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Age-related impairments in memory and in CREB and pCREB expression in hippocampus and amygdala following inhibitory avoidance training

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ABSTRACT

This experiment examined whether age-related changes in CREB and pCREB contribute to the rapid forgetting seen in aged animals. Young (3-month-old) and aged (24-month-old) Fischer-344 rats received inhibitory avoidance training with a low (0.2 mA, 0.4 s) or moderate (0.5 mA, 0.5 s) foot shock; memory was measured 7 days later. Other rats were euthanized 30 min after training, and CREB and pCREB expression levels were examined in the hippocampus, amygdala, and piriform cortex using immunohistochemistry. CREB levels decreased with age in the hippocampus and amygdala. After training with either shock level, young rats exhibited good memory and increases in pCREB levels in the hippocampus and amygdala. Aged rats exhibited good memory for the moderate but not the low shock but did not show increases in pCREB levels after either shock intensity. These results suggest that decreases in total CREB and in pCREB activation in the hippocampus and amygdala may contribute to rapid forgetting in aged rats. After moderate foot shock, the stable memory in old rats together with absence of CREB activation suggests either that CREB was phosphorylated in a spatiotemporal pattern other than analyzed here or that the stronger training conditions engaged alternate mechanisms that promote long-lasting memory.

1. Introduction

As seen in humans, rats and mice exhibit age-related impairments in learning and memory on many tasks. Often, the impairments are characterized in terms of rapid forgetting, in which aged rodents perform similarly to young adult rodents on memory tests soon after training, but have poor memory at later times as compared to young rodents (Barnes, 1991; Foster, 1999; Gold, 2005, 2001; Korol, 2002; Winocur, 1988). There are many examples of accelerated forgetting in aged rodents, with specific time courses that differ by task. In particular, memory for inhibitory avoidance training remains stable in young adult rats for weeks after training but decays within hours to days in old rats. Importantly, the age-related difference in forgetting rate is apparent despite similar or lower foot shock perceptual thresholds in old rats (Foster and Kumar, 2007; Frye et al., 2010; Gold et al., 1981; Morris et al., 2010). Rapid forgetting is also evident in a variety of other tasks, including the water maze (Burke et al., 2008; Gage et al., 1984; Mabry et al., 1996; Rapp et al., 1987), reward reduction (Salinas and Gold, 2005), social transmission of food preference (Countryman and Gold, 2007), visual discriminated avoidance (Gold et al., 1981), Barnes circular maze (Barnes and McNaughton, 1985), spatial reversal (Zornetzer et al., 1982), spontaneous alternation (Da Silva Costa-Aze et al., 2011; McNay and Gold, 2001; Stone et al., 1997), odor-reward association (Roman et al., 1996), and eye-blink classical conditioning (e.g. Solomon et al., 1995; Woodruff-Pak et al., 2007). The breadth of examples of rapid forgetting suggests that this is a key characteristic of age-related changes in memory.

Rapid forgetting seen during aging is analogous to similar findings seen after many treatments that interfere with cell and molecular processes associated with the formation of new memories. Rapidly decaying memory is seen after administration of protein synthesis inhibitors, ERK/MAPK inhibitors, and inhibitors of transcription factors, such as cAMP response element-binding protein (CREB), and is also seen in several knockout and transgenic mice with alterations aimed at these and other molecular targets (e.g. Alberini, 2009; Apergis-Schoute et al., 2005; Costa-Mattioli and Sonenberg, 2008; Goelet et al., 1986; Guzowski et al., 2000; Houpt and Berlin, 1999; Izquierdo et al.,

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2006; Kandel, 2001; McGaugh, 2000; Taubenfeld et al., 2001; Trifilieff et al., 2006). The parallels between the rapid forgetting in these experiments and those seen in aged rodents suggest that similar cellular mechanisms may be involved.

Of particular relevance to the experiments reported here, considerable evidence suggests that CREB is a transcription factor important to the formation of durable memories, perhaps converting rapidly decaying memories and short-term potentiation to more permanent forms (Benito and Barco, 2010; Carlezon et al., 2005; Colombo et al., 2003; Josselyn, 2010; Silva et al., 1998; Taubenfeld et al., 1999; Yin and Tully, 1996). For example, interfering with CREB function via mutations or inhibitors generally disrupts memory assessed at long but not short times after training (Bourtchuladze et al., 1994; Brightwell et al., 2008, 2005; Frankland et al., 2004; Guzowski and McGaugh, 1997; Josselyn et al., 2004; Yin et al., 1994), and these findings are analogous to the rapid forgetting seen in aged rodents. Several studies have examined CREB functions in aged rodents (cf. Lund et al., 2004). One consistent finding is that aged rodents are impaired in training-related activation of phosphorylated CREB (pCREB) in the hippocampus (Countryman and Gold, 2007; Kudo et al., 2005; Monti et al., 2005; Porte et al., 2008; Xu et al., 2010). Several studies of chronically administered treatments have identified substances that can attenuate age-related memory impairments while also enhancing CREB phosphorylation (Assunção et al., 2010; Li et al., 2009; Trofimiuk et al., 2010; Xu et al., 2010). Mouravlev et al. (2006) demonstrated that somatic gene transfer of CREB protein into the hippocampus of young adult rats prevented later formation of age-associated memory impairments. Also, Brightwell et al. (2004) found that aged rats with poor spatial memory have lower hippocampal CREB levels than do those with good spatial memory. However, prior aging studies have apparently not examined whether altering training conditions to promote stable memory formation in aged rodents would reverse deficits in CREB activation.

The present report tested the hypothesis that age-related differences in the expression and activation of CREB and pCREB may contribute to the rapid forgetting that is characteristic of aged rodents. Therefore, we used an inhibitory avoidance task in which the rate of forgetting is accelerated in aged rats. In addition, increases in the aversive component of training result in better maintenance of memory, providing a comparison of conditions in which memory is rapidly or slowly forgotten. Thus, in the experiments reported here, rats were trained with either a low intensity foot shock, which led to age-related rapid forgetting, or a moderate intensity foot shock, which led to stable memory formation in both young and old rats. In past studies of aging and memory, CREB and pCREB expression were assessed only in the hippocampus. In the present experiments, CREB and pCREB expression levels were assessed with immunohistochemistry in brain sections collected 30 min after training from the amygdala and piriform cortex, as well as from hippocampal dentate gyrus, area CA3, and area CA1 (see Fig. 1).

2. Materials and methods

2.1. Subjects

Young adult (3–4 mo.) and old (24–25 mo.) male Fischer-344 rats (Taconic Farms, Germantown, NY) were individually housed in translucent cages with a 12-h light/12-h dark cycle (lights on at 07:00 h) and *ad libitum* access to food and water. Animal pain and discomfort were minimized, and all experiments were conducted

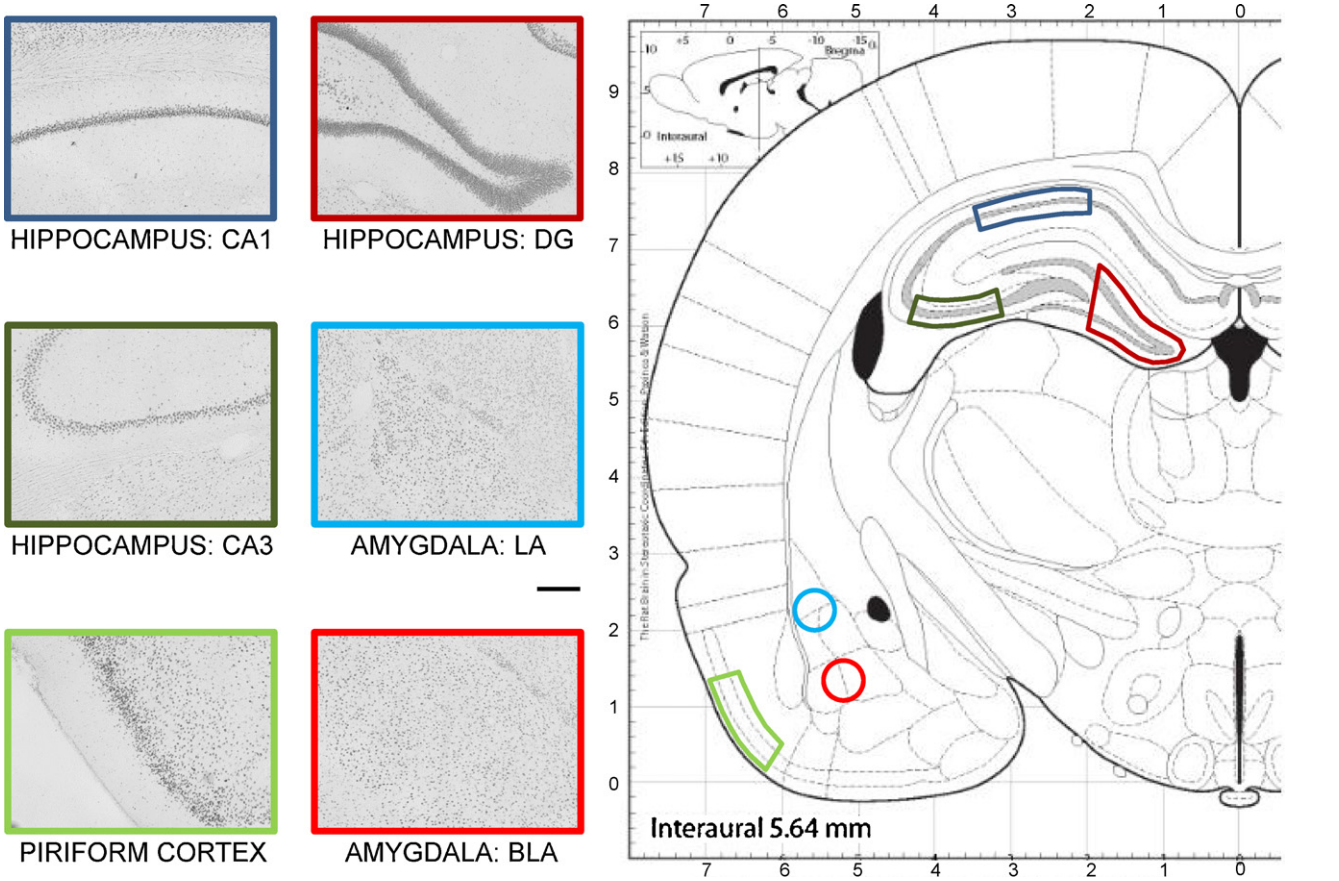

Fig. 1. Illustration of the areas targeted in this study, which were the dentate gyrus (DG), CA3, and CA1 of the hippocampus, the lateral (LA) and basolateral (BLA) nuclei of the amygdala, and the piriform cortex. Photomicrographs show representative CREB immunoreactivity in each region. Scale bar = 200 μm. Adapted with permission from Paxinos and Watson (2003).