ОБЗОРНЫЕ СТАТЬИ

УДК 597.82:612.886

EVOLUTION OF THE VESTIBULOSPINAL SYSTEM'S SOMATOTOPY IN TETRAPODES

V.V.Fanardjian

/L.A.Orbeli Institute of Physiology, National Academy of Sciences of Armenia/
375028, Yerevan, 22 Orbeli Bros.St.

Key words: vestibular system, distribution of vestibulospinal neurons, evolution, frog

The motor structures in anurans is their extensive modification, reflecting the changes in the environmental (partial or full transfer to terrestrial habitat) and development of a tetrapod body form [2, 15, 22, 23, 27, 35]. Being an early stage for such an evolutionary process, anurans possess a less differentiated cerebellovestibular regions to any extant tetrapod[17]. Nevertheless, even at this level, the vestibular nuclear complex (VNC) represent central structures which regulate motor targets [3, 6, 24, 25, 31, 33]. In phylogenic series, the vestibular nuclei are considered to be in the first structures which stand apart from the reticular formation [1, 6, 23] and their tract fibers descending into the spinal cord form one of the old systems of supraspinal control [30]. The arrangement and the topography of the individual nuclei in the VNC of anurans are close to those of other tetrapodes. They are recognized as lateral (LVN), superior(SVN), descending (DVN) and medial (MVN) vestibular nuclei [14, 21, 26, 34, 36].In this respect the amphibians possess a pattern of VNC organization largely similar to the reptiles, birds and mammals. It is possible to guess that the evolution of the VNC has been more conservative than previously thought. According to some authors, the LVN (or ventral vestibular nucleus) is the source of the vestibulospinal tract (VST), the fibers of which descend bilaterally (mainly ipsilaterally) in the ventral funiculus of the spinal cord [6, 7, 8, 9, 14, 27, 35, 36].

In contrast to the sufficient evidence of anatomical and physiological data available for the anurans vestibular nuclei, very little information exists concerning the spatial organization of these nuclei outputs to the spinal cord.

The only morphological study on toads in this direction was carried out by D'Ascanio and Corvaja [9] by means of retrograde axonal transport of horseradish peroxidase. It was shown that within the ventral vestibular nucleus the rostrally located cells, which are mainly concentrated in dorsomedial position, project to the cervical cord; on the other hand, the caudally localized neurons, which are mainly concentrated in ventrolateral division, project down to the lower spinal segments. In others morphological works only fragmentary notices concerning spatial distribution of neurons as a source of the descending vestibulospinal fibers were brought [14, 35, 36].

As it is known, ordered mapping of the body surface onto central neural structures is called somatotopy. Somatotopy represents the important property of the brain which has received its highest development in the man. In the frog, as it was mentioned above, this property represents as one of the earliest stages in evolutionary standing of the brain and therefore it is at the sources of the origin of the somatotopy. In the pres-

ent review the properties of somatotopic organization of VNC of the frog (Rana ridibunda), electrophysiologicaly obtained on the basis of study of neuronal and synaptic activity of the vestibular system by mean of extra- and intracellular recording are considered. The vestibulospinal neurons were identified on the basis of excitatory postsynaptic potentials (EPSP) evoked on the stimulation of the ipsilateral vestibular nerve and antidromic activation from the stimulation of the cervical and lumbar enlargement of the spinal cord [11, 12, 29].

Field potentials in VNC evoked by stimulation of vestibular nerve and spinal cord

Field potentials in the ipsilateral VNC to stimulation of the VIII nerve consisted of two negative components, from which the first one was synchronized activation of vestibular afferent fibers (N_0), and the second one was postsynaptic activation of central vestibular neurons (N_1) [20, 29]. Latency of N_0 component appearance was 0.61–0.89 *ms* (mean 0.73±0.07 ms; n=64). Latency of postsynaptic N_1 component was 1.66–2.92 *ms* (mean 2.18±0.35 *ms*; n=82). Difference between these two indices is synaptic delay was equal to 1.20–2.60 *ms* (mean 1.83 ± 0.38 *ms*; n=41). It is necessary to note that synaptic delay in amphibians is about 1.0 *ms* [5]. The onset of N_0 component appearance peak was 0.77–1.58 *ms* (mean 1.08 ± 0.22 *ms*; n=95); rise time to peak of N_1 was 1.02-3.89 *ms* (mean 2.21±0.54 *ms*; n=92), period of fall up from peak to the half of N_1 amplitude was 0.67-5.48 *ms* (mean 2.85±1.08 *ms*; n=72) [11, 12]. All temporal properties induced by electric activity, like all the other indices, were dependent on temperature at which the experiment was carried out [32].

An increase of stimulation intensity of the vestibular nerve led to an increase of N_0 and N_1 components amplitude. The stability of the latency of N_1 component which confirmed its monosynaptic nature, as well as small shortening of N_1 component rise time and decrease of N_1 component time of fall up from peak to the half of the amplitude were noticed. The latter can be explained by the fact that by increasing stimulation intensity of the vestibular nerve thin afferent fibers start to activate leading to the recruitment of inhibitory interneurons which have a disynaptic inhibitory effect on the second order vestibular neurons [33]. The study of the depth profiles of field potentials demonstrates their major appearance at the depth of 100 *μm* from the surface of the brain stem and a maximum amplitude of N_1 component at the depth of 450–500 *μm* and a decrease of the amplitude in deeper recording sites, which is in agreement with the morphological boundaries of the VNC [21, 26, 34].

In response to the spinal cord stimulation in VNC the antidromic and synaptic field potentials, and more often their complex, which become more pronounced with the increase of stimulation intensity, were recorded. It was of great importance to differentiate between them for selection of antidromic field potentials. The latter consisted of primary positive deflection and a further more expressed sharp negative wave. It was demonstrated that the refractoriness in both tests is less than 1 *ms*, that let us suggest these field potentials as antidromic. On stimulation of the cervical enlargement of the spinal cord the antidromic field potentials appeared with a latency of 0.73–1.50 *ms* (mean 0.94±0.13 *ms*; n=843). They were defined as a result of antidromic activation of vestibulospinal neurons, projecting to or passing through the cervical part of the spinal cord (C-neurons). On stimulation of the lumbar part of the spinal cord, when vestibulolumbar neurons are activated (L-neurons), the antidromic field potentials were evoked with a latency of 1.26–2.43 *ms* (mean 1.61±0.21 *ms*; n=782).

The study of depth profile distribution of the antidromic field potentials for C- and L-neurons showed almost complete coincidence with that of field potentials evoked by

stimulation of the vestibular nerve. Maximal values of all three measurements were recorded at the depth of 500 *μm*. Antidromic field potentials of C- and L-neurons were recorded in MVN, DVN, and predominantly in LVN[13].

Intracellularly recorded from VNC neurons following vestibular nerve and spinal cord stimulation

The intracellular activity was recorded from 244 neurons of VNC. In 142 neurons when cells were impaled, chemically mediated excitatory postsynaptic potentials (EPSPs) were often seen. The latency of these EPSPs was measured by referring to the extracellular control potentials and was equal to 1.5–4.46 *ms* (mean 2.71±0.16 *ms*; n=142). These EPSPs arose sharply with a summit time of 1.5–7.3 *ms* (mean 3.16±1.9 *ms*; n=41), descending phase time (to the half of amplitude) reached 7.85 *ms*. Among these cells in 97 neurons the latency of EPSP does not exceed 3.0 *ms*. The latent period and rising phase of these EPSPs were changed insignificantly at different stimulation intensities of the vestibular nerve which allowed to consider these EPSPs as monosynaptic and recorded cells as second-order vestibular neurons [2, 29, 31, 33]. The onset of the N_1 wave after the vestibular nerve stimulation coincides with that of the monosynaptic EPSPs. Further gradual increase of stimulation intensity often evoked orthodromic excitation of impaled cells, the earliest of which appeared with the latency of 2.46–7.0 *ms* (mean 4.2±1.0 *ms*; n=44).

In response to the spinal cord stimulation in VNC the antidromic and orthodromic field potentials, and more often their complex were recorded [13,29]. When microelectrode was inserted into VNC a prominent fast negativity during stimulation of spinal cord was detected. This negativity developing with a brief latency was supposed to represent antidromic invasion of VST neurons. They were characterized with fixed and short latent period at different intensities of stimulation, short refractory period (1.0–2.0 *ms*), ability to reproduce high frequency stimulation of the vestibular axon, and absence of the preceding slow prepotential. The minimal decrease in the intensity of the threshold stimulation resulted in complete disappearance of the action potential, not a single sign showing any postsynaptic potentials. The cells that could be activated antidromically only by cervical cord stimulation have been designated C cells. This group of neurons includes cells projecting to the cervical, thoracic, and upper lumbar cord. Cells also activated antidromically in result of lumbar stimulation has been termed L cells, which projected to the lumbosacral segments. Antidromic action potentials of C and L neurons were characterized by the latency of 0.57–3.6 *ms* (mean 1.57±1.7 *ms*; n=121) and 1.3–3.9 *ms* (mean 2.18±2.25 *ms*; n=94) respectively. In the VST cells activated antidromically by stimulation at both C and L segments, the axonal conduction velocity was calculated from a distance of 7.0–13.0 mm (mean 9.55±9.66 *mm*; n=97) and the latency difference 0.21–3.2 *ms* (0.83±0.09 *ms*; n = 95) between these segments. In addition, the conduction velocity was determined from the measurements of the latent period of potentials evoked in C neurons by stimulation of the cervical cord allowing necessary modified correction [16]. The distances between the entrance of the microelectrode into the brain stem and sites of stimulation at the cervical level were 3.8–9.9*mm* (6.22±6.34; n=125). The conduction velocity thus determined for C and L cells varied in a wide range from 2.5 to 42.8 *m/s* (mean 13.04±15.085; n=236) with a peak frequency at 6–12 *m/s* [10].

The spatial distribution of antidromic potentials was based on the analysis of the nearly whole VNC in rhombencephalon. Rostrocaudal extension of the tracts recorded occupied an area from the posterior edge of the cerebellum up to the obex with a step

of 100 *μm*. The caudal end of the entry of the VIII nerve was taken as a zero level and positive direction was caudal. The most rostral and the most caudal tracks were recorded at -800 *μm* and 1700 *μm*, respectively. The vestibular nuclei were reconstructed considering the results of our studies and literature data available (*Rana esculenta*, [18, 26] *Rana catesbeiana* [21]). Their correspondence to the structural properties of VNC was given in *Rana ridibunda*, particularly in horizontal and frontal planes.

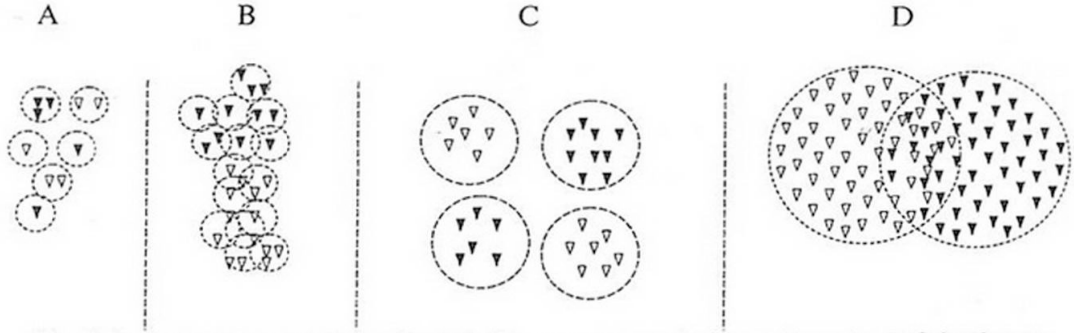

Fig. Schematic representation of patch-like somatotopy in frog (A) and zonal (regional) somatotopy in mammals (D). C and L vestibulospinal neurons are indicated by open and filled triangles respectively. There are partially overlapping of zones in the mammals and separate distribution of single C and L neurons or their small groups in frog. On B and C possible transition stages are given.

The analysis of distribution of antidromic field potentials of C and L neurons demonstrated that they were recorded in MVN, DVN and in LVN. The intracellular activity was recorded from 244 neurons of vestibular nuclear complex, of which 127 are C (52%) and 117 are L-neurons (48%). Antidromic potentials were recorded in the LVN (143 neurons, 58.6%), DVN (75 neurons, 30.7%) and MVN (26 neurons, 10.6%). Ratio between C and L neurons in the LVN was 78 (54.5%) and 65 (45.4%), in the DVN − 41 (54.6%) and 34 (45.3%), in the MVN − 8 (30.7%) and 18 (69.2%).

As was mentioned earlier the conduction velocity along frog C and L vestibulospinal fibers was 2.5 to 42.8 *m/s* (mean 13.04±15.08 *m/s*; n=236). The conduction velocity determined for C neurons is within the limits of 3.6-23.5 *m/s* (10.67±11.54 *m/s*; n=128) and for L neurons 2.5−42.8 *m/s* (15.84±18.42 *m/s*; n = 108). The difference of axonal velocities between C and L neurons was statistically significant ($p<0.001$). The cells having an axonal conduction velocity faster than 14 *m/s* were conventionally called 'fast' cells, and those below 14 *m/s* were called 'slow' cells; 88 cells (36%) were ranked as 'fast' and 156 (64%) as 'slow' cells. Among 127 C neurons 28 cells (22%) were ' fast' and 99 cells (88%) 'slow'. Among 117 L neurons 60 cells (51.2%) were 'fast' and 57 cells (48.8%) 'slow'. There was approximately equal amount of 'fast' and 'slow' L neurons in different vestibular nuclei of VNC: 52.3% and 47.7% for LVN, 50% and 50% for DVN 50% and 50% for MVN correspondingly. However, among C neurons in all the vestibular nuclei 'slow' cells prevailed over 'fast' ones [10].

Thus it became possible to reconstruct spatial distribution of the identified vestibulospinal neurons. However frog VST neurons, sending their axons to different spinal segmental levels, are not grouped together in fields as for mammals, which are characterized by a distinct outline and by considerable overlap between the lumbosacral, thoracic, and cervical regions of Deiter's nucleus. [4, 28]. Our present study has confirmed the assumption done earlier [13] that C and L neurons in the frog VNC, as

a source of vestibulospinal fibers, are scattered separately or more frequently in groups, so that they establish patch-like somatotopy and do not form distinctly designed fields. It is possible to assume that one of the ways of the evolutionary development of the somatotopic organization of brain might be the extension of representation sites of peripherals in the central structures. Eventually, the amount and grouping of neurons in the central structure should determine the qualification and accuracy of movements (Fig.1). There is an impression that the spatial distribution of vestibulospinal neurons in the frog VNC stopped at an early phase where the *topography* is established and didn't transfer to a later phase during which *somatotopy* emerges in mammals [19].

Поступила 21.11.00

ՉՈՐՔՈՏԱՆԻՆԵՐԻ ԱՆԴԱՍՏԱԿԱՅԻՆ ՀԱՄԱԿԱՐԳԻ ՄԱՐՄՆԱԿԵՐՏՎԱԾՔԻ ԷՎՈԼՅՈՒՑԻԱՆ

Վ.Բ. Ֆանարջյան

Անպոչ կենդանիների շարժողական կառույցները, կապված նրանց ապրելակերպի միջավայրի փոփոխության (մասնակի կամ ամբողջական անցում ցամաքային կյանքի) և քառավերջույթային մարմնի զարգացման հետ, ենթարկվել են էական ձևափոխությունների: Անպոչերի գլխվելը էվոլյուցիոն զարգացման վաղ շրջանում բնորոշում է նրանց անդաստրակա-ուղեղիկային հատվածի ամենափոքր տարբերակվածությունը՝ համեմատած այլ չորքոտանիների հետ: Գորտը իրենից ներկայացնում է ուղեղի էվոլյուցիոն ձևավորման վաղ շրջան և կանգնած է մարմնակերտվածքի առաջացման ակունքների մոտ: Աշխատանքում գորտի անդաստրակային կորիզների նեյրոնային և սինապսային կազմավորման էլեկտրաֆիզիոլոգիական ուսումնասիրության հիման վրա ապացուցվում է անդաստրակային համակարգի լաթային մարմնակերտվածքը, որն իրենից ներկայացնում է ավելի բարձրակարգ ողնաշարավորների, այդ թվում կաթնասունների, մարմնակերտվածքի ձևավորման վաղ փուլ:

ЭВОЛЮЦИЯ СОМАТОТОПИИ ВЕСТИБУЛО-СПИНАЛЬНОЙ СИСТЕМЫ У ЧЕТВЕРОНОГИХ

В.В.Фанарджян

Двигательные структуры у бесхвостых подвержены значительной модификации в связи с изменением среды их обитания (частичный или полный переход на сушу) и с развитием четырехконечностного тела. Нахождение бесхвостых на раннем этапе эволюционного развития определяет наименьшую дифференциацию их вестибуло-мозжечковых областей по сравнению с другими четвероногими. Лягушка представляет ранний этап эволюционного становления мозга и стоит у истоков возникновения соматотопии.

В работе на основании электрофизиологического изучения нейронной и синаптической организации вестибулярных ядер лягушки доказывается наличие лоскутной соматотопии вестибуло-спинальной системы, представляющей ранний этап становления зональной соматотопии у более высших позвоночных, включая млекопитающих.

REFERENCES

1. *Akaike T.* J.Physiol., 1983, 259, p.217.
2. *Babalian A.L. , Shapovalov A.I.* Brain Res., 1984, 309, p. 163.
3. *Barale F., Corvaja N., Pompeiano O.* Arch. Ital. Biol., 1971, 109, p. 27.
4. *Brodal A., Pompeiano O., Walberg F.* The Vestibular Nuclei and Their Connections. Springfield, Illinois. 1962
5. *Brookhart J.M., Fadiga E.* J.Physiol., 1960, 150, p.633.

6. *Corvaja N., Grofova I.* Progress in Brain Research , 1972, 37, p.297.
7. *Corvaja N., Grofova I.* Neuroscience, 1978, 3, p.619.
8. *Corvaja N., Grofova I., Pompeiano O.* Brain Behav. Evol., 1973, 7, p.401.
9. *D'Ascanio P., Corvaja N.* Arch. ital. Biol., 1981, 119, p.139.
10. *Fanardjian V.V., Manvelyan L.R., Nasoyan A.M. Proceeds. of the 3d Conference of Armenian IBRO Association. Yerevan, 2000.*
11. *Fanardjian V.V., Manvelyan L.R., Pogossian V.I. et al.* J. Evolt.Biochem., Physiol., 1998, 34, p.471. (In Russian)
12. *Fanardjian V.V., Manvelyan L.R., Zakarian V.L., Nasoyan A.M.* Russian Physiol. Journ., 1999, 84, p.1085.(In Russian).
13. *Fanardjian.V.V., Manvelyan L.R., Zakarian V.L., Pogossian V.I. Nasoyan A.M.* Neuroscience, 1999, 94, p.895.
14. 14. *Fuller P.M.* Brain Behav. Evol., 1974, 10, p.157.
15. *Gona A* . J.comp.Neurol., 1976, 165, p.77.
16. *Ito M., Hongo T., Yoshida M., Okada Y., Obata K.* Jap.J.Physiol., 1964, 14, p.638.
17. *Kappers A.C.U., Huber G.C., Grosby E.C.* The comparative anatomy of the nervous system of vertebrates, including man. New York, 1960.
18. *Kemali M., Braitenberg V.* Atlas of the Frog's Brain. Berlin, Heidleberg, New York., 1969.
19. *Killasckey H.P., Rhoades R.W., Bennett-Clarke C.A.* Trends Neurosci., 1995, 18, p.402.
20. *Kunkel A.W, Dieringer N.* J. Comp. Physiol., 1994, 174, p.621.
21. *Kuruvilla A., Sitko S., Schwartz I.R., Honrubia V.* Laryngoscope, 1985, 95, p.692.
22. *Larsell O.* J. Comp. Neurol., 1925, 39, p.249..
23. *Larsell O.* The Comparative Anatomy and Histology of the Cerebellum from Myxinoids through Birds. Minneapolis, 1967.
24. *Maeda M., Magherini P.C., Precht W.* J. Neurophysiol., 1977, 40, p.225.
25. *Magherini P.C., Precht W., Richter A.* Pflugers Arch., 1974, 348, p.211.
26. *Matesz C.* Neuroscience , 1979, 4, p. 2061.
27. *Montgomery N.M.* Brain Behav. Evol., 1988, 31, p.82.
28. *Pompeiano O., Brodal A.* Arch.ital.Biol., 1957, 95, p.166.
29. *Precht W., Richter A., Ozawa S., Shimazu H.* Expl. Brain Res., 1974, 19, p.377.
30. *Shapovalov A.I.* Neurons and Synapses of Supraspinal Motor Systems. Leningrad, 1975 (in Russian).
31. *Straka H., Biesdorf S., Dieringer N.* J.Neurophysiol., 1997, 78, p.1363.
32. *Straka H., Dieringer N.* Eur. J. Neurosci., 1993, 5, p.251.
33. *Straka H., Dieringer N.* J. Neurophysiol., 1996, 76, p.3087.
34. *Suarez C., Kuruvilla A. Sitko S., Schwartz I.R., Honrubia V.* Laryngoscope, 1985, 95, p.1238.
35. *Ten-Donkelaar H.J., De Boer-Van Huizen R., Schouten F.T.M., Eggen S.J.H.* Neuroscience, 1981, 6, p. 2297.
36. *Toth P., Csank G., Lazar G.* J.Hirnforsch., 1985, 1985, 26, p.365.