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Increase in c-Fos and Arc protein in retrosplenial cortex after memory-improving lateral hypothalamic electrical stimulation treatment

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ABSTRACT

Post-training Intracranial self-stimulation (ICSS) of the lateral hypothalamus (LH), a kind of rewarding deep-brain stimulation, potentiates learning and memory and increases c-Fos protein expression in specific memory-related brain regions. In a previous study, Aldavert-Vera et al. (2013) reported that post-acquisition LH-ICSS improved 48 h retention of a delay two-way active avoidance conditioning (TWAA) and induced c-Fos expression increase in CA3 at 90 min after administration. Nevertheless, this c-Fos induction was only observed after the acquisition session and not after the retention test at 48 h, when the ICSS improving effect was observed on memory. This current study aims to examine the hypothesis that post-training ICSS treatment may stimulate c-Fos expression at the time of the TWAA retention test in retrosplenial cortex (RSC), a hippocampus-related brain region more closely related with long-lasting memory storage. Effects of ICSS on Arc protein, a marker of memory-associated synaptic plasticity, were also measured by immunohistochemistry in granular and agranular RSC. The most innovative results are that the ICSS treatment potentiates the c-Fos induction across TWAA conditions (no conditioning, acquisition and retention), specifically in layer V of the granular RSC, along with increases of Arc protein levels in the granular but not in agranular areas of RSC ipsilaterally few hours after ICSS. This leads us to suggest that plasticity-related protein activation in the granular RSC could be involved in the positive modulatory effects of ICSS on TWAA memory consolidation, opening a new approach for future research in ICSS memory facilitation.

1. Introduction

Intracranial self-stimulation (ICSS) of the lateral hypothalamus (LH), a form of rewarding deep-brain stimulation, potentiates learning and memory, especially when administered immediately post-training. Molecular studies have shown that ICSS increases expression of synaptic plasticity related genes, such as c-Fos and Arc, which are associated with learning and memory consolidation processes in the hippocampus and amygdala memory-related areas (Huguet et al., 2009) (Kadar et al., 2011) (Kádár, Huguet, Aldavert-Vera, Morgado-Bernal, & Segura-Torres, 2013) (Shankaranarayana Rao, Raju, & Meti, 1999) (Takahashi et al., 2009). Behaviorally, ICSS-treated animals require less training to reach the same level of performance than that required for controls (Major & White, 1978) (Redolar-Ripoll, Aldavert-Vera, Soriano-Mas, Segura-Torres, & Morgado-Bernal, 2002). Additionally, the effectiveness of LH-ICSS in reversing severe memory deficits caused by certain brain area lesions such as basolateral (BLA) and lateral (LA) amygdala (Kádár et al., 2014) has been demonstrated, suggesting that mechanisms related to learning may be positively modulated by post-training ICSS. LH-ICSS learning and memory

Abbreviations: Ac, acquisition; AP, antero-posterior; aRSC, agranular retrosplenial cortex; BLA, basolateral amygdala; CA1, CA1 region of the hippocampus; CA3, CA3 region of the hippocampus; CS, conditioned stimulus; DAB, 3,3′-diaminobenzidine; gRSC, granular retrosplenial cortex; ICSS, intracranial self-stimulation; LA, lateral amygdala; LH, lateral hypothalamus; OI, optimum current intensity of ICSS; PBS, phosphate buffered saline; ROI, region of interest; RSC, retrosplenial cortex; Rt, retention; rt, room temperature; TWAA, two-way active avoidance conditioning; US, unconditioned stimulus.

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improvement is hypothesized to be a consequence of simultaneous activity modulation of medial forebrain bundle (MFB) projections to different structures related to memory akin to that produced by learning, with behavioral effects similar to those observed after overtraining (Aldavert-Vera et al., 2013). Thus, ICSS is able to enhance different plasticity-related memory mechanisms, from long-lasting structural changes in the hippocampus (Chamorro-López et al., 2015) to changes in the expression of several genes and proteins related to learning and memory, and to neuroprotection, in several memory systems (Huguet et al., 2009) (Kadar et al., 2011) (Kádár et al., 2013).

A previous work by our group (Aldavert-Vera et al., 2013) showed that LH-ICSS administered immediately after a 5-trial acquisition session of a delay two-way active avoidance (TWAA) conditioning results in an improved retention level assessed at 48 h. Interestingly, we have also shown that combined ICSS and TWAA results in a significantly higher c-Fos increase in the CA3 hippocampal region than that observed for each factor separately. However, this c-Fos increase was only observed immediately after TWAA acquisition and not 48 h later, along with the increase in memory retention. This result led us to believe that memory-consolidation-improving ICSS treatment could also implicate other brain regions, such as the retrosplenial cortex (RSC), more closely related to long-lasting memory consolidation and/or memory retrieval (Todd & Bucci, 2015). The RSC is one of the largest cortical areas in rats, reciprocally connected with many neocortical areas such as the dorsolateral prefrontal cortex, the hippocampus and memory-related thalamic nuclei. There is growing evidence that RSC neurons undergo activity-dependent plastic changes during memory formation and retrieval, at least for spatial and contextual memories, suggesting an important role of the RSC in long-lasting memory storage (Todd & Bucci, 2015). Consistently, it has been proposed that maintenance of the fear-memory trace may depend on late post-training activation of c-Fos in some RSC areas (Katche, Dorman, Gonzalez et al., 2013). While RSC has been considered as a target of the hippocampal-dependent systems that consolidate long-term memory (Miller, Vedder, Law, & Smith, 2014), their involvement in hippocampal-independent tasks, such as delay active avoidance used in this study, has also been suggested (Lukoyanov & Lukoyanova, 2006), but has been less studied.

Interest in RSC is also potentiated by the fact that Arc-protein expression, a marker of memory-related synaptic plasticity (Miyashita, Kubik, Lewandowski, & Guzowski, 2008), is elevated in this area during memory tests at both 1 and 30 days after training in a water maze task (Todd & Bucci, 2015). However, there are no studies analyzing ICSS effects in Arc-RSC expression.

Therefore, in the present study we have evaluated the effects of acquisition and 48-h retention of a TWAA conditioning in a delay paradigm, and the effect of an LH-ICSS treatment on c-Fos protein RSC expression. Complementarily, effects of LH-ICSS on Arc expression in the RSC have been also evaluated.

2. Methods

2.1. Subjects and stereotaxic surgery

A total of 52 (c-Fos study, $n = 34$; Arc study, $n = 18$) male Wistar rats obtained from our laboratory breeding stock were used, with a mean age of 90.23 days (SD = 5.00) and a mean weight of 369.40 g (SD = 25.60) at the beginning of the experiment. The animals were maintained on a 12 h light/dark cycle, and food and water were available ad libitum. All procedures were approved by the Ethics Committee at the *Universitat Autònoma de Barcelona* (protocol 2023).

Under general anesthesia induced by 110 mg/kg Ketolar® *Ketamine chlorhydrate* (Parke-Davis S.L. Pfiezer, Madrid, Spain) and 0.08 ml/100 g Rompun® *Xylazin* 23 mg/ml; i.p. (Bayer, Barcelona, Spain), all rats were implanted with a monopolar stainless steel electrode (150 μm in diameter) aimed at the LH, into the fibers of the medial forebrain bundle, with the incisor bar set at −2.7 mm below the interaural line and according to coordinates from the stereotaxic atlas (Paxinos & Watson, 2007): AP = −2.56 mm; $L = 1.8$ mm (right hemisphere) and $P = -8.5$ mm, with the cranium surface as dorsal reference. ICSS electrodes were anchored to the skull with jeweler's screws and dental cement.

2.2. ICSS behavior and treatment

After a post-surgery recovery period of 7 days, animals were randomly distributed into the experimental groups. Rats in the ICSS groups were taught to self-stimulate by pressing a lever in a conventional Skinner box. Electrical brain stimulation consisted of 0.3 s trains of 50 Hz sinusoidal waves at intensities ranging from 30 to 200 μA. The self-stimulating behavior was shaped, on two consecutive days, to establish optimum-current intensity (OI) of ICSS for each rat. OI was calculated from the mean of the two current intensities producing the highest response rate (responses/min).

ICSS treatment was administered in a single session, consisting of 2500 self-administered trains of electrical stimulation at the OI for each subject. Rats in the groups that would not receive ICSS were equally placed in the ICSS-box for 40 min but did not receive stimulation ("sham treatment"). Treatment duration (min) and total number of lever pressings (total responses) in the ICSS session were recorded. Immediately following either the ICSS-treatment or the sham-treatment session, rats were returned to their home cages.

2.3. c-Fos study

2.3.1. Experimental groups

The effects of a single post-training ICSS-treatment session and/or TWAA acquisition and retention were evaluated on c-Fos expression in the granular (gRSC) and agranular (aRSC) regions of the RSC. For greater clarification, Fig. 1 summarizes the procedure and experimental groups (the same as those used in Aldavert-Vera et al., 2013).

2.3.2. TWAA acquisition and retention

TWAA conditioning was conducted in a 50 × 24 × 23-cm automated two-way shuttle-box (Letica LI-916, Barcelona, Spain) enclosed in a sound attenuating box ventilated by an extractor fan. Acquisition (5 trials, one session) and retention sessions (20 trials, one session 48 h post-acquisition) consisted of the presentation of a 60 dB and 1 kHz tone of 3 s duration (conditioned stimulus, CS) followed by a 0.5 mA electrical foot shock (unconditioned stimulus, US). Both stimuli terminated simultaneously after 15 s according to a delay procedure. The inter-trial interval varied randomly from 50 to 70 s. Crossing into the opposite compartment during the first 3 s of the trial, i.e., before the onset of the shock, was registered as a correct avoidance response. In addition, the total time taken by the rat to enter the opposite compartment from the onset of the conditioned stimulus (response latency) was measured. Just before acquisition and retention sessions, each rat was allowed 10 min of free ambulation in the shuttle box.

2.3.3. c-Fos immunohistochemistry

c-Fos immunodetection was performed as previously described (Aldavert-Vera et al., 2013). Briefly, rats were sacrificed 70 min after the last experimental condition according to each group,