Behavioural Brain Research 323 (2017) 1–10

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Antagonizing the different stages of kappa opioid receptor activation selectively and independently attenuates acquisition and consolidation of associative memories

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HIGHLIGHTS

- Blocking early activation of KOR inhibits acquisition but not consolidation.
- Blocking late activation of KOR inhibits consolidation but not acquisition.
- KOR protein is downregulated upon learning in somatosensory cortex.

ARTICLE INFO

Article history:
Received 16 August 2016
Received in revised form 11 January 2017
Accepted 18 January 2017
Available online 21 January 2017

Keywords:
PMBSF
Primary somatosensory cortex
Learning-induced plasticity
Trace eyeblink conditioning
Whisker

ABSTRACT

Previous work from our laboratory has shown that nonspecific kappa opioid receptor (KOR) antagonism in primary somatosensory cortex (S1) can inhibit acquisition for the forebrain-dependent associative task, Whisker-Trace Eyeblink conditioning (WTEB). Although studies have demonstrated that KOR activation can alter stimuli salience, our studies controlled for these factors, demonstrating that KOR also plays a role in facilitating learning. KOR has two distinct phases of activation followed by internalization/downregulation, that each independently activate kinases and transcription factors known to mediate task acquisition and memory consolidation respectively. The current study demonstrated that antagonism of the initial phase of KOR activation in S1 via local injections of the g-protein inhibitor, pertussis toxin (PTX), blocked initial WTEB acquisition without affecting retention of the association. In contrast, KOR late phase antagonism in S1 via local injections of the GRK3-specific antagonist, guanidinonaltrindole (GNTI), blocked retention of the WTEB association without affecting task acquisition. Consistent with the known mechanism for KOR activation, KOR protein expression in S1 was found to be decreased following WTEB training, further supporting the involvement of neocortical KOR activation with learning. Prior studies have shown that task acquisition and memory consolidation are mediated by distinct molecular processes; however, little is known regarding a potential mechanism driving these processes. The current study suggests that neocortical KOR activation mediates activation of these processes with learning. This study provides the first evidence for a time- and learning-dependent property of neocortical KOR in facilitating acquisition and consolidation of associative memories, while elucidating an unexplored neocortical learning mechanism.

1. Introduction

The opioid system has been extensively examined for its role in pain modulation; however, it has also been shown to play a prominent role in learning and memory. Nonspecific opioid receptor inhibition has been shown by multiple laboratories to impair learning for various behavioral tasks including shuttle avoidance, autoshaping, fear conditioning, Morris water maze, extinction paradigms and eyeblink conditioning [1–5]. More precise studies exploring the specific opioid receptor mediating many of these

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http://dx.doi.org/10.1016/j.bbr.2017.01.032
0166-4328/

learning effects have predominately focused on the mu-opioid receptor (MOR). These studies have extensively explored the role of MOR in acquisition of various learning paradigms such as Morris water maze [4,6,7], radial arm maze [6], and fear conditioning [8]. Likewise, our laboratory has demonstrated that blocking MOR in systemically impairs acquisition for the forebrain dependent associative paradigm Whisker-Trace Eyeblink conditioning (WTEB) [9].

In addition to a role for MOR in learning and memory, studies have recently demonstrated that the kappa-opioid receptor (KOR) also plays a critical role in acquisition of many behavioral tasks. For example, KOR stimulation via dynorphin prevents ischemic- [10] or scopolamine-induced [11] deficits with spontaneous alternation. Likewise, prefrontal injections of KOR agonists impair alcohol reinstatement [12], and withdrawal-induced conditioned place aversion [13]. Furthermore, KOR knockout mice exhibit fewer errors on the radial arm maze, and decreased escape latency across several days of training in the Morris water maze [6]. KOR antagonists have also been shown to block acquisition of contextual fear conditioning in rats [14] and delay eyeblink conditioning in rabbits [15,16]. These studies have suggested that KOR modulation can directly alter acquisition for various learning paradigms. In support of this hypothesis, our laboratory has also demonstrated that neocortical KOR modulation can alter acquisition for the forebrain dependent associative paradigm WTEB [17].

Trace eyeblink conditioning is a well characterized and robust form of associative conditioning in which a neutral conditioning stimulus (CS), is paired with an unconditioned stimulus (US) that elicits an unconditioned response (UR) with a stimulus-free trace interval temporally separating the two. This paradigm is both dependent upon and stimulates learning induced changes in forebrain structures such as S1 and hippocampus [18–23]. Using whisker stimulation as the CS our laboratory has shown that learning results in S1 dendritic spine reorganization, suggesting that S1 is a site of storage for the trace association [24]. In exploring the role of KOR in these forebrain dependent neuronal mechanisms, we have further demonstrated that local infusions of the KOR specific antagonist, NorBNI hinders acquisition for the WTEB association [17]. These data suggest that KOR activation in S1 facilitates acquisition of the trace association.

Interestingly, KOR activation is known to exhibit a biphasic pattern with two conformational states that activate distinct molecular pathways [25]. Upon initial KOR stimulation, the receptor activates Gα and Gβγ subunits that activate the intracellular kinases PI3K, PKCζ, ERK1/2, and JNK [25]. A summary of this process is outlined in Fig. 1. Interestingly, many of these kinases have also been implicated in acquisition of various learning paradigms. For example, PI3K in the hippocampus is required for the acquisition of conditioned place preference [26] and inhibition of PKC is capable of disrupting early memory formation in the mPFC [27]. Additionally, ERK1/2 and its substrates are activated in the cerebellum immediately following eyeblink conditioning in rabbits [28]. These studies suggest that this initial KOR activation could facilitate kinase activation necessary for task acquisition.

With continued KOR stimulation, GRK3 will phosphorylate the KOR receptor and induce arrestin-dependent activation of the transcription factors pCREB and Zif268 [25]. Similar to the kinases, these transcription factors can modulate learning; however, they have been shown to mediate consolidation rather than acquisition. For example, genetically or pharmacologically inhibiting CREB hinders consolidation of fear conditioning and water maze learning without altering acquisition or short term memories [29,30]. Furthermore, Morris water maze studies have demonstrated that zif268 exhibits increased expression in the medial prefrontal cortex during periods of memory consolidation [31]. These studies further suggest that this subsequent phase of KOR activation can drive transcription factor activation mediating memory consolidation. Following this final stage of KOR activation, it undergoes arrestin-dependent internalization where it will either be degraded or recycled back into the membrane [32].

Our initial study of neocortical KOR antagonism on forebrain dependent associative learning blocked both states of KOR activation, hindering our ability to determine the specific role for each state in acquisition and consolidation of the trace association. The current study used pharmacological inhibition for each state of KOR activation to determine their specific role in learning forebrain dependent associations.

2. Materials and methods

2.1. Animals

Seventy-six 3–6 month old male C57BL/6 mice were bred in-house and housed in same-litter groups until surgery, where they were transferred to individual housing in standard (12" × 12" × 12") laboratory cages. All mice were kept on a 12-h light-dark schedule (lights on at 0700) in a temperature controlled room (~21 °C), and provided *ad libitum* access to food and water. All procedures performed were reviewed and approved by the University of Illinois Animal Care and Use Committee and follow the National Institute of Health's animal care guidelines.

2.2. Surgery

Surgeries were performed as previously described [33]. Mice were placed under ketamine (1 mg/kg i.p.) and xylazine (6 mg/kg i.p.) anesthesia. Once anesthetized, a headgear consisting of a plastic strip connector with two Teflon-coated stainless steel wires and one uncoated ground wire were secured to the skull via dental cement. Teflon-coated wires from the headgear were fed under the skin to the periorbital region of the eye, stripped to provide contact, and fastened to the skin. A ground wire was tightly secured to a screw in the skull. For intra-S1 injections, a 26-gauge stainless steel guide cannula (4 mm in length; PlasticsOne, Roanoke, VA) was inserted into S1 contralateral to the periorbital wire stimulators (−0.8 mm AP, 3 mm ML from bregma, and −0.5 mm DV from the dorsal surface of the brain; [34]). The guide cannula was secured to the headgear that was affixed to the skull with dental cement. Upon completion of the surgery, an obdurator was screwed into the guide cannula. All mice were given a minimum of seven days to recover from surgery before onset of training.

2.3. Behavioral training

Mice were placed into standard laboratory cages (12" × 12" × 12") different from their home cage in a sound attenuated chamber. All WTEB training took place between the hours of 0900 and 1400. The headgear described in the above section was connected to a tether that was then connected to a computer running a custom LabView program. The program delivered stimuli (both whisker and shock), as well as monitored eyelid closure. The tether allowed for freedom of mobility during all training procedures. One day prior to training, mice were habituated to the tether and chamber for 10 min. On training days, mice were conditioned as previously described [33]. A presentation of the CS (250 ms whisker stimulation) was paired with a US (100 ms periorbital shock, 0.1–0.5 mA square wave shock, 60 Hz, 0.5 ms pulses). The US shock intensity was tailored to each mouse to generate a detectable blink response with minimal voltage. This also allowed for a subsequent analysis of any drug effects on the shock intensity needed to induce a blink, as discussed in the results section below. The CS and US were separated by a 250 ms stimulus-free trace interval (Fig. 2). Mice were presented with