Structural and functional relationship between the basal forebrain and the medial prefrontal cortex

Erika Gyengési

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Dr. Anna Erdei, D. Sc.

Supervisor:

Dr. László Détári, D.Sc.

Eötvös Loránd University,

Pázmány P. Stny. 1/C

Budapest, 1117

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Introduction

The basal forebrain (BF) has been intensively studied for decades in relation to many physiological processes such as the sleep-wake cycle regulation, attention, learning and memory consolidation (Detari et al., 1984;Lee et al., 2004;Szymusiak, 1995;Zaborszky and Duque, 2003). Among the different neuronal populations, the cholinergic corticopetal projection neurons have received particular emphasis due to their prominent role in the activation of the neocortex (Jones, 2004). The BF cholinergic neuron send abundant innervations to many parts of the brain, including the neocortex and receives numerous inputs from other brain areas; however the prefrontal cortex (PFC) is the only cortical area that sends direct, presumably excitatory projections back to the BF from the higher cortical regions (Zaborszky et al., 1997). The prefrontal cortex is associated with higher cognitive functions, such as attention, planning, working memory and other phenomena like behavioral inhibition, cognitive flexibility, and goal directed control (Goto et al., 2009). Under urethane anesthesia in rats, electroencephalogram (EEG) recorded from the neocortex revealed a characteristic slow (<1 Hz) rhythm, so called cortical Up and Down states (Steriade, 1993). It has been shown that the rhythm of the cortical Up and Down states is transmitted to subcortical structures, including the BF, via direct or indirect pathways and the coupling depends on the strength of cortical synaptic input to these subcortical structures (Bartho et al., 2007). Consequently, the prefrontal cortical input to the BF represents an extraordinary and influential link that has not been extensively studied yet. Hence, this thesis is aimed to examine the functional and anatomical connection between PFC and the BF using electrophysiological and anatomical methods.

Proposal

Electrophysiological experiments

The aim of our experiments presented in this thesis was to investigate the effects of medial prefrontal cortex stimulation and the cortical Up and Down states on BF neurons and further investigate the anatomical nature of the link between the BF and the medial PFC (mPFC). We addressed the following questions in acute, in vivo experiments on urethane anesthetized rats:

- Do the spontaneous changes of cortical activity affect the activity levels of BF neurons? If so, what are the temporal associations between spontaneous cortical Up and Down states and single units in the BF?
- How does the stimulation of the mPFC affect the firing properties of single BF neurons? How is the temporal relationship between the effect of the mPFC electrical stimulation and BF unit activity?
- Do the neuron populations that respond to either PCF stimulation or to spontaneous changes of cortical Up and Down states represent well segregated, anatomically differentiated cell groups in the BF?

Anatomical experiments

Using anatomical tracing methods, electrical lesions and immunohistochemistry, we were looking for the answers of the following questions:

- What is the projection pattern of the descending axons from the mPFC to the BF?
- Is there another neuron population, besides the parvalbumin (PV) containing neurons in the BF that receives direct input from the mPFC?

Methods

Electrophysiology

Animals: Experiments were performed on male Wistar rats (n=31) weighing 250-350 g. Animals were anesthetized with urethane (1.0-1.2 g/kg, i.p.). Supplementary doses of urethane were also given when slow wave cortical activity decreased.

Electrical stimulation of the medial prefrontal cortex: Electrocortical activity (ECoG) was recorded by a transcortical bipolar electrode in urethane anesthetized rats at A: +2.0mm, L: 2.0 mm from the Bregma through a small drilled hole on the right hemisphere. Single unit activity from BF neurons was recorded with glass capillary filled with 1.5-3% Biocytin. Following a 5-10-minute baseline recording three series of stimulus trains each was given at different intensities to the medial prefrontal cortex. After the stimulation was completed, an attempt was made to label the recorded neurons using the juxtacellular filling method (Pinault, 1996).

Immunohistochemistry: Following the experiments, the animals were perfused; the brains were removed and the area from the mPFC to the posterior parts of the BF was cut by Vibratome. Sections processed for fluorescent marker (Streptavidin Cy3) to visualize the Biocytin labeled neurons. When labeled cell bodies were found, sections were further processed for subsequent immunohistochemical staining for: choline acetyltransferase (ChAT), PV, neuropeptide-Y (NPY) and somatostatin (SS). If no cell bodies were found after Cy3 conjugated streptavidine incubation, sections were mounted and Nissl-stained to visualize all the cell bodies and the electrode tracks.

Data analysis: Data processing was generally performed using MatLab7 or Spike software. Baseline spike trains (5-10 min) were analyzed to obtain mean firing rate and coefficient of variation values, to construct inter-spike interval histograms, and to test correlation between ECoG waveforms and unit firing pattern. To determine characteristics of spike shapes, several spontaneous discharges were averaged using the same filtering conditions (300 Hz-10 kHz). Cortical Up states were detected by finding negative deflections below two standard deviations of the EEG. Peri-Event-Time-Histograms (PETHs) were calculated around the local minimum of the Up states. A peak in a PETH was defined significant when at least one of the bins exceeded 95th percentile of the baseline mean (assuming a Poisson distribution, MATLAB 'poissinv' function). Similarly, inhibitory events were considered significant at least one bins were below 5th percentile of the baseline mean.

Anatomy

Anterograde tracing of the descending axons from the mPFC: In order to find anatomical evidence whether or not SS and NPY containing interneurons in the BF receive direct input from the medial PFC, iontophoretic injections of the anterograde tracer BDA were carried out in rats into the medial prefrontal cortical areas of IL, PrL and OF. For light microscopy (LM) procedures, the animals were perfused after 7 days survival time; brains were removed and postfixed overnight. Brains used for light microscopy were immersed into 30% sucrose in PBS overnight, frozen on powdered dry ice, and sectioned at 50 μm with a cryostat. Sections were collected from A: 0.5mm to P: -2.5 mm from the Bregma. For electron microscopy (EM), animals were sacrificed after 3 days of survival time and were transcardially fixed. Brains were removed, post fixed and cut on a Vibratome and the same procedures were carried out. For the

visualization of BDA series of sections were developed with Ni-DAB and precipitate was silver-gold intensified (Gallyas et al., 1980;Liposits et al., 1984). To visualize SS- or NPY-positive structures, the sections already labeled for BDA underwent further immunohistochemical processing using primary antibodies against SS and NPY, followed by biotinylated secondary antibodies, then neurons visualized by DAB reaction.

Electrolytic lesion of the mPFC: After craniotomy above the medial prefrontal areas, a bipolar electrode was lowered to the IL/PrL area (4.5-5 mm from the surface) and 5-10 mA positive current pulses were applied for 1-3 minutes. After 24-48 hours of survival time the animals were perfused. For light microscopy, the degeneration was visualized by using silver staining for terminal degeneration and lysosomes. For electron microscopy, the sections were stained for SS, NPY, calbindin (CB) and calretinin (CR) in generally the same way as the same way as for light microscopic immunoperoxidase labeling. BF areas that contained labeled neurons and processes were selected based on our BDA labeled axon arborization data. Section processed were scanned for degenerating axon terminals and SS or NPY labeled profiles under the electron microscope.

Results

Our experiments provided the following results:

- **Do the spontaneous changes of cortical activity affects the activity levels of BF neurons?** We categorized the recorded neurons based on their relationship to the cortical activity and on their response to tail pinch (TP) stimulation. Units were characterized as F cells if their activity increased due to TP stimulation or spontaneous desynchronization. In contrast, the activity of S cells decreased following TP stimulation and/or was only active during spontaneous cortical slow waves. Out of all the recorded neurons, 41 (72%) increased their discharge rate when LVFA was present in the cortical EEG (F cells) and 9 (15%) showed increased firing rate during SWA (S cells). In addition, 7 neurons (13%) showed no correlation with any EEG pattern
- **How does the stimulation of the mPFC affect the firing properties of single BF neurons?** F and S cells could be further sorted based on their responses to PFC stimuli. We found that 28/41 F and 8/9 S cells responded to PFC stimulation. The majority of the F cells showed excitation (F/+; n=8) then their activity returned to the background level. Another group of F cells (F+/-) showed massive positive response followed by a long depressed period (n=8). In contrast, a

smaller group of F cells (n=6) expressed a short negative, inhibitory response (F/-) while another 6 cells showed a long inhibition (F/--). In the case of S cells we found a group that showed inhibition (n=6) and a smaller, but clearly defined group (n=2) showing excitation in response to PFC stimuli.

- **Do the neuron populations that respond to either PCF stimulation or to spontaneous changes of cortical Up and Down states represent well segregated, anatomically differentiated cell groups in the BF?**

  The same set of neurons was also analyzed regarding their relationship with spontaneous cortical Up and Down states. We found three distinct activity patterns: cells (22/51) fired phase-locked to the cortical Up states (*Up state-on* cells) with a significant excitatory peak on the Up-state triggered PETH and a negative peak on the spike-triggered average; a smaller group of cells (6/51, 11.7%), were tonically active during Down states, while decreased or ceased firing during Up states (*Up state-off* cells), thus displayed a significant inhibitory trough on the PETH and a positive peak on the STA. The rest of the cells (23/51) either did not show any significant changes, or their firing was completely independent of Up and Down states. The changes in the firing rates of the correlated neurons always occurred with a delay compared to the beginning of the Up and Down state transition. In comparison, we found that within the group of Up state-on neurons (n=22) 14 was identified as F cells, 1 as an S cell and 7 were not grouped either. The same analysis was carried out for Up state-off cells (n=6) as well, and we found 4 neurons to fall into the category of F cells and 2 to be S cells.

Anatomical experiments

Using anatomical tracing methods, electrical lesions and immunohistochemistry, we were looking for the answers of the following questions:

- **What is the projection pattern of the descending axons from the mPFC to the BF?**

  Our results confirmed the presence of a significant anatomical connection between the mPFC and the BF. Investigating several injection sites, we found that the axons originating from the PrL/IL tend to project to more medial areas, including the medial septum, ventral pallidum, diagonal band nuclei, substantia innominata, and peripallidal regions while orbitofrontal axons distribute more caudal and lateral areas in the BF. Our electrolytic lesion results also confirmed significant axon arborization in the BF areas, visualized by silver-gold staining of the

degenerating axon terminals. Both the BDA labeled axons as well as the degenerating axon terminals were in close proximity with both SS and NPY containing neurons in the BF.

- **Is there another neuron population, besides the PV containing neurons, in the BF that receives direct input from the mPFC?**

  After examining the anatomical connection between BDA containing descending and lesioned, degenerating axon terminals of the mPFC reaching the BF areas, we found that these axons are probably not directly connecting the SS containing neurons in the BF with mPFC areas. Further investigation is needed to decide whether or not medial prefrontal axons terminate on NPY containing neurons in the BF.

Conclusions

Electrophysiological properties of the connection between the PFC and the BF

From acute, in vivo experiments on urethane anesthetized rats we found the following results:

1. A certain neuron population of the BF is significantly correlated with low voltage fast activity (LVFA) in urethane narcosis in rats, while a different, smaller group of BF neurons are in strong correlation with cortical slow waves (SWS).
2. The spontaneous as well as the stimulus evoked changes of cortical activity result in changes of the activity levels of BF neurons.
3. The electrical stimulation of the mPFC affects the firing properties of single BF neurons. Based on the response for the stimulation of the medial PFC, BF neurons could be further categorized and differentiated.
4. A well categorized group of neurons of the BF are significantly associated with spontaneous cortical Up and Down states which does not necessarily overlap with previous categorizations.
5. Our results revealed a great diversity among F- and S-cells in terms of conduction velocity, spontaneous and evoked neuronal activity, and in terms of correlation between EEG and unit activity, indicating that F- or S-cells are far from being a homogeneous cell population.

Anatomical properties of the prefrontal-basal forebrain connection

1. It is confirmed that the cholinergic areas of the BF receive massive excitatory input from the mPFC.
2. Based on our anatomical results we can conclude that the interneuron population that receives direct input from the mPCF is not likely to be SS-positive. The neurochemical nature of the unlabeled spiny neuron population needs to be identified.

Summary

Our anatomical and electrophysiological studies provided evidence that the basal forebrain actively participates in the prefronto-basalo-cortical circuitry via its input from the prefrontal cortex and its output to distributed, functionally-related cortical areas. Our finding confirmed a close correlation between the BF unit activities and desynchronized and synchronized EEG epochs that supports the notion that BF cholinergic neurons play a considerable role in the desynchronization of the cortical activity. On the other hand, the temporal correlation between the BF and cortical Up and Down states supports the idea of the descending information back to the BF through the medial prefrontal cortex. The fact that both the excitatory and the inhibitory changes of BF single unit activity are modulated by the alteration of cortical Up and Down states proved to be a novelty. In addition, electric stimulation of the medial prefrontal cortex also supported the existence of a functional connection between the BF and the mPFC. Interestingly, despite the excitatory (presumably glutamatergic) nature of the prefrontal input to the BF, we also found significant inhibitory responses in correlation to both the mPFC stimulation and the cortical Up and Down states, which suggests that a significant portion of the medial prefrontal input terminates on inhibitory interneurons in the BF. Thus, by examining the anatomical target of the prefrontal axons in the BF, tried to identify what type of neurons receive direct input. By using various methods we excluded the SS containing cells in the BF, however, stating the final conclusion about various other neuropeptides and calcium binding protein containing neurons is still left to complete.

Publication list

Peer reviewed publications about related to the topic of this thesis:

Gyengési E., Zaborszky L, Détári L. The effect of prefrontal stimulation on the firing of basal forebrain neurons in urethane anesthetized rat. Brain Research Bulletin 75 (2008) 570-580.

Toth A, **Gyengési E**, Zaborszky L, Détári L. Interaction of slow cortical rhythm with somatosensory information processing in urethane-anesthetized rats. Brain Research 1226 (2008) 99-110.

Peer reviewed publications about other unrelated topics:

Nicholas Wallingford, Adam L. Diament, Anna Coppola, **Erika Gyengesi**, Bertrand Perroud, Qian Gao, Kari A. Haus, Xiao-Bing Gao, Zia Shariat-Madar, Fakhri Mahdi, Marvin Nieman, Gretchen LaRusch, Yongming Sun, Julie Blake, Alvin H. Schmaier, Craig H & Sabrina Diano. Warden: Prolylcarboxypeptidase regulates food intake by promoting breakdown of α-MSH. Journal of Clinical Investigation (2009)

Gajda Z, Hermesz E, **Gyengesi E**, Szupera Z, Szente M. The functional significance of gap junction channels in the epileptogenicity and seizure susceptibility of juvenile rats. Epilepsia. 2006 Jun; 47(6):1009-22.

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Kovacs A, Mihaly A, Komaromi A, **Gyengesi E**, Szente M, Weiczner R, Krisztin-Peva B, Szabo G, Telegdy G. Seizure, neurotransmitter release, and gene expression are closely related in the striatum of 4-aminopyridine-treated rats. Epilepsy Res. 2003 Jun-Jul; 55(1-2):117-29.

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