Neurobiology of Learning and Memory 144 (2017) 155–165

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Influence of cued-fear conditioning and its impairment on NREM sleep

Tankesh Kumar, Sushil K. Jha *

School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India

ARTICLE INFO

Article history:
Received 17 November 2016
Revised 30 May 2017
Accepted 15 July 2017
Available online 18 July 2017

Keywords:
Amygdala
Fear-conditioning
Glia
Learning
Memory
Sleep

ABSTRACT

Many studies suggest that fear conditioning influences sleep. It is, however, not known if the changes in sleep architecture after fear conditioning are essentially associated with the consolidation of fearful memory or with fear itself. Here, we have observed that within sleep, NREM sleep consistently remained augmented after the consolidation of cued fear-conditioned memory. But a similar change did not occur after impairing memory consolidation by blocking new protein synthesis and glutamate transmission between glial-neuronal loop in the lateral amygdala (LA). Anisomycin (a protein synthesis inhibitor) and DL-α–amino-adipic acid (DL- α -AA) (a glial glutamine synthetase enzyme inhibitor) were microinjected into the LA soon after cued fear-conditioning to induce memory impairment. On the post-conditioning day, animals in both the groups exhibited significantly less freezing. In memory-consolidated groups (vehicle groups), NREM sleep significantly increased during 2nd to 5th hours after training compared to their baseline days. However, in memory impaired groups (anisomycin and DL- α -AA microinjected groups), similar changes were not observed. Our results thus suggest that changes in sleep architecture after cued fear-conditioning are indeed a consolidation dependent event.

1. Introduction

Many pieces of evidence indicate that sleep facilitates memory consolidation (Maquet, 2001; Peigneux, Laureys, Delbeuck, & Maquet, 2001; Walker & Stickgold, 2004). The significance of sleep in memory consolidation has been investigated by performing sleep-deprivation soon after a training task. A number of studies have reported detrimental effects of sleep deprivation on memory consolidation (Chen, Tian, & Ke, 2014; Chowdhury, Chandra, & Jha, 2011; Graves, Heller, Pack, & Abel, 2003; Kumar & Jha, 2012; Stickgold, Hobson, Fosse, & Fosse, 2001). In addition, studies suggest that total sleep or a specific sleep state [Rapid Eye Movement (REM) sleep or non-REM (NREM) sleep] increased after learning a new task (Fogel, Smith, & Cote, 2007; Hellman & Abel, 2007; Kumar & Jha, 2012; Smith & Rose, 1996). Augmented sleep after learning could also be associated with memory consolidation (Benington & Frank, 2003; Stickgold et al., 2001). However, none of the studies experimentally manipulated memory consolidation and investigated its effect on sleep.

Memory consolidation involves neural events associated with circuit stabilization, and post-learning sleep may facilitate these processes (Aton et al., 2009; Jha, Jones, et al., 2005). Neurons in some brain areas are activated during sleep, in a similar fashion, as they were active while learning (Lee & Wilson, 2002; Wilson & McNaughton, 1994). In cued fear conditioning (CuFC), the induced conditioned-freezing depends on the increased activity of the amygdala neurons to the conditioned stimulus (CS) (Duvarci, Popa, & Pare, 2011; Pape & Pare, 2010). Interestingly, changes in the amygdala neurons are also re-expressed during sleep after conditioning (Hennevin, Maho, & Hars, 1998). These studies suggest that reactivated neuronal assemblies during sleep may contribute to memory stabilization.

In the amygdala, CuFC activates several receptors, (Bauer, Schafe, & LeDoux, 2002; Rattiner, Davis, French, & Ressler, 2004; Rodrigues, Bauer, Farb, Schafe, & LeDoux, 2002), signalling pathways (Ota, Pierre, Ploski, Queen, & Schafe, 2008; Schafe et al., 2000) and "cAMP response element binding" (CREB) protein (Han et al., 2007; Josselyn et al., 2001). The CREB protein plays a significant role in the expression of several genes and eventually new protein synthesis necessary for long-term memory (LTM) formation and selection of neurons for memory processing (Han et al., 2007). In several reports, it has been observed that inhibition of new protein synthesis by anisomycin after training induces memory impairment (Nader, Schafe, & Le Doux, 2000; Schafe & LeDoux, 2000). Hence, a unanimous view is that LTM formation requires new protein synthesis (Kandel, 2001; Schafe & LeDoux, 2000). Anisomycin can, however, also profoundly suppress neuronal activity and neurotransmitter release, which may in turn affect LTM (Canal,

* Corresponding author at: Sleep Research Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India.
E-mail addresses: sushil_1000@yahoo.com, sushilkjha@mail.jnu.ac.in (S.K. Jha).

http://dx.doi.org/10.1016/j.nlm.2017.07.008
1074-7427/

Chang, & Gold, 2007; Qi & Gold, 2009; Sharma, Nargang, & Dickson, 2012), thus contradicting the direct role of *de novo* protein synthesis in LTM formation (Sharma et al., 2012).

Transformation of transient memory to a stable form requires activation of multiple modulatory pathways. For example, distributed synaptic plasticity is induced at the pre- and -post-synaptic sites in the principal neurons and GABAergic plasticity in the GABAergic neurons in the amygdala after CuFC (Pape & Pare, 2010). The theta and gamma oscillations are also generated in the amygdala possibly to synchronize the principal neural activity with inter- and intra-amygdala neurons (Pape & Pare, 2010). Obliteration of these processes altogether, with cytotoxic-lesioning, completely eliminates the induction of conditioned response (Maren, 1999). Therefore, we hypothesized that anisomycin mediated profound alteration in neuronal activity would eliminate the conditioned response to a greater extent compared to merely altering the glutamate-mediated processes of memory consolidation. We also reasoned that NREM sleep (out of total sleep time) will be augmented in the consolidated memory groups, but will not change in the impaired memory groups. To test these hypotheses, we impaired CuFC memory by microinjecting (i) anisomycin and (ii) DL-α-amino adipic acid (DL- α -AA) into the lateral amygdala (LA). DL- α -AA is an inhibitor of glial glutamine synthetase enzyme, which plays a significant role in maintaining an optimal glutamate level at nerve terminals (Laake, Slyngstad, Haug, & Ottersen, 1995) and also in memory consolidation (Kant et al., 2014).

2. Experimental procedures

Animals (male Wistar rats, 250–300 gm) (N = 52) were brought from the university's central animal house to our institutional facility one week before commencement of experiments. They were maintained on a 12:12 light–dark cycle (lights on at 7:00 AM) at 23–24 °C room temperature and food and water were given *ad lib.* All procedures were approved by the Institution's Animal Ethical Committee (IAEC protocol # 9/2011) of Jawaharlal Nehru University, New Delhi. We have also taken utmost care to minimize the use of animals in experiments.

We performed experiments to study the effects of (a) anisomycin (n = 20) and (b) DL-α-AA (n = 20) microinjections into the LA on the consolidation of CuFC memory and sleep.

2.1. Surgical procedures for polysomnographic recordings

Animals were prepared for chronic sleep-wakefulness (S-W) recording using a similar procedure as reported earlier (Jha, Brennan, Pawlyk, Ross, & Morrison, 2005; Kumar & Jha, 2012). The animal was anesthetized with 4% isoflurane, the scalp was shaved, and the head was fixed in the stereotaxic instrument. Skin was cut aside, skull was exposed and two bilateral stainless steel guide cannulae (26 gauge) were implanted in the LA, at brain coordinates (from bregma) AP: 3.2 mm, ML: 5.2 mm, DV: 7.2 mm. Two pairs of stainless-steel screw electrodes were fixed above the frontal and parietal cortices to record electroencephalogram (EEG). Three electrodes were implanted in the dorsal neck muscles to record electromyogram (EMG) (one extra electrode was implanted as a safeguard). One screw electrode was implanted in the nasal bone as a reference. Free ends of all these electrodes were connected to a nine–pin connector, which was cemented to the skull with dental acrylic. Finally, skin was sutured, and the animal was removed from the stereotaxic instrument. Post-operative care was also taken to control infection and brain inflammation. Animals were engaged in the experiments only after one week of recovery.

2.2. Cued fear conditioning

Animals were conditioned to the auditory tone using a similar protocol as has been used before (Kumar & Jha, 2012). Animals were first habituated in the neutral chamber between 10:30 and 11:00 AM on two consecutive days. Thereafter, spontaneous freezing behavior as baseline was recorded in a computer through Freeze Frame software (Coulbourn Inc, USA) using CCTV camera (Sentech USB 2 camera) for 20 min in the neutral chamber on two successive days (Kumar & Jha, 2012). Animals were trained for CuFC in the conditioning chamber by another person and also in a separate room to avoid contextual reminders. A total of ten paired auditory "tone-footshock" stimuli [tone: 2200 Hz, 90 dB, 5 s duration as a conditioned stimulus (CS)] and electrical foot-shock: 0.8 mAmp, 1 s duration as an unconditioned stimulus (US)] were presented over a 10 min period through the Freeze Frame software (Coulbourn Inc USA). The first tone was delivered with a delay of 300 s while the subsequent nine tones were delivered at a regular interval of 60 s. Shock was delivered through the grid floor of the shock chamber for 1 s paired with tone at 4th sec and both the stimuli were co-terminated. Animals' freezing behavior was captured continuously through the camera for 20 min (time matched hour during the baseline) for offline analysis of freezing response. The animals were then randomly divided into four groups after cued fear conditioning, (a) anisomycin (n = 7) and (b) its vehicle microinjection groups (n = 8); (c) DL-α-AA (n = 7) and (d) its vehicle microinjection (n = 8) groups. On the testing day, the animal was re-exposed to the CS alone in the neutral chamber. The tone was presented in a similar way as was presented during the training but without foot-shock. The freezing response was recorded and was analysed offline.

2.3. Polysomnographic recordings

After habituation in the neutral chamber of CuFC, the animals were habituated in the sleep recording chamber. They were tethered through a commutator to the sleep recording set up. After two days of habituation, baseline S-W was recorded for two days between 11:00 AM and 5:00 PM in a computer through the *Somnologica Science software* (Medcare Flaga, Iceland). S-W was also recorded at time matched hours after CuFC training and testing. EEG signals were processed with high-pass 0.1 Hz and low-pass 40 Hz while EMG was processed with high-pass 10 Hz and low-pass 90 Hz filters at 100 Hz sampling rate. The recordings were saved for offline analysis.

2.4. Drugs used

We microinjected anisomycin (190 mM) and DL-α-amino-adipic acid (DL-α-AA) (10 mM) (Sigma-Aldrich) in the LA soon after CuFC training. Anisomycin was dissolved in 40 µl of 1 M HCl, diluted with normal saline and titrated with 1 M NaOH to obtain optimal 7.4 pH. DL-α-AA was dissolved in 3 M HCl, diluted with phosphate buffer saline (PBS) and titrated with 3 M NaOH to obtain optimal 7.4 pH. The vehicles for anisomycin (n = 8) and DL-α-AA (n = 8) were prepared by adding an equal volume of HCl (without the drugs) in normal saline and PBS respectively, and were adjusted to optimal 7.4 pH with NaOH.

A volume of 1.6 µl of either drugs or vehicles was injected at each side in the respective groups. Microinjection was performed with the help of microinfusion pump (Kent Scientific, USA) at a rate of 0.8 µl/min over a two mins period soon after CuFC training. The injector cannula (30 gauge) was left in place for two additional minutes and then removed. In 10 animals [anisomycin (n = 5) and DL-α-AA (n = 5)], both cannulae were outside the LA. Hence, these animals were treated as additional controls.