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Research report

Neocortical prodynorphin expression is transiently increased with learning: Implications for time- and learning-dependent neocortical kappa opioid receptor activation

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ABSTRACT

There are several lines of evidence that indicate a prominent role for the opioid system in the acquisition and consolidation of learned associations. Specifically, kappa opioid receptor (KOR) modulation has been demonstrated to alter various behavioral tasks including whisker trace eyeblink conditioning (WTEB). WTEB is an associative conditioning paradigm in which a neutral conditioned stimulus (CS; Whisker stimulation) is paired following a short stimulus free trace interval with a salient unconditioned stimulus that elicits a blink response (US; Eye shock). Work from our laboratory has shown that WTEB conditioning is dependent upon and induces plasticity in primary somatosensory cortex (S1), a likely site for memory storage. Our subsequent studies have shown that WTEB acquisition or consolidation are impaired when the initial or later phase of KOR activation in S1 is respectively blocked. Interestingly, this mechanism by which KOR is activated in S1 during learning remains unexplored. Dynorphin (DYN), KOR's endogenous ligand, is synthesized from the precursor prodynorphin (PD) that is synthesized from preprodynorphin (PPD). In S1, most PPD is found in inhibitory GABAergic somatostatin interneurons (SOM), suggesting that these SOM interneurons are upstream regulators of learning induced KOR activation. Using immunofluorescence to investigate the expression of PD and SOM, the current study found that PD/SOM expression was transiently increased in S1 during learning. Interestingly, these findings have direct implications towards a time- and learning-dependent role for KOR activation in neocortical mechanisms mediating learning.

1. Introduction

Opioid peptides have a long history of involvement in pain, reward, and learning and memory. Previous reports from our laboratory and others have demonstrated that the general opioid antagonist naloxone, impairs learning of many associative tasks such as delay conditioning, operant lever-pressing behavior, fear conditioning, and trace eyeblink conditioning [1–5]. In exploring the role for specific opioid receptors in learning and memory, our laboratory has shown that antagonizing the kappa-opioid receptor (KOR) impairs acquisition for the associative paradigm whisker-trace eyeblink (WTEB) conditioning [6].

In WTEB conditioning a neutral conditioned stimulus (CS; Whisker stimulation) is paired following a stimulus free trace interval with a salient unconditioned stimulus that elicits an eye-blink (US; Eye shock). This paradigm has been shown to be dependent on, and induce plasticity in primary somatosensory cortex (S1), a likely site for memory storage [7–10]. Our subsequent studies exploring the role of KOR with learning have demonstrated that either systemic or direct S1 KOR inhibition impairs WTEB acquisition [6]. Consistent with our findings, many studies have shown that KOR modulation can alter learning on various tasks such as inhibitory avoidance, spontaneous alternation, water-maze, radial-arm-maze, and conditioned place aversion [11–15]. These studies have strongly suggested that KOR plays a prominent role in mediating various types of learning.

KOR's endogenous ligand and the most likely molecular trigger for these effects on learning is dynorphin. Dynorphin is synthesized from the precursor peptide prodynorphin (PD), which is synthesized in vesicles from the precursor preprodynorphin (PPD) [16]. Similar to KOR, Dynorphin and its upstream precursors have been implicated in various forms of learning. For instance, PD knockout mice exhibit enhanced levels of freezing in a contextual fear conditioning task [17]. Likewise, dynorphin knockout mice display reduced age-related deficits in water

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maze learning [18], while dynorphin injected directly into the hippocampus impairs water maze performance [19]. Although the specific role of dynorphin and its precursors in learning appears to vary depending upon the tasks, these studies collectively suggest that they play a prominent role in the acquisition and consolidation of learning tasks.

Upon exploring the specific cell type expressing these peptides, it has been demonstrated that the dynorphin precursor ligand PPD is primarily found in Somatostatin-containing GABAergic interneurons (SOM) within S1 [20]. These findings suggest that SOM interneurons are a likely upstream cell of KOR activation in S1 with WTEB. SOMs are a major subclass of interneurons in the central nervous system that serve a variety of roles in various species. SOM cells represent a significant proportion of all inhibitory interneurons in the neocortex [21]. Additionally, this population of cells is localized in areas important for learning and memory, such as the neocortex and hippocampus [22], two brain regions critically involved in WTEB acquisition [8,23,24]. In S1, tonically-active SOM cells serve to inhibit neuronal activity, as optogenetic silencing SOM cells causes an increase in firing of pyramidal cells in the area. SOM cells in S1 also receive input from vasoactive intestinal peptide (VIP)-containing inhibitory interneurons. Interestingly, during active whisking, VIP-containing cells cause a decrease in SOM activity [25]. This process does not seem to require active whisking by the animal as passive whisking will also instigate the same mechanism via thalamic relays [26].

Similar to that observed with KOR modulation, studies have strongly suggested a role for SOM cells in learning. Silencing SOM cells in the hippocampus impairs acquisition of contextual-based fear learning [27]. SOM cells are also disinhibited by VIP-containing inhibitory neurons during auditory discrimination [28]. This VIP disinhibition is similar to what is seen in S1 during active [25], or passive whisking [26]. Additionally, SOM cells have been demonstrated to increase in density within somatosensory cortex in a whisker associative paradigm [29]. These studies suggest that neocortical SOM cells regulate learning processes. Furthermore, these studies along with those mentioned above collectively suggest that neocortical SOM cells are regulating learning through KOR modulation. However, the specific role for neocortical SOM regulation of KOR activity with associative learning has never been explored. To explore this molecular pathway and its potential role with associative learning, the current study set out to characterize the expression profile of PD (Dynorphin's precursor) in S1 SOM interneurons during and immediately following WTEB acquisition.

2. Methods

2.1. Animals

Three to six month old male C57BL/6 mice were bred in-house and housed in same litter groups until surgery. After surgery 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.

2.2. Surgery

Surgeries were performed as previously described [30]. 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. 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 different from their home-cage in a sound- and light-attenuated chamber. All WTEB training took place between 0900 and 1400. The headgear described in the surgery section was connected to a tether that was connected to a computer running a custom LabView program. The program delivered both whisker and shock stimuli as well as monitored eyelid closure via a camera attached to the tether. Whisker stimulation was delivered via activation of a piezo-electric strip (Piezo Systems, Cambridge, MA) attached to a comb that was situated directly in front of the whisker pad. This allowed for precise control in stimuli presentation, timing and delivery. For a complete description see Ref. [30]. 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 [30]. 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 eye-blink response with minimal voltage. The CS and US were separated by a 250 ms stimulus-free trace interval (Fig. 1). Mice were presented with the CS-US pairings 30 times per session (day) with an intertrial interval of 15–25 s (mean of 20 s). To monitor eyelid closure a camera on the tether provided a live video feed of the eye that was converted to a binary image in LabView in real time. Upon closure of the eyelid, the size of the visible eye decreased indicating a blink (Fig. 1). A conditioned response (CR) was defined as a 4-standard deviation change in size of the eye binary image from baseline, occurring after CS onset and within 20 ms prior to US onset (Fig. 1). Baseline was defined as the average size of the eye binary Image 60 ms prior to CS onset for each trial. These settings are consistent with that used in other laboratories conducting eyeblink analyses [31–33]. Mice received one training session per day and were identified based on which stage in acquiring the association they achieved. The stages were operationally defined as exhibiting three CRs out of five consecutive trials for the acquisition group [ACQ; behaviorally defined as C1 (one day before behavioral criterion was achieved)], four CRs out of five consecutive trials for the criterion group [CRIT; behaviorally defined as C (the day behavioral criterion was achieved)], and for the overtrained (OT) group as exhibiting four CRs out of five consecutive trials for two consecutive days [behaviorally defined as C + 1 (one day after reaching behavioral criterion)]. Previous studies from our laboratory have demonstrated these behavioral points to be consistent with acquisition of the trace

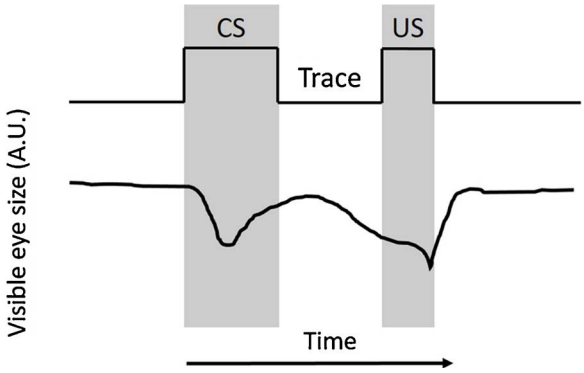

Fig. 1. Schematic of Whisker-Trace Eyeblink (WTEB) paradigm. In WTEB a conditioned stimulus (CS; whisker stimulation), is paired with an unconditioned stimulus (US; periorbital shock) separated by a stimulus free interval (Trace). Upper line shows square wave computer delivered stimuli. Bottom line shows relative visible eye size in arbitrary units (A.U.). A downward deflection of the line represents a decrease in the size of the visible eye due to closure of the eyelid, i.e. a blink. Note, the blink (closing of the eye) during the pre-US interval illustrating a conditioned response (CR).