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Brief Report

BDNF reverses the CTA memory deficits produced by inhibition of protein synthesis

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) is an essential protein synthesis product that has emerged as one of the most potent molecular mediators of not only central synaptic plasticity, but also behavioral interactions between an organism and its environment. Our previous studies on the insular cortex (IC), a region of the temporal cortex implicated in the acquisition and storage of conditioned taste aversion (CTA), have demonstrated that intracortical microinfusion of BDNF induces a lasting potentiation of synaptic efficacy in the projection from the basolateral nucleus of the amygdala (Bla) to the IC of adult rats in vivo. Recently, we found that intracortical microinfusion of BDNF previous to CTA training enhances the retention of this task. In this work, we present experimental data showing that acute intracortical delivery of BDNF (2 μg/2 μl per side) reverses the deficit in CTA memory caused by inhibition of insular cortex protein synthesis due to anisomycin administration (100 μg/μl per side) in male adult Wistar rats. These findings suggest that BDNF is a protein synthesis product essential for neocortical long-term memory storage.

In order to establish long-term memory, synaptic changes must be stabilized by the effects of newly synthesized proteins during a process called consolidation that takes place in areas of the brain like the neocortex, where the modifications that subserve certain forms of learning and memory are likely to reside.

Brain-derived neurotrophic factor (BDNF) has been considered a protein synthesis product, essential for the expression and persistence of long-term synaptic plasticity in the adult brain (Bekinschtein et al., 2008; Pang et al., 2004).

Research on the role of BDNF in learning and memory has focused on hippocampal long-term potentiation and behavioral tasks that are dependent on the hippocampus. In this regard, it has been demonstrated that spatial learning increases the hippocampal mRNA levels of BDNF (Kesslak, So, Choi, Cotman, & Gómez-Pinilla, 1998; Mizuno, Yamada, Olariu, Nawa, & Nabeshima, 2000) as well as its tyrosine kinase B receptor (TrkB) (Gómez-Pinilla, So, & Kesslak, 2001). Alonsoet al. (2002) showed that hippocampal BDNF is required for the formation of both short- and long-term memory and is continuously activated, in a time-dependent manner after consolidation for persistence of long-term hippocampal memory (Alonso et al., 2002). Recently, it has been demonstrated that 12 h after acquisition of a one-trial associative learning task, there is a novel protein synthesis and BDNF-dependent phase in the rat hippocampus that is critical for the persistence of long-term memory storage (Beckinschtein et al., 2007). BDNF and its high affinity receptor TrkB are also abundantly expressed in the neocortex (Yan et al., 1997). BDNF influences the development of patterned connections and the growth and complexity of dendrites in the cerebral cortex (McAllister, 2002). In addition, BDNF induces a long-lasting potentiation of synaptic transmission in the visual cortex of young rats both in vitro (Akaneya, Tsumoto, Kinoshita, & Hatanaka, 1997) and in vivo (Jiang et al., 2001). Regarding the neocortical behavior, increased BDNF and TrkB mRNA expression have been reported in cortical regions during the formation of visual pair-association memory or following the formation of a social recognition memory (Broad, Mimmack, Keverne, & Kendrick, 2002; Tokuyama, Okuno, Hashimoto, Xin Li, & Miyashita, 2000).

The insular cortex (IC) is a region of the temporal cortex in the rat that has been implicated in the acquisition and storage of different aversive motivated learning tasks like spatial maze, inhibitory avoidance and conditioned taste aversion (CTA) (Bermúdez-Rattoni, 2004; Bermúdez-Rattoni & McGaugh, 1991). The IC receives direct projections from the basolateral amygdaloid nucleus (Bla) and it is well established that both the amygdaloid complex and the IC contribute to the formation and retention of taste illness memories (Bermúdez-Rattoni, 2004; Bermúdez-Rattoni & McGaugh, 1991). CTA is a very robust and widely used model for the study of learning and memory processes. In this behavioral model, an animal acquires aversion to a novel taste when it is followed by digestive malaise. In this regard it has been shown that IC microinfusion of anisomycin 20 min before the acquisition of CTA impairs the consolidation of this task (Berman & Dudai, 2001; Rosenblum, Meiri, & Dudai, 1993).

Our previous studies demonstrated that acute microinfusion of BDNF in anesthetized adult rats induces a lasting potentiation of

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synaptic efficacy in the IC (Escobar, Figueroa-Guzman, & Gomez-Palacio Schjetnan, 2003). Moreover, we have recently found that intracortical delivery of BDNF 24 h before CTA training enhances the retention of this task (Castillo, Figueroa-Guzman, & Escobar, 2006). In a recent study, Pang and collaborators showed that BDNF is sufficient to rescue late-phase long-term potentiation (L-LTP) when hippocampal protein synthesis is inhibited (Pang et al., 2004).

In this study, we investigated whether BDNF is capable of reversing the deficit in CTA memory caused by the inhibition of insular cortex protein synthesis.

Sixty-nine male Wistar rats weighing 350–380 g were prepared for this experiment. They were housed individually under a 12/12-h light–dark cycle, with food and water *ad libitum* (except where indicated) and an average room temperature of 22 °C. Animals were implanted bilaterally with 23-gauge stainless steel cannulae under anesthesia (Nembutal, 50 ml/kg i.p.) using standard stereotaxic procedures. The tips of the guide cannulae were aimed to 5 mm above the IC (Castillo et al., 2006; Escobar, Alcocer, & Bermúdez-Rattoni, 2002). For all groups, microinjections were delivered through 30-gauge dental needles as microinjectors that extended 5 mm below the previously implanted guide cannulae (reaching de IC area), as shown in Fig. 1A. Dental needle microinjectors were attached by polyethylene tubing to a 10 μl Hamilton syringe driven by a microinfusion pump (Cole Parmer Co.). Intracortical infusions were given 20 min before the CTA acquisition (Rosenblum et al., 1993). The animals were divided into the following treatment groups: (1) ANI group (ANI, $n = 11$), which received an intracortical microinfusion of anisomycin at a concentration that has been shown to act for 90 min with a 90% of protein synthesis inhibition (100 μg/μl per side, Sigma, St. Louis, MO; Rosenblum et al., 1993); (2) ACSF group (ACSF, $n = 13$), which received an intracortical microinfusion of artificial cerebrospinal fluid (1 μl/min) as anisomycin vehicle; (3) ANIBDNF group (ANIBDNF, $n = 11$), which received the same treatment as ANI group followed immediately by a phosphate buffer solution (PBS)-containing BDNF (2 μg/2 μl per side, Alomone Labs., Jerusalem) (Castillo et al., 2006; Escobar et al., 2003; Jiang et al., 2001); (4) ANIPBS group (ANIPBS, $n = 10$), which received the same treatment as ANI group and PBS (2 μl); (5) CON group (intact control group, $n = 11$), which received the CTA training without any surgical procedure.

Two additional groups (ANIST, $n = 6$) and (ANIST H_2O, $n = 7$), received the same treatment as ANI group and were tested 4.5 h after CTA acquisition session (Ferreira, Gutiérrez, De la Cruz, & Bermúdez-Rattoni, 2002), thus assessing the CTA short-term memory.

A previously described experimental procedure for CTA was used (Castillo et al., 2006; Escobar et al., 2002). Briefly, animals were deprived of water for 24 h and then habituated to drink water from a graduated cylinder twice a day (with an interval of 8 h) during 10-min trials for 3 days, until a stable water consumption baseline was attained. On the acquisition day, water was replaced by saccharin solution 0.1% (Sigma), and 10 min after saccharin consumption the animals received 7.5 ml/kg i.p. of a 0.15 M solution of LiCl, which induces digestive malaise. After three more days of water baseline consumption, water was once again replaced by a 0.1% saccharin solution to test the aversion. The reduction of saccharin consumption with respect to baseline intake was used as a measure of strength of aversion. For groups ANIST and ANIST H_2O the interval of liquid intake was reduced from 8 to 4.5 h during the whole procedure. The group ANIST H_2O received water instead of saccharin during the aversion trial. Upon completing the behavioral experiments, cannulated animals were histologically analyzed in order to verify the injector tip location.

Histological examinations revealed that injectors were placed in the IC in all the groups (Fig. 1A). Three animals with unclear cannulae placements were discarded; two of them were from the ACSF group and the other from the ANI group.

No significant differences among groups were found either in the baseline water intake or during the first presentation of the conditioned stimulus (saccharin flavored solution during CTA acquisition). The average baseline means (±*SEM*) of water intake were (in ml) 16.98 ± 0.44, 16.57 ± 0.54, 15.38 ± 0.57, 15.47 ± 0.41

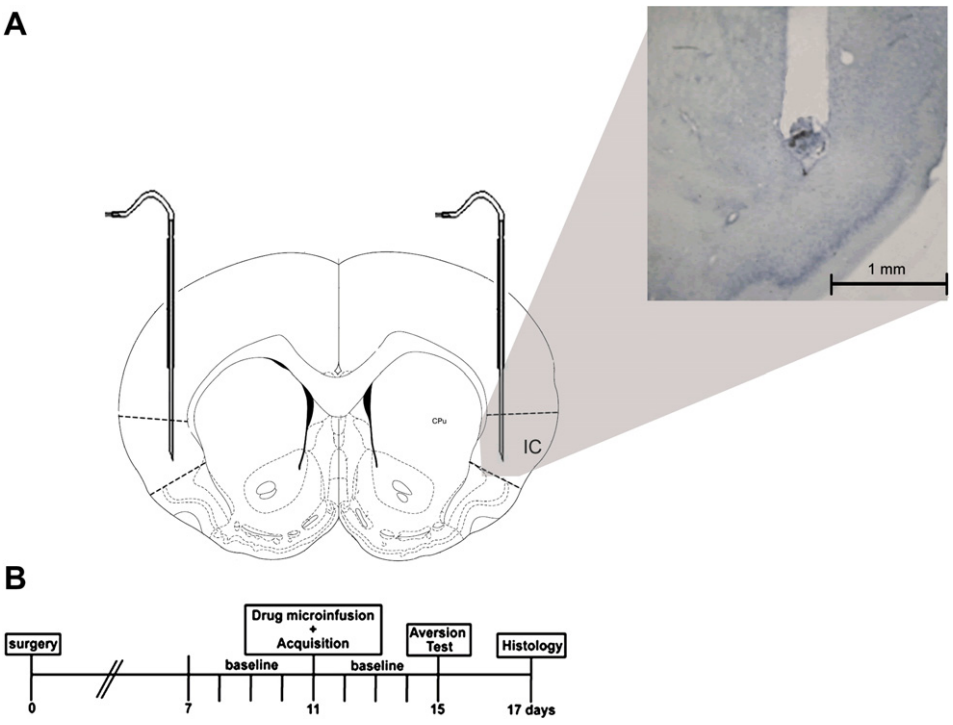

Fig. 1. Schematic representation of the experimental procedure. (A) Schematic diagram of the guide cannulae and microinjectors placement in a coronal plane, discontinuous lines indicate the boundaries of IC area. (B) Diagram of the experimental procedure. IC, insular cortex; CPu, caudate putamen.