Noradrenergic influence on pyramidal cells in the prefrontal cortex: a two-photon laser scanning microscopy study

Doctoral (Ph.D.) Theses

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INTRODUCTION

Working memory is a fundamental building block of cognition: the ability to represent information no longer in the environment, and to use this representational knowledge to guide behavior, thought and affect. Working memory is encoded by persistent activity within networks of prefrontal cortical pyramidal cells with shared stimulus properties, engaged in recurrent excitation. The working memory abilities of the prefrontal cortex are highly dependent on the neuromodulatory environment, whereby loss of catecholamines in prefrontal cortex is as detrimental as destruction of the prefrontal cortex itself. Noradrenaline have “inverted U” influences on prefrontal cortex cognitive functions. Thus, either too little or too much noradrenaline impairs working memory. Noradrenaline has beneficial effects at post-synaptic α_{2A} receptors and detrimental actions at high levels of α_1 and β_1 receptor stimulation.

There is evidence that the hyperpolarization activated cationic channels (HCN channels) have powerful effects on cognitive performance in animals performing working memory tasks and blockade of HCN channels also promoted persistent network activity of prefrontal cortex neurons. HCN channels are particularly dense on pyramidal dendrites of the superficial cortical layers. In monkey prefrontal cortex, these layers also contain the highest concentration of α_{2A}-adrenoceptors, localized in dendritic spines and shafts.

Working memory delay periods are associated with irregularly timed high-frequency burst discharges. High frequency action potential bursts often represent a reliable neural signal through unreliable yet facilitating synapses thereby contributing to the synaptic reverberation in cortical networks. There is evidence that the regenerative signals of distal apical dendrite, named as dendritic spikes, are key factors in the generation of somatic action potential bursting.

MAJOR GOALS

Working memory is based on sustained action potential firing and is associated with irregularly timed high-frequency burst discharges in prefrontal cortical neurons. Noradrenaline has a powerful effect on working memory through α_2-, and β-adrenoceptors. In our experiments we investigated the noradrenergic action on the prefrontal cortex at the *cellular level*: on action potential firing and on dendritic spikes which are important in the burst generation. Our goals to investigate:

- the properties of backpropagating action potentials (bAPs) and the generation of dendritic spikes in prefrontal cortical layer 5 pyramidal cells.
- the role of hyperpolarization activated cationic channels (HCN channels) in the backpropagation of action potentials and dendritic spike generation
- the effect of α_2-adrenoceptors on dendritic spike generation and the possible functional link between HCN channels and α_2-adrenoceptors
- the effect of β-adrenoceptors on the excitability of layer 5 pyramidal neurons in the prefrontal cortex.

METHODS

Acute slice preparation

Slices (300 μm) containing the prefrontal cortex from male 18- to 23-day-old Wistar rats were prepared using a vibratome (Vibratome 3000). Brain slices were placed in artificial cerebrospinal fluid (ACSF) and incubated for 30 minutes at 32 ^{o}C. Slices were left at room temperature for at least 45 min before use.

Electrophysiology

Layer 5 pyramidal neurons in the prefrontal cortex were visualized using video infrared-DIC (Differential Interference Contrast). Patch pipettes were pulled from borosilicate glass (1.2 mm OD, Harvard Instruments, Germany). For current clamp

recordings 2,5-4,5 MΩ electrodes were filled with 125 mM K-gluconate, 20 mM KCl, 10 mM HEPES, 10 mM Di-Tris-salt phosphocreatine, 0.3 mM Na-GTP, 4 mM Mg-ATP, 10 mM NaCl and 100 μM Oregon Green BAPTA-1. Cells with an initial resting membrane potential that was more negative than –60 mV were accepted. In most cases, 5 backpropagating action potentials (bAPs) were evoked by somatic current injections (5 stimuli, 1800 pA, 2-4 ms) to induce dendritic calcium responses. Temperature of the chamber was kept at 32-34^{o}C.

Two-photon imaging

Layer 5 pyramidal cells of the prefrontal cortex were filled with a fluorescent calcium-sensitive indicator (Oregon Green 488 BAPTA-1) through a patch clamp electrode. The fluorescent indicator was excited at 810 nm wavelength. Imaging was performed using a custom-made two-photon laser scanning system consisting of a modified confocal microscope (Olympus Fluoview, Germany). In order to minimize photodamage, the intensity of the excitation laser light was always maintained at the minimum required to attain sufficient signal-to-noise ratio. High-time-resolution fluorescence measurements were obtained in line scan mode (2 ms maximal temporal resolution) after zooming onto a dendritic section. Data recording was started 40 minutes following break-in. At the end of each experiment, a series of images across the depth of the volume encompassing the imaged neuron were taken. Image data were analyzed off-line using a custom-made program written in Matlab.

Calculations

Fluorescent traces are expressed as relative fluorescence changes [$\Delta F/F=(F-F_0)/F_0$] where F_0 is the background-corrected mean pre-stimulus fluorescence, F is the background-corrected fluorescence.

Dye concentration in the cell changes depending on time and distance from the site of the pipette. It has been an extensive debate that what extent this “added calcium buffer” (exogenous calcium buffer) changes calcium homeostasis and the amplitudes of calcium transients.

We have created a mathematical model to investigate the effect of increasing dye concentration on the amplitudes of calcium transients. Our experiments and models show that at low dye concentration (important for measuring at distal dendritic sites) the amplitudes of transients may increase depending on the (i) magnitude of the stimulus and the (ii) parameters of the endogenous buffers and the (iii) initial changes of resting intracellular calcium concentration. Our results help us distinguish between the biological effects on calcium dynamics and the influence of the increasing dye concentration during loading.

RESULTS

Scaling of backpropagating action potential (bAP) evoked calcium transients

In order to map the dendritic scaling of calcium responses evoked by bAPs we applied trains of 5 APs (10-120 Hz) at different frequencies and imaged the apical dendrite of prefrontal cortex layer 5 pyramidal neurons at various distances from the soma. At the most proximal sites (0-50 μm), calcium transients showed weak frequency dependence. Moving distally along the apical dendrite, the amplitude of the evoked calcium transients gradually increased as a function of bAP frequency. At far distal sites (at 600-700 μm from the soma), calcium responses had a binary outcome: low-frequency, subcritical stimuli caused no response but after passing a critical frequency of the bAP train at supracritical frequencies, a stable-amplitude calcium transient was detected. The difference between the smallest and largest transients was maximal and stable at these distal responses. This all-or-none-type feature of distal calcium transients and the existence of the threshold stimulation correspond well with the characteristics of dendritic regenerative events, namely dendritic spikes. Although the critical frequency for one cell was stable during the control experiments, we observed a considerable variation of the critical frequency across different cells.

Dendritic spikes and bAPs utilize different types of voltage-sensitive calcium channels

Application of the T- and R-type voltage sensitive calcium channel selective nickel (100 μM) caused an almost full block of dendritic spikes at distal sites indicating that in the generation of distal dendritic spikes R- and/or T- type channels play an important role. Nickel caused only a small, but significant reduction in the proximal calcium responses to bAP trains measured.

bAP attenuation and dendritic spike initiation: relation to the bifurcation

Corroborating the findings of earlier experiments, at low stimulation frequencies the amplitude of the bAP-evoked calcium transients declined with distance from the soma. However, supracritical calcium transients associated with dendritic spikes showed a substantially different scaling: their amplitudes had a maximum (supracritical maximum) which likely point to the initiation zone of dendritic spikes in the apical dendrite.

Calculation the extinction sites of low-frequency bAP trains from the trendlines of calcium transient amplitudes revealed a relatively narrow section in the apical dendrite.

Different morphologies of layer 5 pyramidal neurons may influence the site of the dendritic spike generation. How are the mean extinction site of low-frequency trains and the peak of supracritical calcium transients related to the localization of the main bifurcation? To address this we mapped distances of extinction sites and distances of supracritical maximum as a function of bifurcation distance. Extinction of subcritical responses was closer to the soma than the main bifurcation in 3 cases, while extinction fell after the bifurcation in 7 other cases. The maximum of the supracritical calcium responses (supracritical maximum) along the dendrite appeared after the bifurcation in half of the cells while in the other half was localized before the bifurcation suggesting that dendritic spike zone did not strictly depend on the exact localization of the bifurcation in spite of the clear overlap of the mean values.

Dendritic spikes reliably invade spines

Assuming a regenerative nature, dendritic spikes should propagate extensively to distal apical dendrites and spines. This issue was not investigated in earlier studies on dendritic spikes. We recorded calcium transients in adjacent dendrite/spine pairs. These experiments revealed a strict correlation between supracritical calcium responses (associated to dendritic spikes) measured in the dendrite and in the adjacent spine suggesting that dendritic spikes reliably invade all spines in distal dendrites.

Hyperpolarization activated cationic current (I_h) shifts the threshold of dendritic spikes

After the characterization of calcium responses associated with distal dendritic spikes, we investigated the possible modulation of frequency profiles of evoked calcium transients.

We applied ZD 7288 to remove I_h and studied the generation of dendritic calcium transients. In accordance with earlier reports, application of ZD 7288 (50 μM) caused hyperpolarization at the soma, confirming its action on I_h in our experiments. The block of I_h by ZD 7288 decreased the frequency-threshold of dendritic spikes: low-frequency bAP trains, which normally fail to evoke the large amplitude, all-or-none-type calcium transients, became able to induce suprathreshold responses. This was particularly evident for the 50 μM dose of ZD 7288; 20 μM ZD 7288 produced a smaller but significant shift in critical frequency. In addition, removal of I_h also increased the amplitude of the calcium transient associated to dendritic spikes.

Under control conditions, two bAPs induced small calcium responses in distal dendrites, but in the presence of 50 μM ZD 7288 they consistently induced large calcium response. Under the block of I_h, even a single bAP became sufficient to produce large calcium responses; this never occurred in control. Thus, the removal of I_h by ZD 7288 facilitated the initiation of dendritic spikes by reducing the threshold number of bAPs.

In addition ZD 7288 caused a large increase in depolarizing afterpotentials associated with dendritic spikes, and we often observed AP bursts in the soma electrical recording. These bursts and increased depolarizing afterpotentials may result from more

effective propagation of dendritic spikes to the soma under the block of I_h supporting earlier findings.

α_2-Adrenergic regulation of dendritic spike initiation

HCN2-4 channels activation is linked to downstream elevation of cAMP level and subsequent intracellular pathways. Thus, neurotransmitters, known to act by changing cAMP levels, are expected to influence I_h and, consequently, the initiation of dendritic spikes. Noradrenaline plays an important role in the prefrontal cortex and prefrontal cortex layer 5 pyramidal neurons express α_2-adrenergic receptors. To address the possibility that activation of α_2-adrenergic receptors can shift the frequency domain of dendritic spike initiation, we applied clonidine, a specific agonist of α_2-adrenergic receptors, at 100 μM concentrations and recorded calcium responses evoked by bAPs with various frequencies. Similarly to the I_h block, clonidine enhanced calcium transients by lowering the threshold for dendritic spike initiation at distal dendritic sites.

Next, we studied whether the effect of clonidine was indeed mediated by specific α_2-adrenergic receptors. 100 μM RX 821002, a specific antagonist of α_2-adrenergic receptors inhibited the effect of clonidine on dendritic spike generation.

The similarities between clonidine and ZD 7288-mediated action suggested the potential role of I_h in the action of clonidine. To investigate this interaction, we perfused ZD 7288 during application of clonidine. Clonidine was not able to change the frequency profile in the presence of 50 μM ZD 7288. The assumption that clonidine acted through the inhibition of I_h was further supported by voltage clamp experiments. I_h was observed during hyperpolarizing voltage steps. This current was reduced by 100 μM clonidine.

Taken together, these data strongly suggest that the α_2-adrenergic receptor agonist clonidine shifted the frequency domain of dendritic spikes to the left by inhibiting I_h.

Noradrenaline increases excitability

Because of the well-known enhancing effect of β-adrenergic receptors on excitability we aimed to explore the involvement of β-adrenergic receptors in the effect of noradrenaline. To estimate excitability of dendrites we induced 650 ms-long currents

with different amplitudes into the cells. These currents induced a different number of APs which traveled up to the basal dendrites and caused calcium responses of variable magnitude. Bath application of 10 μM noradrenaline enhanced the excitability of layer 5 pyramidal cells: the number of APs and the calcium responses of basal dendrites significantly increased in the presence of noradrenaline.

Separating cells into intrinsic bursting and regular spiking groups did not revealed any significant difference between the groups.

β-adrenergic receptors mediate the effect of noradrenaline

In a set of experiments, the non-specific β-adrenergic receptor antagonist, propranolol (10 μM) was applied in the perfusion fluid before and during the noradrenaline treatment. Following a washout the ability of noradrenaline to increase excitability was tested. Propranolol in itself did not cause any change in the number of evoked APs and calcium transients. In the presence of propranolol, the increase in AP number was reduced. Accordingly, the noradrenaline-induced enhancement of evoked calcium transients in the dendrite was prevented by propranolol suggesting the role of β-adrenergic receptors in mediating the effect of noradrenaline.

CONCLUSIONS

- We have explored the distant-dependent scaling of calcium dynamics along the entire length of the apical dendrite at various bAP frequencies. We found that low frequency trains of bAPs were filtered out at a gating region. Above a frequency threshold ("critical frequency") bAP trains avoided filtering and initiated all-or-none-type calcium transients. The all-or-none feature of the large and stable amplitude calcium responses at higher frequency trains suggested regenerative responses that may correspond to dendritic spikes similarly to voltage response characteristics of distal dendrites in other studies.
- We assume that the initiation zone of dendritic spikes is located at the area of the main bifurcation in layer 5 pyramidal cells of the prefrontal cortex.
- Dendritic spikes reliably invade spines in distal dendrites.
- By application of nickel we have shown that R- and/or T- type channels play an important in role in the generation of dendritic spikes.
- The block of HCN-channels by ZD 7288 could shift the frequency threshold and decreased the number of action potentials required to initiate dendritic spikes. The block of HCN channels also increased the amplitude of the dendritic spikes.
- We found that the α_2-adrenoceptors could shift the frequency coding of dendritic spikes to lower frequencies through modulation of HCN-channels.
- Activation of the noradrenergic system increases excitability of layer 5 pyramidal neurons via β-adrenergic receptors.

There has been considerable recent interest in the finding that central synapses are very unreliable. What is meant by unreliable is that an action potential in the presynaptic terminal often fails to produce any postsynaptic response. Dendritic spikes are key factors in the generation of action potential bursts which may represent a reliable neural signal through unreliable yet facilitating synapses thereby contributing to the synaptic reverberation in the prefrontal circuits. This mechanism might be one cellular correlate of the α_2-adrenoceptors mediated actions on working memory. Our data revealed that receptor activation can influence I_h so as to expand or shrink the zone of dendritic spikes in the apical dendrite.

Our data, that activation of the noradrenergic system increases excitability of layer 5 pyramidal neurons via β-adrenergic receptors, may explain at cellular level that noradrenaline at higher rates of release highly alert animals attend to multiple stimuli in the environment and are less able to focus attention on a specific stimulus.

PUBLICATIONS

Publications that form the basis of the Ph.D. dissertation:

Barth, A.M., Vizi, E.S., Zelles, T., & Lendvai, B. (2007)
α_2-Adrenergic receptors modify dendritic spike generation via HCN channels in the prefrontal cortex. *J Neurophysiol*, (doi:10.1152/jn.00943.2007)

Barth, A.M., Vizi, E.S., & Lendvai, B. (2007)
Noradrenergic enhancement of Ca^{2+} responses of basal dendrites in layer5 pyramidal neurons of the prefrontal cortex. *Neurochem Int*, **51**, 323-7.

Lendvai, B., Szabo, S.I., **Barth, A.I.**, Zelles, T., & Vizi, E.S. (2006) Application of two-photon microscopy to the study of cellular pharmacology of central neurons. *Adv Drug Deliv Rev*, **58**, 841-9.