ISSN 2618-1584 Vol. 4, Issue 15: 50-54, DOI: 10.25236/FMSR.2022.041508

Changes in c-FOS Protein Levels in Some Basal Ganglia of Rats before and after Vestibular Stimulation

Li Meihan, Fu Can, Zhang Zhen, Li Meizhi, Sun Liyuan*

Guilin Medical College, Guilin, 541110, China
**Corresponding author*

Abstract: *Objective: To analyse the changes in the levels of c-FOS protein in some basal ganglia ganglia of rats before and after vestibular stimulation. Methods: Rats were selected as the study subjects and non-invasive electrical stimulation was used to understand the function between the vestibular organs and the ganglia and the issue of changes in the receptor mechanism of c-FOS protein in the ganglia. Results: After stimulation of the rat vestibular organ, there was a significant trend towards higher expression of c-FOS positive neurons in the MVN region, along with a similar increase in the number of immunopositive neurons in the PF region. Although no direct nerve fibre connection was found between the MVN and CPu, this index was also observed considering that CPu would be involved in motor regulation, and the results also showed a significant increase in positive neuronal expression in the observation group of rats. The results showed that c-FOS positive neuronal expression in all three regions increased after non-invasive stimulation of the vestibular organ, with statistically significant differences (P<0.05). Conclusion: Stimulation of peripheral vestibular organs can induce functional changes in the basal ganglia and c-FOS protein-positive neuronal expression, giving a good therapeutic reference for future Parkinson's disease and modulating vestibular function in patients.*

Keywords: *Rat, basal ganglia nerve, c-FOS protein, vestibular stimulation*

1. Introduction

The vestibule itself is the body's main organ for the regulation of motor function and balance, while the basal ganglia is the higher centre for the regulation of movement, being the brain region of the cerebral cortex and subcortical neural circuits. Different systems are involved in the regulation and control of motor nerves as the body maintains movement, so basal ganglia lesions can lead to very pronounced motor dysfunction, including hyperkinesia or hypokinesia. Parkinson's disease is typically characterised by a disturbance in motor function or a slowing of voluntary movements to the point where muscle rigidity and postural reflexes are impaired. Non-invasive stimulation of the peripheral vestibular organs can improve certain clinical symptoms in Parkinson's patients, which has very important practical implications for future treatment options for related diseases and is the main entry point for this study.

2. Definition of Relevant concepts

First is the vestibular nerve, which is an important part of the human auditory nerve. Its pivot branch is closely connected with the cochlear nerve. It enters the cranial cavity through the inner ear hole together, and connects the pons through the tail end of the pons. It stops at the pons and different vestibular nuclei in the medulla oblongata. At the same time, a small part of fibers in the vestibular nerve connect with the cerebellum through the lower cerebellar peduncle and stop at the pompons and nodules. The lateral vestibular nucleus has vestibulo spinal tract, which is composed of several fibers. The fibers stop in the ipsilateral anterior horn cells and are related to the balance movement of the human body. The other vestibular nucleus fibers and the medial longitudinal bundle are mainly connected with the III, IV, VI cranial nerves and the anterior column of the upper cervical spinal cord through the internal ear labyrinth, and are related to the reflex adjustment of the position of the eyeball and the activity of the neck. The main function of the vestibular nerve is to ensure the balance of the human body. If dysfunction occurs, it will lead to a sense of imbalance, which may lead to vertigo, nystagmus and other serious situations. The vestibular nerve dysfunction can be detected by cold and hot water perfusion test of the external auditory canal and rotary chair test. In addition, the vestibular nerve is divided into several independent

ISSN 2618-1584 Vol. 4, Issue 15: 50-54, DOI: 10.25236/FMSR.2022.041508

subregions in the bottom of the internal auditory canal, the transverse ridge and the longitudinal ridge, such as the branches of the seventh and eighth cranial nerves. These subregions expand outward through the relevant channels. In the overall distribution, the vestibular nerve middle facial nerve occupies the anterosuperior nerve, the superior nerve occupies the posterior superior nerve, the cochlear nerve occupies the anterior inferior nerve, and the inferior nerve occupies the posterior inferior nerve. There is a single hole behind the transverse ridge, which provides a channel for the single hole nerve. The single hole can be used as a sign to open the outer boundary of the posterior wall of the internal auditory canal, and can prevent the nerve from entering the inner ear labyrinth.Fig. 1 Vestibular nerve

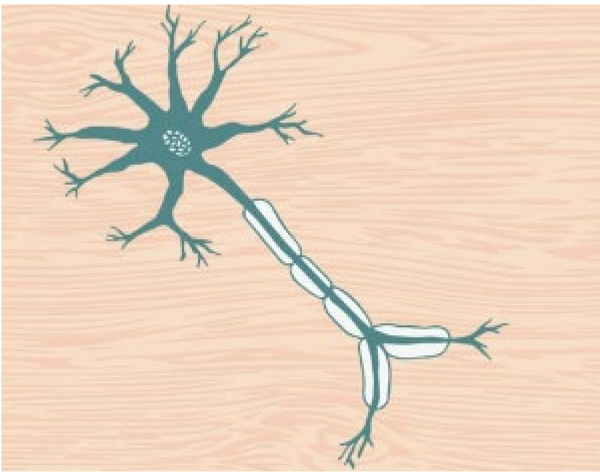

Figure 1: Vestibular nerve

The second is Parkinson's disease, which was previously defined as a nervous system degenerative disease commonly seen in the elderly (people over 60 years old). This definition was overturned in subsequent studies, because the disease also exists in young people (people under 40 years old), but the incidence is relatively low. The vast majority of cases of Parkinson's disease are sporadic cases, and less than 10% of cases have family history. Parkinson's disease itself is not fatal, but it will cause various complications, which are fatal. Parkinson's disease will have pathological changes. The principle of pathological changes is that dopaminergic neurons in the substantia nigra of the midbrain degenerate and die, making DA content in the striatum decrease in a large area, and finally become ill. At present, there is no conclusion on the specific cause of this pathological change, but existing studies have summarized some related factors, including genetic factors, environmental factors, aging, oxidative stress, etc., which may be related to Parkinson's disease. If pathological changes occur in Parkinson's disease, the substantial reduction of DA content in the striatum will induce intracytoplasmic aberrations in the remaining neurons of substantia nigra, and there will be eosinophilic inclusion bodies, that is, Lewy bodies. When Lewy corpuscles appear, the death rate of substantia nigra dopaminergic neurons exceeds 50% clinically, and the non dopaminergic system will also be significantly damaged. Parkinson's disease is closely related to human motor ability, intelligent function, emotional perception and other functions, that is, most patients with Parkinson's disease experience tremor in their limbs after the disease, and the degree will gradually increase, accompanied by the current situation of clumsy activities, and the continued development will lead to the oxidation of static tremor, motor retardation, myotonia and postural gait disorders. At the same time, it is easy to appear the characteristics of intellectual degeneration, emotional depression or apathy. Table 1 Main Symptoms of Parkinson's Disease.

Table 1: Main Symptoms of Parkinson's Disease

Disease	Main symptoms
Parkinson's disease	Static tremor
	Slow gait
	Facial expression disorder
	Myotonia
	One's memory is failing
	Depression
	Pathological palpitation

Combined with Table 1, the most common symptoms are static tremor and myotonia.

Static tremor is the first symptom of Parkinson's disease. According to data statistics, it is the first

ISSN 2618-1584 Vol. 4, Issue 15: 50-54, DOI: 10.25236/FMSR.2022.041508

symptom in 70% of cases. It is common in the distal end of upper limbs on either side of the left and right, that is, the palm. When the tremor is intentionally controlled, it will be reduced or even disappear, but once it is still, it will become obvious. If the nervous tension is more serious, it will disappear during sleep. Static tremor will increase when people walk, and the frequency is about 4 to 6 Hz. In the form, the fingers are slightly curved, and the left and right quivers. Myotonia refers to the obvious resistance of the patient's limbs, neck and trunk during the activity. This sense of resistance will increase with the increase of the applied force during the activity, and the direction of resistance is consistent. Because the feeling is similar to the bending of soft lead pipes, it is also called lead pipe like rigidity.

There are many forms of myotonia. In addition to the conventional resistance to movement, there is also intermittent myotonia, which usually occurs with static tremor. That is, if the patient has static tremor, the tremor may stop for a short time during the tremor process. This phenomenon also belongs to myotonia. Because it is transverse to the gear rotation law, it is called gear like rigidity. Myotonia generally does not occur in the early stage of Parkinson's disease, or it is not easy to detect the disease after it occurs, which is easy to be ignored. If you want to check in this period, you can let the patient move one limb. If you feel the muscle tension of the limb increases, it means myotonia appears.

3. Materials and methods

At present the main means is low click type and the lever type, low click type is on the bottom of the ball through attack the ball flew over obstacles, this method is able to pick the ball’s advantages and makes the energy loss in institutions least, the shortcoming is the ball high requirement of the shape of the electromagnetic valve [1]. Therefore, the development of a high-performance control system of soccer robot has become an urgent desire for soccer robot fans.

Subjects. Ten rats with no damage to the vestibular organs were selected by means of the elevation reflex and rotation test before the start of the study, and were housed in a special animal room with a humidity of approximately 40%-50% and a temperature of 20-26°C. All studies were carried out in accordance with the Regulations on the Management of Laboratory Animals and the "3Rs" principle, taking into account animal welfare and animal ethics.

Research equipment. The research equipment includes electrical stimulator and recording system, electronic balance, drying box, microscope, magnetic stirrer, freezing microtome, rat brain stereotyper, microshaker, pipette, etc. Drugs used for the study include formaldehyde, sodium nitroprusside chloride, tretinoin, etc. Research reagents include hydrogen peroxide solution, acetone, xylene, sodium hydroxide, paraformaldehyde, disodium hydrogen phosphate, etc.

Study methods. The aim of this study was to analyse the effect of the vestibular organ on c-FOS protein. Prior to the experiment, an electrical stimulation electrode implantation procedure needed to be carried out and the optimal stimulation parameters were selected based on the experimental results. A blank control group of 10 rats was surgically exposed to the horizontal semicircular canal for implantation of stimulation electrodes, but no stimulation parameters were given. In the observation group of 10 rats, electrodes were also implanted in the vestibular organs of the rats, but stimulation parameters were given.

It is important to note that there may be different types of stimuli, including temperature, photoelectricity, and chemical factors, which may cause the excitable tissue to respond, so the study used the most common method of electrical stimulation in animal physiology experiments. After all rats were anesthetized by intraperitoneal injection, the skin and other soft tissues were cut open to expose the horizontal semicircular canal, and the tip was inserted into the exposed protective electrode, which was fixed with dental cement for 24h before electrical stimulation was administered. All procedures are performed in a strictly sterile mode and to avoid infection, each rat is given an intramuscular injection of penicillin, followed by brain perfusion and immunohistochemical analysis to observe the results as required.

An electrical stimulation model was established in which electrodes were implanted in the vestibular organs at a current strength of 1mA, frequency of 50Hz, delay of 0.1ms, wave width of 1ms and time of 5min. c-FOS protein changes were analysed at the end of the study in the regions of MVN (medial vestibular nucleus), PF (parafascicular nucleus) and CPu (striatum).

Statistical analysis. The c-FOS protein data were integrated and analysed using SPSS 20.0 statistical software, where the measurement data were expressed as (mean ± standard deviation) and t-test was used for the outcome data between groups. $p<0.05$ indicated that the data differences were statistically

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significant.

4. Results

After stimulation of the vestibular organ, there was a significant increase in the expression of c-FOS positive neurons in the MVN area, while the number of immunopositive neurons in the PF area also increased. Although no direct nerve fibre connection was found between MVN and CPu, this index was also observed considering that CPu would be involved in the process of motor regulation, and the results also showed a significant increase in the expression of positive neurons in the observation group of rats. All three regions within the results showed an increase in c-FOS positive neuronal expression following non-invasive stimulation of the vestibular organ, with statistically significant differences ($p<0.05$). Specific data are shown in Table 2.

Table 2: Differences in c-FOS expression parameters in different regions

Group	Numbers	MVN zone	PF zone	CPu zone
Observation group	10	94.5±2.0	85.6±3.3	37.7±1.4
Control group	10	5.8±0.7	8.2±1.0	5.1±0.3
t	-	132.373	70.982	72.001
P	-	< 0.05	< 0.05	< 0.05

5. Discussion

Parkinson's disease is a neurodegenerative disorder most commonly seen in the middle-aged and elderly, characterised by neurodegeneration and apoptosis of dopaminergic neurons in the dense substantia nigra, as well as the formation of Lewy bodies in the pulp of the remaining neurons. Due to the slow progression of the disease itself, many patients experience only mild stiffness or tremors in the early stages of the disease, progressing to severe difficulty starting and loss of balance in the later stages of the disease. When these symptoms occur, it is an indication of varying degrees of damage to the function of the cerebellum. The etiology of Parkinson's disease is closely linked to genetic and environmental factors, as well as the effects of oxidative stress, excitatory neurotransmitters and immune abnormalities [1]. Oxidative stress, for example, is a typical state of imbalance in the body, manifesting itself as a conflict between oxidative and antioxidant reactions causing various diseases.

In this group of patients, the disease reaches an advanced stage, with severe limitations in locomotion, characterised by disorders of motor function. Physiologically, the body's motor function is a circular circuit of nerve impulses from the cerebral cortex to the basal ganglia, which are then analysed by the thalamus and returned to the cerebral cortex. The striatal system in the ganglia of the body, as part of the extravertebral system, together with the vestibulocerebellar system, regulates the motor function of the upper and lower motor neurons in the body [2]. In humans, as mammals, the cortical layer evolved to form the pyramidal tract, so the striatum plays a key role in motor regulation, receiving nerve impulses from cortical areas and having the ability to regulate the nervous system autonomously. In Parkinson's disease, however, the nigrostriatal lesions themselves can lead to an increase in the body's muscle tone causing difficulty in movement. In recent studies, it has been repeatedly mentioned that stimulation of the vestibular organs can improve motor dysfunction in Parkinson's patients and that MVN nerve fibres can project to the CPu via the PF [3].

Thus, in order to determine the functional links between the vestibular nervous system and the substantia nigra and striatum, this study also focused on c-FOS protein expression to determine whether there is a link between the body's ganglia and the vestibular system, which regulate motor function. The large genus that most closely resembles human disease characteristics was chosen as the model for this study, as it is simple and easy to observe, and can be used as a basis for establishing relatively accurate models of mechanical injury or viral infection.

The basal ganglia are deeper in the brain and there is a link between motor processes and movement processes, which are manifestations of the motor state and motor conduction pathways, and any lesion of the basal ganglia can lead to severe motor dysfunction. This suggests that from a pathophysiological point of view, models of basal ganglia injury lead to the development of motor behavioural deficits in humans, such as involuntary or purposeless hyperactivity, as well as difficulties in motor initiation and slowed movement amplitude. Parkinson's disease is a classic phenomenon of necrosis or hypersecretion of dopaminergic neurons, where many movements cannot be continued or are relatively slow [4].

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From the results of the data in this study, there was a significant trend towards higher expression of c-FOS positive neurons in the MVN area following stimulation of the vestibular organ in rats, along with a similar increase in the number of immunopositive neurons in the PF area. Although no direct nerve fibre connection was found between the MVN and CPu, this index was also observed in view of the involvement of CPu in motor regulation, and the results also showed a significant increase in the expression of positive neurons in the observed rats. This indicates that the vestibular system is involved in motor homeostasis and has a functional link with the ganglia of the body, which is also affected by lesions in the basal ganglia. The human brain contains different types of amino acids, of which glutamate, an excitatory amino acid, is a stimulant in the basal ganglia [5]. The pathophysiological basis of Parkinson's is the weakening of the GABA inhibitory system and the increase in intracellular calcium levels, resulting in cell body destruction and cytoskeletal disruption, leading to neuronal degeneration and death. Calcium-binding proteins in neurons provide some neuronal protection by preventing excessive elevation of calcium ions in the plasma, and after electrical stimulation of the vestibular nerve, some neural pathways in the basal ganglia of rats were altered after electrical stimulation [6].

In conclusion, stimulation of peripheral vestibular organs can induce functional changes in the basal ganglia and c-FOS protein-positive neuronal expression, providing a good therapeutic reference for future Parkinson's disease and modulating vestibular function in patients.

Acknowledgement

The Project to Improve the Basic Research Ability of Young and Middleaged Teachers in Colleges a nd Universities in Guangxi Autonomous Region (2021KY0498, 2020KY12007).

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