



## Neuronal Mechanisms of the Cerebello-Reticular Projection System in Frog

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### Abstract

Intracellular potentials of the medial reticular formation neurons were studied in response to stimulation of ipsilateral auricular lobe of the cerebellar cortex via perfused frog brainstem method. It was shown that stimulation of Purkinje cells evoked mono- and polysynaptic inhibitory postsynaptic potentials in MRF neurons.

**Keywords:** Medial Reticular Formation; Motoneurons; Auricular Lobe of Cerebellum

### Abbreviations

MRF: Medial Reticular Formation; IPSPs: Inhibitory Postsynaptic Potentials; EPSPs: Excitatory Postsynaptic Potentials; LVN: Lateral Vestibular Nucleus; VNC: Vestibular Nuclear Complex

### Introduction

A complex interaction between the motor structures of the brain and spinal cord results the body's motor activity. The medial reticular formation (MRF) and the lateral vestibular nucleus (LVN) play the key role in this interaction. It is known that the LVN originates from the reticular formation. There is strong evidence that impulses coming from the vestibular nuclei to spinal motor neurons can also be mediated through reticulospinal neurons [1,2]. The majority of reticulospinal neurons, regardless of their location in the brainstem, are involved in the transmission of vestibular information to the spinal cord. The reticular formation contains many complex centers. Certain areas of the medulla oblongata affect the motor neurons of the spinal cord. These bulbar neurons are influenced by the overlying areas of the brain.

In the ventrolateral part of the reticular formation of the medulla oblongata, a group of cells was identified that has an inhibitory effect on spinal reflexes. Cells of the dorsal part of the reticular formation provide spinal reflexes [3]. The reticulospinal tract is the most ancient cerebrospinal system.

The aim of this study was to study the functional relationships between the auricular lobe of the cerebellum and MRF. In mammals, inhibitory cerebellar influences on reticular neurons are mediated by well-defined cerebellar-reticular pathways. Such studies in lower vertebrates (frogs) are lacking. There is an opinion about the similarity of the cerebellar-reticular connections in the frog and higher vertebrates, including mammals [4].

### Materials and Method

The experiments were provided on frogs (*Rana ridibunda*) of both sexes using the isolated perfused brain method. Animals were deeply anesthetized with MS-222 solution (0.2g/kg). The chest was opened, and the heart was exposed to inserting a canula for

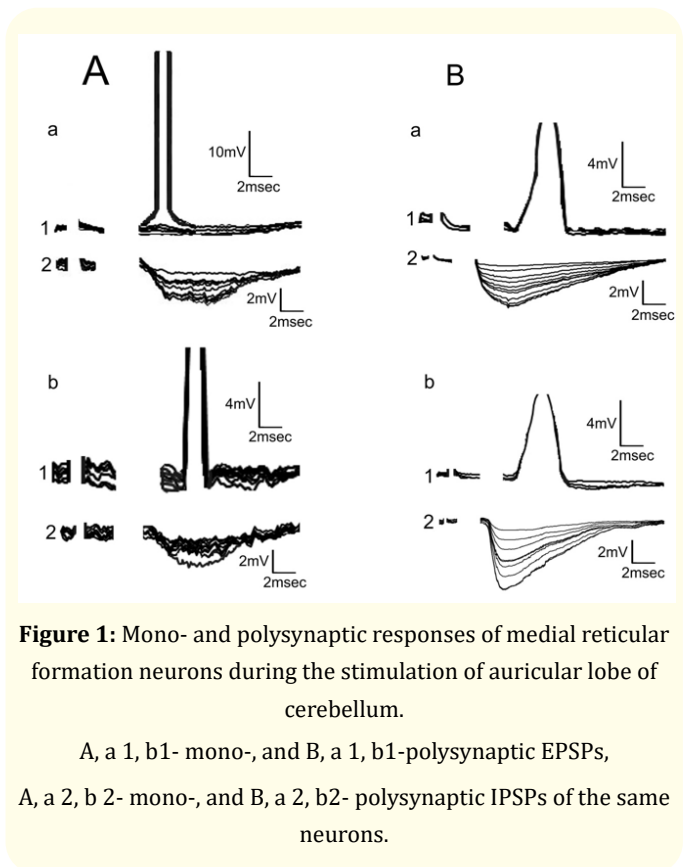
perfusion with Ringer's solution ( $10^{\circ}$ - $18^{\circ}$  C) for poikilotherms, saturated with carbogen. By craniotomy were exposed the skull, the vestibular nerve, and the cerebellum. Electrical stimulation of the anterior branch of the VIII nerve was carried out by single shocks of direct current (0.1 - 0.2 msec; 0.05 - 0.4 mA) using a silver suction electrode. Bipolar silver ball electrodes were carefully applied to the surface of the auricular lobe of cerebellum under visual control. For its electrical stimulation, the same current parameters were used as for the vestibular nerve. For intracellular recording of the electrical activity of MRF neurons, were used worn glass microelectrodes filled with a solution of 2 M potassium citrate (resistance of 10 M $\Omega$ ). Computer analysis of the data was carried out by Origin 8.5 and Multisim programs. The arithmetic mean standard deviations of the indicators are given.

## Results and Discussion

Intracellular activity was registered in 175 reticular neurons. MRF neurons were identified based on excitatory postsynaptic potentials (EPSPs) (Figure 1. A, a 1, b 1, B, a 1, b 1), arising in response to vestibular nerve stimulation. Only 30% of the identified MRF neurons responded to cerebellar stimulation. Amphibians' MRF neurons are abundantly supplied with vestibular fibers [5]. Vestibular afferents also affect the MRF neurons through interneurons localized in the vestibular nuclear complex (VNC) [2].

A single stimulation of the auricular lobe of the cerebellum caused inhibitory postsynaptic potentials (IPSPs). Considering the temporal characteristics of the studied responses, we conventionally divided the recorded IPSPs into two groups: short-latency and long-latency.

The first group included 56 neurons with a latency of 1.65-3.0 msec (on av.  $2.56 \pm 0.33$  msec;  $n = 56$ ). There was no significant change in the latency duration and the time of amplitude rise to the maximum at different stimulation intensities. The latter averaged  $3.72 \pm 0.93$  msec (2.1-6.15 msec;  $n = 52$ ). Their amplitude reached 0.57-3.3 mV (on av.  $1.58 \pm 0.58$  mV;  $n = 52$ ). The total duration varied between 7.46-22.5 msec (on av.  $11.6 \pm 3.16$ ;  $n = 55$ ) (Figure 1. A, a 2, b 2; Figure 2). The noted factors allowed us to consider these IPSP data as monosynaptic.



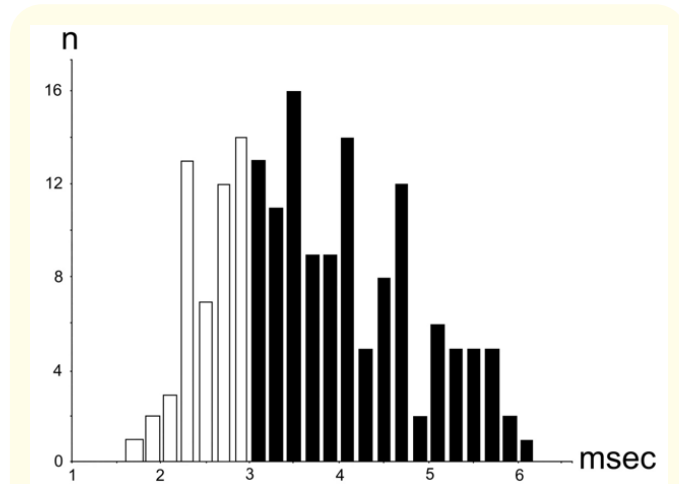
**Figure 1:** Mono- and polysynaptic responses of medial reticular formation neurons during the stimulation of auricular lobe of cerebellum.

A, a 1, b 1- mono-, and B, a 1, b 1-polysynaptic EPSPs, A, a 2, b 2- mono-, and B, a 2, b 2- polysynaptic IPSPs of the same neurons.

There are no morphological studies on the projection of axons of Purkinje cells in the MRF of frogs. Whereas in mammals, a direct connection between the cerebellum and RF was proven either morphologically or electro physiologically. This suggests that short-latency IPSPs were generated monosynaptically in MRF neurons by direct activation of Purkinje cell axons projecting into the MRF, like the existing direct connection between Purkinje cells and VNC [6].

The second group consists of 119 reticular neurons, in which auricular lobe stimulation caused IPSPs with a longer and more unstable latency. They were characterized by a clear shortening of the latencies and the rise time of IPSP hyperpolarization to a maximum with increasing stimulation intensity. Their latency varied between 3.04-6.0 msec (on av.  $4.2 \pm 0.8$  msec;  $n = 119$ ). The duration of the amplitude rise time to the maximum averaged  $5.16 \pm 1.24$  msec (2.22-8 msec;  $n = 87$ ). The amplitude peaked at an

average of  $1.8 \pm 0.62$  mV (0.63-3.5 mV;  $n = 92$ ). The total duration of the IPSPs was within 8.18-27.8 msec (on av.  $15.5 \pm 4.6$  msec;  $n = 112$ ) (Figure 1. B, a 2, b 2; Figure 2). The above-mentioned temporal characteristics of the recorded IPSPs and their dependence on the intensity of stimulation indicate a di- and polysynaptic origin.



**Figure 1:** Mono- and polysynaptic responses of medial reticular formation neurons during the stimulation of auricular lobe of cerebellum.

A, a 1, b1- mono-, and B, a 1, b1-polysynaptic EPSPs,

A, a 2, b 2- mono-, and B, a 2, b2- polysynaptic IPSPs of the same neurons.

It was found that long-latency IPSPs caused by weak stimulation of the cerebellar surface gradually shortened in time with increasing stimulation intensity, i.e., with spatial summation of the inputs of parallel fibers to the dendrites of Purkinje cells. We assumed that the described di- and polysynaptic IPSPs arose not by direct, but by indirect activation of Purkinje cells through parallel fibers, by analogy with those recorded in VNC, in response to stimulation of the auricular lobe of the cerebellar cortex [6,7].

The IPSPs would arise with short-latency or with long-latency depends not only on the intensity of stimulation, but also on the location of the irritating electrode, since some vestibular and reticular neurons respond monosynaptically to very weak stimulation, and the others require a strong stimulus. The small size of the frog cerebellum and the proximity of the auricular lobe to the cerebellar peduncle make it impossible to accurately determine the location of the cerebellar-reticular Purkinje cells.

The best effect of MRF neuron potential discharge in response to stimulation of the vestibular nerve was observed when the electrode was inserted into the area of the bottom of the fourth ventricle, 1.5-2.0 mm caudal to the vestibular nerve, 200-500 $\mu$  lateral to the midline and immersed to a depth of 500-1000 $\mu$  from the dorsal surface. However, it was observed that the incidence of registered IPSPs decreased when the irritating electrode was moved closer to the midline of the cerebellum.

## Conclusion

The mechanism described above also exists in relation to the cerebellar-reticular relationships, considering the registered IPSPs. Servicing and directing the motor act, the cerebellum is actively involved in the regulation of posture and movement of the most diverse nature, cooperating, in addition to the vestibular system, also with the reticular system of the brainstem. We believe that the above-mentioned functions of the amphibian cerebellum are like those of mammals.

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