Neurobiology of Learning and Memory 99 (2013) 10–16

Contents lists available at SciVerse ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Lentivirus-mediated chronic expression of dominant-negative CREB in the dorsal hippocampus impairs memory for place learning and contextual fear conditioning

Balachandar Kathirvelu [a], Brett S. East [b], Amanda R. Hill [b], Clayton A. Smith [b], Paul J. Colombo [a,b,*]

[a] Department of Psychology, Tulane University, New Orleans, LA 70118, USA
[b] Neuroscience Program, Tulane University, New Orleans, LA 70118, USA

ARTICLE INFO

Article history:
Received 25 June 2012
Revised 18 September 2012
Accepted 15 October 2012
Available online 27 October 2012

Keywords:
Lentivirus
CREB
Hippocampus
Place learning
Contextual fear conditioning

ABSTRACT

Extensive research has shown that the transcription factor CREB has an important role during memory formation. In the present study, we tested a new method for chronic, stable expression of a dominant-negative form of CREB (mCREB) in the dorsal hippocampus using lentiviral vectors. In specific, we tested whether lentivirus-mediated chronic expression of mutant CREB impairs memory for two hippocampus-dependent tasks – place training in the water maze and contextual fear conditioning. Two weeks following intra-hippocampal infusion, experimental (mCREB) and control (LacZ and saline) rats were trained for 30 trials in one session on a place task in a water plus-maze and tested for an additional 30 trials on day 2 and on day 7. On day 8, all rats were trained on a contextual fear conditioning task and tested 24 h later. For place learning, there was no difference between treatment groups on day 1, indicating that treatment with the lentiviral vectors did not alter performance or acquisition of the task. In comparisons with controls, mCREB-treated rats were not significantly impaired on day 2, overall, but they showed significant impairment on day 7. Contextual fear memory was impaired in mCREB-infused rats in comparison with controls. At the end of the experiment, total CREB and phosphorylated CREB protein were measured by western blot. Levels of total CREB were increased by approximately 40% among mCREB-treated rats in comparisons with controls, whereas levels of pCREB did not differ between groups, suggesting that the treatment caused significant expression of mCREB. In addition, mCREB infused rats showed a significant reduction in the pCREB to CREB ratio in comparison with controls, suggesting that the memory deficit seen in mCREB rats is most likely due to disruption of gene regulation caused by expression of mutant CREB. Taken together, the present results show that lentivirus expressing mCREB can be used to effectively alter CREB function within the hippocampus and that the treatment impairs memory for hippocampus-dependent tasks.

1. Introduction

cAMP response element-binding protein (CREB) is a transcription factor that regulates gene transcription and synthesis of new proteins necessary for neuronal plasticity and memory formation (Gonzalez & Montminy, 1989; Impey et al., 2004). Several reports have demonstrated the importance of CREB during memory formation in invertebrate (Dash, Hochner, & Kandel, 1990; Kaang, Kandel, & Grant, 1993; Yin et al., 1994) and vertebrate model systems (Bourtchuladze et al., 1994; Guzowski & McGaugh, 1997; Kida et al., 2002; Pittenger et al., 2002). Phosphorylation of CREB, which is a necessary step in CREB-dependent gene transcription (Shaywitz & Greenberg, 1999), has been measured in the hippocampus after training on various spatial and non-spatial tasks including the radial arm maze (Mizuno et al., 2002), plus maze (Colombo, Brightwell, & Countryman, 2003), Morris water maze (Porte, Buhot, & Mons, 2008), socially transmitted food preferences (Countryman, Orlowski, Brightwell, Oskowitz, & Colombo, 2005), contextual fear conditioning (Stanciu, Radulovic, & Spiess, 2001), and inhibitory avoidance (Cammarota et al., 2000), see also, for review (Colombo, 2004). In addition to evidence that learning causes phosphorylation of CREB, systematic genetic manipulations that inhibit CREB function generally cause memory impairment (for exceptions, see Balschun et al., 2003; Gass et al., 1998). These manipulations include the use of knockouts (Bourtchuladze et al., 1994), antisense oligonucleotides (Florian, Mons, & Roullet, 2006; Guzowski & McGaugh, 1997; Pittenger et al., 2002) or acute viral-mediated expression of dominant-negative mutant CREB

* Corresponding author. Address: Tulane University, Department of Psychology, 2007 Percival Stern, New Orleans, LA 70118, USA. Fax: +1 504 862 8744.
E-mail addresses: bkathirv@tulane.edu (B. Kathirvelu), beast@tulane.edu (B.S. East), ahill4@tulane.edu (A.R. Hill), csmith3@tulane.edu (C.A. Smith), pcolomb@tulane.edu (P.J. Colombo).

1074-7427/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nlm.2012.10.008

(Brightwell, Smith, Countryman, Neve, & Colombo, 2005; Brightwell, Smith, Neve, & Colombo, 2008). Among the methods specified above, viral-mediated expression has advantages over other genetic manipulations in that it can be targeted within a specific brain region and cause either acute (Brightwell et al., 2005) or chronic (Mouravlev, Dunning, Young, & During, 2006) transgene expression depending on the viral vector used.

While acute expression of mutant CREB with viral vectors has been used to study the role of CREB during memory formation (Brightwell et al., 2005, 2008; Sekeres, Neve, Frankland, & Josselyn, 2010; Warburton et al., 2005; Yuan, Harley, Darby-King, Neve, & McLean, 2003), it is less well established whether or not chronic expression of mutant CREB has the same effect on memory as acute expression. Indeed, acute and chronic overexpression of wild type CREB may have different effects on memory. For example, acute overexpression of CREB using herpes simplex virus (Brightwell, Smith, Neve, & Colombo, 2007) and Sindbis virus (Restivo, Tafi, Ammassari-Teule, & Marie, 2009) vectors facilitate long-term memory whereas chronic overexpression of CREB using adeno-associated virus had no effect on spatial memory in the Barnes maze or on passive avoidance in rats at three months of age (Mouravlev et al., 2006). It is important to point out, however, that in this longitudinal study overexpression of CREB prevented the onset of age-related memory deficits. Against this background that acute and chronic transgene expression may produce different effects on memory, the current study was designed to test the effects of chronic expression of mutant CREB. Gene expression with herpes simplex virus reaches basal levels by day 7 (Carlezon et al., 1998) whereas expression with lentivirus is stable for months (Greenberg, Lee, Schaffer, & Flannery, 2006; Miyoshi, Takahashi, Gage, & Verma, 1997; Naldini et al., 1996) also see review Barco and Marie (2011). In the current study, we used lentivirus vectors which reportedly produce chronic stable transgene expression, efficient transduction preferentially into neurons, negligible immunogenicity with maintenance of normal cellular function *in vitro* and *in vivo*, and are useful in animal studies that model human diseases (Wong et al., 2006). As indicated by Barco and Marie (2011), there are currently no reports of the effects of lentiviral vectors on chronic expression of mutant CREB or the resultant behavioral effects on memory.

Given the many advantages of lentivirus vectors and the lack of knowledge on the effect of chronic manipulation of mutant CREB, the present study tested the effects of mCREB expression on two different hippocampus-dependent tasks – place learning and contextual fear conditioning – in the same group of animals. Our hypothesis was that chronic expression of mutant CREB in the dorsal hippocampus would impair memory for place learning and contextual fear conditioning. This approach also tests the feasibility of using lentiviral vectors to study various animal models of human neurodegenerative conditions that require chronic treatment.

2. Materials and methods

2.1. Animals

Twenty-nine in-house bred male Long-Evans hooded rats, weighing between 300 and 350 g, were used in this study. All rats were housed individually in plastic-bottom cages with sawdust bedding and *ad libitum* access to food and water in a humidity- and temperature-controlled colony room. The room was set on a 12-h artificial light/dark cycle (lights on at 7:00 A.M.) with behavioral procedures conducted during the light phase of the cycle. All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Tulane University Institutional Animal Care and Use Committee. A timeline of the experiment is shown in the Fig. 1.

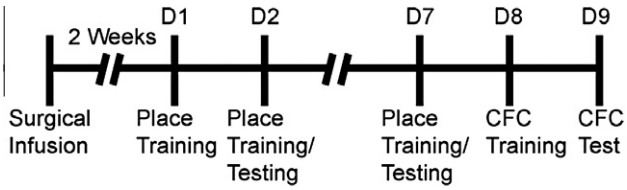

Fig. 1. Schematic representation of experimental design.

2.2. Lentivirus vector-mediated gene transfer

The lentivirus (LV) vectors (LV-mCREB and LV-LacZ) were supplied by Robert Kutner (Neuroscience Center of Excellence, Louisiana State University Health Sciences Center). The cDNA for the dominant negative mutant form of CREB in which Ser133 was replaced by Ala was provided as a gift from Marc Montminy (The Salk Institute of Biological Studies). The lentivirus vector backbone carried the CMV promoter with either the mCREB or LacZ gene. The virus preparation methods are as described previously (Kuroda, Kutner, Bazan, & Reiser, 2008). The estimated titer of these lentivirus vectors was 10^9 infectious units/ml.

2.3. Surgical procedure

Stereotaxic surgery was performed on all rats in a class II biohazard cabinet (Labconco, Fischer Scientific). Rats were anesthetized with a continuous flow of a gaseous mixture containing isoflurane and oxygen and secured in a stereotaxic frame (Kopf Instruments). The skin was incised along the midline of the head and retracted. Holes were drilled through the skull bilaterally above the target coordinates. Immediately before infusion, 2 μl of 20% mannitol (Sigma–Aldrich) were mixed with 4 μl of LV-mCREB, LV-LacZ, or saline. Bilateral infusion needles connected to Hamilton syringes via polyethylene tubing (Plastics One) were loaded with the mixture and inserted through the holes into the brain. The infusion coordinates were (*site* 1: AP = −3.1, ML = ±1.4, DV = 3.8; *site* 2: AP = −4.4, ML = ±3.3, DV = 3.3). The needles were lowered 0.2 mm past the DV coordinate and then raised to the specified DV coordinate to create a potential space to aid diffusion. Then, 6 μl of the mixture were infused at 0.2 μl/min using a dual syringe infusion pump, (Fischer Scientific) and the needle was left in place an additional 5 min to facilitate diffusion. The needles were then removed, the drill holes were sealed with bone wax and the incision closed. All rats were given an intramuscular injection of 0.1 ml (0.006 mg) buprenorphine as a postoperative analgesic. Once removed from the stereotaxic frame, all rats were placed in a recovery box with an overhead heat lamp until ambulatory and were later returned to their home cages in the vivarium. The number of rats in treatment conditions were mCREB = 13, LacZ = 8, and Saline = 8. All rats were given two weeks to recover from surgery as well as for the lentivirus to reach its maximum expression (Kuroda et al., 2008; Sun & Gan, 2011) and then trained on the place task and contextual fear conditioning.

2.4. Apparatus

2.4.1. Water plus-maze

A plus-shaped transparent Plexiglas apparatus with each arm/channel measuring 50 × 21 × 35 cm (L × W × H) was placed inside the center of the water maze (1.83 m diameter and 0.58 m height) filled with water. The maze consisted of Plexiglas walls that