Changes in Hippocampal Arc Protein Expression and Synaptic Plasticity by the Presentation of Contextual Cues Linked to Drug Experience

MARÍA CAROLINA MONTI, ROMINA SOLEDAD ALMIRÓN, ELENA A. BIGNANTE, AND OSCAR ÁNGEL RAMÍREZ*

Instituto de Farmacología Experimental de Córdoba IFEC-CONICET, Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina

KEY WORDS retrieval; contextual cues; hippocampus; Arc protein; LTP

ABSTRACT Contextual cues linked to drug experience have been frequently associated to craving and relapse, with this phenomenon being described in human and experimental animals. Hippocampal synaptic plasticity has been related to learning, memory, and adaptive processes developed during chronic administration of drug abuse. In this study, we investigated if the environmental context associated with withdrawal experience was able to evoke the same behavioral alteration observed after chronic benzodiazepine administration. Furthermore, we studied the hippocampal synaptic plasticity and anatomical expression of Arc protein during withdrawal and the re-exposure to the context associated with anxiety expression (characteristic sign of benzodiazepines withdrawal). It was demonstrated that re-exposure evoked on days 15 and 25 after the first exposure the same behavior. An increased hippocampal synaptic plasticity, expressed as a lower threshold to induce long-term potentiation on dentate gyrus, was observed in animals dependent on diazepam and during retrieval, in the same group, until day 15. This plastic change disappeared 25 days after the first exposure. An overexpression of Arc protein in the dorsal dentate gyrus and CA1 on the first day of withdrawal in the dependent animals was observed. **Synapse 64:39–46, 2010.**

INTRODUCTION

Many studies have indicated that contextual cues associated to drugs, in human ex-addicts, are relevant in craving and relapse (Hyman and Malenka, 2001; Ludwig and Stark, 1974; O'Brien et al., 1977). These drug-conditioned stimuli can also be extended to rodents, with conditioned aspects of addiction leading many authors to suggest the existence of a common neurobiological mechanism mediating drug addiction and memory (Ciccocioppo et al., 2001; Nestler, 2001a, Kelley, 2004; Wolf, 2002). In this aspect, a pivotal role has been attributed to synaptic plasticity at glutamate synapses in different areas of the brain, such as the hippocampus, frontal cortex, ventral tegmental area, and nucleus accumbens. Moreover, an enduring increase in the efficacy of hippocampal glutamatergic synaptic transmission, which is associated with an enhancement in the synaptic plasticity, is now accepted as a molecular mechanism for memory storage in the brain, with contextual cues being relevant (Bliss and Collingridge, 1993; Marin et al., 1996, 1999; Martin et al., 2000). Related to this, in a previous paper, we demonstrated a very close relationship between the behavioral dependence for diazepam (DZ) chronic administration and an enhancement of strength at excitatory synapses on the hippocampal dentate gyrus (Pérez et al., 2002). It is thought that the reward pathway, involving the ventral midbrain, nucleus accumbens, and frontal cortex, is the main neuronal circuitry in the neurobiology of addiction (Eisch et al., 2004). However, other brain areas, such as hippocampus, have been implicated as responsible

*Correspondence to: Dr. Oscar Ángel Ramírez, Haya de la Torre y Medina Allende, Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina. E-mail: oramirez@fcq.unc.edu.ar

Received 3 February 2009; Accepted 6 April 2009

DOI 10.1002/syn.20700

Published online in Wiley InterScience (www.interscience.wiley.com).

for initiation and maintenance of addiction (Morris and Frey, 1997).

Several models of information processing suggest that the hippocampus is engaged in different states of memory such as encoding, consolidating, and retrieving of information (Davis et al., 2004; Kopelman et al., 1999; Reed and Squire, 1998). With respect to this, the storage of recent processing information is temporary in the hippocampal formation (Gluck and Myers, 1998).

Arc or Arg 3.1, which is an effector immediate-early gene (IEGs), is induced in the hippocampal select population of neurons by high-frequency stimulation. This also induces LTP, following a brief behavioral experience involved in long-term memory formation (Guzowski et al., 1999; Link et al., 1995; Lyford et al., 1995). More recently, it has been found that the expression of Arc/Arg 3.1 mRNA, which underlies the recall of recent and remote spatial memory, has an anatomical distribution and time-dependent organization throughout both the dorsal and ventral hippocampus (Gusev et al., 2005).

A convergence of molecular and cellular pathways of drug addiction on one hand and of learning and memory on the other has recently been noted (Kelley, 2004; Nestler, 2001b). Moreover, it is thought that the mechanisms of the adaptive forms of experience-dependent plasticity, such as learning and memory, can be "pathologically" usurped and play a role in the development of addiction (Saal et al., 2003).

Considering all these facts together and bearing in mind our previous results, the major challenge of this work was to find out if whether the memory induced by drugs of abuse, like other kinds of memories, was evoked by the contextual cues linked to the withdrawal experience. We also investigated the participation of the hippocampus in this phenomenon. To do this, we studied synaptic plasticity and the distribution of the expression of Arc protein as a sensitive marker of the neuronal activity that is critically involved in the storage of contextual memory (Guzowski et al., 1999).

MATERIALS AND METHODS

Animals

Male Wistar rats, 37–40 days old and weighing 130–150 g, were used. They were housed in groups of five (which received the same treatment) in their home boxes and kept under a 12:12 L/D cycle (light on at 7 am) at regular temperature conditions (22 ± 1°C). Food and water were available ad libitum.

Elevated plus-maze apparatus

The plus maze (PM) apparatus consisted of two open arms, 50 × 10 cm^2, and two enclosed arms, 50 × 10 × 40 cm^3, with an open roof arranged so that the two open arms were opposite each other. The arms extended from a central platform 10 × 10 cm^2. The apparatus was elevated to a height of 50 cm. Different dependent variables were analyzed using the method reported by Pellow and File (1986). These variables include % of time spent on the open arms, which served as an index of the level of anxiety.

Procedure

Animals were divided into two groups according to the drug treatment and injected daily with either DZ (5 mg/kg/day) or vehicle (distilled water with a drop of Tween 80 and propilenglycol 5%) for 18 days.

Clinical trials have shown that anxiety, muscle spasms, and seizures are major withdrawal signs after the discontinuation of chronic benzodiazepine treatment (Woods et al., 1987, 1992). On the basis of these criteria, we selected our experimental groups to study the activity of the rats in an elevated PM 24 h after the last injection, considering this day the first day of withdrawal. Anxiety was assessed in terms of percentage of time in the open arms, with dependent animals being considered to be those that spent less than 12% of the time there. This percentage was chosen according to the one expressed by control animals.

Previous results from our laboratory shown that, 24 h after the last injection, animals were evaluated in the elevated PM apparatus, as a function of the activity of the DZ group, each subject was assigned to one of two possible groups: animals showing the anxiety sign were named "dependent group" (DEP), while those that received DZ but did not exhibit the anxiety sign were called "nondependent group" (NDEP). This group did not show statistical differences with those animals that received vehicle, "control group" (CTROL) (Pérez et al., 2002). First, we studied the temporal expression of anxiety. Different groups of animals (DZ and CTROL) were evaluated only once in the PM, 1, 2, 3, 4, or 5 days after the last injection of DZ or vehicle, to set up when the animals which received DZ show anxiety.

Animals were divided in two groups and treated with DZ or vehicle for 18 days; the day after that, they were evaluated in the PM and those that received DZ were assigned to the DEP or NDEP group depending on their activity in the PM (as was previously described).

After the elevated PM test, animals were put in their home cages and kept there for either 15 or 25 days. Some groups of animals were re-evaluated in the PM on day 15 and others on day 25, to analyze which ones were able to repeat the same behavior shown in the first PM. We decided to re-expose animals to the same initial context (PM) on day 15 or 25, to evaluate the retrieval of memory, because in these

days animals were not in the withdrawal period characterized by anxiety, which finish the day 4 after the last injection of DZ. Then, animals that show the same initial behavior were renamed as re-exposed-DEP (R-DEP: animals from DEP group), re-exposed-NDEP (R-NDEP: animals from NDEP group), and re-exposed-CTROL (R-CTROL: animals from CTROL group), while animals not repeating the behavior were discarded, as the withdrawal-like behavior retrieval did not take place.

The experimental conditions met the standards for care of laboratory animals as outlined in the NIH Guide for the Care and Use of Laboratory Animals.

Electrophysiological procedures

Animals from the CTROL, DEP, and NDEP and R-DEP, R-NDEP, and R-CTROL groups described in the Procedure section were used for electrophysiological experiments as follows. Immediately after the first or second test, animals were sacrificed by a cervical dislocation. Briefly, rats were sacrificed between 11:00 am and 12:00 pm to prevent variations caused by circadian rhythms or nonspecific stressors. Brains were removed and electrophysiological experiments were carried out using the in vitro hippocampal slice preparation described elsewhere by Ramirez et al. (1988). The hippocampal formation was dissected and transverse slices of ~400 μm thick were placed in a recording chamber (BSC-BU Harvard Apparatus), perfused with standard Krebs solution (ClNa 124.3 mM, ClK 4.9 mM, $SO_4Mg{\cdot}7H_2O$ 1.3 mM, PO_4H_2K 1.25 mM, CO_3HNa 25.6 mM, glucose 10.4 mM, $CaCl_2{\cdot}2H_2O$ 2.3 mM) saturated with 95% O_2 and 5% CO_2. The perfusion rate was 1.6 ml/min, while the bathing solution temperature was kept at 28°C employing a temperature controller (TC-202A Harvard Apparatus). A stimulating electrode was placed in the perforant path, and a recording microelectrode was inserted in the dentate granule cell body layer. Only slices showing a stable response were included in this electrophysiological study. Ten field potentials that responded to the stimuli were sampled at 0.2 Hz. Once no further changes were observed in the amplitude of the response, a tetanus consisting of a train of pulses (0.5 ms) of 2 s duration and of increasing frequency was delivered to the slice by an A310 Accupulser Pulse Generator (World Precision Instruments) at intervals that ranged from 10 min up to 20 min, starting with a 20 Hz tetanus, whose intensity increases from 20 Hz until 200 Hz, with increments of 20 Hz. Five to ten minutes after a tetanus, a new averaged response was recorded; when LTP was not observed, another tetanus at the next higher frequency was applied. LTP was considered to have occurred when the amplitude of the evoked field potentials recorded had risen by at least 30% and persisted for 50–60 min. Once LTP was achieved, no further tetanus was given.

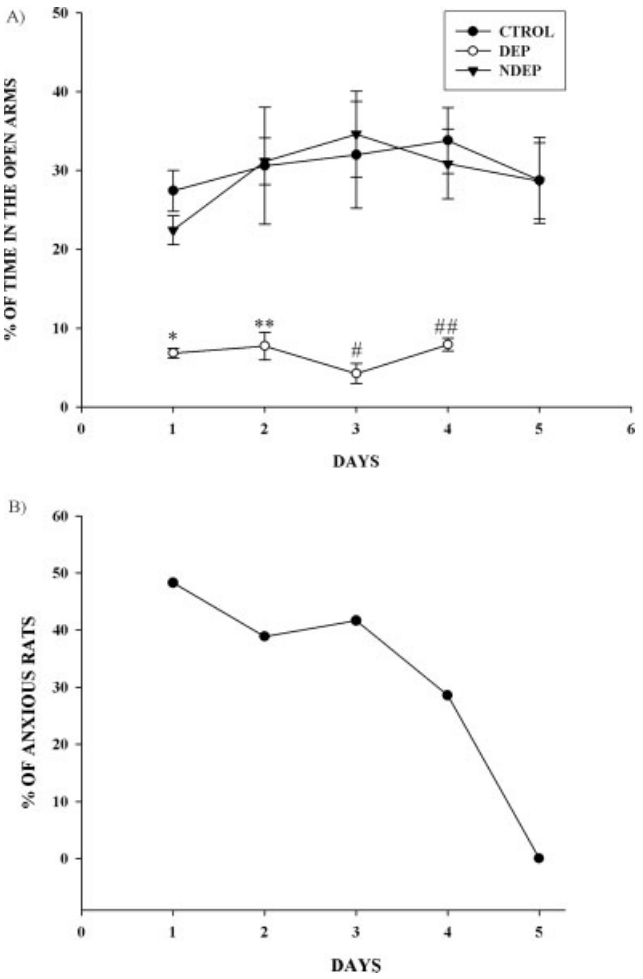

Fig. 1. **A**: The duration of the anxiety sign, expressed as the percentage of time spent in open arms in the plus maze test. This sign was observed in animals treated with diazepam until the fourth day, with no more anxious rats being found from the fifth day. **B**: Percentage of rats showing anxiety for each day.

Immunohistochemical analysis

Rats were deeply anesthetized with chloral hydrate (400 mg/kg ip) and perfused transcardially with a ice-cold solution of saline followed by a solution of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.4. Brains were removed and postfixed in the same fixative overnight at 4°C. They were then placed in 30% sucrose in PBS for 72 h. After this, the brains were sectioned in a cryostat into 40-μm-thick coronal slices, before being immersed in 0.1 M PBS. These were then incubated for 1 h in a solution of 10% methanol and 3% hydrogen peroxide in PBS to eliminate the endogenous peroxidase activity. Later, sections were incubated for 1 h in a blocking solution consisting of 5% bovine-serum albumin (BSA) and

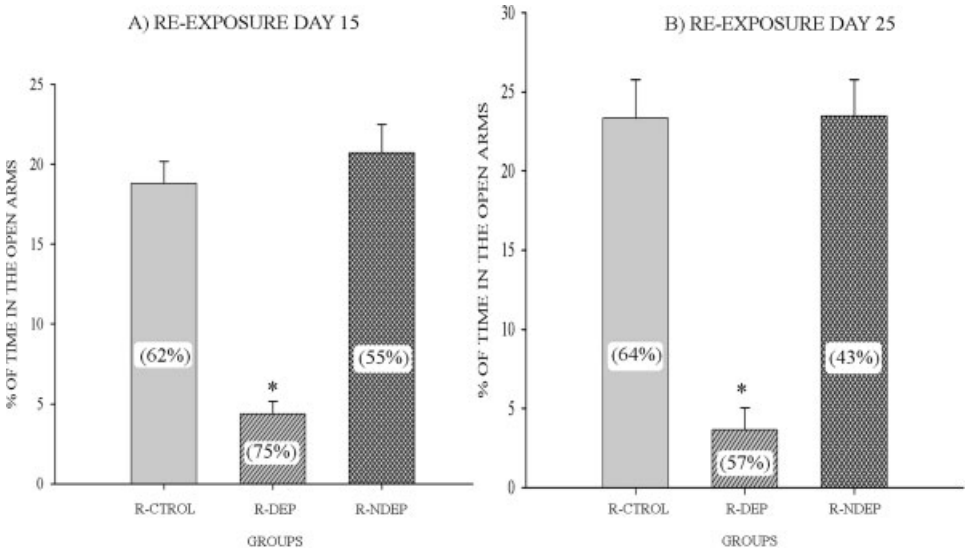

Fig. 2. Percentage of time spent on open arms in an elevated plus maze apparatus in R-CTROL, R-DEP, and R-NDEP groups. Each bar represents the mean and vertical bars ± SEM. Inside the bars, numbers in brackets indicate the percentage of animals that show same initial behavior. **A**: Re-exposure on day 15, $F(5, 111) = 24.57$, $^*P < 0.04$, R-DEP with respect to R-CTROL and R-NDEP groups. **B**: Re-exposure on day 25, $F(5, 84) = 25.6$, $^*P < 0.0002$, R-DEP with respect to R-CTROL and R-NDEP groups.

0.3% triton X-100 in 0.1 M PBS. These were then incubated for 48 h at 4°C in a solution with rabbit polyclonal ARC antibody (H-300) (Santa Cruz Biotechnology, Santa Cruz, CA), diluted 1:800 in 0.1 M PBS containing 1% BSA and 0.1% Tritón X-100. Subsequently, the sections were washed and incubated at room temperature with biotinylated secondary anti-rabbit antibody (Vector Laboratories, Burlingame, CA), diluted 1:200 in 0.1 M PBS containing 1% BSA followed by the avidin–biotin–peroxidase (ABC) complex (Vector ABC kit, Vector Laboratories, Burlingame, CA) for 1 h at room temperature. For visualization, 3′-diamino-benzidine tetrahidocloride (DAB Sigma) was used as chromogen (Sigma fast tablet set): sections were incubated for 5 min with a solution containing 0.05% of DAB and 0.0006% of hydrogen peroxide. The brain sections were then mounted onto slice glass, dehydrated, and cover slipped prior to viewing with light microscopy.

Microscopy and quantification

The positive ARC cells were identified using light field microscopy (Leica DM 4000 B) with Metamorph computer software at a magnification of 10× and counted with computational software (a SCION program from the NIH). The quantification was performed using more or less the same area of the same shape, over the dorsal regions of the DG and CA1 separately. Sample images were taken according to rat brain atlases (Paxinos and Watson, 1986) at the following anteroposterior (AP) coordinates from Bregma: DG dorsal AP 3.14–3.6 mm and CA1 dorsal AP 3.3–3.6 mm.

Statistical analyses

A one-way analyses of variance (ANOVA) was used to evaluate relative (%) time spent in the open arms as an index of the level of anxiety, re-exposure, electrophysiological and inmunohistochemical experimental dates, followed by Newman–Keuls comparisons of means. $P \leq 0.05$ was used to represent a significant difference between groups.

RESULTS

To evaluate if the re-exposure to the context associated with withdrawal experience is important to evoke the anxiety sign as withdrawal-like behavior, first we studied the withdrawal duration to be sure that during the re-exposure animals were not under withdrawal period. A one-way ANOVA test and posterior Newman–Keuls showed that rats expressed anxiety until the fourth day after the last DZ injection, with no more rats showing anxiety from the fifth day, as can be seen in Figure 1A [for the first day $F(1, 35) = 395.11$, $P < 0.000001$; for the second day $F(1, 21) = 106.54$, $P < 0.000001$; for the third day $F(1, 18) = 75.54$, $P < 0.000001$; for the fourth day $F(1, 29) = 74.99$ $P < 0.000001$]. In Figure 1B, we can also observe the decrease in the percentage of rats that showed anxiety over the 5 days. These results indicate that anxiety, characteristic of withdrawal, remained until day 4 after the last administration of DZ.

Figures 2A and 2B show the percentage of time spent in the open arms as an index of anxiety in the PM test, for rats re-exposed to the initial context on

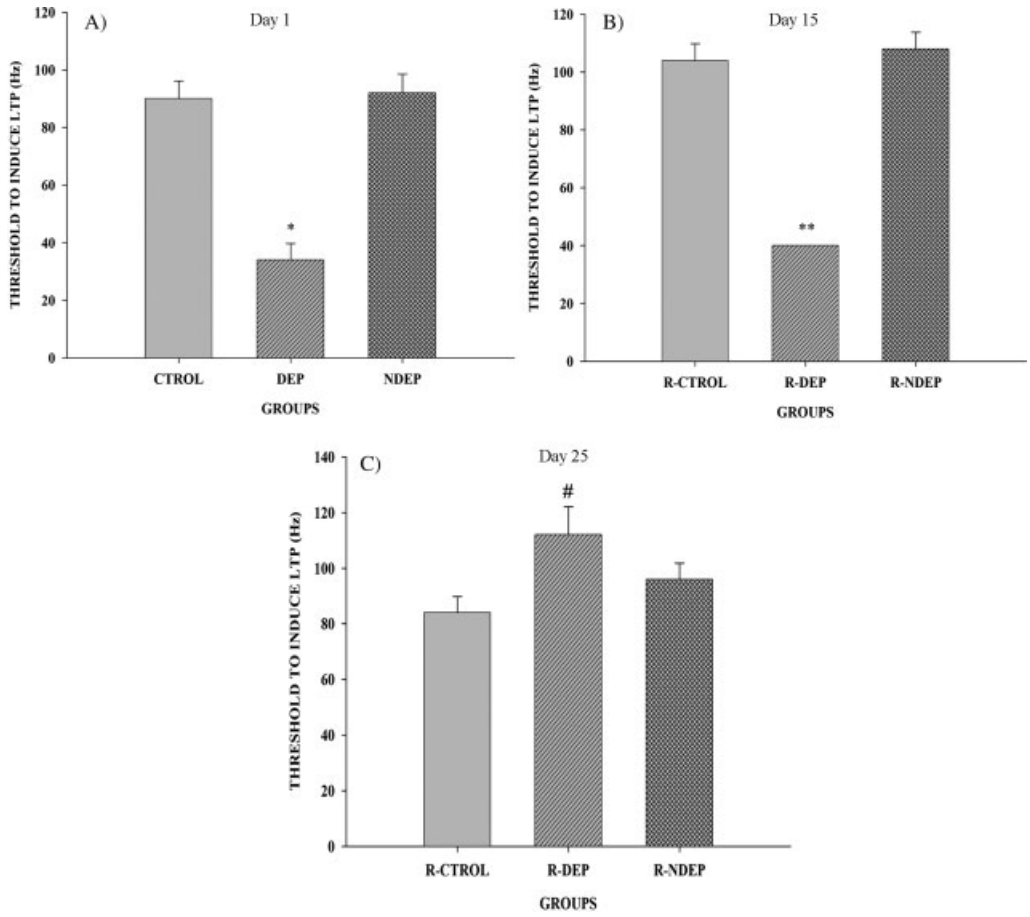

Fig. 3. Threshold to LTP induction in hippocampal dentate gyrus. Bars represent means and vertical bars ± SEM. The number of animals used in each group is indicated within parentheses. A one-way ANOVA test shows differences for the group DEP related to CTROL and NDEP on day 1, $F(2, 12) = 36.1$, $^{*}P < 0.0002$ (**A**) and for the group R-DEP related to R-CTROL and R-NDEP on day 15, $F(2, 12) = 54.6$, $^{**}P < 0.0002$ (**B**). (**C**) A one-way ANOVA test shows differences in the R-DEP group on the day 25 compared to the DEP and R-DEP groups on days 1 and 15, $F(2, 12) = 62.8$, $^{\#}P < 0.0002$.

day 15 and on day 25 after the first exposure. For days 15 and 25, 75 and 57% of animals of the DEP group, respectively, continued expressing anxiety, with no significant differences existing between the exposed and their corresponding re-exposed group.

We decided to study the possible participation of hippocampus in the behavior expressed during the re-exposure to the PM on day 15 or 25. Previous work from our laboratory showed a lower threshold being necessary to induce LTP in the DEP group. Therefore, we studied changes in synaptic plasticity by evaluating the threshold to generate LTP in R-DEP, R-NDEP, and R-CTROL groups on days 15 and 25, to define a possible association with changes observed in DEP, NDEP, and CTROL groups. Figure 3 shows the threshold to induce LTP at day 1 after the last administration of DZ (Fig. 3A), and at days 15 (Fig. 3B) and 25 after the first exposure to the PM (Fig. 3C). We observed a decreased threshold to generate LTP in the DEP group on day 1 after the last DZ injection, with respect to the CTROL and NDEP groups, and on day 15 after the first exposure to the PM; the same change in hippocampal plasticity was observed in the R-DEP group with respect to the R-CTROL and R-NDEP groups. On the other hand, on day 25 no differences were observed among the R-CTROL, R-DEP, and R-NDEP groups.

To investigate a possible association between the expression of Arc protein in the hippocampus and the synaptic plasticity in this area, we decided to measure the expression of this protein in the DEP, NDEP, and CTROL groups, 1 day after the last DZ injection and after the second exposure to the PM test (25 days later) in the R-DEP, R-NDEP, and R-CTROL groups. We studied the topographic expression of Arc protein in dorsal DG and dorsal CA1, and quantified the number of Arc positive cells/mm^2 in these areas. We found an overexpression of Arc protein in dorsal DG (Fig. 4A) and CA1 (Fig. 4B) hippocampal areas on the first day after the last injection, but only in animals showing the anxiety sign, DEP (516.9 ± 20.1 cells/mm^2), compared with NDEP (378.6 ± 13.7 cells/mm^2)

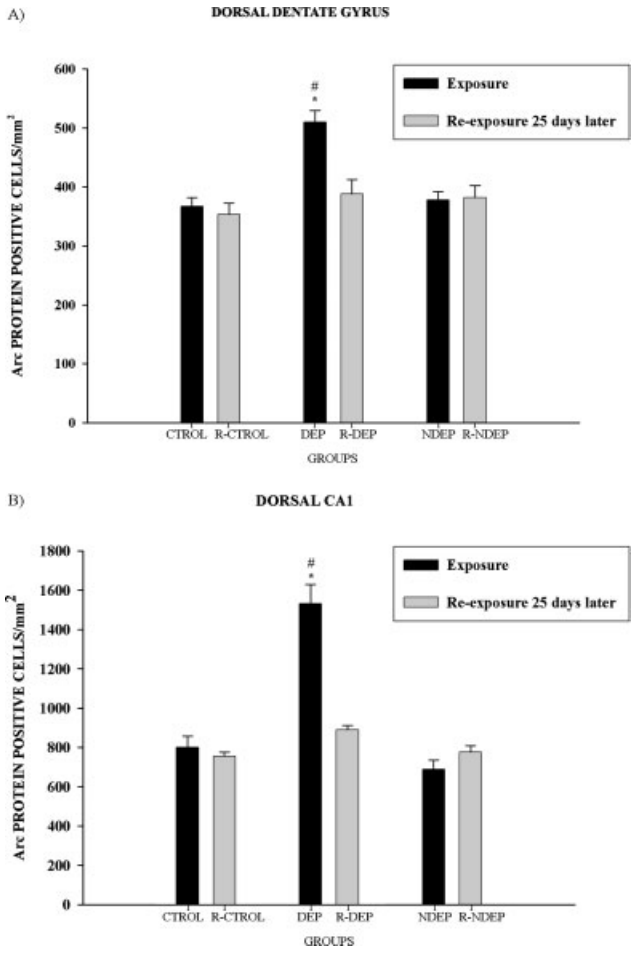

Fig. 4. Quantification of positive Arc protein cells/mm^2 in hippocampus. Bars represent means and vertical bars ± SEM. **A**: A one-way ANOVA test shows differences in dentate gyrus dorsal in the DEP group with respect to the CTROL and NDEP groups, $F(2, 24) = 27.89$, $^*P < 0.0001$, after the first exposure to the plus maze test. DEP group shows statistical difference respect to the R-DEP group (25 days), $F(1, 18) = 17.96$, $^{\#}P < 0.0006$. **B**: A one-way ANOVA test shows differences in CA1 dorsal in the DEP group with respect to the CTROL and NDEP groups, $F(2, 23) = 53.04$, $^*P < 0.0001$ after the first exposure to the plus maze test. DEP group shows statistical difference with respect to the R-DEP group (25 days), $F(1, 13) = 53.19$, $^{\#}P < 0.0002$.

and CTROL (369.4 ± 13.9 cells/mm^2) groups. On the other hand, no changes were observed 25 days after the first exposure to the PM in dorsal DG and CA1 hippocampal.

DISCUSSION

In human ex-addicts, exposure to environmental cues previously associated with drugs of abuse strongly increases the risk of relapse (Hyman et al., 2001; Ludwig and Stark, 1974). The retrieval of memories depends on the relationship between the conditions present during learning and those during the remembrance of them. Moreover, many authors have discussed the possibility that memory and addiction could share neural circuitry and cellular mechanisms (Fuchs et al., 2000; Milekic et al., 2006; Nestler, 2002; Vorel et al., 2001).

Different authors have postulated that hippocampal formation is engaged in different memory processes such as encoding, consolidation, and retrieval (Eldridge et al., 2005). It was reported that damage to the hippocampus not only causes deficits in the learning of information in the environment (Zola-Morgan et al., 1986), but also in the retrieval, particularly for memories from the recent past (Reed and Squire, 1998). However, the specific roles of anatomically distinct hippocampal subfields in short- and long-term memory and retrieval are unknown. Eldridge et al. (2005) have recently reported that DG and CA fields 2 and 3 were selectively active during episodic memory formation. Furthermore, Daumas et al. (2005) have pointed out that although the CA2 and CA3 subregions of the dorsal hippocampus play a role in the acquisition and consolidation of contextual fear memory, they are not necessary for context recognition.

In the present study, we have demonstrated that anxiety, a characteristic sign of withdrawal to benzodiazepines, persists for 4 days after the last injection of DZ (Figs. 1 and 2). It was an important point to choose the days in which animals would be re-exposed to the initial context. After we determined that withdrawal, characterized by anxiety expression, finish the day 4 after the last injection of DZ, we were sure that withdrawal-like behavior observed on days 15 and 25 was due to the retrieval of the memory acquired during the first exposure to the PM.

To temporarily associate this behavior with the hippocampal synaptic plasticity on DG, we determined the threshold to induce LTP on day 1 after the last DZ injection and on days 15 and 25 after the first exposure to the PM. On days 1 and 15, there was a significant decrease in the threshold to generate LTP for DEP/R-DEP groups compared with CTROL/R-CTROL and NDEP/R-NDEP groups (Figs. 3A and 3B). However, on day 25, R-CTROL, R-DEP, and R-NDEP groups did not show any difference in the threshold to induce LTP in hippocampal DG (Fig. 3C). This could be an evidence of the involvement of the hippocampal DG on day 15, through an increase in the synaptic plasticity, in the retrieval of a memory related to the withdrawal experience. However, this structure seems not to be involved in the retrieval of this kind of memory on day 25, which is in agreement with other results that demonstrated the participation of hippocampal DG in the encoding of contextual information up to 25 days (Gluck et al., 1998).

Brain processes like learning and memory are thought to involve plastic changes in synaptic strength. It is therefore reasonable to postulate that

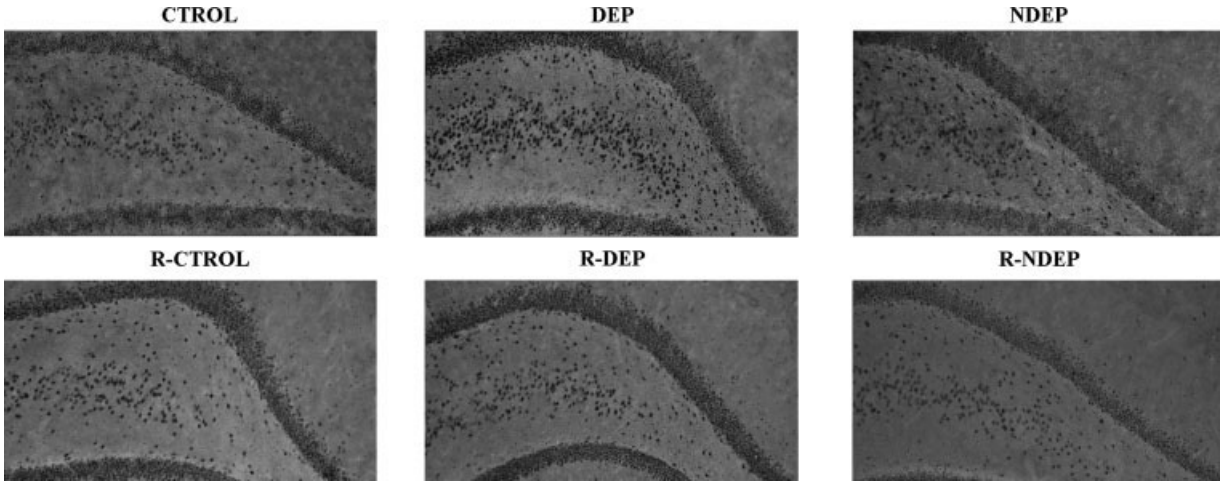

Fig. 5. Photomicrographs (10×) of Arc protein immunoreactivity in hippocampal dentate gyrus dorsal from CTROL, DEP, and NDEP rats chronically treated with vehicle or diazepam (5 mg/kg/day for 18 days) and exposed to the plus maze, and from R-CTROL, R-DEP, and R-NDEP after the re-exposure to the plus maze.

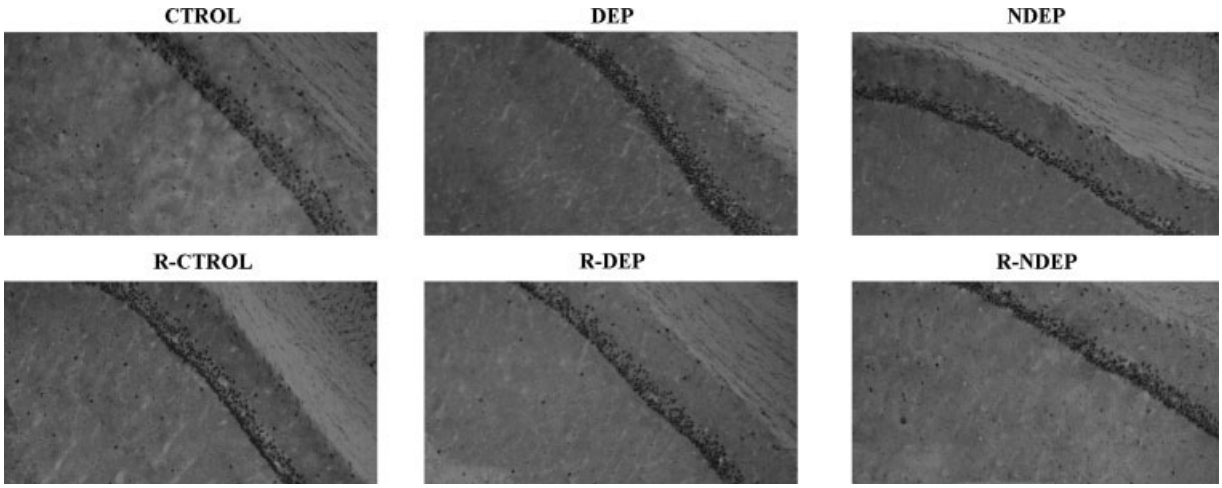

Fig. 6. Photomicrographs (10×) of Arc protein immunoreactivity in hippocampal CA1 dorsal from CTROL, DEP, and NDEP rats chronically treated with vehicle or diazepam (5 mg/kg/day for 18 days) and exposed to the plus maze, and from R-CTROL, R-DEP, and R-NDEP after the re-exposure to the plus maze.

cellular mechanisms supporting these plastic changes might show both spatial specificity and temporal correlation with the stimulus that triggered the plastic change. Despite the abundant evidence connecting synaptic plasticity with gene expression, causal evidence to relate known gene products synthesized in the mammalian cell soma to changes in specific sets of synapses has been missing. The discovery of Arc/Arg3.1 revealed the only activity-induced gene known so far that correlates both temporally and spatially with the stimulus that induced its transcription (Rial Verde et al., 2006). Furthermore, Arc is unique among IEGs in that its mRNA rapidly distributes throughout the dendritic arbor after induction and then localizes to discrete regions that have received direct synaptic stimulation (Guzowski et al., 1999, 2000; Link et al., 1995; Lyford et al., 1995). The hippocampal Arc protein expression plays a critical role in the stabilization, but not in the induction, of LTP and in the consolidation of long-term memory after spatial water task training (Guzowski et al., 1999, 2000). However, a correlation between the expression of hippocampal Arc protein and the encoding and retrieval of information is at present unproved.

For our experimental conditions, we could see an increased expression of Arc protein, in dorsal DG and CA1 during the encoding of the information. This is illustrated in the photomicrographs of Figs. 5 and 6 respectively, where the DEP group shows the increment but not the R-DEP

In conclusion, the results of the present investigation demonstrate that re-exposure to the environmental context associated with the withdrawal experience was able to evoke the same behavior observed during the first PM. This behavior was linked to an increase in the synaptic plasticity, registered in the hippocampal DG, during the encoding and retrieval up to day 15. Moreover, the overexpression of Arc protein in dorsal DG and CA1 24 h after the last injection of DZ is in agreement with the postulation that the expression of Arc protein in hippocampus is induced by incoming information. The behavior evoked by the environmental context associated with the experience of the drug on day 25, but not linked to an increased hippocampal synaptic plasticity or overexpression of Arc protein, may indicate that this behavior on day 25 could have been dependent on other cortical areas of the brain, as usually happens with different information after those time.

ACKNOWLEDGMENTS

This work was supported by the CONICET and SECYT. The authors thank Paul Hobson, native speaker, for his English revision of this manuscript. We would also like to thank Estela Salde for her technical assistance.

REFERENCES

Bliss TVP, Collingridge BL. 1993. A synaptic model of memory: Long-term potentiation in the hippocampus. Nature 6407:31–39.

Ciccocioppo R, Sanna PP, Weiss F. 2001. Cocaine-predictive stimulus induces drug-seeking behaviour and neural activation in limbic brain regions after multiple months of abstinence: Reversal by D_1 antagonists. Proc Natl Acad Sci USA 98:1976–1981.

Daumas S, Halley H, Francés B, Lassalle JM. 2005. Encoding, consolidation, and retrieval of contextual memory: Differential involvement of dorsal CA3 and CA1 hippocampal subregions. Learn Mem 4:375–382.

Davis CD, Jones FL, Derrick BE. 2004. Novel environments enhance the induction and maintenance of long-term potentiation in the dentate gyrus. J Neurosci 29:6497–6506.

Eisch AJ, Mandyam ChD. 2004: Drug dependence and addiction, II: Adult neurogenesis and drug abuse. Am J Psychiatry 161:426.

Eldridge LL, Engel SA, Zeineh MM, Bookheimer SY, Knowlton BJ. 2005. A dissociation of encoding and retrieval processes in the human hippocampus. J Neurosci 13:3280–3286.

Fuchs RA, Weber SM, Rice HJ, Neisewander JL. 2000. Effects of excitotoxic lesions of the basolateral amygdala on cocaine-seeking behavior and cocaine conditioned place preference in rats. Brain Res 929:15–25.

Gluck MA, Myers CE. 1998.Psychobiological model of hippocampal function in learning and memory. In: Martinez J, Kesner R, editors. Neurobiology of learning and memory. San Diego, CA: Academic Press. p 417–448.

Gusev PA, Cui C, Alkon DL, Gubin AN. 2005. Topography of Arc/Arg3.1 mRNA expression in the dorsal and ventral hippocampus induced by recent and remote spatial memory recall: Dissociation of CA3 and CA1 activation. J Neurosci 41:9384–9397.

Guzowski JF, McNaughton BL, Barnes CA, Worley PF. 1999. Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles. Nat Neurosci 12:1120–1124.

Guzowski JF, Lyford GL, Stevenson GD, Houston FP, McGaugh JL, Worley PF, Barnes CA. 2000. Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. J Neurosci 11:3993–4001.

Hyman SE, Malenka RC. 2001. Addiction and the brain: the neurobiology of compulsion and its persistente. Nat Rev Neurosci 2:695–703.

Kelley AE. 2004. Memory and addiction: Shared neural circuitry and molecular mechanisms. Neuron 1:161–179.

Kopelman MD, Stanhope N, Kingsley D. 1999. Retrograde amnesia in patients with diencephalic, temporal lobe or frontal lesions. Neuropsychologia 8:939–958.

Link W, Konietzko U, Kauselmann G, Krug M, Schwanke B, Frey U, Kuhl D. 1995. Somatodendritic expression of an immediate early gene is regulated by synaptic activity. Proc Natl Acad Sci USA 12:5734–5738.

Ludwig AM, Stark LH. 1974. Alcohol craving. Subjective and situational aspects. Q J Stud Alcohol 3:899–905.

Lyford GL, Yamagata K, Kaufmann WE, Barnes CA, Sanders LK, Copeland NG, Gilbert DJ, Jenkins NA, Lanahan AA, Worley PF. 1995. Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron 14:433–445.

Marin R, Salvatierra N, Ramirez O. 1996. Rapid tolerance to benzodiazepine modifies rat hippocampal synaptic plasticity. Neurosci Lett 3:149–152.

Marin RH, Perez MF, Duero DG, Ramirez OA. 1999. Preexposure to drug administration context blocks the development of tolerance to sedative effects of diazepam. Pharmacol Biochem Behav 3:473–477.

Martin SJ, Grimwood PD, Morris RG. 2000. Synaptic plasticity and memory: An evaluation of the hypothesis. Annu Rev Neurosci 23:649–711.

Milekic M, Brown SD, Castellini C, Alberini CM. 2006. Persistent disruption of an established morphine conditioned place preference. J Neurosci 11:3010–3020.

Morris RG, Frey U. 1997. Hippocampal synaptic plasticity: Role in spatial learning or the automatic recording of attended experience? Philos Trans R Soc Lond B Biol Sci 1360:1489–1503.

Nestler EJ. 2001a. Psychogenomics: Opportunities for understanding addiction. J Neurosci 21:8324–8327.

Nestler EJ. 2001b. Neurobiology. Total recall—the memory of addiction. Science 5525:2266–2267.

Nestler EJ. 2002. Common molecular and cellular substrates of addiction and memory. Neurobiol Learn Mem 3:637–647.

O'Brien CP, Testa T, O'Brien TJ, Brady JP, Wells B. 1977. Conditioned narcotic withdrawal in humans. Science 195:1000–1002.

Paxinos G, Watson C. 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, Sidney.

Pellow S, File SE. 1986. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. Pharmacol Biochem Behav 3:525–529.

Pérez MF, Maglio LE, Marchesini GR, Molina JC, Ramírez OA. 2002. Environmental changes modify the expression of Diazepam withdrawal. Behav Brain Res 1:75–81.

Ramirez OA, Orsingher OA, Carrer HF. 1988. Differential threshold for long-term potentiation in the hippocampus of rats with inborn high or low learning capacity. Neurosci Lett 3:275–279.

Reed JM, Squire LR. 1998. Retrograde amnesia for facts and events: Findings from four new cases. J Neurosci 10:3943–3954.

Rial Verde EM, Lee-Osbourne J, Worley PF, Malinow R, Cline HT. 2006. Increased expression of the immediate-early gene arc/arg3.1 reduces AMPA receptor-mediated synaptic transmission. Neuron 3:461–474.

Saal D, Dong Y, Bonci A, Malenka RC. 2003. Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. Neuron 4:577–582.

Vorel SR, Liu X, Hayes RJ, Spector JA, Gardner EL. 2001. Relapse to cocaine-seeking after hippocampal theta burst stimulation. Science 5519:1175–1178.

Wolf ME. 2002. Addiction: making the connection between behavioral changes and neuronal plasticity in specific pathways. Mol Interv 3:146–157.

Woods JH, Katz JL, Winger G. 1987. Abuse liability of benzodiazepines. Pharmacol Rev 4:251–413.

Woods JH, Katz JL, Winger G. 1992. Benzodiazepines: Use, abuse, and consequences. Pharmacol Rev 2:151–347.

Zola-Morgan S, Squire LR, Amaral DG. 1986. Human amnesia and the medial temporal region: Enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. J Neurosci 10:2950–2967.