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Research article

Activation of 5-$HT_{2A/2C}$ receptors reduces the excitability of cultured cortical neurons

Lingli Hu[a,b], Chunhua Liu[a,b], Minyan Dang[a,b], Bin Luo[c], Yiping Guo[a,b,*], Haitao Wang[a,b,d,*]

[a] Key Laboratory of Regenerative Biology, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, Guangdong, 510530, China
[b] Guangdong Provincial Key Laboratory of Stem Cell and Regenerative Medicine, Guangzhou, Guangdong, 510530, China
[c] Department of Otolaryngology, Anhui Provincial Hospital Affiliated to Anhui Medical University, Hefei, 230001, China
[d] Department of Neurobiology and Biophysics, University of Science and Technology of China, Hefei, 230027, China

HIGHLIGHTS

- DOI depresses spontaneous firing of cortical neurons, mimicking the effect of serotonin.
- DOI depresses current-evoked firing and increases neuronal input resistance.
- HCN channels account for DOI-induced increase of input resistance, but not for the decrease of spontaneous firing.
- DOI differentially affects mEPSCs and mIPSCs.

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ABSTRACT

The abundant forebrain serotonergic projections are believed to modulate the activities of cortical neurons. 5-HT_2 receptor among multiple subtypes of serotonin receptors contributes to the modulation of excitability, synaptic transmissions and plasticity. In the present study, whole-cell patch-clamp recording was adopted to examine whether activation of 5-$HT_{2A/2C}$ receptors would have any impact on the excitability of cultured cortical neurons. We found that 2,5-Dimethoxy-4-iodoamphetamine (DOI), a selective 5-$HT_{2A/2C}$ receptor agonist, rapidly and reversibly depressed spontaneous action potentials mimicking the effect of serotonin. The decreased excitability was also observed for current-evoked firing. Additionally DOI increased neuronal input resistance. Hyperpolarization-activated cyclic nucleotide-gated cationic channels (HCN) did not account for the inhibition of spontaneous firing. The synaptic contribution was ruled out in that DOI augmented excitation and attenuated inhibition to actually favor an increase in the excitability. Our findings revealed that activation of 5-$HT_{2A/2C}$ receptors reduces neuronal excitability, which would deepen our understanding of serotonergic modulation of cortical activities.

1. Introduction

The central nervous system receives monoaminergic projections to fulfill its proper function. Serotonergic modulation among these participates in many neural activities including circadian rhythm, mood, learning and memory [1]. The neuromodulatory effect of serotonin is achieved through its multiple receptors [9], and targeted ion channels [1]. Accumulating evidences documented that serotonin influences neuronal excitability and synaptic components [6,14]. Presynaptic inhibition of neurotransmitter release was ascribed to the activation of Gi-coupled 5-HT_1 receptors [8]. Activation of postsynaptic Gq-coupled 5-HT_2 receptors could modulate function and trafficking of receptors [16].

Abbreviations: 5-HT, 5-hydroxytryptamine; ATP, adenosine-5′-triposphate; DIV, days *in vitro*; DOI, 2,5-dimethoxy-4-iodoamphetamine; EGTA, ethyleneglykole-bis-(2-aminoethyl)-tetraacetic acid; GABA, gamma-aminobutyric; GTP, guanosine-5′-triphosphate; HCN, hyperpolarization-activated cyclic nucleotide-gated; HEPES, *N*-2-hydroxyethylpiperaxine-*N*-2-ethanesulfonic acid; mEPSCs, miniature excitatory postsynaptic currents; mIPSCs, miniature inhibitory postsynaptic currents; TTX, tetrodoxin.

* Corresponding authors at: Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, 510530, China.
E-mail addresses: guo_yiping@gibh.ac.cn (Y. Guo), wang_haitao@gibh.ac.cn (H. Wang).

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5-HT_2 receptors mediated a depolarization and induced a tonic firing in neonatal pyramidal neurons [17].

The neurophysiologic processes require the normal serotonergic function, for example, 5-HT_{2A} receptors are involved in slow wave rhythm, while 5-HT_{1A} and 5-HT_{2A} receptors in gamma oscillation. Dysfunction of serotonergic system is implicated in neurological diseases [4]. The ability of 5-HT to modulate the excitability of auditory cortical cells was impaired by early sensorineural hearing loss [13]. Clinically prescribed selective serotonin reuptake inhibitors to elevate endogenous serotonin could modulate the excitability [5–7]. Hence understanding the serotonergic modulation of the cerebral cortex would not only extend our knowledge about information processing, but also help to develop related therapeutic strategy. In the present study whole-cell patch-clamp recording technique was adopted to examine the influence of activation of 5-HT_2 receptors on the membrane excitability.

2. Materials and methods

All protocols were approved by the Animal Subjects Ethics committee at Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences.

2.1. Primary neuron cultures

Primary cultured cortical neurons were obtained from 18-day-old Sprague-Dawley rat embryos [11]. Briefly, the cerebral cortex was dissected and incubated with 0.25% trypsin at 37 °C for 15 min. Cells were then mechanically dissociated using a Pasteur pipette with a fire-narrowed tip in culture medium and plated at a density of 2×10^4 cells/ml on the coverslips pre-coated with poly-D-lysine, and neurons grown up above a uniform astrocyte layer using "sandwich culturing method". Cells were maintained in neurobasal/B27 medium containing 0.5 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin.

2.2. Electrophysiological recording

Whole-cell patch-clamp recordings were obtained at room temperature from cortical neurons 15–23 days after plating [11]. Signals were amplified with a MultiClamp 700 B amplifier, digitized with a Digidata 1440, and acquired with pClamp 10 software (Molecular Devices, USA). The bath solution contained (in mM): 127 NaCl, 5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, and 12 glucose (pH 7.4, 300 mOsm/L). Patch pipettes with resistance between 3 and 5 MΩ were pulled from borosilicate glass (WPI, USA) with a Sutter-97 puller (Sutter, USA). For recording membrane potentials, the pipette was filled with solutions containing (in mM), 145 K-gluconate, 0.2 EGTA, 10 HEPES, 5 NaCl, 1 $MgCl_2$, 4 Mg-ATP, and 0.3 Na-GTP (pH 7.2, 285 mOsm/L). For recording membrane currents, the pipette was filled with solutions containing (in mM), 130 CsCl, 4 NaCl, 1 $MgCl_2$, 10 HEPES, 5 EGTA, 2 Mg-ATP, and 0.2 Na-GTP (pH 7.2, 285 mOsm/L). Stable effect was generally achieved when DOI and 5-HT were bath-applied for 3–5 min.

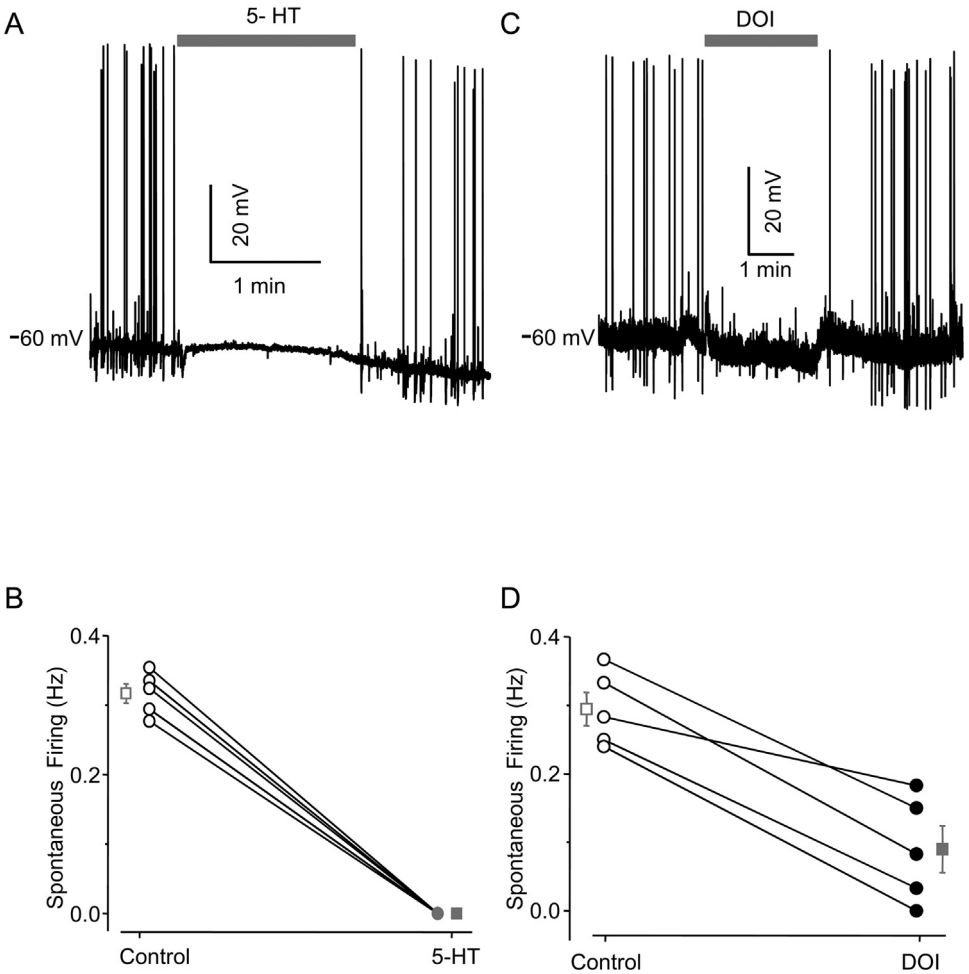

Fig. 1. DOI depressed spontaneous firing of cortical neurons. (A and C), Bath application of 5-HT and DOI depressed spontaneous firing of cortical neurons. Quantitative analysis and individual data revealing the depressive effect of 5-HT (n = 5, B) and DOI (n = 5, D) on the frequency of spontaneous firing. **. $p < 0.01$.