells labeled from afferent vestibular and dorsal root fibers

4. 1. 1. Dye-coupled connections of the vestibular afferent fibers

We have demonstrated a noticeable overlap in the territory of dye-coupled granule cells related to the individual semicircular canals and otolith organs similarly to that we have previously found in the distribution of dye-coupled second order vestibular neurons in the vestibular nuclear complex, which was confirmed by electrophysiological studies. Several morphological experiments described a remarkable overlap in the central termination areas of labyrinthine afferent fibers in frog, pigeon and mammalian species. During physiological studies the lack of clear somatotopy was found in the cerebellum when the otolith and canal afferents were separately stimulated. These data indicate that the topographical representation map characteristic of other sensory systems does not exist in the vestibular system. In this context it is interesting to mention that opinions are still controversial concerning the significance of somatotopical representation in the sensory processing.

Although our results showed a significant overlap in the termination areas, some organotopical segregation related to the receptor organs was found in the rostrocaudal, ventrodorsal and mediolateral directions of the cerebellum.

4. 1. 1. 1. Topography of dye-coupled cerebellar granule cells related to the semicircular canals

In the mediolateral direction dye-coupled granule cells of all the semicircular canals appeared first at the lateral edge of the lobus auricularis. The labeled cells of the anterior and posterior semicircular canals were detected in the corpus cerebelli as well, whereas the cells of the horizontal canal remained within the confines of the lobus auricularis. In the rostrocaudal direction the rostralmost granule cells were coupled to the posterior semicircular canal, while the caudalmost cells were labeled from the nerve of the anterior semicircular canal. In the dorsoventral direction granule cells belonging to the anterior semicircular canal occupied the dorsalmost area while the granule cells of the horizontal semicircular canal were found in the ventralmost part of the cerebellum.

Concerning the canal-related granule cells, the horizontal canal nerve labeling showed the shortest area in all of the three directions, whereas cells of the vertical canals occupied more extensive territory. This difference may reflect the different involvement of horizontal canal and vertical canals in the vestibulo-ocular and vestibulo-spinal reflexes. Selective stimulation of individual labyrinthine nerve branches indicated that the abducens motoneurons received input exclusively from the horizontal canal in the frog.

4. 1. 1. 2. Topography of dye-coupled cerebellar granule cells related to the otolith organs

Contrary to the mediolateral organization of the canal nerve-related dye-coupled cells, all otolith organs were represented by their labeled granule cells both in the lobus auricularis and in the corpus cerebelli. In the rostrocaudal direction the lagenar and saccular neurons localized more rostrally than neurons of the utricle, which were found in the caudal half of the cerebellum. The dye-coupled neurons of the lagena occupied the longest rostrocaudal extension whereas the cells of the utricle were found in the shortest area. Neurons of the saccule appeared in the longest dorsoventral extension followed by a shorter expansion of dye-coupled neurons of the lagena and then by the cells of the utricle.

A well known functional difference may be expressed in this topographical separation of the representation areas of otolith organs. The utricle is a pure vestibular organ both in frog and mammalian species and its afferent fibers terminate exclusively in the vestibular nuclei. Additionally this is the only otolith organ in the frog that activates the vestibulo-

ocular reflex. In contrast to the utricule, the saccule and the lagena have a dual function in frog, although, with a differential weighting in the vestibular and acoustic sensation. The frog saccule is predominantly an acoustic organ, as evidenced from the projection of saccular fibers to the classically defined acoustic nucleus that is named nucleus sacculari and suggested that it is equivalent with the mammalian nucleus cochlearis ventralis. The functional role of the saccule is characterized by the absence of a convergence between afferent saccular signals and afferent signals from other labyrinthine organs and by the absence of saccular signals from vestibulo-ocular, maculo-ocular and postural reflexes. Contrary to the saccule, the lagena is predominantly a vestibular organ as evidenced from the projection of lagenar fibers to all vestibular but not to auditory nuclei. The vestibular role of the lagena is further strengthened by its contribution to the postural reflexes. As a conclusion of the previous and our present work it can be summarized that the lagena is predominantly a vestibular organ and the saccule is primarily related to acoustic sensation in the frog. It was demonstrated in our earlier experiments that first order vestibular afferent fibers of the lagena and saccule project into the superior olive, the hindbrain acoustic nucleus of the frog, which strengthens the dual function of them. The major functional role of labyririth organs have changed during the evolution so the receptors with the same name in different vertebrate taxa may have different function. For instance, the saccule in lower vertebrates plays a remarkable role in the acoustic sensation, while in mammalian species it functions as an almost pure vestibular receptor. In amphibians the saccule and the lagena with dual function are considered as evolutionary transitions between the two basic types of labyrinth receptors.

It should be mentioned that focusing merely to the cerebellar organotopy of vestibular receptors might not give full information about some other kinds of organization of them. It is known that hair cells in the central and peripheral parts of the crista ampullaris of semicircular canals and the macula of utricle and lagena present different physiological properties. There are also differences in size of fiber diameters of afferent nerves connected to the receptor cells and in their convergence with a different contribution onto second order vestibular neurons. Unfortunately, our technique is not adequate for such high resolution in labeling the individual labyrinthine afferent fibers and showing the probable organized arrangement of their dye-coupled granule cells in the cerebellum.

It remains questionable of whether a single cerebellar granule cell receives multiple input form the canals, otolith organs and somatosensory receptors either through gap

junctions or chemical synapses. Convergence of vestibular input was recorded from Purkinje cells that could be co-stimulated from the horizontal and from the posterior canal of the frog. Similarly, the combination of canal and otolith inputs to the Purkinje cells produced an increase in the mean rate of cell discharge suggesting that the cerebellum is an important place for the integration of multiple canal and otolith inputs. A considerable convergence of inputs onto the second order vestibular neurons was recorded following application of natural stimuli to different vestibular receptors in mammalian species. Contrary to these findings, other physiological studies showed that about 90 % of second order vestibular neurons received monosynaptic excitatory inputs from only one semicircular canal nerve in non-mammalian and mammalian species suggesting a one receptor – one neuron coupling.

4. 1. 2. Dye-coupled connections of the spinal dorsal root fibers

The majority of dye-coupled granule cells related to dorsal root fibers of the spinal cord were situated mostly in the corpus cerebelli. We have detected an almost complete separation in the rostrocaudal distribution of dye-coupled cerebellar granule cells of cervical versus lumbar spinal segments, whereas their territories in the mediolateral and dorsoventral directions were almost entirely overlapping. Our results confirm several previous morphological and physiological experiments that showed remarkable overlapping between the cerebellar termination areas of spinal dorsal root fibers related to the upper and lower limbs. The common termination area of mossy fibers from the brachial and lumbar spinal segments was reported in the mediolateral and ventrodorsal directions when the limb innervating dorsal roots were selectively labeled with cobalt. A detailed study on the cerebellar localization of somatosensory impulses was carried out in the frog by recording the responses of Purkinje cells to natural or electrical stimuli concluding that no clear somatotopic organization of the upper and lower limb was found in the frog cerebellum either for the mossy or climbing fibers.

4. 1. 3. Convergence between the vestibular and proprioceptive inputs in the cerebellum

The common areas shared by different combinations of labyrinthine organs and dorsal root fiber-related granule cells were found in all main directions of the cerebellum. The

partial overlap of the vestibular and spinal cord related dye-coupled granule cells presented here is in accordance with our previous findings when we have found a widespread projection of the frog vestibular nucleus into those parts of the nervous system that also receive proprioceptive impulses suggesting a convergence of sensory modalities involved in the sense of balance.

4. 1. 4. The role of electric transmission underlying locomotion

According to electrophysiological studies the electric transmission between primary afferents and their second order neurons subserves the depolarization of inactive fibers related to the target cells and activation of them. This process may serve to synchronize the afferent impulse activity and to boost synaptic activation of cells by increasing the number of active fibers. This suggests that electric transmission plays an important role in the coordination of extremly rapid, goal-directed movements, which are relevant to the survival of animals. The physiological studies were supported with morphological experiments in which low molecular weight tracers (Neurobiotin, Lucifer yellow) were injected into the postsynaptic neuron. The dyes were found in the terminals of afferent fibers that suggest that the gap junctions in these primary afferents are not only involved in fast anterograde synaptic transmission but also provide the substrate for a retrograde intercellular communication.

In earlier experiments, stimulation of vestibular nerve resulted in two monosynaptic field potentials in lumbar part of the spinal cord in the frog, the first of which appeared with a very short latency. Since the first order vestibular afferent fibers do not project directly to the spinal cord, electric transmission was postulated in the vestibular nuclei. It was then verified with physiological methods and dye-coupled connections between the first order afferent vestibular fibers and second order vestibular neurons were demonstrated. Cerebellar granule cells dye-coupled to the first order vestibular afferent and dorsal root fibers presented here provide further evidence for the importance of electrical coupling in the function of vestibular system in the frog.

Summarizing the previous and our present results we can assume that the afferent inputs of vestibular and proprioceptive fibers mediated by gap junctions to the cerebellar granule cells subserve one of the possible morphological background of a very rapid modification of the motor activity in the vestibulo-cerebello-spinal neuronal circuit. In case of body displacement, current is generated both in the labyrinthine and in the limb muscle

receptors. Labyrinthine receptors provide mixed gap junctional/chemical synapses to the vestibular nuclei and the coupling current at gap junctions may depolarize the adjacent inactive terminal(s) and boosts up the effectiveness of the vestibular input by promoting the recruitment of new fibers. Consequently, it may synchronize the afferent impulses, enhances the synaptic activation of the second order vestibular neurons which, in turns, process the command to spinal motoneurons by the vestibulospinal tract. Due to the combination of the chemical and electrical impulse transmission it provides a mechanism by which motoneurons can be activated sequentially. However, in case of very rapid displacement of the body, the propagation of signal from the vestibular receptors to the motoneurons and then to the muscle may be too slow to control the movements in time and properly. In order to obtain the required muscle contraction a control mechanism may be implemented based on the electrical coupling of labyrinthine and muscle receptors to the cerebellum through the mossy fiber-granule cell pathway. The electrical coupling may synchronize and amplify the afferent signals and, via the axons of granule cells, it may produce a very fast activation of large number of Purkinje cells in wide area of the cerebellum. The inhibitory action of Purkinje cells modulates the firing pattern of the second order vestibular neurons directly or indirectly and it may result an increase or decrease in the level of activation of motoneurons. The input from the vestibular and proprioceptive fibers may coincide in the granular layer of the cerebellum providing further amplification of signals.

4. 2. Organization of last-order premotor interneurons related to the protraction and retraction of the tongue

4. 2. 1. Distribution of the last-order premotor interneurons

We have demonstrated different distribution of premotor interneurons related to the protractor and retractor muscles of the tongue.

4. 2. 1. 1. Distribution of the last-order premotor interneurons related to protractor muscles of the tongue

By applying of BDA with iontophoretic injection into the dorsomedial subunit of the hypoglossal nucleus containing the motoneurons of protractor muscles of the tongue, we

could identify a large number of retrogradely labeled cells in different areas of the brainstem. The overwhelming majority of labeled neurons were detected at the ipsilateral side of injection both in the rostral and caudal directions. The majority of labeled neurons were distributed in the nucleus reticularis medius of the medial reticular zone of the rhombencephalic reticular formation. Labeled neurons were also found in the nucleus raphes of the median reticular zone, in the nucleus reticularis inferior of the medial reticular zone, in the lateral reticular zone on both sides, in the nucleus prepositus hypoglossi, in the nucleus of the solitary tract, in the vestibular nuclei, in the spinal nucleus of the trigeminal nerve and in the dorsal column nuclei. It is proven that the pattern generator centers related to the tectobulbar and tectobulbospinal pathways play an important role in the function of motor circuits of medulla and spinal cord. In our earlier study, by applying Phaseolus vulgaris leucoagglutinin (PHA-L) with iontophoretic injection into the lateral vestibular nucleus we have identified a large number of fiber terminals in the areas occupied by last-order premotor interneurons. These findings suggest that the vestibular sytem has a remarkable role in the integrated muscle contraction underlying pray-catching behaviour.

4. 2. 1. 2. Distribution of the last-order premotor interneurons related to retractor muscles of the tongue

In order to examine the distribution of last-order premotor interneurons related to the retractor muscles of the tongue we have applied BDA with iontophoretic injection into the ventrolateral subunit of the hypoglossal nucleus. We could identify retrogradely labeled cells in different areas of the caudal brainstem and cervical segment of the spinal cord at the ipsilateral side of injection both in rostral and caudal directions. The majority of labeled neurons were found in the intermediate gray matter, which recive a large number of primary afferent fibers from the trigeminal nerve and the lateral vestibular nucleus. Our results are in accordance with the earlier studies indicating that the owerwhelming majority of the primary afferent fibers and descending pathways form indirect contact with motoneurons through the last-order premotor interneurons. We could identify retrogradely labeled cells in a smaller number in the spinal nucleus of the trigeminal nerve and in the dorsal horn of the cervical spinal cord. Some larger cells were found ipsilaterally in the ventral part of the lateral funicule of the spinal cord corresponding to the marginal or Hoffman nucleus.

4. 2. 1. 3. Morphological properties of the last-order premotor interneurons

The premotor interneurons related to the protractor and retractor muscles of the tongue showed similar morphological features. The diameter of cell bodies varied in the range of 10-12 μm, the substantial number of them possessed round or ovoid-shape perikarya and only a few neurons showed pyramidal-shaped cell body. Applying BDA with iontophoretic injection into the ventrolateral subunit of the hypoglossal nucleus we found labeled cells with large perikarya in the marginal nucleus. On the basis of dendritic arborization pattern we have classified the last-order premotor interneurons of the hypoglossal motoneurons into two groups. In one group of neurons, the perikarya gave three or more stem dendrites arborizing profusely. In the second group, the neurons possessed one or two stem dendrites that were branching once or twice. The overwhelming majority of cells belonged to the second group. Similar morphological characterization was described on the last-order premotor interneurons of lumbosacral motoneurons of the rat. It was previously suggested that characteristic geometry of dendritic arbor of spinal cord and brainstem motoneurons provides a preferred target for one array of fibers over another. In the present study, the last-order premotor interneurons with different morphological features may be in favor of receiving synaptic input from various parts of the brain. There are no data in the literature about the neurochemical character of the premotor interneurons of the motor cranial nerve nuclei in the frog. In the chicken lumbosacral spinal cord, the last-order premotor interneurons were positive either for glycin or GABA and many of them were negative for these inhibitory neurotransmitters. Premotor interneurons of the rat hypoglossal motoneurons were heterogeneous with respect to their neurotransmitter phenotypes. Further experiments are needed to decide the specific neurochemical character of the last-order premotor interneurons of the hypoglossal nucleus.

4. 2. 2. Retrograde neuronal labeling technique

It is known that the BDA is taken up by axon terminals, dendrites and perikarya and transported in both retrograde and anterograde directions. Applying a neuronal tracer, it always remains some uncertainty about the specificity of labeling. One of the possible sources of non-specific labeling is the fibers of passage that can also take up the BDA as it was previously demonstrated. Contrary to these findings, it has been shown that the tracer can be taken up and transported only by damaged fibers. Other experiments showed that

the uptake of BDA by intact fibers is negligible if the tracer is applied by iontophoretic injection. To the best of our knowledge, there are no traveling fibers among the XII motoneurons; therefore, the chance of non-specific labeling of fibers of passage can be excluded. Since the site of injection was restricted to the perikarya and proximal dendrites of motoneurons, it is very improbable that the fibers of medial longitudinal fascicle (MLF), running in close vicinity of the XII nucleus, are labeled accidentally. Moreover, in case of the unintentional labeling of MLF, we should have seen the cells of origin in the nucleus of MLF but it was not the case. Similarly, the false labeling of the medial lemniscus is also improbable because we have never seen ascending fibers traveling in the direction of thalamus. Labeled cells in the reticular formation may be the cells of origin of reticulospinal projections filled up through their dendrites extending into the hypoglossal nucleus. This possibility can be rejected by the absence of any labeled axons and terminals at the level of spinal cord. Consequently, we can regard the vast majority of retrogradely labeled neurons as the last-order premotor interneurons that establish direct synaptic contact with the somatodendritic compartment of hypoglossal motoneurons supplying the protractor and retractor muscles of the tongue.

4. 2. 3. Neuronal circuit underlying prey-catchig behaviour

There is no morphological evidence for the direct connection between the optic tectum and the hypoglossal motoneurons innervating protractor and retractor muscles of the tongue. It is certain that during the chain of events of the prey-catching behavior the tectal command is conveyed mainly via the crossed tectobulbar tracts to the hypoglossal nucleus. Following lesions of tectobulbar pathways, degenerated terminals were exclusively shown among small neurons in the rhombencephalic grey matter surrounding the hypoglossal nucleus, but never in the vicinity of the motoneurons themselves. These findings indicate the polysynaptic pathway between tectal efferents and hypoglossal motoneurons. The last-order premotor interneurons summarizing and conveying the informations from the different parts of central pattern generators to motoneurons forming a station of this polysynaptic pathway. Our results provided additional informations about the convergence of different sensory modalities that play a role in the feeding motor program of the frog.

On the basis of present results and of the earlier findings, the neuronal circuitry related to the hypoglossal nucleus underlying prey-catching behavior can be summarized as follows. The tectal output is transmitted to the the last-order premotor interneurons through

the crossed, and in smaller extent via the uncrossed tectobulbar tracts. The area of reticular formation containing the last-order premotor interneurons receives significant input from the descending fibers of lateral vestibular nucleus, from the proprioceptive fibers of jaw closing muscles and from the medial lemniscus. In addition, a large number of protractor and retractor-related last-order premotor interneurons show a significant overlap with the termination area of cerebellar efferents and hypoglossal afferents that coordinate the timing of tongue protraction with jaw opening. These observations suggest that tectal, vestibular, tactile and proprioceptive signals are integrated by the last-order premotor interneurons and their pattern of activity is processed to the motoneurons of the hypoglossal nerve, supplying the protractor and retractor muscles of the tongue.

5. Summary

Applying different neuronal labeling techniques we have studied the morphological background of the sensorimotor system underlying the control of prey-catching behavior in the frog.

We have mapped the dye-coupled granule cells related to the nerves of individual labyrinthine organs and of dorsal root fibers of limb-innervating segments of spinal cord. The difference in the extension of territories of the vertical and horizontal canals may reflect their different involvement in the vestibuloocular and vestibulospinal reflexes. We could demonstrate only slight overlap between dye-coupled cells related to the lagena and saccule and the termination area of the utricular fibers in rostrocaudal direction. This separation is supportive of the dual function of the lagena and the saccule. We have described that the territories of granule cells related to the cervical and lumbar segments of the spinal cord were almost completely separated along the rostrocaudal axis of the cerebellum. In spite of the partial segregation we demonstrated a significant overlap in the related areas of termination that suggests a remarkable convergence of the afferent input of the vestibular and prorioceptive fibers on the cerebellar granule cells.

Applying BDA injection into the dorsomedial and ventrolateral subnucleus of the hypoglossal nerve we have examined the distribution and morphological features of the last-order premotor interneurons related to the protractor and retractor muscles of the tongue. We have described that the majority of them were distributed ipsilateral to the site of injection and extended in rostral and caudal directions. Labeled neurons related to the protractor muscles were found mainly in the rhombencephalic reticular formation, whereas labeled neurons related to the retractor muscles were located mainly in the intermedier gray matter of the caudal brainstem and cervical spinal cord. We could demonstrate morphologically heterogenous populations of the last-order premotor interneurons that suggest the different origin of their affrent inputs. These results strengthen the earlier studies that suggest indirect transmission between the tectum opticum and the hypoglossal motor neurons.

Our experiments revealed that the convergence of sensory modalities related to the tectum opticum, vestibular system, proprioceptors and cerebellum has a significant importance during the coordination of the prey-catching and feeding behavior. These results can help to understand the underlying sensory and motor processes of different behaviours.

Key words: sensorimotor integration, neuronal labeling, brainstem, vestibular system cerebellum, hypoglossal nucleus

Publications

This thesis is based on these in extenso publication:

Éva Rácz, Tímea Bácskai, Gábor Halasi, Endre Kovács, Clara Matesz. 2006. Organization of dye-coupled cerebellar granule cells labeled from afferent vestibular and dorsal root fibers in the frog Rana esculenta. J Comp Neurol 496:382-94. **IF: 3,855.**

Éva Rácz, Tímea Bácskai, Gábor Szabó, György Szekely, Clara Matesz. 2008. Organization of last order premotor interneurons related to the protraction of tongue in the frog, Rana esculenta. Brain Res 1187:111-115. **IF: 2.341.**

Other in extenso publications:

Tímea Bácskai, Gábor Veress, Gábor Halasi, Ádám Deák, **Éva Rácz**, György Szekely, Clara Matesz. 2008. Dendrodendritic and dendrosomatic contacts between the oculomotor and trochlear motoneurons of the frog, Rana esculenta. Brain Res Bull 75:419-423. **IF: 1.684.**

Clara Matesz, Gabriella Kovalecz, Gábor Veress, Ádám Deák, **Éva Rácz**, Tímea Bácskai. 2008. Vestibulotrigeminal pathways in the frog, Rana esculenta. Brain Res Bull 75:371-374. **IF: 1.684**.

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Abstracts:

Éva Rácz, Tímea Bácskai, Gábor Halasi, Clara Matesz. 2004. Dye-coupled connections of the primary afferent vestibular fibers in the cerebellum of the frog. 1st International Conference on Basic and Clinical Immunogenomics, Budapest. Tissue Antigens 64:436. **IF: 1,737.**

Éva Rácz, Endre Kovács, Tímea Bácskai, Gábor Halasi, Clara Matesz. 2005. Dye-coupled connections of the primary afferent fibers in the cerebellum of the frog. Congress of Hungarian Neuroscience Society, Pécs. Clinical Neuroscience 58(1):78.

Éva Rácz, Gábor Halasi, Tímea Bácskai, György Szekely, Clara Matesz. 2006. Vestibular-lesion induced changes in the expression of tenascin, fibronectin and phosphacan in the frog. IBRO International Workshop, Budapest. Clinical Neuroscience 59(1):55.

Ádám Deák, Tímea Bácskai, Gábor Veress, **Éva Rácz**, Klára Matesz. 2006. Vestibular afferents to the brainstem: morphological substrate for vestibulo-autonomic interaction. IBRO International Workshop, Budapest. Clinical Neuroscience 59(1):19.

Éva Rácz, Zoltán Mészár, Gábor Veress, László Módis, György Szekely, Klára Matesz. 2007. Composition of the perineuronal net of the motoneurons in the brainstem. Congress of Hungarian Neuroscience Society, Szeged. Clinical Neuroscience 60(1):54.

Ádám Deák, Tímea Bácskai, **Éva Rácz**, Klára Matesz. 2007. Vestibular lesion-induced changes in the expression of hyaluronan in the rat brainstem. Congress of Hungarian Neuroscience Society, Szeged. Clinical Neuroscience 60(1):16.

Éva Rácz, Tímea Bácskai, Gábor Szabó, György Szekely, Clara Matesz. 2007. Organization of last order premotor interneurons related to the protraction of tongue in the frog, Rana esculenta. 5th ECCN, Paris.

Klára Matesz, Gabriella Kovalecz, Gábor Veress, Ádám Deák, **Éva Rácz**, György Szekely, Tímea Bácskai. 2007. Vestibulotrigeminal pathways in the frog, Rana esculenta. 5th ECCN, Paris.

Tímea Bácskai, Gábor Veress, Gábor Halasi, Ádám Deák, **Éva Rácz**, Klára Matesz. 2007. Dendrodendritic and dendrosomatic contacts between the oculomotor and trochlear motoneurons of the frog, Rana esculenta. 5th ECCN, Paris.

Éva Rácz, Botond Gaál, Andrea Hunyadi, György Szekely, Klára Matesz. 2008. Distribution of extracellular matrix in the vestibular nuclear complex of the frog. IBRO International Workshop, Debrecen. Clinical Neuroscience 61(1):53.

Botond Gaál, **Éva Rácz**, Zoltán Mészár, László Módis, Klára Matesz. 2008. Composition of the perineuronal net of the motoneurons in the frog. IBRO International Workshop, Debrecen. Clinical Neuroscience 61(1):28.

Andrea Hunyadi, **Éva Rácz**, Gábor Veress, György Szekely, Klára Matesz. 2008. Direct connection between the vestibular afferents and abducens motoneurons in the frog. IBRO International Workshop, Debrecen. Clinical Neuroscience 61(1):35.

Éva Rácz, Botond Gaál, Gábor Veress, Andrea Hunyadi, György Szekely, Klára Matesz. 2008. Organization of extracellular matrix in the vestibular nuclear complex and cerebellum of the frog, Rana esculenta. 6th FENS, Genf.

Klára Matesz, Andrea Hunyadi, **Éva Rácz**, Gábor Veress, György Szekely. 2008. Vestibular afferent fibers establish direct connection with the abducens motoneurons of the frog, Rana esculenta. 6th FENS, Genf.