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# Noradrenergic actions in the basolateral complex of the amygdala modulate Arc expression in hippocampal synapses and consolidation of aversive and non-aversive memory

Jayme R. McReynolds [a], Kelly M. Anderson [b], Kyle M. Donowho [c], Christa K. McIntyre [c,*]

[a] Department of Biomedical Sciences, Marquette University, Milwaukee, WI 53201-1881, United States
[b] Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390-9004, United States
[c] School of Behavioral and Brain Sciences, University of Texas at Dallas, Richardson, TX 75080-3021, United States

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ABSTRACT

The basolateral complex of the amygdala (BLA) plays a role in the modulation of emotional memory consolidation through its interactions with other brain regions. In rats, memory enhancing infusions of the β-adrenergic receptor agonist clenbuterol into the BLA immediately after training enhances expression of the protein product of the immediate early gene Arc in the dorsal hippocampus and memory-impairing intra-BLA treatments reduce hippocampal Arc expression. We have proposed that the BLA may modulate memory consolidation through an influence on the local translation of synaptic plasticity proteins, like Arc, in recently active synapses in efferent brain regions. To date, all work related to this hypothesis is based on aversive memory tasks such as inhibitory avoidance (IA). To determine whether BLA modulation of hippocampal Arc protein expression is specific to plasticity associated with inhibitory avoidance memory, or a common mechanism for multiple types of memory, we tested the effect of intra-BLA infusions of clenbuterol on memory and hippocampal synaptic Arc expression following IA or object recognition training. Results indicate that intra-BLA infusions of clenbuterol enhance memory for both tasks; however, Arc expression in hippocampal synaptoneurosomes was significantly elevated only in rats trained on the aversive IA task. These findings suggest that regulation of Arc expression in hippocampal synapses may depend on co-activation of arousal systems. To test this hypothesis, a "high arousal" version of the OR task was used where rats were not habituated to the testing conditions. Posttraining intra-BLA infusions of clenbuterol enhanced consolidation of the high-arousing version of the task and significantly increased Arc protein levels in dorsal hippocampus synaptic fractions. These findings suggest that the BLA modulates multiple forms of memory and affects the synaptic plasticity-associated protein Arc in synapses of the dorsal hippocampus when emotional arousal is elevated.



## 1. Introduction

It is frequently observed that emotionally arousing events are better remembered than non-emotionally arousing events (Cahill et al., 1996; Hamann, Ely, Grafton, & Kilts, 1999; McGaugh, 2000). Extensive evidence indicates that the basolateral complex of the amygdala (BLA) modulates memory through an influence on efferent brain regions such as the dorsal hippocampus (McGaugh, 2004; McIntyre et al., 2005; Packard, Cahill, & McGaugh, 1994; Roozendaal, Nguyen, Power, & McGaugh, 1999). One way that the BLA likely influences efferent brain regions is by modulating plasticity or expression of plasticity-related proteins in candidate brain regions (Huff et al., 2006; Ikegaya, Nakanishi, Saito, & Abe, 1997; McIntyre et al., 2005). The protein product of the immediate early gene, activity-regulated cytoskeletal-associated protein (Arc/*Arg* 3.1), commonly used as a marker of neuronal plasticity, is of particular interest because it has been shown to undergo local translation (Waung, Pfeiffer, Nosyreva, Ronesi, & Huber, 2008; Yin, Edelman, & Vanderklish, 2002) and its mRNA is localized to discrete regions in hippocampal dendrites that have received direct synaptic stimulation (Steward, Wallace, Lyford, & Worley, 1998). Arc protein expression signifies more than just synaptic activity; blockade of Arc protein expression in the dorsal hippocampus impairs maintenance, but not induction, of hippocampal late-phase long-term potentiation and long-term, but not short-term hippocampus-dependent memory, indicating

* Corresponding author. Address: 2601 North Floyd Rd, GR 41 Richardson, TX 75080, United States. Fax: +1 (972) 883 2491.
*E-mail address:* christa.mcintyre@utdallas.edu (C.K. McIntyre).

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that Arc expression plays a functional role in long-term plasticity and memory (Guzowski et al., 2000; McIntyre et al., 2005; Messaoudi et al., 2007). Noradrenergic activation of the BLA increases Arc protein expression in the dorsal hippocampus following training on the inhibitory avoidance task in a post-transcriptional manner (McIntyre et al., 2005) and noradrenergic manipulation of the BLA can influence corticosterone-induced Arc protein expression in dorsal hippocampal synaptic-enriched tissue following training on the inhibitory avoidance task (McReynolds et al., 2010) suggesting a role for BLA modulation of synaptic Arc protein expression. Taken together with evidence that Arc and other plasticity-related mRNAs can be translated in isolated synaptoneurosomes (Dong et al., 2003; Dziembowska et al.; Richter & Lorenz, 2002; Shin, Kundel, & Wells, 2004; Yin et al., 2002) and Arc is found specifically in stimulated regions of dendrites (Farris, Lewandowski, Cox, & Steward, 2014; Huang, Chotiner, & Steward, 2007; Steward & Worley, 2001; Steward et al., 1998), we have proposed the hypothesis that actions in the BLA may modulate the local translation of plasticity-related mRNAs in downstream synapses that are engaged by the training experience (McIntyre et al., 2005; McReynolds & McIntyre, 2012). This hypothesis is supported by evidence that memory enhancing or impairing drug infusions into the BLA influence levels of Arc and another locally translated protein, calcium-calmodulin-dependent kinase IIα (CaMKIIα), but not the somatically localized immediate early gene c-Fos, in the rostral anterior cingulate cortex (Holloway-Erickson, McReynolds, & McIntyre, 2012). However, it is unclear whether BLA modulation of synaptic mRNAs could be considered a general rule of memory consolidation. Here, we examine whether the described effects are isolated observations associated with inhibitory avoidance memory.

The BLA is involved in the memory modulation of aversive tasks such as inhibitory avoidance (Da Cunha, Roozendaal, Vazdarjanova, & McGaugh, 1999; Ferry, Roozendaal, & McGaugh, 1999; LaLumiere, Buen, & McGaugh, 2003; Roozendaal et al., 1999), conditioned taste aversion (Miranda, Quirarte, Rodriguez-Garcia, McGaugh, & Roozendaal, 2008), auditory fear conditioning (Roozendaal et al., 2006), and spatial and cued water maze (Packard et al., 1994). The BLA also plays a role in memory for appetitive behaviors, such as conditioned place preference (McIntyre, Ragozzino, & Gold, 1998) and conditioned cue preference (Ferbinteanu & McDonald, 2001). Although less is known about the role of the BLA in the formation of long-term memory for tasks with little to no emotional arousal, Roozendaal and colleagues demonstrated that intra-BLA infusions of norepinephrine enhanced memory for a relatively non-arousing object recognition task (Roozendaal, Castello, Vedana, Barsegyan, & McGaugh, 2008). This task does not require any external motivation but exploits a rat's innate exploratory behavior. To avoid novelty-related anxiety, rats can be extensively habituated to the experimental apparatus prior to training. In fact, posttraining corticosterone injections only enhance long-term memory formation for this task when rats have no prior habituation (Okuda, Roozendaal, & McGaugh, 2004) and this effect is dependent upon arousal-induced norepinephrine (Roozendaal, Okuda, Van der Zee, & McGaugh, 2006). Though there is some discrepancy in the literature about the extent of the involvement of the hippocampus in the object recognition task, there is very strong evidence that the hippocampal system is engaged during training and testing and is critical for long-term memory for this task (Broadbent, Squire, & Clark, 2004; Clarke et al., 2008; Cohen et al., 2013; da Silveira, Furini, Benetti, Monteiro Sda, & Izquierdo, 2013; Hammond, Tull, & Stackman, 2004; Jobim et al., 2012). To examine whether the effect of noradrenergic actions in the BLA on hippocampus-dependent memory and Arc protein expression is generalized across multiple classes of memory, and whether these effects are dependent upon training-induced emotional arousal, we examined the effect of immediate posttraining intra-BLA administration of the β-adrenoceptor agonist, clenbuterol, on memory for the aversive inhibitory avoidance task and the relatively non-arousing novel object recognition task, and quantified synaptic Arc protein levels in the dorsal hippocampus following training.

## 2. Materials and methods

### 2.1. Subjects

Eighty seven male Sprague–Dawley rats (250–275 g at the time of arrival), obtained from Charles River Breeding Laboratories (Wilmington, MA), were housed individually in a temperature-controlled (22 °C) colony room, with food and water available *ad libitum*. Rats were maintained on a 12 h light–12 h dark cycle (7:00–19:00 h, lights on) and kept in the animal colony room for one week before surgeries or behavioral procedures. All experimental procedures were in compliance with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee (University of Texas at Dallas).

### 2.2. Surgery

Rats were anesthetized with isoflurane (1% in $O_2$) (Western Medical Supply) and the skull was positioned into a stereotaxic frame (Stoelting, Wood Dale, Il). For animals used for the intra-BLA cannula experiment, two 15-mm-long stainless steel guide cannulas (23 gauge; Small Parts, Miramar, Fl) were implanted bilaterally 2 mm above the BLA [coordinates: anteroposterior (AP), −2.7 mm from Bregma; mediolateral (ML), ±5.2 mm from midline; dorsoventral (DV), −6.4 mm below skull surface; incisor bar, −3.3 mm from interaural line (Paxinos & Watson, 2005)]. The guide cannulas were fixed in place with acrylic dental cement and two small anchoring screws. Stylets (15-mm long insect dissection pins) were inserted into each cannula to maintain patency. After surgery, rats were given 2.0 mL of saline to prevent dehydration. Rats were allowed to recover a minimum of 7 days before the commencement of behavioral training and testing.

### 2.3. Inhibitory avoidance

Following recovery from surgery, rats were handled for 2 min per day for five consecutive days before training in order to habituate rats to the experimental procedures. Rats were then trained on an inhibitory avoidance task. The inhibitory avoidance apparatus consisted of a trough-shaped alley (91 cm long, 15 cm deep, 20 cm wide at the top and 6.4 cm wide at the floor) that was divided into two compartments, separated by a manually controlled sliding door that opened by retracting into the floor. The starting "light" compartment (31 cm long) was white and illuminated, whereas the shock "dark" compartment (60 cm long) was made of two dark electrifiable metal plates and was not illuminated. The rats were placed in the light compartment and allowed to cross to the dark shock compartment. After a rat stepped completely into the dark compartment, the sliding door was closed and a single inescapable footshock (0.38 mA, 1 s) was delivered. The rat was removed from the dark compartment 15 s later and, after drug treatment, returned to the home cage. Some rats received a retention test 48 h after training. During the retention test, rats were returned to the light compartment of the inhibitory avoidance apparatus and the latency to reenter the dark compartment with all four paws (maximum latency 600 s) was measured. Memory of the training experience was inferred from longer crossing latencies on the retention test. No shock or drug was delivered dur-



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