საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 2, №2, 2008
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 2, no. 2, 2008

Human and Animal Physiology

Antidromic Responses of Nucleus Accumbens Neurons to the Ventral Pallidum and Subpallidal Area Stimulation

Gogi Todua

I. Beritashvili Institute of Physiology, Tbilisi

(Presented by Academy Member T. Oniani)

ABSTRACT. 49 neurons in the nucleus accumbens (NA) were activated antidromically by electrical stimulation of the ventral pallidum (VP) and subpallidal area (SP). 6 units were activated antidromically from both structures, suggesting axonal bifurcation with projections to both stimulation sites. The antidromic spikes had constant latencies, between 8.5 and 25.5 ms. The relation of these findings to limbic influences on locomotor activity is discussed.

Key words: *antidromic response, latency, collision test, collateral.*

It is well established that forebrain limbic structures are important in the development and expression of emotional and motivational processes, but little is known about the neural mechanisms by which limbic processes gain access to the motor system. Graybiel [1] proposed that the NA is a key structure in the functional link between limbic forebrain structures and the motor system. It projects to the ventral analog of the globus pallidus, known as the VP and SP. In the present study projections of NA neurons to "limbic striatum" were explored using the technique of antidromic stimulation.

9 adult wistar rats, weighing 250-350 g. and anesthetized with uretan (1.15-1.2 g/kg. i.p.) were used as subjects. Both the femoral vein (for injection of drugs) and the femoral artery (for measurement of the arterial blood pressure) were cannulated and the animal was placed in a stereotaxic frame. The skull overlying the left amygdala, pallidum and NA was removed. The dura was excised over these areas to allow placement of electrode and the exposed cortical surface was covered with paraffin oil. Stainless steel concentric electrodes (0.5 mm. diameter, 0.5 mm. tip separation) were used for electrical stimulation. Monophasic square pulses of 0.1 ms. duration and 6-20 V intensity (corresponding to currents of 150-800 μA) were used. Stimulating electrodes were inserted into VP, SP and basolateral amygdala. An indifferent electrode was placed on the frontal bone. Effective stimulation sites were studied by moving the electrode systematically. Stereotaxic coordinates for stimulating and recording electrodes were taken with reference to the atlas of Pellegrino L. J., et al.

Extracellular recordings from the neurons in NA were obtained, using glass microelectrodes. They were filled with 2.5 M NaCl solution. Microelectrode impedance was 3-6 MΩ measured at 100 Hz in saline. Potentials were amplified and displayed on an oscilloscope for visual observation and photography.

After terminating the experiment lesions were made through stimulating electrode by passing direct current (300 μA for 10 sec). The animals were killed with an overdose of nembutal and perfused with formaline. The brain was then removed from the skull, fixed with 10% formaline and frozen for sectioning.

Action potentials recorded from individual neurons were classified on the basis of responses as being either orthodromic or antidromic in nature. Criteria for identification of antidromic responses were: 1. Stability of latency; 2. Faithful responses to high rates of stimulation (above 300Hz); 3. Collision of the antidromic response with an orthodromically traveling action poten-

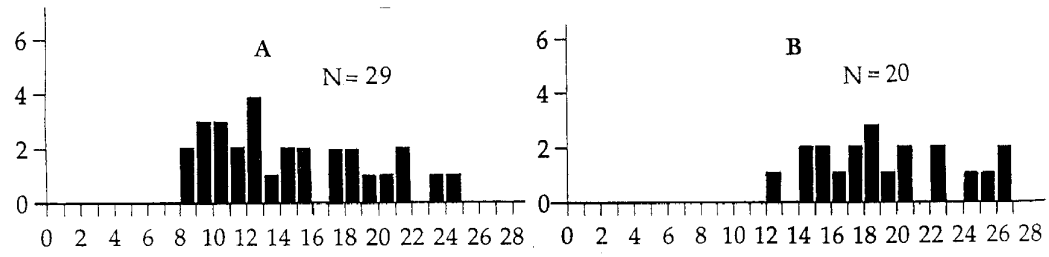

Fig. 1. Latency histograms of antidromic responses of NA units to VP (A) and SP (B) stimulation. Abscissa latency in ms, ordinate number of units.

tial. As antidromically activated units did not fire spontaneously, in order to perform a collision test the action potentials in response to amygdala stimulation were used to trigger the SP and VP stimulus. Biphasic spikes of duration of less than 1 ms., indicative of recording from fibers of passage, were not included in the result.

Recordings were made from 180 neurons in the NA. A total of 49 of these neurons were activated antidromically. The antidromic action potentials were of 1.0-3.2 ms duration, 0.5-2.5 mV amplitude and positive-negative in configuration. Most of the responses showed an IS-SD break which suggest that recordings were made from soma. The antidromic spike of 11 units did not differ in their configuration from orthodromic spikes.Possibly the interval between the two components may be too brief for detection by our relatively crude recording procedure. Antidromic responses had constant latencies between 8.5 to 25.5 ms. (Fig. 1. A, B) Typical antidromic action potentials elicited in VP and SP stimulation are shown in Fig. 2. Units responded regularly to a brief train of stimuli, developed at high frequency (A1,B1). 5 superimposed sweeps indicate the stability of latency. In many cases only the initial segment component remained even in cells that responded to 1 Hz stimulation by a full spike 100% of the time. The B spike followed frequencies of up to 300 Hz in all units and more than 400 Hz in a few units. The antidromic nature of these responses was further verified by their colliding with orthodromic spikes, occurring in response to basolateral amygdala stimulation (A 2.3; B 2.3). Fig. 2 illustrates the responses of 3 units to SP stimulation, following faithfully high frequency stimulation by a pair of pulses, delivered 2.5 ms apart (corresponding to 400 Hz stimulation). Antidromic spikes were followed by an inhibition phase. With intensities of stimulation just above the threshold evoking the antidromic spike, the duration of inhibition was 70-110 ms. Increasing the stimulus intensity resulted in prolongation of the inhibitory phase up to 150-180 ms. The conduction velocity, based on the distance of 3.0-4.2 mm between the stimulating and recording electrodes, was established to be 0.8-1.4m/sec for 24 neurons. Conduction time of axons was estimated by subtracting 0.2ms (latent period for spike generation).

6 neurons were activated antidromically by stimulation of both the VP and SP, suggesting axonal bifurcation with projections to both stimulation sites. Recordings from one of these neurons are shown in Fig. 3. This unit responded to VP and SP stimulation with stable latency of 10.5 and 14.5 ms. respectively Fig. 3 A1, B1. with a suitable delay orthodromic excitatory response of the same silent NA neuron to basolateral amygdala stimulation "collided" with antidromic spikes elicited by VP and SP stimulation (Fig. 3 A2.3, B2.3). The diagrammatic cell with branched axon illustrates the explanation of these findings (Fig. 3C). To measure the conduction time along the branches the following parameters are determined: 1. Latent period of antidromic activation to stimulation of axon branches (L_B and L_C); 2. The reciprocal collision time (I_{BC} and I_{CB} when the stimulus order was reversed). The reciprocal collision time is the maximal interval between the stimuli, applied to each site which produces a blockade of the second antidromic spike (in our examples 18.6 and 23.4 ms respectively Fig. 3 D1,2, E1,2); 3. The duration of absolute refractory period to stimulation of axonal branches.Output units with branched axons responded orthodromically to basolateral amygdala stimulation and the absolute refractory period of their axon was estimated using Swadlow procedure [2] which allows determination of the refractory period at the stimulating site. This method con-

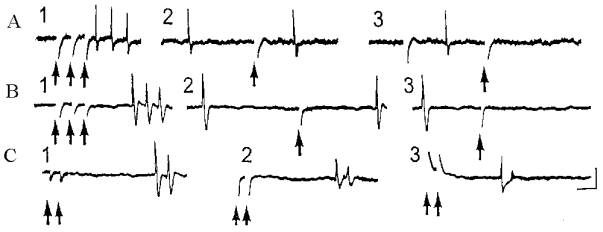

Fig. 2. Responses of NA neurons to VP (A.B) and SP (C) area stimulation. A1,B1. Responses to VP stimulation with trains of three stimuli, delivered 3 ms apart. Five sweeps were superimposed. Application of the collision test. A2,B2. When the interval between orthodromic spike and antidromic stimulation was more than the critical delay period antidromic spikes occured. A3,B3. Disappearance of antidromic spike by collision with orthodromic spike. C1,2,3. Antidromic spikes faithfully followed double stimulus to 2.5 ms interval with constant latency. Upward deflection is positive. Calibration 0.2 mV 4 ms.

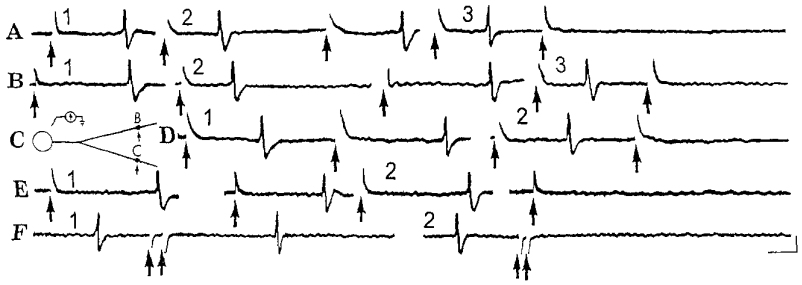

Fig. 3. NA cell invaded antidromically by stimulation of both VP (A) and SP (B). A1B1. Superimposed oscilloscope traces illustrate constant latency of responses. A2B2. Antidromic responses of these units when interval was more than critical. A3B3. Antidromic responses of NA neurons to VP and SP stimulation "collided" with orthodromically evoked spikes from amygdala, when the delay interval between orthodromic spike and antidromic stimulation was shortened. C. schematic drawing of stimulation of two axonal branches (B and C – points of stimulation). D. E. Measurement of collision time between SP and VP branches. D1. Neuron responded to SP stimulus, when it was applied 20 ms. after the VP stimulus. D2. Neuron did not respond to SP stimulus when the interval between the SP and VP stimuli was 18.6 ms. E. when the order of stimuli was reversed to VP stimulation neuron responded at an interstimulus interval of 25 ms [1], but when interval was 23.5 ms a response to test stimuli did not occur [2]. Four superimposed traces. F. Refractory period test conducted with Swadlow's procedures. The interval between the orthodromic action potential and the C pulse was 5 ms. Note the absence of a C pulse response due to collision. T pulse response was observed at 2 ms interval between C-T stimuli [1], but not at 0.9 ms [2]. Calibration 0.2 mV 4 ms.

sists in delivering a pair of antidromic pulses; conditioning (C) and test (T) pulses strongly (half of the antidromic latency) after the initiation of the orthodromic spike. The antidromic potential, initiated by C pulse always collides with the orthodromic action potential and never invades the cell body/initial segment. Such a stimulation procedure allows the cell body /initial segment to fully recover from refractoriness before the action potential initiated by T pulse reaches that neuronal segment. When the interval between the C and T pulses is reduced to less than the duration of the refractory period at the site of stimulation no spikes will be seen at the site of stimulation. The utility of this test is illustrated in Fig. 3F. When two stimuli were applied at interval of 2.0 ms a response to T stimulus occurred. A T pulse response disappeared when the C-T interval was shortened to 0.9ms. Thus, for the above cell the refractory period of stimulated axonal segment as determined by this method was 0.9ms. and significant discrepancy was seen to exist between the refractory period as determined traditionally. In our experiments refractory period estimates obtained with Swadlow procedure ranged from 0.7 to 1.6ms. When traditional standard double pulse stimulation technique is used action potentials initiated by both the C and T pulses invade the cell body/initial segment.The failure to record a T pulse response may be due to refractoriness of the cell body/initial segment, rather than the stimulation site.

We concluded the existence of the axon collateral when the reciprocal collision time was greater than the difference between the latencies of the two antidromic responses plus the refractory period of the second axonal branch stimulation. If the same neuronal branch was stimulated at proximal and distal sites the collision time should be equal and not greater than the sum with maximal estimated error of 0.3ms. The conduction time X_C between the branching point of axon and the site of stimulation in VP may be calculated as follows: $X_C=½ [I_{BC}+L_C-L_B-R_C]$. When the sequence of C and T stimuli was reversed the conduction time in the second branch of axon from the branching point to the site of stimulation in SP was calculated using the following formula $X_B=½[I_{CB}+L_B-L_C-R_B]$ [3]. In our experiments for all units with axon collaterals the value X_C was 4-8.3 ms and the conduction time X_B ranged from 5.2-13.6 ms.

NA can be considered to be a "paralimbic" structure [4]. Although its cytoarchitectonic appearance resembles that of the caudate nucleus, it does receive important input from the amygdaloid complex, hippocampus and other limbic structures [5-7]. It has strong projections to structures involved in motor programing – to VP and SP [8.9]. VP was considered to project to the subthalamic nucleus and the thalamus component of the motor system. SP projects to the regmental pedunculopontine nucleus, which is a mean component of the mesencephalic locomotor region [10]. Electrical stimulation of these structures has been shown to elicit locomotor activity [11, 12]. It has been suggested that subpallidal-pedunculopontine neurons contribute to the locomotor activity of behaviors, associated with the limbic system and have a role in limbic control integration.

Findings from this study support suggestions that the NA may be a functional interface between limbic and motor systems. As identified by antidromic activation to single pulse stimulation of SP and VP in NA 27% and 14% of units responded antidromically. The application of reciprocal collision test suggests that at least some NA neurons give terminals to both structures. Projections from NA to SP and VP may be functionally significant pathways by which limbic structures may influence the motor system.

ადამიანისა და ცხოველთა ფიზიოლოგია

N. accumbens-ის ნეირონთა ანტიდრომული პასუხები ვენტრალური პალიდუმის და სუბპალიდური მიდამოს გაღიზიანებაზე

გ. თოდუა

ი.ბერიტაშვილის ფიზიოლოგიის ინსტიტუტი, თბილისი

(წარმოდგენილია აკადემიკოს თ. ონიანის მიერ)

ნაშრომში შესწავლილია N. accumbens-ის ნეირონთა ანტიდრომული პასუხები ვენტრალური პალიდუმის და სუბპალიდური მიდამოს გაღიზიანებაზე. ანტიდრომულ პასუხთა იდენტიფიკაცია ხდებოდა ტრადიციული კრიტერიუმების საფუძველზე. 49 ანტიდრომულად მოპასუხე ნეირონიდან 6 განიცდიდა ანტიდრომულ აქტივაციას ორივე სტრუქტურიდან, რაც მიუთითებს N. accumbens-ში დატოტვილაქსონიანი ნეირონების არსებობაზე. ი. შინოდას და სხვა ავტორთა მიერ მიღებული განტოლებების მეშვეობით გამოთვლილ იქნა აქსონთა სხვადასხვა უბნებში აგზნების გასატარებლად საჭირო დრო. აქსონებში აგზნების გატარების სიჩქარე ცვალებადობდა 0.6-0.9 მს-ის ფარგლებში. N. accumbens-ის მჭიდრო ფუნქციური კავშირები ერთი მხრივ წინა ტვინის ლიმბურ სტრუქტურებთან, ხოლო მეორე მხრივ ექსტრაპირამიდულ სტრუქტურებთან მიუთითებს, რომ იგი წარმოადგენს ერთ-ერთ მნიშვნელოვან შუალედურ რგოლს, რომლის მეშვეობითაც ხდება თავის ტვინის ლიმბური სტრუქტურების გავლენა მამოძრავებელ სისტემაზე.

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Received April, 2008