Neurobiology of Learning and Memory 136 (2016) 228–235

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Cannabinoid modulation of memory consolidation within the cerebellum

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ARTICLE INFO

Article history:
Received 3 August 2016
Revised 1 November 2016
Accepted 2 November 2016
Available online 3 November 2016

Keywords:
Cannabinoid receptors
2-AG
Cerebellum
Eyeblink conditioning
Consolidation

ABSTRACT

Cannabinoid receptors contribute to learning and synaptic plasticity mechanisms. The cerebellum contains a high density of cannabinoid receptors and manipulations of cannabinoid receptors affect synaptic plasticity within the cerebellar cortex. *In vivo* studies have found that cannabinoid agonists impair learning of cerebellum-dependent eyeblink conditioning in rodents and humans. However, the role of cannabinoid receptors or endocannabinoids in memory consolidation within the cerebellum has not been examined. In the current study, we examined the role of cannabinoid receptors and endocannabinoids during learning and consolidation of eyeblink conditioning in rats. Administration of the cannabinoid receptor agonist WIN55,212-2 or drugs that increase/decrease endocannabinoid levels directly into the cerebellar cortex before each training session resulted in marked learning impairments. When administered 1 h after each training session, during memory consolidation, the cannabinoid inverse agonist SR141716A or the endocannabinoid suppressor THL impaired memory. In contrast, increasing endocannabinoid levels with JZL-184 or infusion of WIN55,212-2 within the cerebellar cortex facilitated memory consolidation 1 h post-training. Intracerebellar manipulations of cannabinoid receptors or endocannabinoid levels had no effect on memory consolidation when administered 3 or 6 h after each training session. The results demonstrate that cannabinoids impair cerebellar learning, but facilitate memory consolidation mechanisms within the cerebellar cortex 1–3 h after training.

1. Introduction

Cannabis sativa is the most widely used psychoactive drug in the United States with rates continuing to increase over the past decade (Substance Abuse, Results from the 2013 National Survey on Drug Use, NSDUH Series H-48, & MD: Substance Abuse, 2014). The major active ingredient in cannabis, Δ^9-tetrahydrocannabinol, binds to G-protein coupled cannabinoid receptors CB1R and CB2R (Devane, Dysarz, Johnson, Melvin, & Howlett, 1988; Gaoni & Mechoulam, 1964). CB1Rs are the most abundant G-protein coupled receptor in the mammalian brain with the highest density of receptors within the cerebellar cortex (Herkenham et al., 1990, 1991). Two endogenous cannabinoids (endocannabinoids) have been discovered, 2-Arachidonoy lglycerol (2-AG) and Anandamide. Cannabinoid receptors have many diverse roles throughout the brain including synaptic plasticity during learning and memory. CB1Rs within the cerebellar cortex have been demonstrated to be important for the induction of long-term depression (LTD) in parallel fiber synapses with Purkinje cells *in vitro* (Lévénès, Daniel, Soubrié, & Crépel, 1998; Safo & Regehr, 2005; van Beugen, Nagaraja, & Hansel, 2006). In addition, systemic or genetic CB1R manipulations, which impair CB1R function throughout the brain, result in decrements in the rate of eyeblink conditioning, a type of associative learning that depends on the cerebellar cortical plasticity (Edwards et al., 2008; Kishimoto & Kano, 2006; Skosnik et al., 2008; Steinmetz & Freeman, 2010, 2011, 2013; Steinmetz et al., 2012). The hypothesis drawn from these studies is that the decrement in acquisition of eyeblink conditioning is the result of impaired synaptic plasticity mechanisms within Purkinje cells.

Less is known about the role of cerebellar cannabinoid receptors in memory consolidation. Studies examining the role of CB1Rs and endocannabinoids during consolidation have been conducted primarily in the hippocampus, amygdala, and prefrontal cortex (Tan et al., 2011; Wise, Thorpe, & Lichtman, 2009). Each of these areas plays an important role in memory consolidation for different tasks, but contains moderate levels of CB1Rs as compared to the cerebellar cortex. To date, there have been no studies examining the role of

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http://dx.doi.org/10.1016/j.nlm.2016.11.002
1074-7427/

CB1Rs during memory consolidation within the cerebellum. As mentioned above, the cerebellar cortex contains the highest density of CB1Rs and also is an important site of synaptic plasticity underlying motor learning (Safo & Regehr, 2005). Manipulations of cannabinoid receptors or endocannabinoid levels should therefore affect memory consolidation within the cerebellum.

The current study employed cerebellum-dependent delay eyeblink conditioning to examine the role of CB1Rs and endocannabinoids during learning and memory consolidation. Eyeblink conditioning involves the presentation of a conditioned stimulus (CS) that does not elicit eyelid closure prior to learning (e.g., a tone), followed by an unconditioned stimulus (US) that elicits eyelid closure before learning (e.g., periorbital stimulation). After repeated CS-US pairings an adaptively timed eyelid closure conditioned response (CR) develops. The cerebellum, specifically the cerebellar cortex and anterior interpositus nucleus, is essential for acquisition and retention of the CR (Freeman & Steinmetz, 2011; McCormick & Thompson, 1984). In rats, the cerebellar cortical area at the base of the primary fissure is necessary for the acquisition of eyeblink conditioning (the eyeblink conditioning microzone, Steinmetz & Freeman, 2014). In the current study, rats received 5 daily sessions of eyeblink conditioning in which a CB1R agonist (WIN55,212-2), CB1R inverse agonist (SR141716A), monoacylglycerol lipase inhibitor (JZL-184; increases endocannabinoid levels), or diacylglycerol lipase inhibitor (THL; decreases endocannabinoid levels) was infused into the eyeblink conditioning microzone before (learning manipulation) or after (consolidation manipulation) each training session. Activating CB1Rs or increasing endocannabinoid levels within the eyeblink conditioning microzone of the cortex were predicted to impair memory consolidation since CB1R agonists impair learning when given before training sessions.

2. Materials and methods

2.1. Subjects

The subjects were 113 male Long-Evans rats (250–300 g). The rats were housed in the animal colony in Spence Laboratories of Psychology at the University of Iowa (Iowa City, IA). All rats were maintained on a 12 h light/dark cycle and given *ad libitum* access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Iowa.

2.2. Surgery

One week before training, rats were anesthetized with isoflurane. After the onset of anesthesia, the rats were fitted with differential electromyography (EMG) electrodes implanted into the upper left orbicularis oculi muscle. The reference electrode was a silver wire attached to a skull screw. The EMG electrode leads terminated in gold pins inserted into a plastic connector. A bipolar stimulating electrode for delivering the periorbital stimulation US was implanted subdermally, caudal to the left eye. A 23 gauge guide cannula was implanted 1 mm dorsal to the base of the primary fissure of the cerebellar cortex. A 30 gauge stylet was inserted into the guide cannula. The stereotaxic coordinates taken from bregma for the cannula was 11.4 mm posterior, 3.0 mm lateral, and 3.2 mm ventral. The plastic connector housing the EMG electrode leads, bipolar stimulating electrode, the guide cannula, and skull screws were secured to the skull with bone cement.

2.3. Microinjections

Before each infusion, the stylet was replaced with a 30 gauge infusion cannula that extended 1.0 mm beyond the guide cannula. The infusion cannula was connected to polyethylene tubing (PE 10), which was connected to a 10 μl syringe. The syringe was placed into an infusion pump and 0.5 μl of each drug was infused over 5 min at a rate of 6.0 μl/h. After the infusion, the cannula was left in place for 3 min in order to allow diffusion of the drug.

2.4. Drugs

The CB1R/CB2R agonist WIN55,212-2 ([R]-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenylmethanone), CB1R inverse agonist SR141716A [5-(4-Chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide], the monoacylglycerol lipase inhibitor JZL-184 [4-nitrophenyl-4-[bis(1,3-benzodioxol-5-yl)(hydroxy)methyl]piperidine-1-carboxylate] or the diacylglycerol lipase inhibitor THL [(*S*)-((*S*)-1-((2*S*,3*S*)-3-Hexyl-4-oxooxetan-2-yl)tridecan-2-yl) 2-formamido-4-methylpentanoate] were administered intracranially to rats. WIN55,212-2 and THL were dissolved in a vehicle of 1:1:18 solution of ethanol, cremophor, saline and SR141716A and JZL-184 were dissolved in a vehicle of 1:1:18 solution of ethanol, tween 80, saline. WIN55,212-2, JZL-184 and THL were purchased from Sigma/RBI. SR141716A was a generous gift from NIDA Drug Supply Program (Rockville, MD).

2.5. Apparatus

A detailed description of the apparatus has been published (Nicholson & Freeman, 2000). Briefly, conditioning occurred within small-animal operant chambers (BRS/LVE). The electrode leads from the rat's head stage was connected to peripheral equipment by lightweight cables that allowed the rat to move during conditioning. Computer software controlled the delivery of stimuli and the recording of eyelid EMG activity (JSA Designs, Raleigh, NC).

2.6. Conditioning procedure

The rats were allowed to adapt to the training environment for 5 min before each training session. Paired training sessions occurred in which 5 blocks of 9 paired CS–US presentations and 1 CS-alone probe trial were presented. Fewer trials were administered than previous studies from our laboratory in order to minimize within-session memory consolidation. The CS was a 400-ms tone (2 kHz; 85 dB) and terminated with a 25-ms shock US. The US intensity was set such that a rat would elicit eyelid closure and slight head movement (2.5 mA). CRs were defined as EMG activity that exceeded a threshold of 0.4 units (amplified and integrated units) above the baseline mean during the CS period after 80 ms. EMG responses that exceeded the threshold during the first 80 ms of the CS period were defined as startle responses. On CS-alone probe trials, the duration for scoring CRs was extended beyond the CS to the end of the trial period (1.0 s). Measurements of CR amplitude, onset latency, and peak latency were taken from CS-alone probe trials. URs were defined as responses that crossed the threshold after the onset of the US.

2.7. Experimental design

The rats received either vehicle, WIN55,212-2 (10 μg/μL), SR141716A (1.0 μg/μL), JZL-184 (0.5 ng/μL), or THL (1 ng/μL) infusions into the cerebellar cortex. Additionally, rats given WIN55,212-2 or SR141716A were randomly assigned to receive the infusions at different time points. These time points were immediately before the session (pre), immediately following the session (0 h), 1 h following the session (1 h), 3 h following the session (3 h), or 6 h following the session (6 h). JZL-184 and THL