საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 2, №3, 2008
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 2, no. 3, 2008

Human and Animal Physiology

Antidromic Identification of Output Units of Basal Nucleus of the Amygdaloid Complex Projected to Hippocampus: A Study Combined with Intracellular Staining

Gogi Todua

P. Anokhin Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow
I. Beritashvili Institute of physiology, Tbilisi

(Presented by Academy Member T. Oniani)

ABSTRACT. Extra and intracellular recordings were made from antidromically identified neurons of basal nucleus [BN] of the amygdaloid complex, due to ammonic subfields stimulation. For identification of antidromic responses traditional criteria were used. Most effective was CA1 subfield stimulation. 12 units were recorded intracellularly. Among them 9 units were stained with biocytin and analyzed morphologically. The somata of identified neurons were pyramidal or polygonal in shape and the mean size was 22.5 x 17.2 μm. It is proposed that connections between amygdaloid complex and hippocampus may be important in the limbic memory and learning system. *© 2008 Bull. Georg. Natl. Acad. Sci.*

Key words*: basal nucleus, hippocampus, antidromic response, latency, learning, memory.*

Extensive study in both man and experimental animals has demonstrated that the medial temporal lobe has an important role in emotional behavior, learning and memory. Recent experiments with monkeys indicate that normal learning and memory are dependent upon the conjoint functions of three medial temporal lobe structures: the hippocampal formation, amygdaloid complex and entorhinal cortex [1, 2]. The hippocampus (i.e. the dentate gyrus, the ammonic subfields CA1-CA4, the presubiculum and subiculum) has been long considered the critical structure for learning and memory [3, 4], as bilateral pathology in men has been associated with a severe and lasting memory deficit. On the other hand, there is also substantial neuropathological and experimental evidence demonstrating an important role for the amygdala in memory function [5, 6]. Experimental studies in animals have demonstrated that lesions of each structure alone produce a significant memory deficit and combined lesions of two of these structures (i.e. amygdaloid complex plus the hippocampal formation) produce a more severe memory deficit. Taken together, these studies suggest that normal learning and memory is dependent upon the conjoint function of at least the hippocampus and amygdala. In order to determine the potential morphological basis of this interaction we have investigated the projections from BN of the amygdaloid complex to the ammonic subfields. In the present study the output units in BN were identified via antidromic stimulation of CA1-CA4 regions. Main attention has been devoted to the distribution and characterization of some electrophysiological properties and morphological features of these neurons.

The data were obtained from experiments performed on 14 unanesthetized adult cats immobilized with d-tubocurarine chloride (1.8 mg/kg i.v.). The surgical operations were performed under ether anesthesia. Analgesia was then discontinued and at least 2 h. elapsed before recordings were made. The head was fixed in a stereo-

taxic instrument. Atropine (0.05 mg/kg i.m.) was administered to prevent secretion. The body temperature was maintained at 37-38° C with heating pad. Cranium was exposed, the dura retracted and brain covered with pool of refined oil. Wound edges and pressure points were anesthetized with 2% lidocaine solution. Three stimulating electrodes (outer diameter 0.3 mm, tip distance 0.2 mm) were inserted sterotaxically into ammonic subfields according to the stereotaxic atlas [7]. The hippocampus was stimulated with pulses (100-350 μA, 0.1 ms, 0.1 Hz) produced from Grass S88 constant current stimulator. Glass micropipettes, with tip diameter less than 1.0 μm filled with 3M KCl solution and 2-8MΩ impedance were used for extra and intracellular recordings. Signals from microelectrodes were filtered, amplified and displayed on an oscilloscope for visual observation and photography.

The criteria for the antidromic responses were as follows: constant latency, a short refractory period (usually of less than 2 ms.), no preceding prepotentials, ability to follow high frequency repetitive stimulation and collision with an orthodromic spike.

Following electrophysiological analysis biocytin was injected iontophoretically with 2nA depolarizing current (300 ms. duration, 1-2 Hz) for 2-10 min. At the end of experiments animals were deeply anesthetized with nembutal (40 mg/kg i.p.) and perfused with buffered 7% formalin through the ascending aorta. Brains were dissected immediately and stored in 20% sucrose buffer solution overnight. Serial sagittal sections at 60 μm in thickness were cut on a freezing microtome. The sections were processed with biotin-avidin complex to visualize the biocytin-injected neurons by HRP histochemical reaction [8]. The morphology of stained neurons was examined with light microscopy and those thus selected were reconstructed with camera lucida drawing.

Studies were made of 39 BN output neurons, identified by their antidromic response to hippocampal formation stimulation. Among them 30 units were recorded extracellularly and 9 intracellularly. All of these units were tested for their response to electrical stimulation of different ammonic subfields. Effective stimulation sites were studied by moving the electrode systematically. The final site of stimulation was then marked and verified histologically. Most effective was CA1 subfield stimulation. CA3 and CA4 subfields were ineffective.

Extracellular antidromic action potentials were of 1.2-2.1 ms. duration, 0.5-1.2 mV amplitude and positive-negative in configuration. Most of these responses showed an 1S-SD break, which suggests that recorded action potentials were soma-dendritic and not of axonal origin. Fig. 1 displays examples of extracellularly recorded antidromic spikes. These responses satisfied all criteria for antidromic activation. These units followed two (AB) and three (C) shocks applied at 400 Hz frequency. Each record shows 5 superimposed sweeps. The antidromic nature of these responses was shown further by collision test. Collision was achieved by applying stimulation of ammonic subfields at progressively shorter interval following an orthodromic action potential, until cancellation of CA1-CA2 stimulus evoked response occurred. This interval was compared with the predicted critical interval, which is equal to the latency plus the refractory period of this neuron. That is C = L + R.

Collision between antidromic spike and spontaneously occurred action potentials are rarely observed, because in our experiments the spontaneous activity of BN units is very low to absent. In order to perform the collision test, action potentials in response to orbital gyrus or posterior sylvian gyrus stimulation were used to trigger. Fig. 1 D.E. demonstrates the application of the collision test. Unit responded to CA1 region stimulation with stable latency of 6.6 ms. Fig. 1 D shows control response, where both orthodromic and CA1 responses were elicited. When CA1 stimuli were delivered in a period of less than 6.9 ms after preceding orthodromic spike CA1 response failed to occur [E]. 8 units did not respond to cortical stimulation and collision test could not apply to these units, but they satisfied the remaining criteria. Fig. 2 illustrates an example of antidromic action potential, recorded intracellularly. Superposition of 5 raw traces shows the stability of the antidromic spike latency. The spike rose to its peak in around 0.5 ms., with a spike duration of

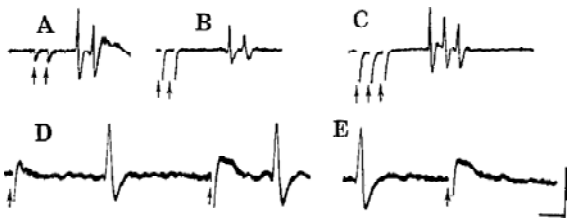

Fig. 1. Antidromically evoked spikes recorded extracellularly. The response of BN neurons to stimulation of hippocampal formation with twin [A. B] and three [C] pulses, with interstimulus pulses of 2.5 ms. [400 Hz frequency]. Superposition of 5 raw traces showing the stability of the antidromic spike latency. D.E. Collision of action potential elicited by hippocampal formation stimulation with action potential elicited synaptically in the neuron by orbital gyrus stimulation. When the orthodromic spike was followed 8 ms. later by stimulation of hippocampus antidromic spike was elicited [D]. The hippocampus stimulating pulse collides with orthodromic spike at an interval of 6.6 ms. Calibration bar 1 mV, 5 ms. Upward deflections are positive.

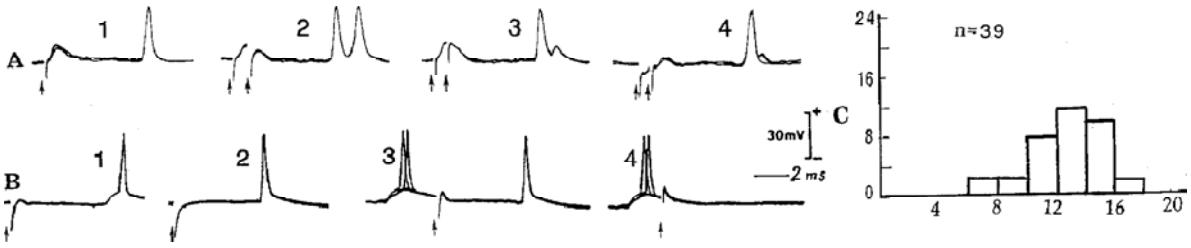

Fig. 2. Intracellular recordings from antidromically activated BN units.
A1. Five superimposed sweeps illustrate constant latency of antidromic spike. Donward oblique arrows indicate 1S-SD inflection. Note the absence of prepotentials. A2-4 responses to antidromic double stimulation with interstimulus interval of 2 ms. [A2] 1.6 ms. [A3] and 1.2 ms. [A4] stimulus strength was at thresholds. B. Application of the collision test. unit responded orthodromically to orbital gyrus stimulation [B1] and antidromically from CA1 region stimulation [B2]. Stimulus applied 9 ms. after orthodromic spike consistently evoked antidromic spike [B3] Stimulus applied 8 ms. after orthodromic spike did not evoke antidromic spike, therefore consistent collision [B4].
C. Latency histogram of antidromic spikes in response to hippocampal formation stimulation. Abscissa: latency in ms, Ordinate: number of units.

slightly more than 1 ms. Refractoriness of this neuron was studied by a double shock technique (A2-4). With time interval of 2 ms. between shocks applied to the CA1 region the IS-SD infection of the second spike became more prominent and the spike duration was prolonged (A2). With 1.6 ms. shock interval the test SD spike was blocked, leaving only the IS spike [A3]. The IS spike was at threshold of firing at 1.2 ms. time interval [A4]. Fig. 2B shows the response of another unit. This unit responded to orbital gyrus stimulation orthodromically [B1]. In Fig. B4 the orthodromic spike precedes the antidromic ones by a time interval of 8 ms, preventing the appearance of the antidromic spike.

The latencies of antidromic responses ranged from 6.5 to 16.2 ms. The latency histogram of antidromic spikes is shown in Fig. 2C. Based on the estimated distance between stimulating and recording electrodes, the response latencies of these cells corresponded to conduction velocities of 0.8-1.1 m/sec.

In the present study 8 neurons were stained with an intracellular injection of biocytin in order to provide morphological information about the population from which recordings were obtained. 6 neurons were analyzed morphologically in detail. In Table 1 the values of the somatic size, cell volume and the radius of the dendritic domain are listed with electrophysiological values.

Fig. 3 shows photomicrographs of intracellularly stained BN neurons in the frontal [A-B] and sagittal section [C-D]. Their somata were pyramidal or polygonal and the sparsely spinous dendrites extended radially. The average size of the dendritic fields varied among neurons. Mean size was 180-260 μm. The mean somatic size was 22.4 ± 5 x 16.2 ± 4.3 μm.

The present investigation describes the efferents that directly connect the amygdala to hippocampal formation. By using complementary physiological and morphological methods the specific sites of termination as well as the cells of origin of these efferent projections have been determined. It is well established that the hippocampus plays an essential role in memory function and emotional behaviors. Studies investigating brain system and memory in rats, monkeys and humans pro-

Table 1

Morphological features and electrophysiological properties of morphologically identified neurons

№ of unit	Cell size LxS μm	Cell volume μm^3	Dendritic radius μm	Antidromic latency ms	Absolute refractory period ms	Relative refractory period ms
1	24 x 11	3637	190	6.2	1.1	1.7
2	26 x 10	3991	280	7.9	1.2	1.9
3	24 x 22	5339	230	7.1	1.1	2.1
4	20 x 18	3610	210	8.2	1.0	1.7
5	22 x 20	3920	300	11.5	1.1	1.6
6	26 x 12	1862	198	13.8	1.4	1.8

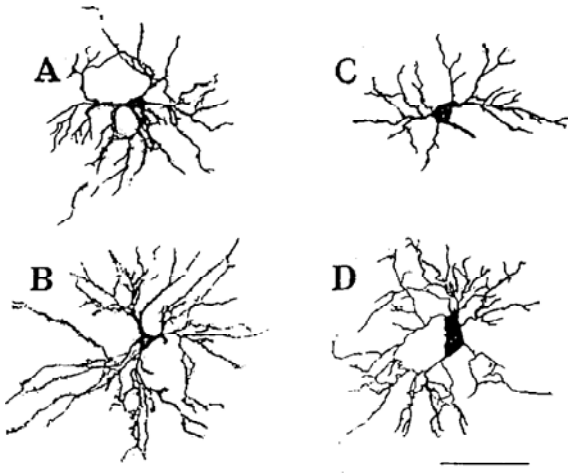

Fig. 3. Photomicrographs of biocytin-stained BN neurons. A-B Photomicrographs of BN neurons in the frontal plane. C-D Photomicrographs of BN neurons in the sagittal plane. Calibration length 100 μm.

vide evidence suggesting that the hippocampus is critical for only one "kind of memory" or one form of memory representation. The hippocampus is critically involved in cognitive [4], spatial [9], or declarative [10] memory. It has been suggested that the amygdala and in particular the basolateral amygdala modulates hippocampus dependent memory system [11, 12]. Priming stimulation of the basolateral group of the amygdala resulted in an enhanced long-term potentiation in the dentate gyrus, to prefrontal path stimulation. There is abundant evidence that emotional stress can either improve or impair learning, depending on the severity and context [13-15]. An intact basolateral part of amygdala is required for memory modulating processes initiated by infusion of drugs administered into the hippocampus [16, 17]. Lesions of the basolateral amygdala but not central nucleus attenuated the induction of population spike of long-term potentiation in dentate gyrus in vivo [17]. A. Akirav and G. Levin established that the amygdala has a biphasic effect on hippocampal plasticity and immediate excitatory effect and long-lasting inhibitory effect [18]. Direct connections between the amygdala and hippocampus, described in the present study may provide an anatomical basis for the influence of amygdaloid complex on hippocampal dependent memory.

ადამიანის და ცხოველთა ფიზიოლოგია

ჰიპოკამფში პროეცირებული ამიგდალური კომპლექსის ბაზალური ბირთვის ნეირონთა ანტიდრომული იდენტიფიკაცია: გამოკვლევა შიგაუჯრედული შეღებვის თანხლებით

გ. თოდუა

პ. ანოხინის სახელობის უმაღლესი ნერვული მოქმედების და ნეიროფიზიოლოგიის ინსტიტუტი, რუსეთის მეცნიერებათა აკადემია, მოსკოვი
ი. ბერიტაშვილის ფიზიოლოგიის ინსტიტუტი, თბილისი

(წარმოდგენილია აკადემიკოს თ. ონიანის მიერ)

ამონის რქის გაღიზიანებაზე ანტიდრომული პასუხების საფუძველზე იდენტიფიცირებული ამიგდალური კომპლექსის ბაზალური ბირთვის ნეირონები შესწავლილ იქნა უჯრედგარე და უჯრედშიგა გამოყვანით. ანტიდრომულ პასუხთა იდენტიფიკაციისთვის გამოიყენებოდა ტრადიციული კრიტერიუმები. ყველაზე ეფექტური იყო CA1 ველის გაღიზიანება. 12 უჯრედი შესწავლილ იქნა შიგაუჯრედული გამოყვანით. მათგან

9 შეღებილ იქნა ბიოციტინით და შემდეგში განხორციელდა მათი მორფოლოგიური ანალიზი. იდენტიფიცირებულ ნეირონებს ახასიათებდათ პირამიდული ან პოლიგონალური ფორმის სხეული და მათი საშუალო ზომა შეადგენდა 22.5 x 18.2 მკმ-ს. განხილულია ამიგდალურ კომპლექსსა და ჰიპოკამფს შორის არსებული პირდაპირი კავშირების შესაძლო როლი ლიმბური სისტემის მონაწილეობით მიმდინარე მეხსიერებისა და დასწავლის პროცესებში.

REFERENCES

1. *R.C. Saunders, E.A. Murray, M.I. Mishkin* (1984), Neuropsichologia, **22**: 785-96.
2. *E.A. Murray, M.I.Mishkin* (1986), J. of Neurosci., **6**:1991-99.
3. *D.M. Bannerman, J.M. Rawlins, S.B. Hugh, R.M. Deakon* (2004), Neurosci. Behav. Rev., **28**: 273-83.
4. *M.A. Trivedy, G.D. Coove* (2004), Neurobiol. Learn. Mem., **81**: 172-184.
5. *A.L. Cahil., J.L. McGaugh* (1996), Corr. Opin. Neurobiol., **6**: 237-42.
6. *M.B. Potent, C.A. Tomaz* (1992), Behav. Neurosci., **106**: 791-99.
7. *H.A. Jasper, A.N. Ajmone-Marsan* (1954), Stereotaxic atlas of cat's brain, Ottawa.
8. *K.A. Aoricawa, W.A. Armstrong* (1988), J. Neurosci. Meth., **25**: 1-11.
9. *R.E. Hirsh* (1973), Behav. Biol., **12**: 421-442.
10. *J.K. O'Keefe, L.M. Nade* (1978), The hippocampus as a cognitive structure. New York.
11. *N.A. Cohen Squire L*. (1980), Science, **210**: 207-209.
12. *M.G. Packard, L.M. Cahil, J.A. McGaugh* (1994), Proc. Natl. Acad. Science,USA, **91**: 8477-81;
13. *B.A. Roozentaal, J.A. McGaugh* (1996), Neurobiol. Learn. Mem., **65**: 1-8.
14. *H.A. Anisman, G. Bignami* (1978), Psychopharmacology of aversively motivated behavior. New York;
15. *J.I. Martinez, R.A. Jensen, R. Messing* (1981), Endogenous peptides, learning and memory system. New York;
16. *B.A. Roozentaal, J.A. MsGaugh* (1997), Neurobiol. Learn. Mem., **67**: 176-79.
17. *Y.A. Ikegaya, H. Saito, K. Abe* (1994), Br. Res., **656**: 157-64.
18. *I.A. Akirav, G.R. Levin* (1999), J. of Neurosci., **19**, 23: 10530-10535.

Received May, 2008