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ORIGINAL ARTICLE

Requirement of an Early Activation of BDNF/c-Fos Cascade in the Retrosplenial Cortex for the Persistence of a Long-Lasting Aversive Memory

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Abstract

During the past few years, there has been growing interest in the role of the retrosplenial cortex (RSC) in memory processing. However, little is known about the molecular changes that take place in this brain region during memory formation. In the present work, we studied the early post-training participation of RSC in the formation of a long-lasting memory in rats. We found an increase in c-Fos levels in the anterior part of the RSC (aRSC) after inhibitory avoidance (IA) training. Interestingly, this increase was associated with memory durability, since blocking c-Fos expression using specific antisense oligonucleotides (ASO) impaired long-lasting retention 7 days after training without affecting memory expression 2 days after training. In addition, we showed that BDNF is one of the upstream signals for c-Fos expression required for memory persistence, since blocking BDNF synthesis prevents IA training-induced increase in c-Fos levels in aRSC and affects memory persistence. In addition, we found that injection of BDNF into aRSC around training was sufficient to establish a persistent memory and that this effect was prevented by *c-fos* ASO infusion into the same structure. These findings reveal an early post-training involvement of aRSC in the processing of a long-lasting aversive memory.

Key words: IEG, inhibitory avoidance, memory persistence, rat

Introduction

The retrosplenial cortex (RSC) comprises the entire posterior cingulate cortex in rodents (Vogt and Peters 1981) and it has almost half of the length of the rat cerebrum. Situated at the crossroads between the hippocampal formation and many neocortical areas including the visual cortices and the medial prefrontal cortex, it has attracted much attention especially for its involvement in cognition and spatial navigation (Vann et al. 2009; Nelson et al. 2014). There is a growing body of evidence suggesting that RSC dysfunctions participate in the earliest stages of Alzheimer's disease and in anterograde amnesia (Minoshima et al. 1997; Nestor et al. 2003; Villain et al. 2008; Pengas et al. 2010; Aggleton 2014). Moreover, recent studies have shown several alterations in resting-state connectivity in the RSC and hippocampal formation in people at high risk for Alzheimer's disease (Drzezga et al. 2011; Sheline and Raichle 2013).

Although reversible or irreversible inactivation of RSC produces memory alterations resembling those caused by hippocampal lesions (Cooper and Mizumori 2001; Vann and Aggleton 2004; Keene and Bucci 2008a, 2008b, 2008c; Robinson et al. 2011, 2012; Katche, Dorman, Slipczuk, et al. 2013; Nelson et al. 2014), the role of this area in memory processing is still poorly understood (Ranganath and Ritchey 2012).

Long-lasting storage of information is a hallmark feature of brain circuits. While much attention has been paid to studying the mechanisms of long-term memory (LTM) formation, much less is known about the mechanisms that underlie the persistence

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of memory storage over time (see for references Katche, Cammarota, et al. 2013). We and others have previously described the requirement of a late phase of protein synthesis and gene expression in several brain regions including the anterior part of the RSC (aRSC), 12 h after training, for the persistence of contextual fear conditioning and inhibitory avoidance (IA) LTM (Bekinschtein et al. 2007; Ou et al. 2010; Katche, Dorman, Gonzalez, et al. 2013; Nakayama et al. 2015). In the hippocampus, we also demonstrated that BDNF signaling involving the activation of c-Fos and Zif-268 is critical for the establishment of long-lasting IA memory (see for references Bekinschtein et al. 2014). In the aRSC, we recently found that 2 different protein synthesis inhibitors infused during a critical time window around 12 h after IA training caused a deficit in memory maintenance without affecting LTM formation; similar findings were obtained when antisense oligonucleotides (ASO) for *c-fos* were delivered 8 h after IA training (Katche, Dorman, Gonzalez, et al. 2013). Taking into account that c-Fos is rapidly and transiently expressed during different learning tasks (Izquierdo and Medina 1997; Tischmeyer and Grimm 1999; Guzowski 2002; Kubik et al. 2007) and that inhibition of c-Fos expression impaired memory formation (Dudai 1996; Tischmeyer and Grimm 1999; He et al. 2002), in the present study we first determined whether there is an early post-training activation of c-Fos protein expression in the aRSC and then we tested its role in the formation and maintenance of IA LTM. In addition, we studied BDNF expression in RSC as an upstream signal for c-Fos expression in memory persistence.

Materials and Methods

Animals

Experiments were conducted in male Wistar rats (UBA, Argentina) weighting 220–250 g and 2.5 months old. Animals were housed 5 to a cage at 23°C, with water and food ad libitum, under a 12 h light/dark cycle (lights on at 7:00 AM). The procedures followed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committees of the University of Buenos Aires.

Inhibitory Avoidance Training and Testing

Animals were trained in a one-trial step-down IA task as previously described (Bekinschtein et al. 2007). Briefly, the apparatus was a 50 × 25 × 25 cm acrylic box with a 5-cm-high, 7-cm-wide, and 25-cm-long platform on the right end of a series of stainless steel bars that made up the floor of the box. For training, animals were gently placed on the platform; as they stepped down to the grid they received either a 3-s, 0.7-mA scrambled foot-shock (strong training) or a 3-s, 0.3-mA scrambled foot-shock (weak training). Rats were tested for retention 2 or 7 days after training. All animals were tested only once. In the test sessions, the foot-shock was omitted. In all experiments, the animals were trained between 7:00 AM and 9:00 AM.

Open Field and Elevated Plus Maze Tests

The open field was a 50 × 50 × 39 cm arena with black plywood walls and floor divided into 9 squares by black lines. The number of line crossings and rearings was measured during a 5-min-long test session. To evaluate their anxiety state, animals were exposed to an elevated plus maze. The total number of entries into the 4 arms, the number of entries, and time spent in the open arms were recorded over a 5-min session.

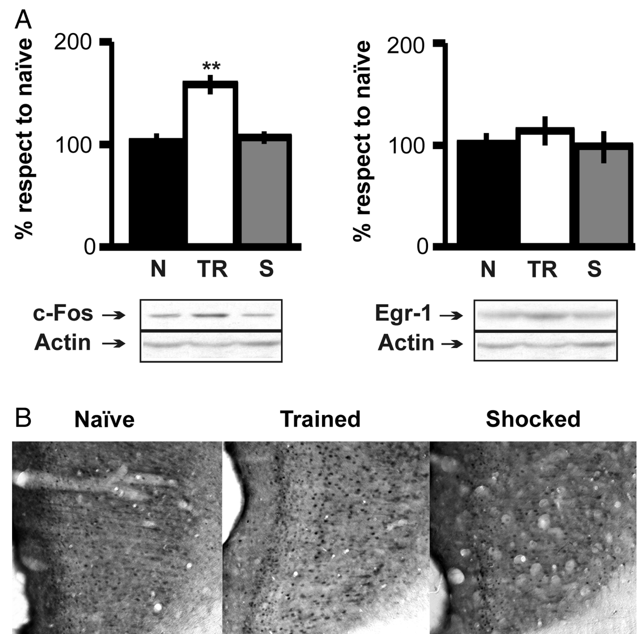

Figure 1. Memory processing is associated with an early increase in c-Fos expression, but not Egr-1 expression, in aRSC. (*A*,*B*) Change in aRSC c-Fos (left) and Egr-1 (right) levels after strong IA training. Bars indicate the percentage of change respect to the naïve (N, black bar) group for rats trained (TR, white bar) and Shocked (S, gray bar), and sacrificed 1 h after the behavioral procedure. Data are expressed as mean ± SEM. $^{**}P < 0.01$, Newman–Keuls after ANOVA, $n = 5$ per group. (*B*) IA training induced an increased in c-Fos immunoreactivity in aRSC. Rats were sacrificed 1 h after IA training, coronal sections of the brain were subjected to immunohistochemical assays using antibodies against c-Fos.

Immunoblot Assays

The animals utilized in the biochemical experiments (Figs 1A and 4A) were divided in 3 experimental groups: 1) animals trained in the IA task and killed 1 h after training (Trained group, TR); 2) animals received a foot-shock identical to that given to the trained ones but were not submitted to the IA training procedure (the platform was not inside the box, and the animals were put directly over the grid) and killed 1 h after shock as the trained group (Shocked group, S); and 3) animals withdrawn from their home cages at the same time point than the other 2 groups and killed immediately thereafter (Naïve group, N). Animals were killed by decapitation, brains were isolated and the aRSC was dissected out on ice from slices between anterior (A) −2.3 to −6.3 and lateral (L) ± 0.5 coordinates of the atlas of Paxinos and Watson (1997) and rapidly homogenized in ice-chilled buffer [20 mM Tris-HCl (pH 7.4), 0.32 M sucrose, 1 mM EDTA, 1 mM EGTA, 1 mM PMSF, 10 µg/mL aprotinin, 15 µg/mL leupeptin, 50 mM NaF and 1 mM sodium] as described previously. Tissue was homogenized and samples of homogenates were subjected to SDS-PAGE as described before. PVDF membranes were incubated first with anti-BDNF (1:1000; Santa Cruz Biotechnology), anti-c-Fos (1:3000; Santa Cruz Biotechnology) or anti-Egr1 antibody (1:2000; Santa Cruz Biotechnology), then stripped and incubated with anti-Actin antibody (1:5000, Santa Cruz Biotechnology). Image densitometry analysis was performed by using Gel-Pro Analyzer (version 4.0, Media Cybernetics, Inc., MD, USA).

Immunohistochemistry

Rats were anesthetized with ketamine/xylazine anesthesia (100 and 20 mg/kg, respectively) 1 h after IA training or shock or

Naïve and perfused transcardially with 100 mL of 0.9% saline, followed by 300 mL of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were isolated and transferred to a solution of 30% sucrose in 0.1 M phosphate buffer at 4°C for 48 h. Brains were frozen and cut (50 µm) in the frontal plane. The brain sections were subjected to an immunohistochemical assay with an anti-c-Fos antibody (1:1000; Santa Cruz Biotechnology). The reaction product was then visualized using the nickel-DAB technique (Vector Laboratories).

Surgery and Infusion Procedures

Rats were implanted under deep ketamine/xylazine anesthesia (100 and 5 mg/kg, respectively) with 22-g guide cannulae in the aRSC at coordinates A −4.3, L ± 0.5, V −1.8 of the atlas of Paxinos and Watson (1997). The cannulae were fixed to the skull with dental acrylic. Obturators were then inserted into the cannulae to prevent blockage. After 4 or 5 days of recovery from surgery, the animals were handled once a day for 2 days and then trained in IA. In all cases, infusions were bilateral and had a volume of 1 µL. The entire infusion procedure took ~4 min, the infusion rate was 1 µL/min. Injectors were left in place for an additional minute following infusion before they were removed carefully. Histological examination of cannula placements was performed and only the behavioral data from animals with the cannula located in the intended site were included in the final analysis (218 of 240 animals; 9% of the animals were discarded).

Drugs

Oligonucleotides (ODN) (Genbiotech, S.R.L.) were high-performance liquid chromatography purified phosphorothioated end-capped 15/18-mer sequences, resuspended in sterile saline to a concentration of 2 nmol/µL. *c-fos* ASO 5′-GAA CAT CAT GGT CGT-3′, *c-fos* missense oligonucleotides (MSO) 5′-GTA CCA ATC GGG ATT-3′. *bdnf* ASO, 5′-TCTTCCCCTTTTAATGGT-3′; *bdnf* MSO, 5′-ATACTTTCTGTTCTTGCC-3′. ODN sequences were subjected to a BLAST search on the National Center for Biotechnology Information BLAST server using the Genbank database. ASO are specifics for rat *c-fos* or *bdnf* mRNA, respectively. Control MSO sequence, which included the same 15/18 nucleotides as the ASO but in a scrambled order, did not generate any full matches to identified gene sequences in the database. To determine the degree of inhibition of c-Fos protein expression 1 h after IA training, *c-fos* ASO or MSO were infused 4 h and *bdnf* ASO or MSO were infused 2 h before trained animals in IA task, and immunoblots assays were carried out as described above. BDNF blocking antibody was infused at a dose of 0.5 µg per side (Chemicon) and Human recombinant BDNF (Alomone) was infused at a dose of 0.25 µg per side; both were dissolved in sterile saline.

Data Analysis

All data were tested for normality distribution and for homogeneity of variance (Leven's test). In most of the behavioral experiments, statistical analysis was performed by unpaired Student's *t*-test comparing mean step-down latencies of drug-treated group and vehicle group at each time point studied. When data did not meet the above ANOVA assumptions, we used the Kruskal–Wallis analysis of ranks for multiple comparisons. Immunoblot data were analyzed by one-way ANOVA followed by Newman–Keuls multiple comparison test. All data are presented as mean ± SEM.

Results

IEG Expression in the aRSC after IA Training

To determine whether IA training results in a learning-specific alteration in c-Fos protein expression in the aRSC, we measured c-Fos levels by immunoblotting in homogenates of aRSC following a strong training protocol that induces a long-lasting IA LTM (Bekinschtein et al. 2007; Katche, Dorman, Gonzalez, et al. 2013). As shown in Figure 1A, there was a significant increase in c-Fos levels in the aRSC 1 h after training (+ 58% with respect to naïve rats; $n = 5$; $P < 0.01$, Newman–Keuls after ANOVA). This was confirmed by immunohistochemistry (Fig. 1B). No change in c-Fos levels was observed in animals that received a shock only (+ 3% in comparison with naïve rats, $n = 5$, Fig. 1A). Given that no alterations in c-Fos expression were seen 3 or 6 h after IA training (Katche, Dorman, Gonzalez, et al. 2013), the present findings suggest that IA training is associated with an early phase of c-Fos expression in the aRSC. In addition, no modifications were found in Egr-1 levels in the aRSC (Fig. 1A).

c-Fos Expression Requirement in Memory Formation and Persistence

Although the experiments presented above demonstrate that IA training results in an increase in c-Fos protein expression in aRSC, they do not address the question of whether the early post-training activation of c-Fos is required for memory formation and/or persistence. To do that, we utilize an ASO to specifically block de novo c-Fos expression in the aRSC (Dragunow et al. 1993). In our previous findings, 2 nmol of *c-fos* ASO infused into the aRSC was detected 2 h, but not 24 h, after injection (Katche et al. 2010; Katche, Dorman, Gonzalez, et al. 2013). The bilateral infusion of *c-fos* ASO into the aRSC (Fig. 2A) 4 h before IA training blocked learning-induced increase in c-Fos protein expression in comparison with trained rats infused with a *c-fos* scrambled MSO (Fig. 2B), indicating that a single infusion of *c-fos* ASO is sufficient to block de novo c-Fos expression induced by IA training.

The bilateral intra-aRSC infusion of *c-fos* ASO, but not *c-fos* MSO, 4 h before training caused marked retention impairment 7 days after, but left memory retention intact at 2 days post-training (Fig. 2C; $P < 0.01$, $n = 9$, ASO vs. MSO, Student's *t*-test). These findings indicate that inhibition of the early wave of c-Fos expression in the aRSC does not affect IA LTM formation, but profoundly impaired the persistence of IA memory. On the other hand, the immediate post-training infusion of *c-fos* ASO in the aRSC caused no effect on memory at 2 or 7 days test sessions (Fig. 2C).

To evaluate if *c-fos* ASO can potentially affect performance during IA test sessions by inducing changes in locomotor activity and anxiety, animals that received *c-fos* ASO 4 h before IA training were subjected to the open field and elevated plus maze tests 7 days later. *c-fos* ASO did not affect anxiety state or exploratory behavior in a novel environment and did not modify basal locomotor activity (Fig. 3), suggesting that the observed memory deficit is directly caused by inhibition of c-Fos expression that is required for persistence of the memory trace. In addition, the effect of *c-fos* ASO on retention scores at 7 days post-training appears not to be due to alterations in training performance that could affect memory formation because no changes in memory retention were found when animals were tested 2 days post-training. Moreover, it is not likely that the lower retention scores observed at 7 days post-training were caused by modifications in behavioral performance, because administration of *c-fos* ASO immediately after training did not cause any deficit in memory retention.

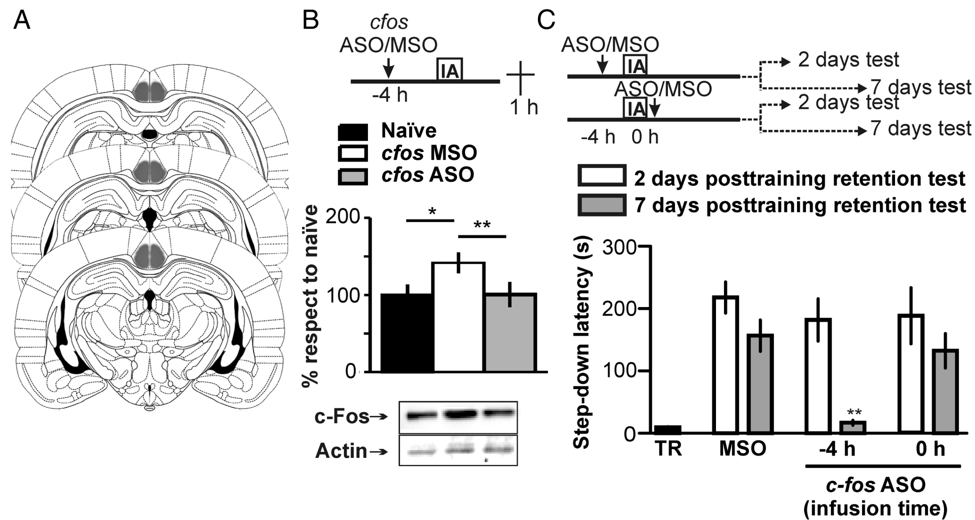

Figure 2. Early c-Fos expression is required for maintenance of memory in aRSC, but not for memory formation. (*A*) Schematic representations of rat brain sections at 3 rostrocaudal planes (−3.80, −4.30, and −4.80 from bregma) taken from the atlas of Paxinos and Watson (1997). (*B*) (Upper) Intra-aRSC c-fos ASO infusion 4 h before training prevented the learning-associated increase in c-Fos 1 h after training. Bars show the normalized mean percentage levels (±SEM) with respect to the naive group. $^*P < 0.05$, $^{**}P < 0.01$, in Newman–Keuls test after ANOVA, $n = 5$. (Lower) Representative blots showing c-Fos and Actin levels. (*C*) Animals were infused into aRSC with *c-fos* MSO (left) or *c-fos* ASO (2 nmol/1 µL/side; right), −4 or 0 h after training. Data are expressed as mean ± SEM of training (TR, black bar) or test session step-down latency, 2 days (white bars) or 7 days (gray bars) after IA training. $^{**}P < 0.01$ versus Veh; two-tailed Student's *t*-test, $n = 8$–12 per group.

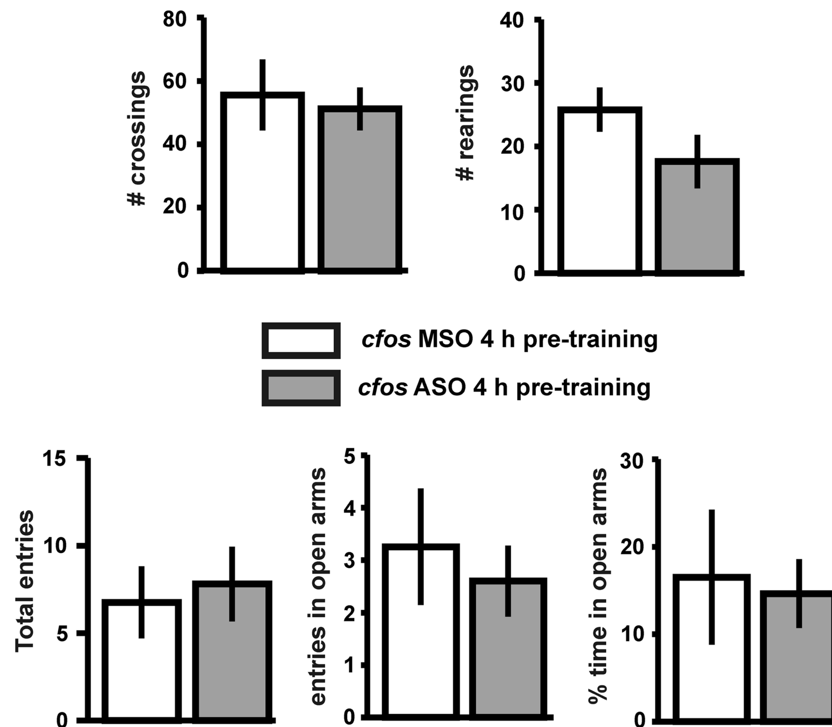

Figure 3. Infusion of *c-fos* ASO 4 h before IA training in aRSC does not affect locomotor activity, anxiety state, or exploratory behavior. (Upper) Number of crossings (left) and rearings (right) during a 5-min open field (OF) session for animals that had received *c-fos* MSO (white bars) or *c-fos* ASO (gray bars; 2 nmol/µL; 1 µL/side) in aRSC 4 h before IA training, 7 days before the OF session. Data are expressed as mean ± SEM number of crossings or rearings ($n = 6$). (Lower) Total number of entries (left), number of entries into the open arms (center), and time spent in open arms (right) during a 5-min plus maze session for rats that had received bilateral intra-aRSC infusion of *c-fos* MSO (white bars) or *c-fos* ASO (gray bars) 7 days before ($n = 5$).

BDNF: As Upstream Modulator of c-Fos Expression in aRSC

Having established a critical role of an early postacquisition expression of c-Fos in aRSC in maintaining long-lasting memory, we next addressed the question of what could be the upstream extracellular signal that is involved in c-Fos activation during IA training. Given that BDNF is required for memory persistence and controls the expression of IEGs including c-Fos (Bekinschtein et al. 2007; Katche et al. 2010; Cohen et al. 2011), we explored whether BDNF in the aRSC is an upstream activator of c-Fos

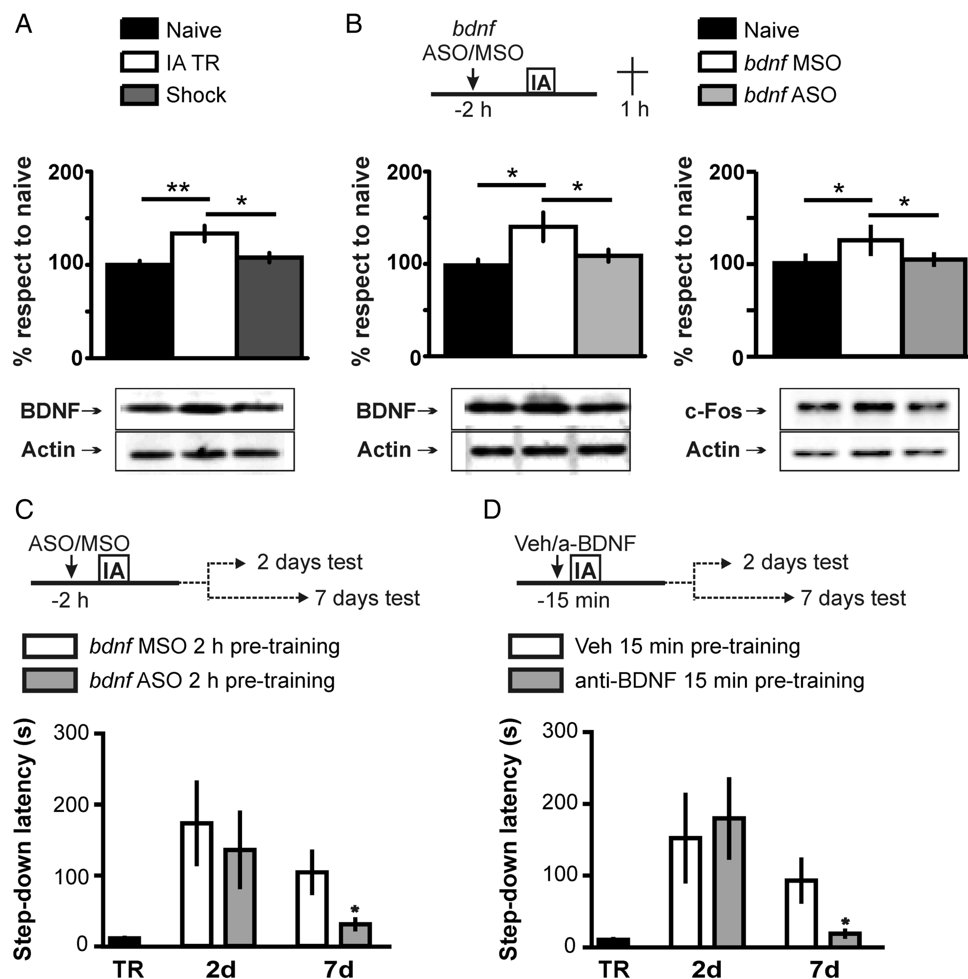

Figure 4. Early BDNF expression in aRSC is required for learning-induced c-Fos expression and for LTM persistence. (*A*) (Upper) Change in aRSC BDNF levels after strong IA training. Bars indicate the percentage of change respect to the naïve (N, black bar) group for rats trained (TR, white bar) and shocked (S, gray bar), and sacrificed 1 h after the behavioral procedure. *$P < 0.05$, **$P < 0.01$, in Newman–Keuls test after ANOVA, $n = 5$. (Lower) Representative blots showing BDNF and Actin levels. (*B*) (Upper) Intra-aRSC *bdnf* ASO infusion 2 h before IA training prevented the learning-associated increase in BDNF (left) and in c-Fos (right) 1 h after training. Bars show the normalized mean percentage levels (±SEM) with respect to the naive. *$P < 0.05$, in Newman–Keuls test after ANOVA, $n = 5$. (Lower) Representative blots showing BDNF (left), c-Fos (right) and Actin levels. (*C*) Animals were infused into aRSC with *bdnf* MSO (white bars) or *bdnf* ASO (2 nmol/1 µL/side; gray bars), 2 h before training. Data are expressed as mean ± SEM of training (TR, black bar) or test session step-down latency, 2 days (left) or 7 days (right) after IA training. *$P < 0.05$ versus Veh; two-tailed Student's *t*-test, $n = 8–12$ per group. (*D*) Animals were infused into aRSC with vehicle (Veh) or anti-BDNF antibody (0.5 µg/1 µL/side; gray bars), 15 min before training. Data are expressed as mean ± SEM of training (TR, black bar) or test session step-down latency, 2 days (left) or 7 days (right) after IA training. *$P < 0.05$ versus Veh; two-tailed Student's *t*-test, $n = 8–12$ per group.

expression during memory processing. We first determined whether IA training results in a learning-specific alteration in BDNF protein levels. By immunoblotting homogenates of aRSC from rats subjected to a strong training protocol that induces a long-lasting IA LTM (Bekinschtein et al. 2007), we observed a significant increase in BDNF levels in the aRSC 1 h after training (BDNF: +33.7% with respect to naïve rats, $n = 4$, $P < 0.05$, Newman–Keuls after ANOVA). No change in BDNF levels was observed in animals that received a shock only (BDNF: +7.8% in comparison with naïve rats, $n = 4$, Fig. 4A). Then, based on previous findings showing that intrahippocampal infusions of *bdnf* ASO inhibited BDNF synthesis 2 h later without lowering basal BDNF levels (Bekinschtein et al. 2007), we injected *bdnf* ASO (2 nmol/side) or MSO into the aRSC 2 h before IA training and determined the levels of aRSC BDNF and c-Fos expression by immunoblotting. As shown in Figure 4B, pretraining infusion of *bdnf* ASO prevented the IA-induced increase in BDNF and in c-Fos expression ($P < 0.01$ with respect to *bdnf* MSO; $n = 5$; Newman–Keuls after ANOVA). In addition, the pretraining infusion of *bdnf* ASO into the aRSC selectively affected the memory retention scores at a 7-day test session, without altering memory performance at a 2-day test session ($P < 0.05$ in comparison with *bdnf* MSO; $n = 8$; Student's *t*-test) (Fig. 4C). Together, these findings indicate that BDNF synthesis in the aRSC is important for the early activation of c-Fos and for the persistence of IA memory. To further analyze early BDNF requirement on IA LTM persistence, we neutralized endogenous BDNF biological activity by delivering function-blocking anti-BDNF antibodies into aRSC 15 min before IA training. As shown in Figure 4D, there is memory impairment at 7 days, but not 2 days after IA training ($P < 0.05$ in comparison with Veh; $n = 8–12$; Student's *t*-test). This result demonstrates that endogenous BDNF activity is required around training for the persistence of LTM.

BDNF and the Promotion of Memory Persistence in aRSC

To further substantiate the involvement of aRSC BDNF in memory persistence, we trained animals using a weak protocol that

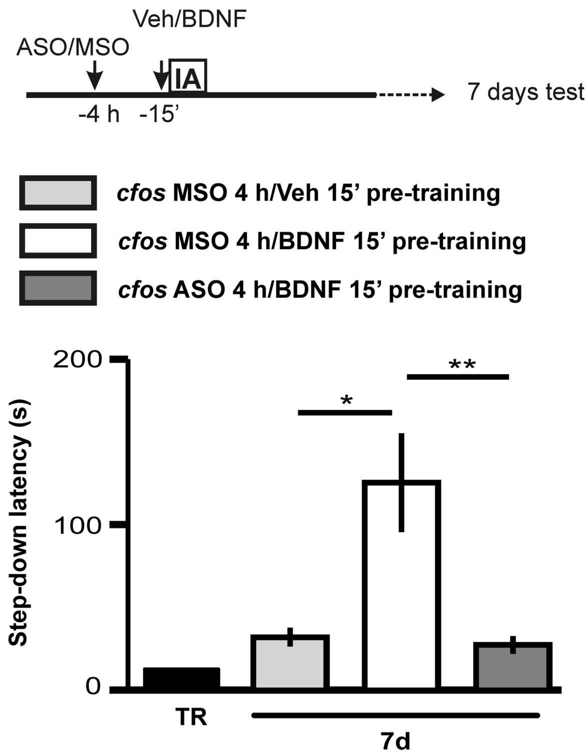

Figure 5. *cfos* ASO abolished the BDNF-dependent promotion of memory persistence. Animals were infused into aRSC with *c-fos* MSO (light gray and white bars) or *c-fos* ASO (2 nmol/1 µL/side; dark gray bar), 4 h before training and then, with hrBDNF (light gray and dark gray bars) or Veh (white bar) 15 min before training, data are expressed as mean ± SEM of training (TR, black bar) or test session step-down latency, 7 days after IA training. *$P < 0.05$, **$P < 0.01$, in Kruskal–Wallis, $n = 10–12$ per group.

generates a non-lasting IA LTM (Bekinschtein et al. 2008; Katche et al. 2010; Katche, Dorman, Gonzalez, et al. 2013), 15 min before training they received human recombinant BDNF (0.25 µg/per side) into the aRSC and tested 7 days later. As shown in Figure 5, BDNF was sufficient to promote the persistence of IA memory ($P < 0.05$, $n = 11–12$, Kruskal–Wallis). Importantly, BDNF-induced maintenance of memory was prevented by the infusion of *c-fos* ASO into the aRSC 4 h before training ($P < 0.01$; $n = 12$, Kruskal–Wallis). These results indicate that BDNF is an upstream signal to c-Fos activation necessary for the establishment of a long-lasting IA LTM.

Discussion

The results presented here provide compelling evidence supporting the hypothesis that the aRSC is critically involved in the maintenance of a long-lasting fear-motivated LTM. First, we demonstrated that IA training is associated with a rapid increase in the levels of retrosplenial c-Fos. Second, knock-down experiments using a *c-fos* ASO showed that the early activation of this IEG in the aRSC is required for the persistence, but not the formation, of IA LTM. Third, retrosplenial BDNF is also important for maintaining IA LTM and appears to be an upstream extracellular signal involved in c-Fos-induced long-lasting memory. The present findings, together with those demonstrating a late post-training phase of consolidation in the aRSC involved in LTM persistence (Katche, Dorman, Gonzalez, et al. 2013), indicate that this cortical region is important for maintaining LTM.

Our results that show an increase in c-Fos protein expression in aRSC after IA training are consistent with those reporting an increased expression of c-Fos in RSC following contextual fear conditioning (Robinson et al. 2012; Tanaka et al. 2014), spatial learning in the Morris water maze (Czajkowski et al. 2014), and after classical conditioning (Radwanska et al. 2010). These authors also reported that glucose utilization in the RSC is elevated after a single-cue delayed fear conditioning. In addition, Arc protein expression is elevated in RSC following fear conditioning (Robinson et al. 2012) and after exploration of a novel environment (Spulber et al. 2009). These early post-training activations of aRSC cells associated with different learning tasks suggest that aRSC is part of a functional circuitry responsible for processing associations among environmental stimuli (Radwanska et al. 2010; Robinson et al. 2012, 2014; Katche, Dorman, Gonzalez, et al. 2013; Cowansage et al. 2014; Czajkowski et al. 2014; Tanaka et al. 2014).

There is widespread evidence that elevated expression of IEGs, including *c-fos*, is tightly coupled to cellular activity and reflects their involvement in different brain processes (Lanahan and Worley 1998). The rapid expression of IEGs has been associated with the first transient transcriptional wave in memory formation (Kandel 2001; Guzowski 2002; Alberini 2009; Liu et al. 2012). To transiently knock-down the expression of this IEG (Wickstrom 1986) and determine its role in memory formation of different learning tasks, we and others have previously utilized an *c-fos* ASO strategy (Lamprecht and Dudai 1996; Grimm et al. 1997; Katche et al. 2010; Kemp et al. 2013). In this context, it is unexpected that an early activation of c-Fos in the aRSC is critical for the persistence, but does not affect IA LTM formation.

Recent findings suggest that Fos functions at enhancers to control of activity-dependent gene expression in the brain (Malik et al. 2014). They found that an unexpected large proportion of activity-dependent enhancers bind Fos, and that this Fos binding is almost exclusively on enhancers; in this way, Fos mediates neuronal activity by enhancing a wide range of genes that control synaptic function. These results support the significance of Fos expression in memory processing, showing that it does no function only as a neuronal activity marker, but it is a modulator of the expression of several genes involved in synaptic plasticity at the translation level.

BDNF exerts diverse roles in regulating neuronal structure and function (Schinder and Poo 2000; Tyler et al. 2002). In particular, it appears to be critical for synaptic plasticity and memory processing in the adult brain (Castren et al. 1993; Ma et al. 1998; Tokuyama et al. 2000; Tyler et al. 2002; Cunha et al. 2010; Peters et al. 2010; Edelmann et al. 2014; Panja and Bramham 2014; Nakayama et al. 2015). It has been recently reported that BDNF is a major mediator of the molecular mechanisms underlying the persistence of LTM storage in the hippocampus (Bekinschtein et al. 2007; Nakayama et al. 2015), amygdala (Ou et al. 2010), and insular cortex (Martinez-Moreno et al. 2011). The present findings expand this list to include the aRSC, and suggest that not only BDNF is necessary, but sufficient to induce a long-lasting IA LTM. Consistent with these findings, it has been shown that BDNF acts on dendrites to induce ERK 1/2 activation and upregulation of the expression of the IEGs *Arc* and *c-fos* (Cohen et al. 2011; Nakayama et al. 2015).

The persistence of memory storage is not the only stage of fear memory processing affected by manipulations of the RSC. Lesions of RSC hindered avoidance learning (Gabriel et al. 1991) and reversible inactivation of RSC impaired IA recent and remote memory retention (Katche, Dorman, Slipczuk, et al. 2013). It has also been demonstrated that RSC is involved in retrieval of recent

and remote contextual fear memories (Corcoran et al. 2011; Katche, Cammarota, et al. 2013; Katche, Dorman, Gonzalez, et al. 2013; Cowansage et al. 2014; Tanaka et al. 2014), and for extinction of recent and remote context fear memories (Corcoran et al. 2013). Interestingly, PKA activity in the RSC appears to be important for both remote memory retrieval and extinction (Corcoran et al. 2013). In this context, the storage of long-lasting memory is modulated by D1/D5 dopamine receptors in aRSC (Katche, Dorman, Gonzalez, et al. 2013).

Despite a wealth of evidence regarding a role of RSC as an important hub of information flow between the hippocampus and neocortical areas, RSC is now seen as a functional integrator of memory information coming from extra-hippocampal sites beyond the medial temporal lobe (Aggleton 2014). In this context, it has been recently proposed that the RSC, together with prefrontal cortices and several nuclei of the thalamus, consistently participate in, and are required for, several features of episodic memory (Pergola and Suchan 2013; Aggleton 2014; Robinson et al. 2014). Based on the present findings, RSC appears to have an important role in the persistence of long-lasting fear-motivated memory.

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Notes

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