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5-HT_{2C} serotonin receptor blockade prevents tau protein hyperphosphorylation and corrects the defect in hippocampal synaptic plasticity caused by a combination of environmental stressors in mice

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ABSTRACT

Exposure to multimodal sensory stressors is an everyday occurrence and sometimes becomes very intense, such as during rave parties or other recreational events. A growing body of evidence suggests that strong environmental stressors might cause neuronal dysfunction on their own in addition to their synergistic action with illicit drugs. Mice were exposed to a combination of physical and sensory stressors that are reminiscent of those encountered in a rave party. However, this is not a model of rave because it lacks the rewarding properties of rave. A 14-h exposure to environmental stressors caused an impairment of hippocampal long-term potentiation (LTP) and spatial memory, and an enhanced phosphorylation of tau protein in the CA1 and CA3 regions. These effects were transient and critically depended on the activation of 5-HT_{2C} serotonin receptors, which are highly expressed in the CA1 region. Acute systemic injection of the selective 5-HT_{2C} antagonist, RS-102,221 (2 mg/kg, i.p., 2 min prior the onset of stress), prevented tau hyperphosphorylation and also corrected the defects in hippocampal LTP and spatial memory. These findings suggest that passive exposure to a combination of physical and sensory stressors causes a reversible hippocampal dysfunction, which might compromise mechanisms of synaptic plasticity and spatial memory for a few days. Drugs that block 5-HT_{2C} receptors might protect the hippocampus against the detrimental effect of environmental stressors.

1. Introduction

Several lines of evidence indicate that exposure to a combination of environmental stressors might be detrimental to neurons and interfere with mechanisms of activity-dependent synaptic plasticity. For example, high ambient temperature is permissive to excitotoxic brain damage [1–5], and rhythmic light stimulation causes EEG alterations in humans [6]. In addition, long-term exposure to strong noise causes cognitive impairment and dendritic spine alterations [7–9], and overcrowding causes anxiety and loss of type-1A 5-hydroxytryptamine (5-HT_{1A}) receptors in rodents [10]. The hippocampus is particularly sensitive to the detrimental effect of combined multimodal stressors. Accordingly, mice exposed to a combination of restraint stress, overcrowding, and visual and

Abbreviations: EDTA, ethylenediaminetetraacetic acid; fEPSPs, field excitatory postsynaptic potentials; GSK-3β, glycogen synthase kinase-3β; FJB, fluoro-jade B; GFAP, glial fibrillary acidic protein; HFS, high frequency stimulation; 5-HIAA, 5-hydroxyindoloacetic acid; HPLC, high-performance liquid chromatography; 5-HT, serotonin; LTP, long-term potentiation; MDMA, 3,4-methylendioxymethamphetamine; PFA, paraformaldehyde; PPF, paired-pulse facilitation; PVDF, polyvinylidene difluoride; RAWM, radial arm water maze; RS-102,221, 5-HT_{2C} receptor antagonist; TDZD-8, 4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione; TTBS, tween-tris buffer solution.

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acoustic stress for 5 h show an impairment of hippocampal long-term potentiation (LTP) and spatial memory associated with a reduction of dendritic spines in CA3 neurons [11].

Overcrowding, high ambient temperature, visual and acoustic stress, and physical stress by compulsive dancing are hallmark features of rave parties, in which there is also a widespread use of ethanol, cannabis, and club drugs, such as 3,4-methylendioxymethamphetamine (MDMA), γ-hydroxybutyrate, and ketamine [12]. It is generally believed that is the use of club drugs that makes rave parties particularly harmful to young people causing both short- and long-term consequences involving both the cardiovascular system and the CNS. This is certainly true, and may involve mechanisms that are not classically described with the most popular drugs of abuse. For example, acute injection of MDMA in mice, at doses that are roughly equivalent to those recreationally used by humans, causes a transient hippocampal dysfunction and memory deficit, which depends on the inhibition of the Wnt pathway, with ensuing activation of glycogen synthase kinase-3β (GSK-3β), and increased phosphorylation of tau protein [13]. The harmful effects of environmental stressors are only considered within the context of their association with drugs of abuse because loud noise, bright lights, densely packed crowds of dancers, and high ambient temperature are known to amplify the neurotoxic effects of MDMA and other club drugs [14–23]. An important question is whether combined sensory and physical stressors may cause learning impairment and cognitive dysfunction on their own, and whether the underlying mechanisms are drug targetable.

We now report that exposure to a combination of stressors that are reminiscent of those experienced by rave attendants enhances tau protein phosphorylation in CA1 and CA3 hippocampal regions, and causes a transient impairment of LTP at the Schaffer-collateral-CA1 synapses associated with a defect in spatial memory. Remarkably, these effects were mediated by 5-HT acting at 5-HT_{2C} receptors, and a single systemic injection of a selective 5-HT_{2C} receptor antagonist was sufficient to reverse the "pathological" phenotype caused by the combination of environmental stressors.

2. Methods

2.1. Materials

3,4-Methylendioxymethamphetamine (MDMA) was kindly provided by National Institute on Drug Abuse (NIDA) (Bethesda, MD). 4-Benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione (TDZD-8) was purchased from Calbiochem EMD Millipore (Darmstadt, Germany). Fenfluramine was purchased from Sigma (St. Louis, MO). RS-102,221 and ketanserin were purchased from Tocris Bioscience (Bristol, UK).

2.2. Animals

Experiments were performed using 8-week old male and female C57BL/6N and CD1 mice (Charles River, Calco, CO, Italy). All mice were kept under environmentally controlled conditions (room temperature = 22°C, humidity = 40%) on a 12-h light/dark cycle with food and water *ad libitum*. All experiments were carried out according to the European (86/609/EEC) and Italian (D. Lgs 116/92) guidelines of animal care. The specific experimental protocol was reviewed by the internal animal care and use committee and approved by the Italian Ministry of Health (D. Leg. 93/2011-B project #2). All efforts were made to minimize animal suffering and the number of animals used.

2.3. Experimental design

Male adult C57BL/6N mice exposed to a combination of environmental stressors and their unstressed controls (see below) were used in all experiments. Different groups of mice were used for (i) assessment of neuronal damage in the hippocampus and corpus striatum (at 36 h and 30 days after exposure to stress); (ii) assessment of tau protein phosphorylation in the hippocampus and striatum by immunohistochemical and Western blot analysis (up to 10 days after stress); (iii) measurements of 5-HT and 5-HIAA levels; (iv) measurements of 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor expression in the hippocampus (only unstressed mice); (iii) induction of LTP in the hippocampus at 24–36 h and 10 days after stress; and (iv) evaluation of spatial learning and memory during the 36 h following exposure to stress. Additional groups of stressed and unstressed mice were treated i.p. with each of the following drugs (or their respective vehicles): the GSK-3β inhibitor, TDZD-8 (10 mg/kg, i.p.), the 5-$HT_{2A/C}$ antagonist, ketanserin (1 mg/kg, i.p.), the selective 5-HT_{2C} receptor antagonist, RS-102,221 (2 mg/kg, i.p.), or MDMA (12.5 mg/kg, i.p.). In stressed mice, all these drugs were administered 2 min prior to the onset of the stress session. The 5-HT-relasing agent, fenfluramine (20 mg/kg) or a higher dose of MDMA (50 mg/kg, subdivided into two doses of 25 mg/kg administered with a 2-h interval) were injected i.p. only to unstressed control mice. RS-102,221 was injected in a saline solution containing 50% dimethylsulfoxide; all other drugs were dissolved in saline. Finally, other groups of mice were exposed to a second 14-h stress session 10 days after the first stress session. These groups of mice were used for neurochemical, electrophysiological, or behavioral analysis, as specifically indicated.

2.4. Stress session

Male C57BL/6N mice were exposed for 14 h to a multi-stressor condition consisting in multimodal combined stimuli: overcrowding, light stimuli and loud noise (Fig. 1A). The overcrowding was created by placing 18 mice of two strains (CD1 and C57BL/6N) and both sexes (4/5 female and 4/5 male for strain) in a small cage (13 cm (H) × 22 cm × 16 cm). Male and female mice of the two strains were placed in the same cage during exposure to stress to mimic the heterogeneity typical of rave events. No aggressive behavior was observed at least during the first two hours of stress exposure, and no animal was injured at the end of the stress session. Neurochemical, electrophysiological and behavioral experiments were carried out exclusively on male C57BL/6N mice. Light stimuli were administered using a 60 watt bulb system giving 2000 lux with intermittent intervals of 3 s. The continuous loud noise was generated playing a techno music track with an amplitude of 80 dB. The mean temperature measured inside the cage during the rave exposure was 27 ± 1 °C. Mice were exposed to stress for 14 h from 6 p.m. to 8 a.m.; therefore, exposure to stress included the whole dark phase of the light-dark cycle when the postsynaptic response to serotonin is known to be higher [24]. Control mice were placed in a 13 × 22 × 16 cm cage but with no overcrowding (five C57BL/6N male mice per cage) and were not exposed to visual or acoustic stressors.

2.5. HPLC analysis of 5-HT and 5-HIAA

Mice were killed at different times (from 2 h to 10 days) after the onset of stress. Hippocampi were dissected out, homogenized by sonication in 0.6 ml of ice-cold 0.1 M perchloric acid, and centrifuged in a microfuge at maximal speed. Twenty microlitres of the supernatant were injected into a HPLC equipped with a programmable solvent module 126 (Beckman, Instruments, Fullerton, CA), an analytical C18 reverse-phase column kept at 30 °C