Early Stress Prevents the Potentiation of Muscarinic Excitation by Calcium Release in Adult Prefrontal Cortex

Éliane Proulx, Deepika Suri, Scott P. Heximer, Vidita A. Vaidya, and Evelyn K. Lambe

Background: The experience of early stress contributes to the etiology of several psychiatric disorders and can lead to lasting deficits in working memory and attention. These executive functions require activation of the prefrontal cortex (PFC) by muscarinic M1 acetylcholine (ACh) receptors. Such $G\alpha_q$-protein coupled receptors trigger the release of calcium (Ca^{2+}) from internal stores and elicit prolonged neuronal excitation.

Methods: In brain slices of rat PFC, we employed multiphoton imaging simultaneously with whole-cell electrophysiological recordings to examine potential interactions between ACh-induced Ca^{2+} release and excitatory currents in adulthood, across postnatal development, and following the early stress of repeated maternal separation, a rodent model for depression. We also investigated developmental changes in related genes in these groups.

Results: Acetylcholine-induced Ca^{2+} release potentiates ACh-elicited excitatory currents. In the healthy PFC, this potentiation of muscarinic excitation emerges in young adulthood, when executive function typically reaches maturity. However, the developmental consolidation of muscarinic ACh signaling is abolished in adults with a history of early stress, where ACh responses retain an adolescent phenotype. In prefrontal cortex, these rats show a disruption in the expression of multiple developmentally regulated genes associated with $G\alpha_q$ and Ca^{2+} signaling. Pharmacologic and ionic manipulations reveal that the enhancement of muscarinic excitation in the healthy adult PFC arises via the electrogenic process of sodium/Ca^{2+} exchange.

Conclusions: This work illustrates a long-lasting disruption in ACh-mediated cortical excitation following early stress and raises the possibility that such cellular mechanisms may disrupt the maturation of executive function.

Key Words: Acetylcholine, calcium imaging, development, maternal separation, muscarinic M1 receptor, slice electrophysiology

The prefrontal cortex (PFC) is one of the last brain regions to mature (1–3), and accordingly, peak performance of executive function is only achieved in mid to late adolescence (4–6). Executive function is essential for performing complex tasks (7–9). Disruptions in PFC activity and impairments in executive function are observed in several psychiatric disorders (8,10), including depression (11,12), schizophrenia (13–15), bipolar disorder (16), and attention-deficit/hyperactivity disorder (17), all of which may have developmental origins (18–22). Brain regions requiring protracted development may be especially vulnerable to the effects of early stress (23), which in itself is a risk factor for PFC dysfunction, cognitive deficits, and psychiatric illness (23–25). Yet, much remains unknown about the normal maturation of the PFC and the cellular mechanisms underlying its vulnerability to disruption.

Acetylcholine (ACh) modulation of the PFC is essential for executive functions such as working memory and attention (26–29) and dysregulation of the cholinergic system has been implicated in the executive deficits prevalent in psychiatric disorders (29,30). At the cellular level, the ability of PFC neurons to sustain persistent activity forms the basis of working memory (31) and involves muscarinic acetylcholine receptors (32–34). Layer V pyramidal cells of the PFC receive a dense cholinergic innervation arising from the basal forebrain (35,36) and respond to ACh principally via M1 muscarinic receptors (37). Activation of these $G\alpha_q$-coupled receptors exerts robust excitatory actions (37–40) in addition to releasing calcium (Ca^{2+}) from inositol 1,4,5-trisphosphate (IP_3)-sensitive stores (41). Although the ionic mechanisms underlying these responses have been extensively studied, they remain incompletely understood. In particular, the role of agonist-induced Ca^{2+} release in shaping muscarinic excitation is not known. Although intracellular Ca^{2+} likely plays an important role in shaping persistent activity (33,42,43), no study to date has simultaneously examined the release of intracellular Ca^{2+} together with the magnitude and timing of muscarinic excitation. Furthermore, it is unknown how these aspects of PFC cholinergic signaling change developmentally or if they are vulnerable to disruption by environmental factors such as early stress.

Using multiphoton Ca^{2+} imaging with concurrent whole-cell electrophysiological recordings in brain slices of PFC, we demonstrate for the first time that agonist-induced Ca^{2+} release from intracellular stores significantly potentiates the excitatory effects of muscarinic ACh receptors. We further provide mechanistic insight into the source of these excitatory effects. Importantly, we show that such potentiation of muscarinic receptor activity is associated with key developmental stages for executive function and is subject to disruption by early stress.

Methods and Materials

Animals

Sprague Dawley rats were used for all experiments, which were approved by the University of Toronto Animal Care

From the Department of Physiology (EP, SPH, EKL), University of Toronto, Toronto, Ontario, Canada; Tata Institute of Fundamental Research (DS, VAV), Mumbai, India; Heart and Stroke/Richard Lewar Centre of Excellence in Cardiovascular Research (SPH), and Department of Obstetrics and Gynaecology (EKL), University of Toronto, Toronto, Ontario, Canada.

Address correspondence to Evelyn K. Lambe, Ph.D., University of Toronto, Department of Physiology, 1 King's College Circle, Toronto, ON M5S 1A8, Canada; E-mail: evelyn.lambe@utoronto.ca.

Received Jun 25, 2013; revised Oct 2, 2013; accepted Oct 21, 2013.

0006-3223/$36.00
http://dx.doi.org/10.1016/j.biopsych.2013.10.017

Committee or the Tata Institute of Fundamental Research Animal Ethics Committee. The characterization of the normal interaction between the ACh-elicited Ca^{2+} release and the ACh-elicited inward current (I_{ACh}) was conducted in 31 young adult male rats (age: 60 ± 13 days, range 40–95 days). The second set of experiments examined how the above interaction matures and whether it is susceptible to disruption by early stress. For these experiments, we used repeated maternal separation to elicit early stress (44,45), and control animals were born and raised within the same animal room during the same time period. Recordings were performed in adolescence (postnatal day [P]30–P45; $n = 5$ control rats, $n = 4$ early stress [ES]), young adulthood (P60–P100; $n = 9$ control rats, $n = 6$ ES), or adulthood (P130–P175; $n = 6$ control rats, $n = 8$ ES). The third set of experiments employed the same maternal separation paradigm and examined gene expression in the PFC at either P21 ($n = 7$–8 control rats, $n = 8$–9 ES) or P60 ($n = 7$ control rats, $n = 7$–8 ES).

Early Stress Paradigm

Pregnant primiparous dams delivered pups within the animal housing facility and litters were randomly assigned to ES or control groups on P1. Pups in the ES litters were separated from their dams for a period of 3 hours at the same time each day from P2 to P14. Control litters were left undisturbed during this time. All litters were handled briefly at 3- to 4-day intervals to allow for cage cleaning and weighing. Once weaned, all pups were housed in same-sex sibling groups of two to three rats.

Brain Slice Preparation and Recording Conditions

Each brain was rapidly cooled with 4°C oxygenated sucrose artificial cerebrospinal fluid (254 mmol/L sucrose substituted for sodium chloride). Coronal slices (400 μm) of the PFC were cut on a Dosaka Linear Slicer (SciMedia, Costa Mesa, California) and were transferred to 30°C oxygenated artificial cerebrospinal fluid (containing, in mmol/L: 128 sodium chloride, 10 D-glucose, 26 sodium bicarbonate, 2 calcium chloride, 2 magnesium sulfate, 3 potassium chloride, 1.25 monosodium phosphate, pH 7.4) in a prechamber (Automatic Scientific, Berkeley, California) and allowed to recover for at least 1.5 hours before the beginning of an experiment. Slices were placed in a chamber on the stage of an upright microscope for whole-cell recordings. Artificial cerebrospinal fluid was bubbled with 95% oxygen and 5% carbon dioxide and flowed over the slice at 30°C with a rate of 3 mL to 4 mL per minute.

Electrophysiological Recordings and Multiphoton Ca^{2+} Imaging

Layer V pyramidal neurons were patched under visual control using infrared differential interference contrast microscopy in the cingulate and prelimbic regions. Intracellular patch solution contained (in mmol/L): 120 K-gluconate, 5 potassiumchloride, 2 magnesium chloride, 4 dipotassium adenosine triphosphate, .4 disodium-guanosine triphosphate, 10 disodium-phosphocreatine, and 10 4-(2-hydroxyethyl)piperazin-1-yl ethanesulfonic acid (HEPES) buffer (adjusted to pH 7.33 with potassium hydroxide). The high-affinity Ca^{2+} dye Oregon Green BAPTA-1 (OGB-1, 100 μmol/L; Molecular Probes, Life Technologies, Burlington, Ontario, Canada) was included in the pipette along with the Ca^{2+} insensitive dye Alexa-594 hydrazide (20 μmol/L; Molecular Probes), which was used for the visualization of the neuron and subsequent morphological reconstruction (Supplementary Methods & Materials in Supplement 1). Currents were recorded with an Axopatch 200b (Molecular Devices, Sunnyvale, California), acquired and low-pass filtered at 2 kHz with pClamp10.2/Digidata1440 (Molecular Devices).

Multiphoton imaging was performed using a Ti:sapphire laser (Newport, Irvine, California) tuned to wavelength 800 nm and an Olympus (Richmond Hill, Ontario, Canada) Fluoview FV1000 microscope with a 60× water-immersion .90 numerical aperture objective. The emitted fluorescence was separated into green (OGB-1 signal) and red (Alexa-594 hydrazide signal) channels with a dichroic mirror at 570 nm and filtered (green barrier filter: 495–540 nm; red barrier filter: 570–620 nm) before detection. Images were acquired at a rate of ~10 frames per second and analyzed with Fluoview software (Olympus). A pansomatic area of interest was selected for analysis and green fluorescence increases were calculated relative to baseline fluorescence (dF/F_0). Calcium responders (ACh_{Ca2+}) were identified as cells where the ACh-elicited Ca^{2+} increase was at least five times the standard deviation of the baseline fluorescence signal; whereas, cells that lacked such a response to ACh were considered nonresponders ($ACh_{No\ Ca2+}$). Pseudocoloring was achieved post hoc for illustrative purposes with look-up tables adjusted to the maximal signal bandwidth in Fiji (ImageJA v.1.45b; http://fiji.sc/Fiji).

Intrinsic cell properties were assessed in current clamp mode. Calcium responders (ACh_{Ca2+} cells) were not significantly different from $ACh_{No\ Ca2+}$ cells as assessed by membrane potential (ACh_{Ca2+}: −80 ± 2 mV, $n = 16$; $ACh_{No\ Ca2+}$: −81 ± 1 mV, $n = 14$; $p = 0.9$), input resistance (ACh_{Ca2+}: 118 ± 12 MΩ, $n = 16$; $ACh_{No\ Ca2+}$: 144 ± 14 MΩ, $n = 14$; $p = .2$), and membrane capacitance (ACh_{Ca2+}: 180 ± 10 pF, $n = 16$; $ACh_{No\ Ca2+}$: 167 ± 9 mV, $n = 14$; $p = .4$). Examination of cholinergic currents was performed in voltage-clamp mode at a liquid junction potential-corrected holding potential of −75 mV.

Pharmacology

Acetylcholine chloride and caffeine were obtained from Sigma (Oakville, Ontario, Canada). Pirenzepine, thapsigargin, and KB-R7943 were obtained from Tocris (R&D Systems Inc., Minneapolis, Minnesota). All drugs were bath applied.

Quantitative Polymerase Chain Reaction

We assessed the developmental expression of genes involved in Ca^{2+} signaling using quantitative polymerase chain reaction (qPCR). Control and ES animals were killed by decapitation and the PFC dissected in ice-cold phosphate buffered saline. RNA was extracted from tissue samples using Trizol reagent (Sigma) and 2 μg from each sample was reverse-transcribed (high-capacity complementary DNA reverse transcription kit, Applied Biosystems, Life Technologies, Carlsbad, California). The synthesized complementary DNA was subjected to qPCR with primers specific to the genes of interest and data analyzed using the ΔΔCt method described previously (46) with normalization to the endogenous housekeeping gene hypoxanthine-guanine phosphoribosyltransferase (*Hprt*). Results were compared with age-matched control animals to examine the influence of early stress on gene expression within a particular age or to the P21 control group to assess the influence of early stress on the developmental profile of gene expression. Results were expressed as fold change ± SEM.

Statistics

Results are expressed as mean ± SEM, and all statistical comparisons were made at a significance level of .05 (Prism versions 5.0d/6.0, GraphPad Software, La Jolla, California). Average current