Neurobiology of Learning and Memory 119 (2015) 34–41

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Memory-enhancing intra-basolateral amygdala clenbuterol infusion reduces post-burst afterhyperpolarizations in hippocampal CA1 pyramidal neurons following inhibitory avoidance learning

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ARTICLE INFO

Article history:
Received 14 August 2014
Revised 12 November 2014
Accepted 19 December 2014
Available online 19 January 2015

Keywords:
Emotional memory
Afterhyperpolarizations
Medium AHPs
Slow AHPs
Amygdalo-hippocampal circuitry
Beta agonist

ABSTRACT

Activation of the basolateral amygdala can modulate the strength of fear memories, including those in single-trial inhibitory avoidance (IA) tasks. Memory retention, measured by the latency to re-enter a dark-compartment paired 24 h earlier with a footshock, varies with intensity of this aversive stimulus. When higher intensity footshocks were used, hippocampal CA1 pyramidal neurons exhibited reduced afterhyperpolarizations (AHPs) 24 h post-trial, an effect blocked by immediate post-trial inactivation of the basolateral complex of the amygdala (BLA). Similar AHP reductions in CA1 have been observed in a number of learning tasks, with time courses appropriate to support memory consolidation. When less intense footshocks were used for IA training of Sprague-Dawley rats, immediate post-trial infusion of the β-adrenergic agonist clenbuterol into BLA was required to enhance hippocampal Arc protein expression 45 min later and to enhance memory retention tested 48 h later. Here, using Long-Evans rats and low-intensity footshocks, we confirmed that bilateral immediate post-trial infusion of 15 ng/0.5 μl of the β-adrenergic agonist clenbuterol into BLA significantly enhances memory for an IA task. Next, clenbuterol was infused into one BLA immediately post-training, with vehicle infused into the contralateral BLA, then hippocampal CA1 neuron AHPs were assessed 24 h later. Only CA1 neurons from hemispheres ipsilateral to post-trial clenbuterol infusion showed learning-dependent AHP reductions. Excitability of CA1 neurons from the same trained rats, but from the vehicle-infused hemispheres, was identical to that from untrained rats receiving unilateral clenbuterol or vehicle infusions. Peak AHPs, medium and slow AHPs, and accommodation were reduced only with the combination of IA training and unilateral BLA β-receptor activation. Similar to previous observations of BLA adrenergic memory-related enhancement of Arc protein expression in hippocampus, increased CA1 neuronal excitability in the fear-modulated IA task was activated by immediate post-trial β-receptor activation of the ipsilateral BLA.

1. Introduction

Emotional arousal can enhance memory formation, with stressful emotional stimuli creating strong, lasting memories (McIntyre, Power, Roozendaal, & McGaugh, 2003). Stress from fear activates the basolateral complex of the amygdala (BLA), which in turn modulates consolidation of memory via the hippocampus and other synaptically-connected brain regions (McIntyre et al., 2003). Bilateral infusions of D-amphetamine into the amygdala immediately following training on a hippocampal-dependent task enhance learning compared to vehicle infusions (Packard, Cahill, & McGaugh, 1994). The basal amygdalar adrenergic system has been found to be important in the process of modulating memory (Ferry & McGaugh, 1999; McIntyre, Hatfield, & McGaugh, 2002; McIntyre et al., 2005). More specifically, β-adrenergic modulation of the BLA during memory consolidation can enhance or impair the formation of strong emotional memories (McIntyre et al., 2005). Norepinephrine (NE) release in the BLA immediately post-training on an emotionally arousing single-trial inhibitory avoidance (IA) task is positively correlated with subsequent retention performance (McIntyre et al., 2002). Additionally, post-trial infusion of the β-adrenergic agonist clenbuterol into the BLA dose-dependently enhanced memory (i.e. increased the latency of rats to avoid entry into the dark-compartment of an IA chamber paired in a single trial with a low-intensity footshock) (Ferry & McGaugh, 1999).

Arc protein, an immediate early gene product signaling a short-term increase in neuronal activity, is transiently expressed in the hippocampus following acquisition of a number of tasks

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http://dx.doi.org/10.1016/j.nlm.2014.12.004
1074-7427/

(McIntyre et al., 2005). After a single IA training trial using a low-intensity aversive footshock, Arc expression was enhanced in CA1 of hippocampus 45 min after immediate post-trial clenbuterol infusions into the BLA; using a higher-intensity footshock, immediate post-trial lidocaine infusions into the BLA reduced CA1 Arc expression 45 min post-trial (McIntyre et al., 2005). Similarly, 24 h after acquisition of an IA task, in which a high-intensity footshock was paired with the dark compartment, hippocampal CA1 post-burst afterhyperpolarizations (AHPs) were significantly reduced, i.e. intrinsic excitability was enhanced (Farmer & Thompson, 2012), while immediate post-trial BLA lidocaine infusions blocked this learning-dependent plasticity in CA1 AHPs. Learning-dependent reductions in CA1 neuron AHPs are a reliable and highly replicable cellular mechanism expressed in hippocampus, with a time-course appropriate to support memory consolidation (Disterhoft & Oh, 2006; Farmer & Thompson, 2012; McKay, Mathews, Oliveira, & Disterhoft, 2009; Moyer, Thompson, & Disterhoft, 1996; Oh, Kuo, Wu, Sametsky, & Disterhoft, 2003; Oh, Oliveira, & Disterhoft, 2010; Thompson, Moyer, & Disterhoft, 1996).

After learning new tasks, CA1 pyramidal neurons exhibit transient reductions in AHPs (Farmer & Thompson, 2012; Moyer et al., 1996; Oh et al., 2010). Consistent with a hypothesis that AHP reductions serve as a cellular learning mechanism in hippocampal pyramidal neurons, drugs that reduce CA1 AHP amplitudes/durations improve acquisition in several different memory tasks (Disterhoft & Oh, 2006; Donzis & Thompson, 2014; Moyer, Thompson, Black, & Disterhoft, 1992). Normal aging, which is accompanied by enhanced AHPs (reduced excitability) in CA1 neurons, impairs learning (Disterhoft & Oh, 2006; Moyer et al., 1992; Oh et al., 2010). After acquisition of new spatial (Disterhoft & Oh, 2006; Oh et al., 2003) or trace eyeblink (Moyer et al., 1996; Thompson et al., 1996) learning tasks, reductions in the amplitude and area or duration of AHPs are seen in CA1 pyramidal neurons prepared *in vitro* up to 72 h post-learning. AHP reductions are observed 1–24 h after IA learning in CA1 and CA3 pyramidal neurons (Farmer & Thompson, 2012). AHPs can be further segregated into fast (generated by BK channels), medium (generated by apamin-sensitive SK channels), and slow (generated by apamin-insensitive, currently unknown channels) AHPs (Farmer & Thompson, 2012; Oh et al., 2010; Sah & Faber, 2002). Time-course analyses of AHPs show fast (peak), medium (mAHP) and slow (sAHP) AHPs are all transiently reduced after learning an IA task with a high-intensity footshock used as an aversive stimulus (Farmer & Thompson, 2012). Learning-dependent reduction of AHPs can also reduce accommodation to a sustained stimulus, an effect observed in several tasks, including trace eyeblink conditioning, fear conditioning, and single-trial IA training (Disterhoft & Oh, 2006; Farmer & Thompson, 2012; McKay et al., 2009; Moyer et al., 1996; Thompson et al., 1996).

In the current study, after rats underwent a single IA training trial, they were immediately infused (bilaterally) with clenbuterol into the BLA, and dose-dependent effects on memory retention (latency to enter the compartment of the apparatus paired with an aversive footshock) were assessed 24 h later. In a second experiment, 24 h after post-trial BLA clenbuterol infusion, intrinsic excitability (measures of AHPs and accommodation) were assessed in hippocampal CA1 neurons *in vitro*. Intrinsic excitability of CA1 neurons from IA-trained BLA clenbuterol-infused hemispheres was compared to that of neurons from contralateral control (vehicle-infused) hemispheres, as well as to neurons from untrained litter-mate control rats which also received respective unilateral BLA clenbuterol and BLA vehicle infusions.

2. Materials and methods

2.1. Subjects

Experiments were performed using male Long-Evans rats (2–3 mo). Rats were locally bred, with litter-mates maintained in our animal facility under conditions approved by the UT Dallas Institutional Animal Care and Use Committee on a 12/12 h light/dark schedule prior to testing. Rats were handled daily for 5 min for 5 d prior to experimental use. All behavioral testing took place in low ambient light conditions. Rats were trained on an IA task (Experiments 1 and 2), or served as litter-mate untrained controls prior to subsequent brain slice recordings (Experiment 2). The experimenter was blind to experimental conditions for all dose-response testing and data analysis (Experiment 1). While data in Experiment 2 was not collected blindly (the same researcher infused drugs, prepared slices, and recorded from them), data analysis was carried out in a blind fashion. Only one emotionally-arousing experience and one class of assay (behavioral or neurophysiological) was carried out per animal.

2.2. Cannula implantation

Rats were anesthetized with isoflurane, and stereotaxically implanted bilaterally with cannula (15 mm, 23 ga.) into the BLA (−2.7 mm AP, ±5.1 mm ML, −7.4 mm DV) 5 d prior to training and subsequent *in vitro* recordings. 1 mg/kg, i.m., of antibiotic (enrofloxacin) was given post-surgery. Fig. 1 illustrates the timeline of the methods used in the two following experiments.

2.3. Histology

Following behavioral or neurophysiological experiments, cannula placement was verified (see Fig. 2). In experiment 1, rats were anesthetized with isoflurane, decapitated and brains were rapidly

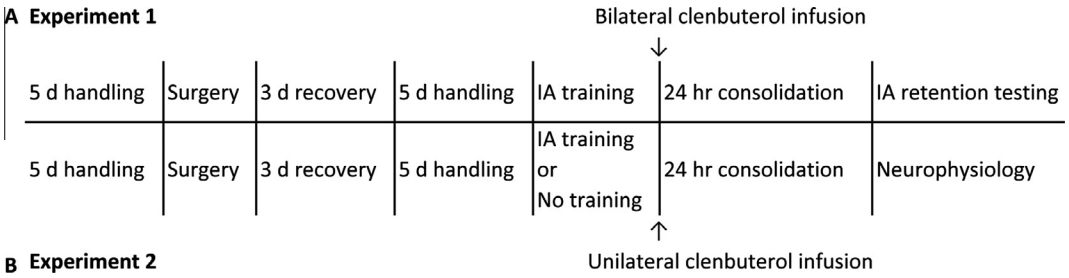

Fig. 1. Timelines for behavioral and neurophysiological assessment of BLA clenbuterol infusions. (A) The timeline used to determine a bilateral memory-enhancing dose of clenbuterol delivered immediately after a single-trial inhibitory avoidance (IA) task. Cannula implanted rats were trained on the IA task with a low-intensity aversive footshock. Immediately post-trial, they were infused bilaterally into the BLA with vehicle or doses of clenbuterol. Memory retention was tested 24 h later. (B) The timeline used to assess the effects of BLA infusion of clenbuterol (into one hemisphere) or vehicle (contralateral hemisphere) on CA1 neuron intrinsic excitability in IA trained and untrained control rats.