Phosphorylation state of CREB in the rat hippocampus: A molecular switch between spatial novelty and spatial familiarity?

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Abstract

The activation of cAMP response element-binding protein (CREB) after a learning experience is a common feature in the formation of several associative memories. We recently demonstrated that the increase in the hippocampal phosphorylated CREB (pCREB) levels 1 h after a short exploration of an open field (OF) was associated to detection of spatial novelty and was not related to the memory formation of habituation in this non-associative learning paradigm. Moreover, after a long training of three OF sessions, hippocampal pCREB levels were below to that observed in control rats. The present results show that such decrease does not correlate with memory retrieval or improvement in long-term memory of habituation. Instead, it is associated with the familiarity to the arena. Our experiments revealed that the relevant variable to induce CREB deactivation was the prolonged exploration of the arena (30 min). A 15 min OF exploration was ineffective. Furthermore, the last 5 min period of a prolonged exploration was crucial to change CREB phosphorylation state: when exploration took place in a novel arena the level of pCREB increased; in contrast, when it was performed in the familiar OF, pCREB levels decreased. Taken as a whole, our results suggest that CREB phosphorylation state in the hippocampus switches in response to exposure to a novel or to a familiar spatial environment.

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1. Introduction

Novelty detection plays an important role in both adapting to environmental changes and avoiding dangers. A novel stimulus provokes a response that declines or habituates as the stimulus becomes familiar (Sokolov, 1963). When rats are submitted for the first time to an environment they display a natural tendency to actively explore it; in parallel the animals compare this experiences with stored memories of places previously explored to judge its novelty (Montag-Sallaz, Welz, Kuhl, Montag, & Schachner, 1999). However, when the animals are exposed to the same context for a second time, there is a decrease in the exploratory response which becomes a good memory index for spatial habituation (Vianna et al., 2000; Winograd & Viola, 2004). This decrease in the exploratory response also involves the discrimination of spatial familiarity. The efficiency and speed of novelty detection confer an evolutionary advantage that provide a reason for the existence of a familiarity discrimination network in addition to those networks used for recollection (Bogacz & Brown, 2003; Brown & Aggleton, 2001).

The hippocampal region is an essential component of the circuit that detects and responds to new stimuli (Grunwald, Lehnertz, Heinze, Helmstaedter, & Elger, 1998; Knight, 1996). The novelty-related activity occurs regardless of the attentional status and habituates rapidly (Yamaguchi, Hale, D’Esposito, & Knight, 2004). Moreover, biochemical and plastic changes associated with
novelty can be detected in the hippocampus (Acquas, Wilson, & Fibiger, 1996; Giovannini et al., 2001; Izquierdo et al., 2001; Winograd & Viola, 2004; Xu, Anwyl, & Rowan, 1998). In addition, the hippocampal region plays a critical role in remembering both the environmental context in which an event occurs (Kim & Fanselow, 1992; Maren & Fanselow, 1997; Nadel & Moscovitch, 1997), and where an event takes place within allocentric space (Eichenbaum, 2000; O’Keefe & Nadel, 1978). Pharmacological or surgical manipulation of the hippocampus impairs memory formation of several spatial learning tasks, including spatial habituation (Broadbent, Squire, & Clark, 2004; Kesner, Dakis, & Bolland, 1993; Morris, Garrud, Rawlins, & O’Keefe, 1982; Nakazawa, McHugh, Wilson, & Tonegawa, 2004; Pittenger et al., 2002; Riedel et al., 1999; Vianna et al., 2000). Taken into account the above body of evidence, the role of hippocampus is essential in novelty detection and in processing of spatial memories.

From mollusk to mammals, numerous evidences support the involvement of cAMP response element-binding protein (CREB) in formation of associative, aversive or spatial memories (Bernabeu, Cammarota, Izquierdo, & Medina, 1997; Bouritchaludze et al., 1994; Cammarota et al., 2000; Guzowski & McGaugh, 1997; Josselyn, Kida, & Silva, 2004; Kida et al., 2002; Pittenger et al., 2002; Silva, Kogan, Frankland, & Kida, 1998; Taubenfeld, Wiig, Bear, & Alberini, 1999; Viola et al., 2000; Yin, Del Vecchio, Zhou, & Tully, 1995; Yin et al., 1994; but see Balschun et al., 2003). We have recently initiated a series of studies aimed to determine the role of CREB activation in the hippocampus of rats submitted to a free exploration in an open field (OF). We found that pCREB levels increased between 1 and 2 h after a single 5 min OF exploration, returning to basal levels 3 h after training (Vianna et al., 2000). This increase is not related with formation of the non-associative spatial memory of habituation; it is indeed associated with the detection of a novel environment (Winograd & Viola, 2004). This conclusion is based on the following results: hippocampal pCREB levels increased after a brief novel exploration that does not induce the formation of memory of habituation and also increased in rats amnesic for spatial memory. Indeed, pCREB levels did not increase in rats re-exposed to the context. In contrast, there was a decrease in pCREB levels in rats subjected to a long training protocol in the OF (Winograd & Viola, 2004). When the animals are submitted to a long training protocol, consisting of three consecutive OF session (5 min–20 min–5 min) separated by 3 h intervals, a decrease in the exploratory behavior (observed in the third session respect to the second one) is accompanied by a decrease in pCREB levels in the hippocampus. On the other hand, when all sessions lasted 5 min (short training protocol) those changes were not observed (Winograd & Viola, 2004).

Considering that hippocampal pCREB levels increase after novelty detection, the goal of the present work was to determine whether the deactivation of CREB correlates with retrieval of a prominent spatial memory, with formation of a better long-term memory of habituation, or with discrimination of spatial familiarity.

2. Materials and methods

2.1. Subjects

Male Wistar rats (age, 2 months; weight, 180–210 g) from our own breeding colony were used. The animals were housed five to a cage, with water and food ad libitum, under a 12 h light/dark cycle (lights on at 7:00 a.m.) at a constant temperature of 23°C. All behavioral testing was conducted during the light phase of the cycle.

The experimental protocols for this study 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 Committee of The University of Buenos Aires.

2.2. Apparatus and measurements

Different behavioral protocols were done in either a square and/or a circular open field (OF). The square OF was a 50 cm high, 50 cm wide, and 39 cm deep arena with black plywood walls and a brown floor divided into nine squares by black lines. The circular OF was 50 cm diameter, 50 cm deep with red plywood walls and black floor with nine divisions. Number of line crossings and rearings were measured in blocks of 5 min and their decrement in subsequent sessions was used as an index of memory of spatial habituation (Winograd & Viola, 2004).

2.3. Behavioral procedures

To avoid unnecessary emotional stress, all rats were previously handled daily during 3 min for 3 days. Within each cage, rats were randomly assigned to naïve or to different experimental group. Naïve animals never explored the OF. Experimental animals were submitted to single or multiple OF sessions and after each training session they were placed back into their holding cage.

Behavioral protocols were graphically summarized in the upper part of each figure to provide a clearer training description. To test whether the decrease of pCREB levels was dependent on the exploration to a familiar environment, rats were submitted to a 5 min exposure to a square OF followed by a 20 min exposure to the same arena 3h later. After an interval of 3 h, the animals explored during 5 min either the square OF (Fig. 1A) or a different circular OF (Fig. 1B). Another behavioral protocol included the sequence described above and the addition of further 5 min square OF session 3 h after the exposure to the circular one (Fig. 4A).

In other experiment, we examined if the relevant variable that induces CREB deactivation was the OF time exposure. Thus, rats were submitted to a single 30 min (Fig. 2A) or a single 15 min (Fig. 3A) square OF sessions.

Finally, we tested the long-term memory of spatial habituation 1 day after the short and long training protocols. In the short one, animals were submitted to three 5 min sessions in the square OF, separated by 3-h intervals. The long training protocol was identical to the short one except that the second training session lasted 20 min. The test session, performed 24 h after training, consisted in a 5 min exploration in the square OF (Fig. 6A).

To performed biochemical assays, animals were sacrificed by decapitation 1 h after the end of the training protocol and their hippocampi were dissected out. To take into account the biological variation attrib-
utable to circadian rhythm, experimental and naive animals were sacrificed at the same time.

2.4. Biochemical procedures

Biochemical assays were performed in the nuclear fraction of the hippocampus. All the procedure was carried out at 4 °C. After sacrifice, the brains were immediately removed, the hippocampi were dissected out, pooled, and 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 aprotonin, 15 μg/ml leupeptin, 50 mM NaF, and 1 mM sodium orthovanadate). The homogenate was centrifuged 10 min at 900 g and the obtained nuclear pellet resuspended in buffer (20 mM Tris–HCl, pH 7.4, 1 mM PMSF, 50 mM NaF, and 1 mM sodium orthovanadate.) The samples were stored at −70 °C until used.

Samples of nuclear fraction (30 μg of protein) were subjected to SDS-PAGE (10% gels) and immunoblots were performed as previously described (Cammarota et al., 2000). Membranes were incubated with the following antibodies: anti CREB (1:1.500; Cell Signaling Technologies, Beverly, MA, USA), anti pCREB (1:1.000; Cell Signaling Technologies), and anti α catalytic subunit of cAMP-dependent protein kinase (PKA) (1:8000, Santa Cruz Biotechnology). The activated mitogen-activated protein kinases (pMAPKs) were detected in hippocampal homogenates using the antibody anti phospho-p42/p44 MAPKs (1:4000, Cell Signaling Technologies).

Fig. 1. Exposure to a novel circular open field (OF), following habituation to a square OF, reverses the decrease in exploratory activity and increases hippocampal pCREB levels. (A) Flow diagram of the training protocol. The rats were submitted to square OF (rectangles) during 5 or 20 min. (†) Time of sacrifice. Exploratory activity depicted as the number of crossings (black bars) and rearings (grey bars) in the initial 5 min of each OF session (1s, first square OF; 2s, second square OF; 3s, third square OF). ***p < .001 respect to the first OF sessions; **p < .01 and ***p < .001 respect to the second OF session. Newman–Keuls multiple comparison test after one-way repeated measures ANOVA, n = 8. (B) Flow diagram of the training protocol. The rats were submitted to square OF (rectangles) and a novel circular OF (circle) during 5 or 20 min. (†) Time of sacrifice. Exploratory activity depicted as the number of crossings (black bars) and rearings (grey bars) in the initial 5 min of each OF session (1s, first square OF; 2s, second square OF; 1c, first circular OF). *p < .05; ***p < .001 respect to the other OF sessions. Newman–Keuls multiple comparison test after one-way repeated measures ANOVA, n = 9. (C) Representative Western blot films and densitometric analysis of hippocampal CREB (grey bars) and pCREB (black bars) levels 1 h after the third OF session performed in a familiar arena (familiar) or in a novel circular OF (novel). *p < .05; **p < .01 respect to naïve animals; ***p < .001 respect to familiar group, Newman–Keuls multiple comparison test after one-way repeated measures ANOVA, n = 6–7.

Densitometric analysis of the films was performed by using MCID Image Analysis System (5.02v, Image Research, Ontario, Canada). Western blots were developed for linearity within the range used for densitometry.

Protein phosphatases total activity was detected in hippocampal nuclear samples by a colorimetric method using a Ser/Thr phosphatase assay kit (Upstate Biotechnology) according to the directions supplied by the manufacturer. Briefly, after removing free phosphate from nuclear sample, the assay was initiated by the addition of phosphorylated hexapeptide substrate and quenched 15 min later by the addition of an acidic malachite green solution. The green color was quantitated spectrophotometrically at 650 nm against a phosphate standard curve (Santoro et al., 1998).
2.5. Data analysis

Newman–Keuls multiple comparison test after repeated measures one-way analysis of variance (ANOVA) was applied for the statistical analysis of behavioral data. Non-paired Student’s t test was used when two independent groups were compared.

3. Results

3.1. Hippocampal pCREB levels increased after spatial novelty

Confirming our previous results (Winograd & Viola, 2004), rats trained with a long protocol in the square OF gradually decreased their exploratory activity over consecutive sessions (Fig. 1A; \( p < .001 \)). As expected, hippocampal pCREB levels 1 h after this training were lower than those observed in control rats (Fig. 1C, \( p < .01 \)). No changes in the total amount of CREB protein were observed. We wanted to test whether this phenomenon was dependent on the exposure to a familiar environment. Thus, we trained rats with the long protocol, except that this time the third exposure took place into a novel circular OF (Fig. 1B). Predictably, the animals actively explored the circular arena, displaying a number of crossings and rearings similar to those observed during the first session in the square OF (Fig. 1B). In parallel, CREB phosphorylation state in

Fig. 2. Reduced hippocampal pCREB levels are observed after a 30 min exposure to an open field (OF), when animals were familiarized with the environment. (A) The rectangle represents the 30 min session in a square OF. (†) Time of sacrifice. Exploratory activity depicted as the number of crossings (B) and rearings (C) in 5 min blocks until completion of the 30 min session. As can be seen, the major exploratory activity occurs during the first 5 min and then diminishes gradually until the last 10 min when minimal activity is registered. *** \( p < .001 \) respect to the 5 min block; ** \( p < .01 \), and *** \( p < .001 \) respect to the 10 min block; Newman–Keuls multiple comparison test after one-way repeated measures ANOVA, \( n = 9 \). (D) Representative Western blot films and densitometric analysis of hippocampal CREB (grey bars) and pCREB (black bars) levels 1 h after a 30 min OF session. *** \( p < .001 \); Student’s t test, \( n = 6–7 \).

Fig. 3. Hippocampal pCREB levels do not change after a 15 min square open field (OF) session. (A) The rectangle represents a 15 min session in a square OF. (†) Time of sacrifice. (B) Exploratory activity is depicted as the number of crossings and rearings in 5 min blocks. *** \( p < .001 \) vs. the 5 min block; ## \( p < .01 \) vs. the 10 min block; Newman–Keuls multiple comparison test after one-way repeated measures ANOVA, \( n = 8 \). (C) Representative Western blot films and densitometric analysis of hippocampal CREB (grey bars) and pCREB (black bars) levels 1 h after the 15 min OF. \( p > .05 \); Student’s t test, \( n = 6–7 \).
hippocampal nuclear fraction increased 1 h after submitting rats to the novel circular OF (p < .01). No changes in total amount of CREB protein were detected (Fig. 1C). Thus, the decrease in pCREB levels following the long training protocol was not attributable to a “fatigue” of CREB phosphorylation mechanisms, because CREB was activated when rats were subjected to a subsequent novel circular environment. Also, the changes in pCREB levels after the third exposure to the arena were not induced by previous training sessions. We observed that CREB phosphorylation state did not differ at 7 h after the first OF session or at 4 h after the second one (Winograd & Viola, 2004).

Owing that a short training protocol, consisted of three 5 min sessions, abolished the pCREB increment associated with first exploration spatial novelty, but did not decrease the pCREB levels with respect to naïve group (Winograd & Viola, 2004), we suggest that exposure to an extensively explored territory is required for CREB deactivation below basal levels.

3.2. A single long open field session, but not a short one, decreases pCREB levels

Next, we examined if the relevant variable that induces CREB deactivation was the time that rats spent exploring the arena. It is conceivable that a longer training session may lead to an enhanced spatial familiarity and decrease motivation to explore the field. Thus, we decided to subject rats to a single 30 min session in an OF, so that the animals spent the same time in the arena than those subjected to the long protocol (Fig. 2A). We observed that the number of crossings and rearings gradually decreased and reached a plateau in the last 10 min of the trial, reflecting a minimal exploratory behavior (Figs. 2B and C, p < .001 with respect to the first 10 min of the session). As expected, pCREB levels measured in the hippocampus 1 h after training were significantly reduced in comparison to naïve rats (Fig. 2D, p < .001). No changes in the total amount of CREB protein were detected (Fig. 2D, p > .05). Moreover, a single 15 min OF exploration, the same period of time elapsed in a short training protocol (Fig. 3A), was unable to modify neither the hippocampal pCREB nor CREB protein levels (Fig. 3C, p > .05). As shown in Fig. 3B, rats actively explored the square OF during the initial 5 min, then the exploratory behavior decreased as a function of time (p < .001 respect to 5 min block; p < .01 respect to 10 min block).

Based on these experiments, the decrease in CREB phosphorylation after the long OF training protocol may not be associated with retrieval of a “good” memory of habituation, because this phenomenon was also observed after a single long OF protocol that lacks a test session (Fig. 2D).

3.3. Exploration of a familiar environment decreases pCREB levels

To further examine the possibility that CREB deactivation specifically occurs in response to the presentation of a familiar environment, we trained rats to explore the square OF two times followed by the exploration to a novel circular OF (as in Fig. 1B); after this, the animals were exposed for a third time to a 5 min session in the square OF (Fig. 4A). The first exposure to a location (square or circu-
lar OFs) induced the largest exploratory behavior of rats, while the exposure to a repeated place produced a decline in the number of crossings and rearings (Figs. 4 B and C; \( p < .01 \)–.001, 3 s session respect to 1 s and 1 c). Confirming our hypothesis, hippocampal pCREB levels decreased when the animals detected the place explored as familiar (Fig. 4D; \( p < .05 \)). No changes in total amount of CREB protein were found (Fig. 4D; \( p > .05 \)).

To elucidate the signaling cascades involved in CREB deactivation, we begin to study protein phosphatases activity and the activation of some protein kinases in the hippocampus of animals subjected to spatial familiarization (Fig. 5A). No changes in nuclear protein phosphatases total activity, nuclear α catalytic subunit of cAMP-dependent protein kinase (PKA) levels, or phosphorylated mitogen-activated protein kinase (phospho-p42/p44 MAPKs) levels were found 1 h after a long training protocol in comparison to naive rats (Figs. 5B–D).

3.4. The decrease in pCREB levels is not associated with an improvement in the long-term memory of spatial habituation

Finally, we wanted to test if the fall of pCREB levels in the hippocampus, initially observed after the long training protocol, could correlate with an improvement in the long-term memory of spatial habituation. We trained rats in a short or a long training protocol and registered the exploratory behavior 24 h later (Fig. 6A). Figs. 6B and C show the number of crossings and rearings corresponding to each training session and to the 5 min test session performed the following day. As expected, the exploratory behavior in the second visit to the OF was lower than that in the first exposure to the arena. The decrease was similar in both experimental groups. But, as previously shown (Winograd & Viola, 2004), at the third training session, a lower exploratory activity was evident in rats trained with the long training protocol compared to that observed in rats trained
with the short training protocol (Figs. 6B and C, \( p < .01 \)). In spite of this, the memory of spatial habituation expressed in the test session did not differ between the experimental groups (Figs. 6B and C, \( p > .05 \)). This demonstrates that the prominent exploratory habituation showed during training was not stored in a long-term memory. In other words, the decrease in exploratory behavior and the associated decrease in pCREB levels observed at the final session of a long training protocol were not related to the formation of a better long-term memory of spatial habituation.

4. Discussion

Recognition is one of the aspects involved in the ability to remember. It requires the identification and judgment of prior occurrences of whatever has been identified (Brown & Aggleton, 2001). In particular, spatial recognition memory is observed in rats through the preference for exploring a novel place over a familiar one (Hannesson, Vacca, Howland, & Phillips, 2004). We propose that in an OF paradigm, memory of spatial habituation includes the discrimination of a familiar place.

We designed a series of behavioral protocols consisting in the submission of rats to single or multiple sessions of exposure to an open field. The animals decreased their exploratory activity if they had previously explored the arena, expressing the spatial memory. After training, we registered the hippocampal pCREB nuclear levels. CREB activation has been consistently used as a marker of associative memory processing (Silva et al., 1998). It was observed that pCREB levels were increased only after a short exposure to a novel place (Fig. 1C). This result is in agreement with our previous report where it was experimentally determined that this phenomenon was independent of the animal ability to form memory of habituation, suggesting that the detection of a novel context was the triggering stimuli for CREB phosphorylation (Winograd & Viola, 2004).

On the other hand, the more lasting exposure to an open field induced a decreased in the pCREB levels. The main goal of this work was to study if such decrease was associated with retrieval of the memory of habituation, formation of a long-term memory or discrimination of the spatial familiarity.

The decrease in hippocampal pCREB levels measured after the long OF training protocol was not related to memory retrieval. This conclusion was based on two results: (1) pCREB levels diminished after a single 30 min OF protocol that lacks a test session (Fig. 2D) and (2) no changes in pCREB levels were observed after a second or third 5 min OF session, when animals retrieved the information storied earlier, (Winograd & Viola, 2004). In addition, lower pCREB levels were not related to the formation of a better long-term memory of spatial habituation since memory expression at the following day was similar in groups of rats trained with short or long training protocols (Fig. 6).

Alternatively, our results suggest that CREB deactivation is associated to environmental familiarity, expressed by a lower number of crossings and rearings performed by rats, and clearly dependent on the time the rat spent in the OF (Figs. 2 and 3). In that sense, the behavioral spatial habituation at final stage of single 15 min OF session was moderate, being greater than that achieved in a 5 min session but lower than the spatial habituation occurred at the end of a 30 min session. Thus, a few seconds to 5 min exposure to a novel place causes an increase in pCREB levels in the hippocampus; 15 min exposure does not alter CREB phosphorylation state, but a 30 min exposure to the arena significantly decreases it (see Fig. 7). We think that this gradient of environmental familiarity is well correlated with changes in pCREB levels; thus, the increase in the exploration time to the arena reversed the CREB activation attained by spatial novelty. Moreover, re-exposure to a familiar OF, after exploration of a novel one is also associated with a decrease in pCREB levels (Fig. 4D).

In our paradigm, the changes in hippocampal pCREB levels might be a response to spatial novelty–familiarity detection and not to memory formation of spatial habituation. Probably an “off switch” mechanism that terminates with learning-induced plasticity could prevent a sustained CREB activity that does not contribute to memory formation. This results might be related with those of

![Fig. 7. Hippocampal CREB phosphorylation levels switches in response to spatial novelty and environmental familiarity. Figure depicts the different training protocols, submitting rats to square and circular open field (OF), and their associated pCREB levels (Winograd & Viola, 2004) and present findings. Sizes represent the session length while the filling intensity depicts exploratory activity in each session. All inter-session times were 3 h long, and pCREB levels were measured 1 h after the last OF session (being those: novel; short or familiar). Increase (↑), decrease (↓), and no changes (=) in hippocampal pCREB levels with respect to naive group.](image-url)
Colombo and colleagues (2003) who recently shown an initial increase of pCREB levels in the hippocampus and the neostriatum of rats trained on an appetitively motivated cross maze task. Those levels remained elevated in the hippocampus and were switched off in the neostriatum of rats who adopted a “place search strategy”, while the inverse result was observed in rats who adopted a “response search strategy.” Thus, memory systems may be activated in parallel during training for processing different aspects of the information acquired (Colombo, 2004).

Could changes in pCREB levels be a response to changes in rat locomotor activity? When an animal is exposed for first time to an OF displays a great exploratory activity (including a great locomotor activity) that diminishes as it gathers information about the environment. Thus, rats tend to explore it extensively if the OF is a novel one, a little less if the OF is repeated, and very scarcely if it is well known (familiar). However, changes in pCREB levels are not probably due to locomotor activity per se because: (1) the locomotor activity in the last 5 min of the second OF in the long protocol (25 min of total exploration) was similar to the last 5 min of a single 30 min OF exposure; however, no changes in pCREB levels were observed after the former protocol, while a large decrease was found after the single 30 min OF (Fig. 2D and (Winograd & Viola, 2004)). (2) The rise in pCREB levels after exposure to a 5 min novel OF was similar to the rise in pCREB levels observed after a 20 s one (Fig. 1C and (Winograd & Viola, 2004)); however, the animals displayed a great locomotor activity along the 5 min (Fig. 1B) but they could only perform few crossings in the brief 20 s exposure. In sum, a careful analysis of the results shows that changes in pCREB levels are associated with the degree of familiarity to the OF and not with locomotor activity per se.

It has been demonstrated that phosphorylation of CREB in Ser-133 serves to recruit CREB-binding protein (CBP) facilitating gene transcription (Chrivia et al., 1993) that could lead synaptic plasticity and behavioral changes. However, a plethora of events can regulate CREB mediated gene expression. Some of these events include CREB phosphorylation in Ser-142 and methylation of CBP and CRE sequence. Gene expression also depend on signaling pathways inducing CREB activation, on the combination of different members of ATF/CREB family to form different class of dimers or on the requirement of additional regulatory partners for stable recruitment of cofactors (Lonze & Ginty, 2002; Shaywitz & Greenberg, 1999; Zhang et al., 2005).

Nevertheless, our findings agree with the work of Kinney and Routtenberg (1993). They found that exposure to a novel radial maze enhanced the binding of hippocampal transcription factors to their CRE consensus DNA recognition element. This increased binding was observed 1 h after a short (4 min) exploration, but not with a 15 min exposure. The authors suggest that lack of novelty could lead to the deactivation of the transcription factor, probably by a post-translational mechanism.

Recently, involvement of pCREB in synaptic plastic processes within perirhinal cortex was studied in a visual recognition memory paradigm in rats (Warburton et al., 2005). Familiarity discrimination has been identified as the reduction in the responses of these neurons when the stimulus is re-encountered. The authors registered neuronal activation by novel and familiar pictures measured by fos and pCREB-stained nuclei in perirhinal cortex. They observed a normal pattern with greater expression of both proteins evoked by novel than familiar pictures. Moreover, adenoviral transduction of a dominant-negative inhibitor of CREB before training, disrupted this normal pattern and impaired the preferential exploration of novel over familiar objects, providing evidence for the involvement of pCREB in long-term recognition memory. Based on these results, we cannot rule out the possibility that the increase of pCREB observed after the exploration of a novel place, could be related to the storage of information about the category of the context (never explored before = novel).

In addition, c-fos, one of the gene products regulated by CREB (Impye et al., 2004) responded according to the contextual stimuli. By immunohistochemical detection of c-fos protein, Zhu, McCabe, Aggleton, and Brown (1997) reported that activation of hippocampal neurons was ascribed to the novelty of the environment. After exposure to a familiar OF only a few nuclei stained for this protein were observed. Also, a strong c-fos production in hippocampus was observed 1 h after exposure of mice for 3 min to a novel spatial context, but a pre-exposure resulted in a reduction of this protein (Radulovic, Kammermeier, & Spiess, 1998).

In a similar way, auditory novelty in birds is also associated with changes in membrane gene expression. The paradigm consisted in the presentation of birdsong playbacks that trigger an overt behavioral response, which habituated after song repetition (Stripling, Milewski, Kruse, & Clayton, 2003). The authors reported a rapid and transient increase in the zenk mRNA expression after an initial exposure to birdsong. However, when the song presentation continued for more than 30 min, zenk mRNA levels were indistinguishable from controls (Mello, Nottebohm, & Clayton, 1995). The habituated genomic response was reactivated when at the final part of the long acoustic pattern the speaker location was changed (Kruse, Stripling, & Clayton, 2004). The results suggest that changes in the attentional state or in the stimulus salience may be a critical factor for zenk activation, which might be associated with the integration of new information. Again, the degree of zenk induction appears to be inversely correlated with stimulus-context familiarity and may be linked to a neural circuit engaged in song recognition and discrimination (Kruse et al., 2004; Mello et al., 1995).

An increase in pCREB level was recently observed in the nucleus accumbens after training rats in a food search spatial task. This increase was also observed in untrained rats submitted for a first time to the food search apparatus; thus the activation of CREB may be mainly triggered...
by detection of spatial novelty (Alvarez-Jaimes, Centeno-Gonzalez, Feliciano-Rivera, & Maldonado-Vlaar, 2005). Similarly to our results, a 15 min spatial novelty did not increase the pCREB level in the hippocampus. However, the authors observed an increase of pCREB levels after a further 15 min exploratory session performed the following day. Therefore, they argue that prolonged exploration of this complex environment, composing of a square field (1 m x 1 m x 46 cm high) with 16 holes baited in the floor, might induce immediate CREB phosphorylation in the hippocampus.

In Drosophila it was postulated the role of CREB as a molecular switch for the formation of long-term memory (Tully, 1997; Yin & Tully, 1996; but see Perazzona, Isabel, Preat, & Davis, 2004). Overexpression of an activator isoform of CREB enhances the formation of long-term memory in an olfactory paradigm, while overexpression of a repressor isoform induces the opposite effect (Yin et al., 1995, 1994).

Based on our present findings, we propose that CREB in the hippocampus behaves as a molecular switch for spatial recognition. When rats explore a new place for up to 5 min, the increases in pCREB levels are associated with novelty. But if the animals explore the same place for 15 min, pCREB levels do not change. On the other hand, the decrease in pCREB levels after 30 min exploration of an environment (using different long training protocols) accompanies spatial familiarity (Fig. 7).

A relevant issue, partially resolved, is the study of molecular pathways involved in the observed changes in CREB phosphorylation state. A first insight to this question was done by Vianna et al. (2000) who showed that in association with the increase in pCREB levels, found after a single 5 min OF session, there was a sequential rise in PKA activity, phosphorylated p42 and p44 MAPKs and phosphorylated α subunit of calcium calmodulin-dependent protein kinase (pCAMKIIα) levels in hippocampus. On the other hand, the molecular mechanisms involved in pCREB decrease after rat OF familiarization are still unclear. Here, we showed that no changes in nuclear protein phosphatases total activity, nuclear PKAα catalytic subunit levels, or phospho-p42/p44 MAPKs levels were found in hippocampus 1 h after environmental familiarization (Fig. 5). Different experiments are being carried out to elucidate the signaling cascades involved in CREB deactivation.

In conclusion, our results suggest that pCREB levels in hippocampus could be considered a molecular marker for spatial discrimination, and that bi-directional change of the phosphorylation state of CREB is associated with the exploration of a novel or familiar environment.

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