

Rodent Neural Activation in the Possessing and Infralimbic Frontal Cortex

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Abstract

The interconnection between the medial prefrontal cortex (mPFC) and the dorsal raphe nucleus (DR) is involved in mood regulation and stress tolerance. The infralimbic (IL) compartment of the mPFC is the rodent equivalent of the ventral anterior cingulate cortex, which is closely associated with the pathophysiology/treatment of major depressive disorder (MDD). Increased excitatory neurotransmission in the IL, but not in the prelimbic cortex PrL, causes depressive- or antidepressant-like behaviors in rodents associated with altered serotonergic (5-HT) neurotransmission. Therefore, we examined regulation of 5-HT activity by both his mPFC subsets in anesthetized rats. Electrical stimulation of IL and PrL at 0.9 Hz similarly inhibited 5-HT neurons (53% vs. 48%). However, stimulation at higher frequencies (10–20 Hz) revealed a higher proportion of 5-HT neurons that were more sensitive to IL than PrL stimulation (86% vs. 59% at 20 Hz), suggesting that GABAA Different involvement (not 5). -HT1A) receptor. Similarly, electrical and optogenetic stimulation of IL and PrL enhanced 5-HT release in the DR in a frequency-dependent manner, with a greater increase following IL stimulation at 20 Hz. It differentially regulates serotonin activity, apparently playing a superior role. IL, an observation that may help clarify brain circuits involved in MDD.

Keywords: Infralimbic cortex; Prelimbic cortex; 5-HT neurons; In vivo electrophysiology; Intracerebral microdialysis

Introduction

The prefrontal cortex (PFC) is the highest level association cortex. It is dedicated to the expression, planning, and execution of actions according to temporal patterns and is deeply involved in higher brain functions such as perception, attention, memory, language, intelligence, consciousness, and emotion changes in neuropsychiatric disorders [1]. In particular, the dorsolateral PFC (eg, Brodmann's area 46) plays an important role in cognitive processes such as working memory and executive function. The ventral anterior cingulate cortex – vACC (including Brodmann's area 25) – is involved in processing emotional information. The PFC shows extensive interconnections with most cortical and subcortical structures, with the exception of the basal ganglia, which feed back to the PFC via the thalamus. This connectivity allows the PFC to achieve the highest level of integration among all cortical regions and to control behavior top-down by selecting one of the possible scenarios represented internally [2].

In particular, the PFC correlates with monoaminergic nuclei (raphe nucleus, 5-hydroxytryptamine or serotonin (5-HT), locus coeruleus, norepinephrine (NA), ventral tegmental area, dopamine (DA)). , adjust. Interestingly, the PFC dorsal raphe (DR) circuit is involved in responding to challenging situations, such as during stressful work [3].

vACC is deeply implicated in the pathophysiology and treatment of stress-related disorders. Thus, it has been reported that a patient with major depressive disorder (MDD) has an abnormally increased activity of his vACC, and deep brain stimulation (DBS) of the vACC has been associated with clinical improvement in patients with treatment-resistant MDD. brought. Similarly, a recent neuroimaging study found that MDD patients had higher task-induced vACC hyperactivity than controls, which normalized after a single dose of the fast-acting antidepressant ketamine [4].

Although there are significant differences in the complexity of human and rodent brains in terms of connectivity and function, the infralimbic (IL) and prelimbic (PrL) cortices are distinct from his vACC and dorsal cortex in humans, respectively. It is believed to be the rodent homologue of the lateral PFC. In particular; vACC and IL are strongly implicated in anxiety suppression and antidepressant effects.

Thus, optogenetic activation of ILs in rats results in immediate and sustained antidepressant effects similar to ketamine. Moreover, topical application of ketamine or the selective serotonin reuptake inhibitor (SSRI) citalopram in the IL mimics their systemically administered antidepressant-like effects. Dense populations of IL and PrL layer V pyramidal neurons project to the DR. Anatomical evidence suggests that PrL projects more densely to the DR than IL, but functional The data suggest that ILs more specifically control DR activity. PrL. Thus, acute increases in rat IL glutamatergic neurotransmission induced by blockade of the astroglial glutamate transporter GLT-1 elicit immediate antidepressant effects that are temporally associated with enhanced 5-HT release. Did. Both effects were offset by prior inhibition of 5-HT synthesis, suggesting the involvement of her 5-HT system in affecting behavior. Interestingly, when GLT-1 blockade was performed with PrL, no neurochemical or behavioral effects occurred, but it induced a comparable increase in extracellular glutamate similar to IL [5].

On the other hand, a sustained increase in glutamatergic activity in the IL induced by small interfering RNA (siRNA) strategies towards the astroglial glutamate transporters GLAST and GLT-1 triggered a depressive-like phenotype in mice, which was reversed by citalopram and ketamine. These behavioral alterations were accompanied by decreased 5-HT function and reduced hippocampal BDNF expression. Again, the application of siRNAs in the PrL, despite reducing GLAST and GLT-1 expression, as occurred in the IL, did not induce depressive states nor reduced 5-HT function. Overall, these observations add further support to the relevance of the IL (and of the vACC in humans) in mood control and strongly suggest a differential modulation of serotonergic activity by the IL and PrL. Given the different role of

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the IL and PrL in terms of their antidepressant effects, depressive-like behaviors, and their associated changes in serotonergic function, here we examined the control exerted by the IL and PrL cortices on serotonergic function in the DR under the working hypothesis that each area may distinctly modulate 5-HT neuronal activity, possibly via differential excitatory inputs onto 5-HT and GABA neurons within the DR. To this end, we analyzed the effects of electrical IL and PrL stimulation on neuronal 5-HT activity, and the effects of electrical and optogenetic stimulation of IL and PrL on 5-HT release in the DR as a total neuronal measure [6].

Materials and Methods

Drugs

8-OH-DPAT (5-HT_{1A}-R agonist, 30 µg/kg), maleate WAY-100635 (5-HT_{1A}-R antagonist, 10 µg/kg) and picrotoxin (GABA_A-R antagonist, 2 mg/kg) were purchased from Sigma/RBI (Natick, MA, USA). 8-OH-DPAT and WAY-100635 were dissolved in saline and stored at -20°C until use. Picrotoxin was dissolved in 10% DMSO and prepared on the day of the experiment. Doses are expressed as free base and were selected according to previous studies by our laboratory and others [11, 42,62]. Both compounds were injected intravenously (i.v.) via the femoral vein in a volume of 1 ml/kg [7].

In vivo microdialysis during electrical or optogenetic stimulation

Extracellular 5-HT concentrations in the DR during electrical or optical stimulation of IL or PrL were measured under chloral hydrate anesthesia by in vivo micro dialysis as previously described [11, 42,62]. It was done. Briefly, the probe placed in the DR was continuously perfused with artificial spinal fluid (aCSF) at 3.28 µL/min and 10 min fractions were collected. After six basal samples (no stimulation), two fractions were collected during electrical or optogenetic IL or PrL stimulation. After the last stimulation, 3 or 6 more samples (no stimulation) were collected. 5-HT concentrations were analyzed by high performance liquid chromatography (HPLC) with electrochemical detection (Waters 2465) at +0.75 V with a detection limit of 1-2 fmol/sample [8].

Immunohistochemistry

Coronal sections (30 µm coronal) were cut with a Microm HM500M cryostat and stored at 4°C in PBS containing 0.02% sodium azide. Anti-GFP (1: 500, Invitrogen, #11122, RRID: AB_221569) antibody was used to assess the expression of the AAV ChR2 viral construct. Floating sections were washed with PBS, permeabilized, and blocked for 15 minutes with PBS containing 0.3% Triton X-100, 0.2% gelatin, and 5% normal donkey serum (S30-100 ML Merck Millipore, Burlington, MA) [9]. Sections were washed again with PBS and incubated overnight at 4°C with primary antibodies. Wash brain sections and apply secondary antibody (1:200 donkey vs rabbit A555 (A-31572, Life Technologies, Carlsbad, CA, USA)) GFP visualization only. Sections were washed again with PBS, incubated in Hoescht 33342 1/1000 (H3570, Life Technologies) for 10 min, and mounted on microscope slides using

Entellan (Sigma-Aldrich, St. Louis, MO, USA) [10].

Discussion

In this study, do infralimbic (IL) and prelimbic (PrL) inputs to the DR elicit different responses in 5-HT neurons, as suggested by the distinct roles of the two mPFC subdivisions in emotional regulation? (See introduction). This study was also prompted by previous data from our laboratory. This indicates that acute stimulation of her AMPA-R of IL (but not PrL) elicited an immediate antidepressant-like response in rats, which was associated with her 5-day potentiation. HT release associated. Current electrophysiological observations showed that both IL and PrL electrical stimulation at low frequency (0.9 Hz) elicited global inhibitory effects comparable to DR 5-HT neurons. Regional differences were found when IL and PrL were stimulated at higher frequencies, indicative of phasic mPFC activity, resulting in a higher proportion of serotonergic neurons and an average firing rate of IL compared to PrL stimulation. (80% vs 64% at 10 Hz, 86 % vs. 59% at 20Hz). Responses of 5-HT neurons to 20 Hz IL stimulation were sensitive to GABA_A (but not 5-HT_{1A}) receptor blockade, implicating local GABA_A-R inputs in the control of DR-5-HT neuronal activity. I was. We also showed that both IL and PrL electrical and optogenetic stimulation enhanced 5-HT release in the DR, with a greater effect of IL compared to PrL stimulation. To our knowledge, no previous studies have examined the possible differential regulation of serotonin activity by ILs and PrL. Given the critical role of the PFC and 5-HT system in MDD, our current observations may help us better understand the brain circuits involved in the pathophysiology and management of this psychiatric disorder.

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