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Short Communication

Methylphenidate has long-lasting metaplastic effects in the prefrontal cortex of adolescent rats

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HIGHLIGHTS

- Two week's methylphenidate (MPH) treatment enhanced LTP in the rat PFC *in vivo*.
- 15–18 days after the end of MPH treatment LTP remained strongly enhanced.
- Five months later LTP was no longer augmented.
- Could not be induced after high MPH doses.
- Doses of MPH that improved maze-learning also enhanced LTP in the same animals.

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ABSTRACT

Methylphenidate (MPH) is widely used as a "nootropic" agent and in the treatment of disorders of attention, and has been shown to modulate synaptic plasticity *in vitro*. Here we present *in vivo* evidence that this MPH-induced metaplasticity can last long after the end of treatment. MPH (0, 0.2, 1 and 5 mg/kg) was administered daily to male rats from postnatal day 42 for 15 days. The animals were tested daily in a radial maze. Long-term potentiation (LTP), a marker of neural plasticity, was induced *in vivo* in the prefrontal cortex after 2–3 h, 15–18 days or 5 months without treatment. The behavioral performance of the 1 mg/kg group improved, while that of animals that had received 5 mg/kg deteriorated. In the 1 and 5 mg/kg groups LTP induced 2–3 h after the last MPH treatment was twice as large as in the controls. Further, 15–18 days after the last MPH administration, in groups receiving 1 and 5 mg/kg, LTP was about fourfold higher than in controls. However, 5 months later, LTP in the 1 mg/kg group was similar to controls and in the 5 mg/kg group LTP could not be induced at all. No significant changes of LTP were seen in the low-dose group of animals (0.2 mg/kg). Thus, firstly, doses of MPH that improve learning coincide approximately with those that augment LTP. Secondly, MPH-induced increases in LTP can last for several weeks, but these may disappear over longer periods or deteriorate at high doses.

Methylphenidate (MPH) is the most frequently used substance in the treatment of Attention Deficit Hyperactivity Disorder (ADHD; [1]). It is also widely used as a performance enhancing drug [2], helping to focus attention and maintain concentration particularly among adolescents and young adults [3]. Prolonged repetitive ingestion appears to modulate behaviors in adolescents in a lasting way [4]. In this context, it is possible that MPH induces lasting plastic changes in the neural circuitry underlying these modulations.

Animal behavioral studies have shown that MPH facilitates the acquisition of certain learning tests, albeit within a quite limited dose range. Berridge and Devilbiss [5] reported that in rats, a dose of 0.5 mg/kg improved performance of a delayed response task. But,

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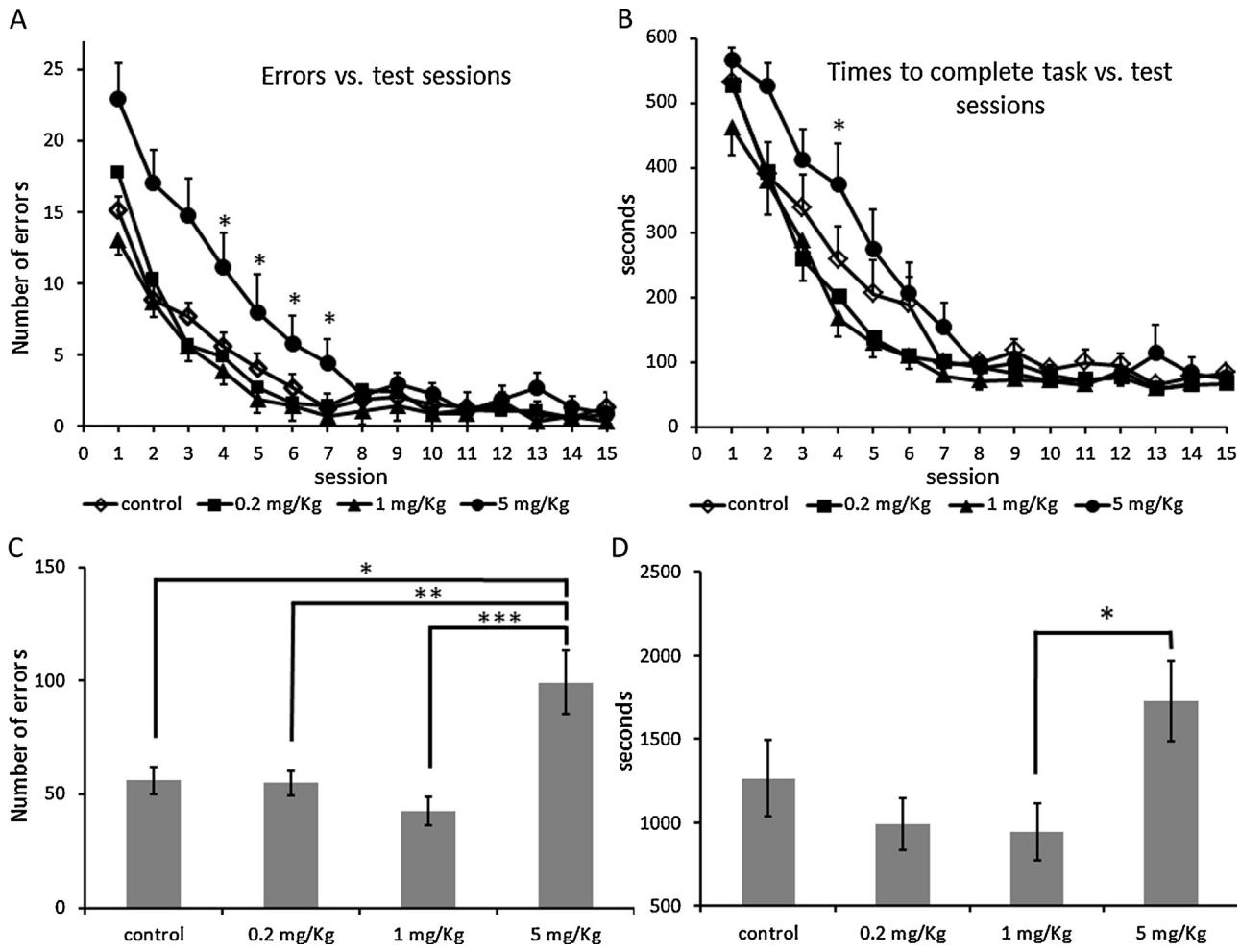

Fig. 1. MPH influences learning in the radial maze. (A) and (B) Controls and 3 groups that had been injected with MPH (0.2, 1 and 5 mg/kg) 30–60 min before testing continuously reduce the number of errors and times taken to complete the task until a plateau is reached. Individual trials display significant differences between the 5 mg/kg and 1 mg/kg groups. (C) and (D) Accumulated errors and times taken to complete the task for all sessions ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; $n = 12$ for all groups; HSD of Tukey; error bars: S.E.M.).

after a relatively small increase of the dose (2.0 mg/kg) performance deteriorated. Another study showed that orally administered MPH (3 mg/kg) improved performance in a radial maze behavioral learning task [6]. The underlying mechanism for such effects could be that MPH increases concentrations of dopamine and noradrenaline in brain structures relevant for these types of tasks [7,8] and modulates the underlying synaptic plasticity [9,10]. It is possible that MPH influences such cognitive abilities by way of a metaplastic effect. If this were so, modulation of synaptic plasticity should occur at similar doses that enhance performance in behavioral tasks.

Recently, it has been shown that MPH changes synaptic plasticity as assessed by its capacity to influence the induction of long-term potentiation (LTP) and/or long-term depression (LTD) in the hippocampus [11] and in the prefrontal cortex (PFC) [12]. The latter region is where MPH appears to exert effects on the expression of ADHD-related features such as the control of impulsivity [13]. Thus, in relevant brain structures MPH *in vitro* appears to fulfill the requirements of inducing metaplasticity (*i.e.* the modulation of plasticity) as has been characterized by Abraham and Bear [14]. But, it is not clear, however, whether MPH-induced metaplasticity also occurs *in vivo* and, if so, for how long it may last.

We designed our study of rats in order to model the repetitive administration of MPH in adolescent/young adult people and to correlate measurements of synaptic plasticity with performance on a learning test. To achieve this goal, we combined a radial maze test with the *in vivo* induction of LTP in the PFC. MPH was administered to rats shortly before daily training trials in a radial arm maze starting at postnatal day (PND) 42 and lasting for 15 days. At the end of behavioral testing, LTP was induced in the PFC *in vivo* at three time-points after the last administration of the drug. Details of the methods have been described in Burgos et al. [15]. The experiments were approved by the Ethics Committee of the University of Santiago de Chile.

Seventy four rats (male Sprague-Dawley; aged 21 PND) were purchased from the rearing facility of the Catholic University in Santiago. They were accustomed to the laboratory environment housing 4 animals per cage and maintained at a temperature of 20 °C on a 12/12 h light/dark cycle for 14 days. Six of these received no treatment or training and were fed *ad libitum* for four more weeks. Then they were subjected to the induction and recording of LTP as described below serving as "naïve" controls (Fig. 3B).

The remaining 68 animals were familiarized with the eight arm radial maze starting at PND 35 for 5 days without administering MPH. They were trained and treated with MPH from PND 42 for 15 days once a day in the morning (between 10 and 12 am). MPH (Andromaco Laboratories, Santiago de Chile) was dissolved in physiological saline and injected *ip* daily for 15 days, 30–60 min prior to each training session (0, 0.2, 1 and 5 mg/kg). One week before testing the *ad libitum* feeding regime was adjusted to maintain animals on average at 80% of the body mass observed with free food