Pharmacology, Biochemistry and Behavior 118 (2014) 46–50

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Opioid antagonism impairs acquisition of forebrain-dependent trace-associative learning: An eyeblink conditioning analysis

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ARTICLE INFO

Article history:
Received 23 July 2013
Received in revised form 30 December 2013
Accepted 9 January 2014
Available online 16 January 2014

Keywords:
PMBSF
Naloxone
Whisker–trace–eyeblink
WTEB
Hippocampus
μ-opioid

ABSTRACT

While the opioid system is predominantly known for its properties governing nociception, it has also been found to play a role in learning and memory. Opioid involvement in task acquisition and retention has been examined using various associative paradigms. These analyses have demonstrated that depending upon the associative paradigm and timing of opioid modulation relative to the task, it can either impair acquisition or facilitate memory consolidation. However, opioid involvement in forebrain-dependent trace-associative learning paradigms has never been examined. In associative paradigms, a subject learns to associate two stimuli, while in trace paradigms the two stimuli are separated in time, which is thought to increase task difficulty due to utilization of forebrain structures. The current analysis utilized the trace paradigm whisker–trace–eyeblink (WTEB) conditioning with a trace interval of 250 ms, in conjunction with pre- and post-training opioid inhibition with naloxone, a well-characterized nonspecific opioid antagonist. Naloxone administration prior to training (pre-training) was found to significantly impair acquisition of the WTEB association; however, administration following training (post-training) did not significantly differ from saline controls. These findings demonstrate that opioid inhibition impairs acquisition of forebrain-dependent trace-associations, further suggesting that opioid activation plays a modulatory role in trace-acquisition. Prior behavioral analyses have suggested that hippocampal μ-opioid receptors are most likely facilitating this effect; however, subsequent analyses will be needed to determine the specific brain region(s) and opioid receptor subtype(s) mediating this effect.

1. Introduction

Associative learning paradigms are some of the oldest and most extensively used paradigms for examination of behavioral and biochemical mechanisms underlying learning and memory. In associative learning, a neutral conditioned stimulus (CS) is paired with a salient unconditioned stimulus (US) eliciting an unconditioned response (UR). After repeated CS–US pairings, the CS predicts the onset of the US, thus eliciting a learned conditioned response (CR). In delay conditioning, the CS is presented and co-terminates with the US. This form of conditioning is forebrain independent, in that removal of the hippocampus or neocortex does not impair acquisition (Mauk and Thompson, 1987; Norman et al., 1977; Oakley and Russell, 1977; Theios and Brelsford, 1966). Rather, delay conditioning is dependent upon brainstem and cerebellar processing (Clark et al., 1984; Mauk and Thompson, 1987). In trace-conditioning, the CS and US are temporally separated by a stimulus-free trace interval, which recruits higher brain regions. For example, pre-training hippocampal or neocortical lesions impair acquisition of trace–eyeblink associations (Galvez et al., 2007; Kim et al., 1995; Moyer et al., 1990; Solomon et al., 1986; Takehara et al., 2002; Weiss et al., 1999). Furthermore, anatomical and biochemical analyses have demonstrated various forms of plasticity in both the hippocampus and neocortex during and following trace–eyeblink conditioning (Chau et al., 2013; Galvez et al., 2006; Gierdalski et al., 2001; Gruart et al., 2012; Moyer et al., 2000; Power et al., 1997; Thompson et al., 1996a,b). These and other analyses have provided much insight into the underlying mechanism for acquisition of trace-associations.

Behavioral analyses from other paradigms have further suggested that the opioid system is intimately involved in learning and memory. Pharmacologically inhibiting or genetically removing opioid receptors have been shown to impair acquisition on various behavioral paradigms, such as Morris Water Maze and 8-arm radial arm maze (Jamot et al., 2003; Jang et al., 2003; Sanders et al., 2005). Furthermore, studies using paradigms which are more typically viewed as delay-associative, such as shuttle-avoidance, extinction, and cued fear conditioning, have also demonstrated that opioid inhibition both before and after training impairs acquisition (Izquierdo, 1980; Kim and Richardson, 2009; McNally et al., 2004; Meilandt et al., 2004; Messing et al., 1989). Interestingly, μ-opioid activation has been found to significantly retard acquisition of delay-eyeblink conditioning in rabbits (Aloyo et al., 1993). However, opioid inhibition prior to delay-eyeblink training in

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0091-3057/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pbb.2014.01.005

rabbits showed no effect on acquisition but rather significantly facilitated extinction (Hernandez and Powell, 1983). These and other analyses have strongly suggested a role for the opioid system in acquisition of various learning tasks; however, opioid involvement in acquisition of neocortical dependent trace-associative paradigms has not been examined.

One trace-associative paradigm used in laboratory analyses of learning and memory is trace–eyeblink conditioning. In trace–eyeblink conditioning, a subject learns to associate a CS (e.g. sensory stimulation such as light, tone, or in rodents, whisker deflection) with a US that causes the subject to blink. Eyeblink conditioning is one of the few behavioral paradigms that are routinely used in various species, including humans, greatly facilitating the translational ability of these and subsequent findings across species. The following study used the opioid antagonist, naloxone, in conjunction with the well-established 250 ms trace-associative paradigm, whisker–trace–eyeblink (WTEB), to determine if the opioid system plays a modulatory role in acquisition of forebrain-dependent trace-associative learning.

2. Materials/methods

2.1. Animals

Thirty-six 3 to 6 month-old male C57BL/6J mice were individually housed on a 12-h light–dark schedule with lights on at 0700. Mice were provided access to food and water ad-libitum. All procedures performed were reviewed and approved by the University of Illinois Animal Care and Use Committee.

2.2. Surgery

The surgical procedure was performed as previously described (Galvez et al., 2009). Briefly, mice were anesthetized with a ketamine (1 mg/kg IP) xylazine (6 mg/kg IP) cocktail. Once anesthetized, a plastic strip connector with two Teflon-coated stainless steel wires and one uncoated ground wire was affixed to the head (headgear). The two coated wires were fed through the skin and left exposed at the periorbital region of the right eye. The ground wire was secured to a ground screw in the skull. Dental acrylic cement secured the headgear to the skull. All mice were given a minimum of five days to recover from surgery prior to WTEB training.

2.3. WTEB procedure

Mice were placed into standard (12″x12″) laboratory cages in a sound-attenuated chamber. All procedures took place between 0900 and 1400. Mice were connected via their headgear to a tether that allowed free mobility while within the training cages. One day prior to testing, mice were habituated to the training cage and tether for 10 min. After habituation, mice were randomly assigned to receive either pre-training naloxone ($n = 10$), post-training naloxone ($n = 10$), pre-training saline ($n = 9$), or post-training saline ($n = 7$). At the time of training, the tether was connected to a computer running a custom LabView program that delivered stimuli (whisker stimulation and periorbital shock), and acquired data (blink response and properties).

On each training day, mice were conditioned as previously described (Galvez et al., 2009). Briefly, mice were presented with a CS consisting of 250 ms whisker stimulation delivered via a custom whisker stimulator (Galvez et al., 2009), paired with a 100 ms periorbital shock US (0.1 to 0.5 mA periorbital square wave shock, 60 Hz, 0.5 ms pulses, Fig. 1a). The shock intensity was tailored for each animal to generate a detectable blink response (Fig. 1b). Mice were given 30 trials per day with a 15 to 30 s (mean of 20 s) inter-trial interval. An optic sensor that was attached to the tether was used to record closure of stimulated eyelid. A CR was defined as a 4-standard-deviation change in voltage from baseline occurring 20 ms prior to US onset (Fig. 1a). Mice were trained with 30 trials per day for 8 days or until criterion, defined as 4 CRs out of 5 consecutive trials. The mean number of days for the combined saline groups to reach criterion was 3.70 days, and all saline mice reached criterion by 6 days of training. A subset of the naloxone mice did not reach criterion while on the drug after 8 days of training. These mice were assigned a criterion day of eight; however, to ensure that they were capable of learning the association, drug administration was discontinued while continuing training. All of these mice reached criterion within 3 days of subsequent training while not receiving naloxone.

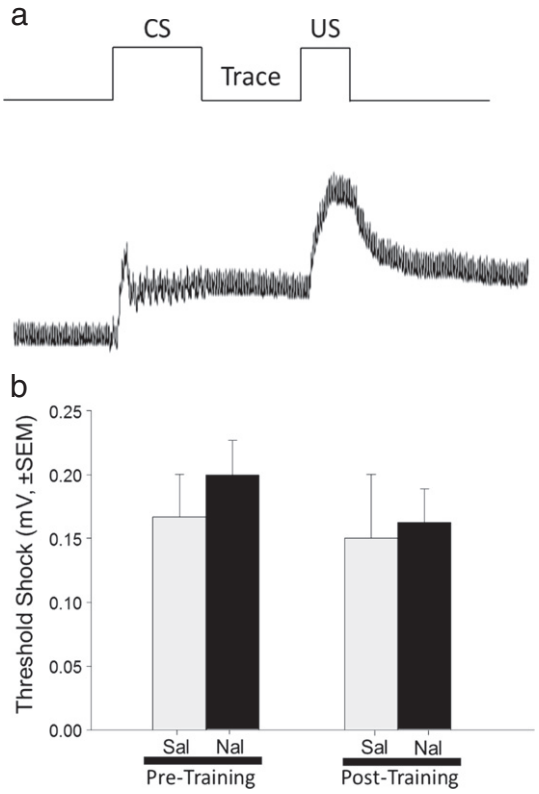

Fig. 1. Schematic of conditioning paradigms and shock intensity levels. a) Top: Schematic illustrating the conditioned stimulus (CS; 250 ms), trace interval (250 ms), and unconditioned stimulus (US; 100 ms) administration. Bottom: A typical blink response exhibiting a conditioned response (CR) at the onset of the CS, and an unconditioned response (UR) at the onset of the US, during training. b) Mean shock intensity required to cause the mouse to blink. There was no significant difference across groups suggesting that opioid antagonism did not significantly alter blink induction. SEM = standard error of the mean; Sal = Saline; Nal = Naloxone.

2.4. Drugs/dosing

Mice were randomly assigned to either pre-training naloxone, post-training naloxone, pre-training saline or post-training saline conditions. On the day of training, mice were injected with naloxone (5 mg/kg IP; Sigma–Aldrich, St. Louis, MO) or saline either 8 min prior to (pre-training) or immediately following (post-training) WTEB conditioning. Prior associative learning paradigms have demonstrated that 5 mg/kg of naloxone IP administered approximately 8 min prior to training impairs acquisition of fear conditioning extinction in young mice (Kim and Richardson, 2009). Additionally, lower doses given 5–10 min prior to training have been shown to exert similar effects in rats (Izquierdo, 1980; Messing et al., 1989). The half-life of naloxone in a mouse brain is approximately 30 min (Kishioka et al., 2013), suggesting that even with an 8 min pre-training injection, the mice will be under the influence of the drug during the entire training session. Training takes approximately 20 min. All mice received one injection per day on each day of training.