

# Noradrenergic enhancement of $\text{Ca}^{2+}$ responses of basal dendrites in layer 5 pyramidal neurons of the prefrontal cortex

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## Abstract

Although the excitatory effects of noradrenaline have been thoroughly studied in the central nervous system, there is relatively little known about the adrenergic effects on  $\text{Ca}^{2+}$  dynamics of dendrites. In the present study, we imaged basal dendrites of layer 5 pyramidal neurons in the prefrontal cortex using two-photon microscopy. In our experiments noradrenaline, applied in the bath, enhanced excitability of layer 5 pyramidal neurons. The number of evoked action potentials following current injection to the soma increased by 44.7% on average. In the basal dendrites and spines the evoked  $\text{Ca}^{2+}$  responses were also markedly enhanced. Noradrenaline-induced effects could be blocked by the  $\beta$ -adrenergic blocker propranolol. Our data, that activation of the noradrenergic system increases excitability of layer 5 pyramidal neurons via  $\beta$ -adrenergic receptors and enhances  $\text{Ca}^{2+}$  signaling in basal dendrites, suggest a cellular site of action for noradrenaline to improve the integrative capabilities of dendrites.

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## 1. Introduction

Noradrenaline is thought to play modulatory roles in a number of physiological processes and has potent and long-lasting ionic effects on cortical neurons. In cortical pyramidal cells, activation of  $\beta$ -adrenergic receptors results in an enhanced excitability and responsiveness to depolarizing stimuli through a marked suppression of a slow  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  current, which underlies slow after hyperpolarization and spike frequency adaptation (c.f. McCormick et al., 1991). On the other hand, noradrenaline can reduce synaptic transmission in layer 1 (Law-Tho et al., 1993). Activation of  $\beta$ -adrenergic receptors can increase population spike amplitude supporting the hypothesis that activation of these receptors enhances pyramidal cell excitability (Mueller et al., 1982). Other adrenergic receptors may combine their action to modulate neural function at the cellular level: for example, prefrontal cortex (PFC) layer 5 pyramidal neurons express  $\alpha_2$ -adrenergic receptors in the apical dendrite (Aoki et al., 1998). GABAergic

interneurons of the rat frontal cortex can be excited via  $\alpha$ -adrenoceptors which results in an increased inhibition of pyramidal neurons (Kawaguchi and Shindou, 1998).  $\alpha_1$ -Adrenoceptor activation enhances excitatory postsynaptic currents (Marek and Aghajanian, 1999), neuronal firing rate in cerebral cortex (Bradshaw et al., 1981) and produces vigilance (Harsing et al., 1989). Differentiation of neocortical neurons might be one important target of noradrenergic afferents during early postnatal development of the neocortex (Rorig et al., 1995).

The noradrenergic system densely innervates the PFC (Lewis and Morrison, 1989), and plays a specific role in visual attention (Rossetti and Carboni, 2005; Viggiano et al., 2004; Dalley et al., 2001). In particular, it has been suggested that attention deficits may be linked to PFC dysfunctions (Robbins, 1997). Neuropsychological studies suggest that the PFC also has a control function on working memory and episodic long-term memory in humans (Shimamura, 1995; Ranganath et al., 2003). At the cellular level, learning and memory functions are thought to be linked to synaptic plasticity (Lynch, 2004). Plasticity of dendrites are shaped by the interaction of backpropagating action potentials (bAPs), synaptic events, and the generation of local spikes (Hausser et al., 2000).

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In this report, we aimed to explore the noradrenergic influence on bAP-evoked  $\text{Ca}^{2+}$  dynamics of basal dendrites in layer 5 pyramidal neurons. To estimate the excitatory effect of locally released noradrenaline, we characterized the change in excitability by recording  $\text{Ca}^{2+}$  responses in basal dendrites of PFC neurons at various distances from the soma. We also investigated the involvement of  $\beta$ -adrenergic receptors in the noradrenaline-induced increase in excitability.

## 2. Materials and methods

### 2.1. Slice preparation

Slices (300  $\mu\text{m}$ ) containing the prefrontal cortex from male 18–21-day-old Wistar rats were prepared using a vibratome (Vibratome 3000). Brain slices were placed in ACSF containing (mM) 127 NaCl, 25  $\text{NaHCO}_3$ , 25 D-glucose, 2.5 KCl, 2  $\text{CaCl}_2$ , 1  $\text{MgCl}_2$ , and 1.25  $\text{NaH}_2\text{PO}_4$ , and incubated for 30 min at 32 °C. Slices were left at room temperature for at least 45 min before use.

### 2.2. Electrophysiology

Layer 5 pyramidal neurons in the PFC were visualized under video infrared-DIC. Patch pipettes were pulled from borosilicate glass (1.2 mm O.D., Harvard Instruments, Germany). For current clamp recordings 3–6 M $\Omega$  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 112  $\mu\text{M}$  Oregon Green BAPTA-1. Cells with an initial resting membrane potential that was more negative than  $-60$  mV were accepted. Square-wave somatic current pulses (650 ms) with increasing intensity (100–300 pA) were used to evoke action potentials (APs). Spike half-width did not change significantly during the experiment. Data acquisition and analysis were performed using pClamp8 (Axon Instruments, USA). Instantaneous frequencies were calculated from the intervals between evoked APs within the train. Normalized instantaneous frequencies were expressed as the ratio of frequencies calculated for the actual and the last intervals of APs.

### 2.3. Two-photon imaging

Imaging was performed using a custom-made two-photon laser scanning system consisting of a modified confocal microscope (Olympus Fluoview, Germany) and a titanium-sapphire laser (Millenia/Tsunami, SpectraPhysics, USA), which has been described previously (Rozsa et al., 2004; Lendvai et al., 2006). Briefly, fluorescent indicators were excited at 810 nm wavelength. Detection was performed in both epi- and trans-fluorescence mode using external photomultiplier tubes (R3896, Hamamatsu, Germany). In order to minimize photodamage, the intensity of the excitation laser light was always maintained at the minimum required to attain a sufficient signal-to-noise ratio. High-time-resolution fluorescence measurements were obtained in line-scan mode (2 ms temporal resolution) after zooming onto a dendritic section. Data recording was started 30 min 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. Fluorescence traces are expressed as relative fluorescence changes  $[\Delta F/F = (F - F_0)/F_0]$  where  $F_0$  is the background-corrected pre-stimulus fluorescence. Amplitude of the  $\text{Ca}^{2+}$  transient was determined by averaging 30 data points around the largest  $\Delta F/F$  value of the transient.

## 3. Results

### 3.1. bAP-evoked $\text{Ca}^{2+}$ transients in basal dendrites of layer 5 pyramidal neurons

Layer 5 pyramidal neurons were identified under the guidance of infrared differential interference contrast microscopy. Cells

were filled with the  $\text{Ca}^{2+}$ -sensitive fluorescent dye Oregon Green BAPTA-1 through a patch clamp electrode. Fluorescence images of the neurons were obtained by collapsing the three-dimensional two-photon image stacks (Fig. 1A). Fine details of basal dendrites were then revealed in higher magnification. Depolarizing current pulses through the patch clamp electrode induced action potentials at the soma (Fig. 1A–C). Two-photon imaging was used in the line scan mode; a single line on the two-dimensional image of the cell and the dendritic arbor set the target of imaging (Fig. 1A and D). Sampling at a high rate (2 ms temporal resolution) precisely revealed the rising phase and amplitudes of the evoked  $\text{Ca}^{2+}$  responses at basal dendritic sites. Types of layer 5 pyramidal neurons were also identified based on their firing pattern in response to 300 pA pulses. Three of 13 cells were found intrinsically bursting (IB), the others (10 of 13 cells) showed regular spiking (RS) activity. Bursting was observed at the beginning of the current injection in IB cells (Fig. 1F).

Plotting the instantaneous frequency (calculated from the intervals between action potentials) revealed that following high frequency bursting, characteristic for the first few spikes,

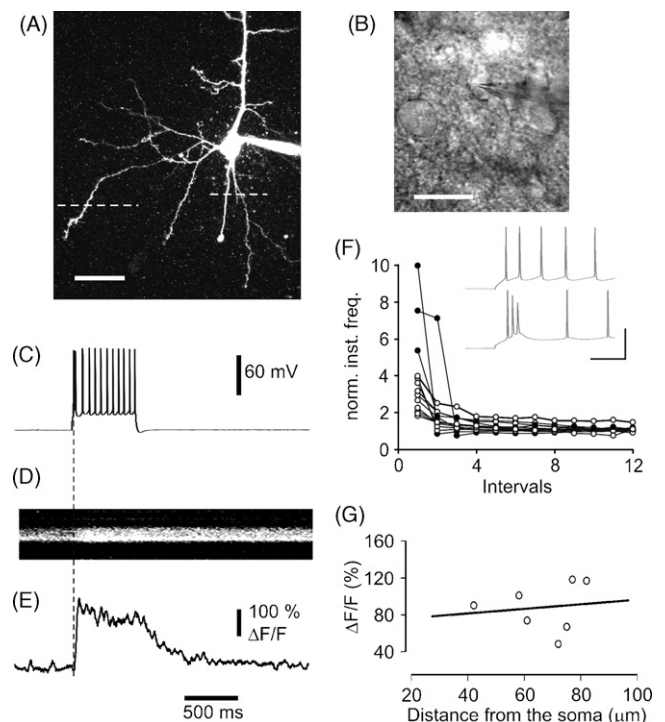


Fig. 1.  $\text{Ca}^{2+}$  responses of basal dendrites in layer 5 pyramidal neurons: (A) two-photon image of a layer 5 pyramidal cell of PFC filled with 112  $\mu\text{M}$  Oregon Green BAPTA-1. Scale bar, 40  $\mu\text{m}$ , horizontal white dashed lines indicate the sites of line scans; (B) IR-DIC image of layer 5 pyramidal cells of the PFC, scale bar, 40  $\mu\text{m}$ ; (C) somatic patch clamp recording shows action potential train evoked by 650 ms depolarizing current injection; (D) the corresponding line scan image from basal dendritic segment reveals the  $\text{Ca}^{2+}$  influx by increasing fluorescence intensity; (E)  $\text{Ca}^{2+}$  transients were calculated by the change in fluorescence intensity ( $\Delta F/F$ ); (F) electrophysiological characteristics of pyramidal cells. Plot of the normalized instantaneous spike frequency against interspike intervals reveals two groups of pyramidal cells. Inset shows electrophysiological responses of the different types of pyramidal cells to somatic current injection, scale bar, 50 ms, 60 mV; (G) the amplitudes of  $\text{Ca}^{2+}$  transients. Dendritic scaling of  $\text{Ca}^{2+}$  transients evoked by two action potentials at 100 Hz in basal dendrites.

IB cells produce firing at a lower mean frequency (17.1 Hz) than RS cells (25.7 Hz). We compared the effects of two bAPs at 100 Hz in basal dendrites. High-frequency stimulation caused virtually identical  $\text{Ca}^{2+}$  responses at different distances ranging from 40 to 85  $\mu\text{m}$  (Fig. 1G) suggesting that dendritic sites investigated in this study can be compared after summarizing.

### 3.2. Noradrenaline enhances excitability

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 (Fig. 2A and B) which traveled up to the basal dendrites and caused  $\text{Ca}^{2+}$  responses of variable magnitude (Fig. 2A and B). Bath application of 10  $\mu\text{M}$  noradrenaline significantly enhanced the excitability of basal dendrites, which was reflected by two important response types: the evoked APs and the  $\text{Ca}^{2+}$  responses of basal dendrites. Separating cells into IB and RS groups did not reveal any significant difference between the groups ( $p = 0.7$ ). The number

of APs significantly increased in the presence of noradrenaline (Fig. 2C). The noradrenaline-induced enhancement in excitability did not depend on the stimulus; the enhancement of responses was not statistically different at different amount of current injected ( $p = 0.2$ , Fig. 2A–D). The mean increase in AP number by noradrenaline was 44.7% ( $\pm 11.6\%$ ,  $n = 13$ ). In line with the effect on AP firing, the amplitudes of evoked  $\text{Ca}^{2+}$  transients in basal dendrites were also markedly enhanced (Fig. 2D). The mean enhancement of  $\text{Ca}^{2+}$  dynamics was 40.3% ( $\pm 13.2\%$ ,  $n = 12$ ). We obtained data that the effect of noradrenaline on excitability was evident in single spines of basal dendrites (Fig. 2E), which are the computational units of neural integration (Yuste and Denk, 1995) and sites of single synapse activation (Mainen et al., 1999). The noradrenaline-induced enhancement of excitability was lower in spines ( $21.6 \pm 8.6\%$  increase compared to control,  $n = 4$ ).

### 3.3. $\beta$ -Adrenergic receptors mediate the effect of noradrenaline

Because of the well-known enhancing effect of  $\beta$ -adrenergic receptors on excitability we aimed to explore the involvement of  $\beta$ -adrenergic receptors in the effect of noradrenaline. In a set of experiments, the non-specific  $\beta$ -adrenergic receptor antagonist, propranolol (10  $\mu\text{M}$ ) 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  $\text{Ca}^{2+}$  transients (Fig. 3A). In the presence of propranolol, the increase in AP number was reduced. Accordingly, the noradrenaline-induced enhancement of evoked  $\text{Ca}^{2+}$  transients in the dendrite was prevented by propranolol ( $n = 3$ , Fig. 3B) suggesting the role of  $\beta$ -adrenergic receptors in mediating the effect of noradrenaline.

## 4. Discussion

In this report, we explored the enhanced dendritic  $\text{Ca}^{2+}$  responses as an adrenergic receptor function in layer 5

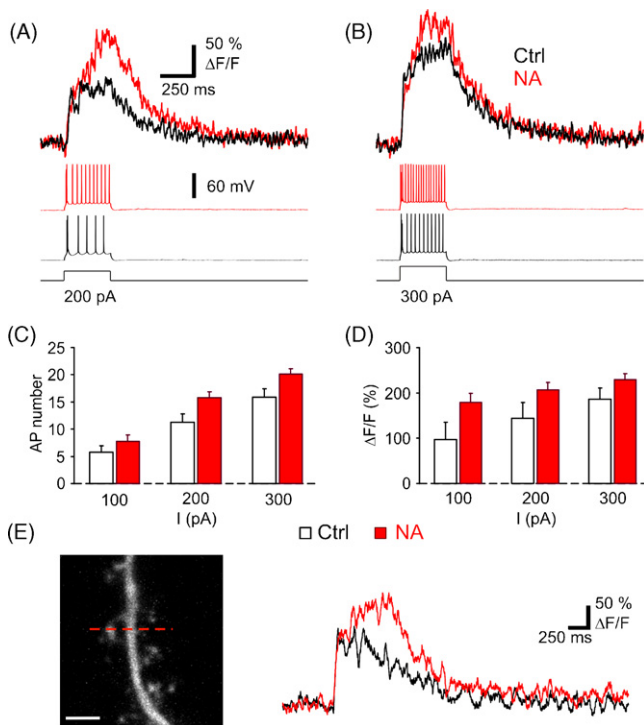


Fig. 2. Increased excitability and enhanced  $\text{Ca}^{2+}$  dynamics of basal dendrites following the application of noradrenaline: (A, B)  $\text{Ca}^{2+}$  transients (upper traces) in basal dendrites and the corresponding somatic patch clamp recordings (from upper second and third traces). At 200 pA (A, lower trace) and at 300 pA (B, lower trace), current pulse the number of evoked action potentials and the amplitudes of corresponding  $\text{Ca}^{2+}$  transients increase. Black, control; red, in the presence of noradrenaline (NA); (C) summarized data show noradrenaline-induced increase in the number of action potentials. Action potentials were evoked by current injections of different magnitude (100–300 pA); (D) mean amplitudes of  $\text{Ca}^{2+}$  responses evoked by various magnitude current injections (100–300 pA) (error bars are S.E.M.). Two-photon image of a spiny basal dendritic segment. Scale bar, 3  $\mu\text{m}$ , horizontal red dashed line indicates the site of line scan. (Right) In spine application of NA increases the amplitude of  $\text{Ca}^{2+}$  transients evoked by somatic depolarizing current pulse. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

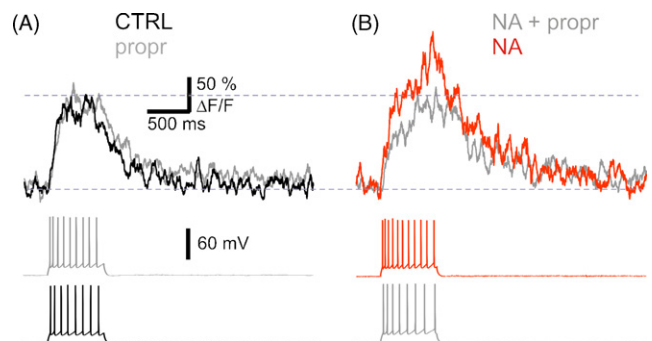


Fig. 3. Involvement of  $\beta$ -adrenergic receptors in the effect of noradrenaline: (A, B)  $\text{Ca}^{2+}$  transients (upper traces) in basal dendrites and the corresponding somatic patch clamp recordings (from upper second and third traces); (A) application of the  $\beta$ -adrenergic receptor antagonist propranolol (10  $\mu\text{M}$ ) does not change the backpropagating action potential evoked  $\text{Ca}^{2+}$  transients (black: in control conditions, gray: in the presence of propranolol); (B) NA did not influence  $\text{Ca}^{2+}$  transient amplitude in the presence of propranolol (gray).

pyramidal neurons of the PFC. It has been shown that noradrenergic axon terminals predominantly do not make synaptic contact in the cortex and  $\alpha_2$ -adrenergic receptors are often localized to nonsynaptic sites (Aoki et al., 1998). Thus, the noradrenergic system mostly operates as a nonsynaptic system (Vizi, 2000). Nonsynaptic transmission is thought to be the central target system of psychoactive drugs (Vizi, 2000). Extrasynaptic adrenergic receptors can sense low levels of endogenous noradrenaline and adrenergic drugs (Vizi et al., 2004). The released noradrenaline can inhibit its own release and the release of other transmitters, such as ACh, predominantly through  $\alpha_2$ -adrenoceptors (Vizi and Kiss, 1998; Umeda et al., 1997; Harsing and Vizi, 1991). In contrast,  $\beta$ -receptors mediate mostly excitatory actions at the cellular level. Indeed, it has been shown in the cerebellum that noradrenaline depolarizes the basket cells through a  $\beta_2$ -adrenoceptor-mediated manner that ultimately leads to facilitation of GABAergic synaptic activity (Saitow and Konishi, 2000).

Based on the present results we suppose that  $\beta$ -adrenergic receptor activation by noradrenaline results in increased excitability of layer 5 pyramidal neurons that is reflected by the enhanced dendritic  $\text{Ca}^{2+}$  dynamics. In particular, the probability of inducing strong  $\text{Ca}^{2+}$  responses (that is to activate subcellular cascade mechanisms) by depolarizing inputs considerably increases in case of higher noradrenergic activity of the PFC. Taken together that the PFC play a role in cognitive processes and noradrenaline enhances excitability of PFC neurons, we can conclude that noradrenaline is positively involved in cognitive functions. Indeed, noradrenaline has been shown to play an important role in regulating the working memory and attention of the PFC (c.f. Ramos and Arnsten, 2007).

Basal dendrites play a specific role in neural information processing. Communication between pyramidal neurons of the neocortex is established almost exclusively through inputs arriving the basal dendrites (Markram, 1997; Feldmeyer et al., 2002; Lubke et al., 2003). Basal dendrites are also important target of extracortical areas (Herkenham, 1980; Agmon and Connors, 1991; Kuroda et al., 1998). Thus, the noradrenaline-induced excitability, which results in more responsive dendrites, can enhance the integration of various cortical and subcortical inputs at the level of basal dendrites. Drugs acting on  $\beta$ -adrenergic receptors can interact with memory, but the outcome depends on the type of  $\beta$ -adrenergic receptor. Endogenous activation of the  $\beta_1$ -adrenergic receptor impairs PFC cognitive function (Ramos et al., 2005). Thus, it seems very likely that noradrenaline acted via  $\beta_2$ -adrenergic receptors to enhance firing and evoked  $\text{Ca}^{2+}$  dynamics in basal dendrites of layer 5 PFC neurons in our study.

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