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Differential Arc protein expression in dorsal and ventral striatum after moderate and intense inhibitory avoidance training

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ABSTRACT

Intense training refers to training mediated by emotionally arousing experiences, such as aversive conditioning motivated by relatively high intensities of foot-shock, which produces a strong memory that is highly resistant to extinction. Intense training protects memory consolidation against the amnestic effects of a wide variety of treatments, administered systemically or directly into brain structures. The mechanisms of this protective effect are unknown. To determine a potential neurobiological correlate of the protective effect of intense training, rats were trained in a one-trial step-through inhibitory avoidance task using different intensities of foot-shock (0.0, 0.5, 1.0, and 2.0 mA). Some rats from each group were sacrificed 45 min after training for immunohistochemical Arc protein detection in dorsal and ventral striatum; other rats were tested for extinction during six consecutive days, starting 48 h after training. The results showed that training with 1.0 and 2.0 mA produced optimal retention scores, which were significantly higher than those of the 0.5 and 0.0 mA groups. Also, a higher resistance to extinction was obtained with 2.0 mA than with the other intensities. A high number of neurons expressed Arc in ventral, but not in dorsal striatum in both the 1.0 and 2.0 mA groups, with a larger area of Arc signal in the latter group. We conclude that an increased Arc expression may be related to enhanced synaptic plasticity in the ventral striatum, suggesting that it may be one of the physiological substrates of enhanced learning.

1. Introduction

Information derived from different learning experiences are not similarly stored and retrieved. Compared with events with a neutral connotation, emotional or aversive events are recalled better due to the release of stress hormones, which interact with endogenous neurotransmitters to facilitate memory consolidation (McGaugh, 2013; Schwabe, Joëls, Roozendaal, Wolf, & Oitzl, 2012). Furthermore, stronger consolidation is produced by enhanced learning. Examples of enhanced learning are post-traumatic stress disorder (PTSD) (Parsons & Ressler, 2013), addiction-motivated learning (Hyman, 2005; Torregrossa, Corlett, & Taylor, 2011), and learning produced by intense training (Prado-Alcalá, Medina, López, & Quirarte, 2012). The latter refers to learning mediated by a high number of trials or training sessions, or to one-trial aversive conditioning motivated by high intensities of foot-shock (Prado-Alcalá, 1995; Prado-Alcalá et al., 2007, 2012). The present work focused on intense training.

Intense training produces a strong memory of inhibitory avoidance (IA) which is quite resistant to extinction (Bello-Medina, Flores, Quirarte, McGaugh, & Prado Alcalá, 2016; Garín-Aguilar et al., 2012; Prado-Alcalá, Haiek, Rivas, Roldan-Roldan, & Quirarte, 1994), and to a wide variety of amnestic treatments, administered either systemically or directly into brain structures; these include sodium channel blockers (Garín-Aguilar, Medina, Quirarte, McGaugh, & Prado-Alcalá, 2014; Salado-Castillo, Sánchez-Alavéz, Quirarte, Martínez García, & Prado-Alcalá, 2011), protein synthesis inhibitors (Díaz-Trujillo et al., 2009), and neurotransmitter receptor antagonists (Cobos-Zapiaın et al., 1996; Durán-Arévalo, Cruz-Morales, & Prado-Alcalá, 1990;

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Solana-Figueroa, Salado-Castillo, Quirarte, Galindo, & Prado-Alcalá, 2002).

The protective effect of intense training has been studied in several brain structures involved in memory consolidation, such as the hippocampus (Quiroz et al., 2003), amygdala (Salado-Castillo et al., 2011), substantia nigra (Cobos-Zapiaín et al., 1996; Salado-Castillo et al., 2011), and striatum (Prado-Alcalá & Cobos-Zapiaín, 1977; Pérez-Ruiz & Prado-Alcalá, 1989). Importantly, the striatum also has been linked to PTSD (Goodman, Leong, & Packard, 2012), and to the reward-reinforcement learning involved in drug addiction (Yager, Garcia, Wunsch, & Ferguson, 2015).

Growing evidence supports a functional heterogeneity of the striatum. This has led to anatomically dissect the striatum into distinct functional regions or compartments. Taking into account the reciprocal and unidirectional connections with the hippocampus, amygdala, and motor cortical areas, the striatum has been divided into dorsolateral, dorsomedial and ventral regions (Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004), albeit other groups suggest a functional distinction between the dorsomedial and dorsolateral regions because the former is predominantly involved in spatial/contextual learning, whereas the latter enables the formation of procedural learning (Devan & White, 1999; Lozano, Serafín, Prado-Alcalá, Roozendaal, & Quirarte, 2013; Packard & Knowlton, 2002; White & McDonald, 2002). We have also reported that there is a differential involvement of its medial and lateral regions in memory consolidation of inhibitory avoidance learning (Salado-Castillo, Dıaz del Guante, Alvarado, Quirarte, & Prado-Alcalá, 1996), and that intense training increases dendritic mushroom spines in dorsomedial, but not in the dorsolateral striatum (Bello-Medina et al., 2016). In addition, cholinergic blockade of dorsal striatum induced retention deficits of an aversively motivated task while blockade of ventral striatum facilitated retention of this task (Neill & Grossman, 1970).

Given the functional heterogeneity of the striatum, the aim of this study was to determine whether different intensities of foot-shock used in inhibitory avoidance training induce distinct patterns of neuronal activation. One strategy to visualize neuronal activation is to measure the expression of immediate-early genes (IEGs). IEG expression is a well-recognized powerful tool for evaluating neuronal activity in the brain; detection of IEG mRNA or protein products in the brain provides information about where and when neurons were activated (Okuno, 2011; Ramírez-Amaya et al., 2005). One such IEG is Arc (Activity-regulated cytoskeletal associated protein, also known as Arg3.1) (Link et al., 1995; Lyford et al., 1995), whose expression is highly dynamic and is induced by robustly patterned synaptic activity, including natural stimuli, seizures, LTP, and memory-related processes (Guzowski, 2002; Guzowski, Setlow, Wagner, & McGaugh, 2001; Guzowski et al., 2000; Lyford et al., 1995; Ramírez-Amaya et al., 2005).

Exploration of new environments induces strong Arc expression in the hippocampus as well as in related neocortical areas such as the parietal and entorhinal cortex in rats and mice (Guzowski, McNaughton, Barnes, & Worley, 1999; Ramírez-Amaya et al., 2005). Arc involvement in learning and memory processes is further supported by the deterioration of long-term potentiation and spatial water maze learning produced by inhibition of its expression with infusion of antisense oligodeoxynucleotides directly into the hippocampus (Guzowski et al., 2000); this inhibition also impairs long-term, but not short-term memory of inhibitory avoidance (McIntyre et al., 2005). Furthermore, it was found that in the hippocampus and cortex of rats the proportion of cells expressing Arc protein is the same as that of cells that express Arc mRNA after spatial exploration (Ramírez-Amaya et al., 2005).

Although Arc mRNA expression in dorsomedial or dorsolateral striatum alone did not correlate with the stage of training in a dual-solution task (a task that can be solved using either place or response strategies), the ratio of expression in the dorsomedial striatum to that in the dorsolateral striatum was relatively high among rats that used a place strategy early in training, as compared with the ratio among over-trained response rats (Gardner et al., 2016). Together, these data stress the importance of Arc protein in the consolidation of different types of memory, as well as its usefulness for mapping neuronal networks that underlie information processing.

In sum, the studies described above showed that there is a regional differentiation within the striatum regarding the processing of information involved in memory of diverse tasks, mediated by moderate or intense training. In the present work we hypothesized that moderate training of inhibitory avoidance induces the expression of Arc protein in different striatal regions; we also predicted that intense training will induce a higher expression of this protein in different striatal regions.

In order to test these hypotheses, we evaluated the expression patterns of the IEG Arc in the dorsomedial, dorsolateral, ventromedial, and ventrolateral regions of the striatum after training rats in the inhibitory avoidance task with different foot-shock intensities. Additionally, as an indication of the extent of emotional arousal produced by the task, we quantified blood serum corticosterone (CORT) concentration, which correlates positively with the intensity of aversive training (Cordero, Merino, & Sandi, 1998).

2. General methods

This section describes the procedures common to the different experiments of this study.

All experimental procedures were approved by the Animal Ethics Committee of the Instituto de Neurobiología, Universidad Nacional Autónoma de México, which complies with the Mexican Council Norm (SAGARPA NOM-062-ZOO-1999) and the NIH Guidelines for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.1. Subjects

We studied 98 adult male Wistar rats (250–350 g; 8–9 weeks old on arrival to the laboratory vivarium), which were maintained in a room with a 12/12 h light-dark cycle (lights on at 7:00 h) and housed individually in acrylic cages with food and tap water ad libitum. The temperature of the room where the animals were housed was kept at 21 °C.

2.2. Handling

Rats were handled, once a day, for 5 min across 3 days prior to the experiments. The handling procedure consisted of gently touching and holding the rat with both hands. All animals were handled and transported from the vivarium to the handling room, including a group that never experienced the inhibitory avoidance task nor the training room (group Cage). All the experiments were performed between 9:00 h and 13:00 h.

2.3. Apparatus

The rats were trained in an inhibitory avoidance apparatus consisting of two compartments separated by a sliding door. The safe compartment (30 × 30 × 30 cm) had a lid and walls made of red-colored acrylic, with a floor made of stainless steel bars (6 mm in diameter, 9 mm apart). This compartment was illuminated by a 10 W light bulb located in the center of its lid. The other, non-illuminated shock compartment (30 cm long) had front and back walls and a floor made of stainless steel plates with side walls