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Sleep deprivation impairs synaptic tagging in mouse hippocampal slices

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ABSTRACT

Metaplasticity refers to the ability of experience to alter synaptic plasticity, or modulate the strength of neuronal connections. Sleep deprivation has been shown to have a negative impact on synaptic plasticity, but it is unknown whether sleep deprivation also influences processes of metaplasticity. Therefore, we tested whether 5 h of total sleep deprivation (SD) in mice would impair hippocampal synaptic tagging and capture (STC), a form of heterosynaptic metaplasticity in which combining strong stimulation in one synaptic input with weak stimulation at another input allows the weak input to induce long-lasting synaptic strengthening. STC in stratum radiatum of area CA1 occurred normally in control mice, but was impaired following SD. After SD, potentiation at the weakly stimulated synapses decayed back to baseline within 2 h. Thus, sleep deprivation disrupts a prominent form of metaplasticity in which two independent inputs interact to generate long-lasting LTP.

1. Introduction

The ability to learn and remember, and thereby adapt behavior based on past experience, is critical for survival. These processes are thought to be mediated in large part by synaptic plasticity, in which the strength of particular synaptic links between neurons is modified and then maintained for varying amounts of time in this modified state (Bliss and Collingridge, 1993, Hebb, 1949, Martin, Grimwood, & Morris, 2000). Strengthening a synaptic connection is called potentiation, and when this alteration lasts more than a few minutes, it is referred to as long-term potentiation (LTP) (Bliss and Lomo, 1973, Lomo, 2003). LTP can occur in many locations throughout the nervous system, but in rodents it has been studied most thoroughly in the hippocampus (Huang, Nguyen, Abel, & Kandel, 1996, Nicoll, 2017). LTP is typically induced by applying tetanic, or high frequency, trains of stimulation, either once or repeatedly, to a bundle of axons that form synapses onto the neurons of interest. LTP in the CA1 region of the hippocampus has the property of being input-specific, in the sense that applying tetani to a population of neurons via one set of synaptic inputs does not cause non-specific potentiation in other non-tetanized inputs (Andersen, Sundberg, Sveen, & Wigström, 1977).

However, in the last 25 years, it has become increasingly clear that modulation of one set of synapses can in fact influence the ability of other synapses to undergo plasticity. This “plasticity of plasticity” has been termed metaplasticity (Abraham and Tate, 1997). When this phenomenon occurs due to prior activity at the same synapse undergoing potentiation, this is called homosynaptic metaplasticity, whereas when prior activity at one synapse can influence the ability for plasticity at other synapses, this is called heterosynaptic metaplasticity (Hulme, Jones, Raymond, Sah, & Abraham, 2014, Young and Nguyen, 2005, Sharma and Sajikumar, 2015). A prominent example of heterosynaptic metaplasticity is synaptic tagging and capture (STC), in which induction of a long-lasting form of plasticity in one set of synapses causes conversion of short-lasting plasticity at another set of synapses into a long-lasting form. This is thought to occur because induction of either short-lasting or long-lasting forms of synaptic plasticity leaves a molecular “tag” at the affected synapses, which allows those synapses to “capture” the plasticity-related proteins created in response to the induction of long-lasting plasticity (Frey and Morris, 1997, Frey and Frey, 2008). This cellular phenomenon was originally described in hippocampal slices (Frey and Morris, 1997), but has also been observed in intact animals (Shires, Da Silva, Hawthorne, Morris, & Martin, 2012), and has potential behavioral correlates, such as the ability to remember otherwise innocuous details better when in the context of a traumatic event (Moncada and Viola, 2007, Reymann and Frey, 2007, Viola, Ballarini, Martínez, & Moncada, 2014). It is clearly important to better understand how an animal’s experiences and environmental and internal conditions influence these processes of metaplasticity.

Sleep deprivation (SD) is one particular condition that affects all too many of us. SD has been shown to impair hippocampus-dependent

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memory tasks such as contextual fear conditioning, object recognition, and water maze navigation (Graves, Heller, Pack, & Abel, 2003, Guan, Peng, & Fang, 2004, Palchykova, Winsky-Sommerer, Meerlo, Durr, & Tobler, 2006, Peigneux, Laureys, Delbeuck, & Maquet, 2001, Smith and Rose, 1996). In other tasks, such as the Y-maze or 8-box spatial task, performance is more or less maintained in the face of sleep deprivation, but the strategy the animal uses shifts away from a hippocampus-dependent mode (Bjorness, Riley, Tysor, & Poe, 2005, Hagewoud, Havekes, Tiba, et al., 2010b). SD also has a negative effect on synaptic plasticity in the hippocampus (Campbell, Guinan, & Horowitz, 2002, Davis, Harding, & Wright, 2003, Ishikawa et al., 2006, Kim, Mahmoud, & Grover, 2005, Kopp, Longordo, Nicholson, & Luthi, 2006, Marks and Wayner, 2005, McDermott et al., 2003, Ravassard et al., 2009, Tartar et al., 2006, Vecsey et al., 2009). Relevant to the current study, we have shown in mice that a 5-h period of total SD specifically impairs long-lasting forms of LTP that depend on the second messenger cyclic AMP (cAMP) and its target, protein kinase A (PKA) (Vecsey et al., 2009).

Although there have been many studies of the effects of SD on LTP, we are unaware of any published tests of SD on metaplasticity. Therefore, in the current study, we examined the effects of 5 h of total SD on synaptic tagging in mouse hippocampal slices.

2. Materials and methods

2.1. Subjects

All experiments were carried out on young adult (2–3 months old) male C57BL/6J mice from Jackson Laboratories. Mice were individually housed in a temperature-controlled environment on a 12 h/12 h light/dark schedule, with *ad libitum* access to food and water. All experiments were conducted according to National Institutes of Health Guidelines for Animal Care and Use and were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

2.2. Sleep Deprivation

Sleep deprivation was carried out as described previously (Vecsey et al., 2009, Vecsey, Park, Khatib, & Abel, 2015). Briefly, mice were handled for 2–3 min per day for 6 days prior to sleep deprivation. Sleep deprivation was carried out for 5 h beginning at zeitgeber time (ZT) 0–1 by the gentle handling method (Havekes, Vecsey, & Abel, 2012) to achieve nearly complete sleep loss.

2.3. Electrophysiology

Studies of synaptic tagging were carried out by an experimenter blinded to the condition (sleep-deprived or non-sleep-deprived) of the animal. Protocols were based off of previously published work (Huang, McDonough, & Abel, 2006, Park et al., 2014). Mice were killed by cervical dislocation, and hippocampi were rapidly dissected in ice-cold, oxygenated artificial cerebrospinal fluid (aCSF – pH 7.4, containing 124 mM NaCl, 4.4 mM KCl, 1.3 mM $MgSO_4$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 2.5 mM $CaCl_2$, and 10 mM D-glucose bubbled with 95% O_2/5% CO_2). Transverse slices (0.4 mm thick) were prepared using a tissue chopper and placed in an interface recording chamber maintained at 30 °C (Fine Science Tools, Foster City, CA). ACSF was constantly perfused over slices at a rate of approximately 1 ml/min. Following a recovery time of at least 1.5 h, field excitatory postsynaptic potentials (fEPSPs) were elicited from Schaffer collateral (area CA3 to area CA1) synapses using bipolar nichrome wire (0.5 mm; AM Systems, Carlsborg, WA) extracellular stimulating electrodes placed in stratum radiatum of CA1. For synaptic tagging experiments, two stimulating electrodes were positioned in such a way as to activate two separate sets of inputs (S1 and S2) onto the same postsynaptic population of neurons (Fig. 1A). Pathway independence was assessed by the absence of paired-pulse facilitation (50 ms interval) between the two pathways. Extracellular fEPSPs in the apical dendrites were recorded using a glass micropipette (1.5 mm OD; AM Systems, Carlsborg, WA) electrode filled with aCSF with a resistance of 2–4 MΩ. Data were acquired using ClampEx 9.2 and a Digidata1322 A/D converter (Axon Instruments, Union City, CA) at 20 kHz and low pass filtered at 2 kHz with a 4-pole Bessel filter. To examine basal synaptic transmission, input-output curves were generated by measuring the initial slope of the fEPSP in response to systematic increases in the strength of the stimulus. Slices that had maximum amplitude responses of less than 4 mV were rejected. Stimulus strength was set to elicit approximately 40% of the maximum initial fEPSP amplitude. Paired pulse facilitation was then examined at interpulse intervals between 25 and 300 ms. For synaptic tagging long-term potentiation (LTP) experiments, test pulses were delivered to Schaffer collaterals once every minute for 20 min. Slices that did not have stable baseline responses for 20 min were rejected. After 20 min, LTP was induced electrically by using one of two protocols. A massed 4-train protocol (four 1 s, 100 Hz tetanic stimulus trains delivered 5 s apart) was used to induce long-lasting L-LTP in input pathway S1. 30 min later, a 1-train protocol (one 1 s, 100 Hz) in input pathway S2 was used to induce short-lasting S-LTP (see Vecsey et al. (2007): Figs. 1A and 4A/B/C/D, Isiegas et al. (2008): Fig. 3A, Vecsey et al. (2009): Fig. 1D, Bridi et al. (2013): Fig. 4A/B, and Bridi, Hawk, Chatterjee, Safe, and Abel (2017): Figs. 3A and A) for examples demonstrating the decremental nature of this form of LTP in isolation). After induction of synaptic potentiation in S2, test pulses were delivered once per minute for two hours to assess the efficacy of the synaptic tagging and capture process.

2.4. Statistics

We performed a mixed-model ANOVA using Sleep Condition (SD vs. NSD) as a between-subject factor, and Time Point (average of first 20 min vs. average of last 20 min) and Input Pathway (S1 vs. S2) as within-subject factors. When significant overall effects and interactions were found, Student's *post hoc* tests were used to look for specific differences. JMP11 was used for all statistical analysis.

3. Results

In our synaptic tagging protocol, we chose to use massed 4-train 100 Hz LTP as our strong stimulus because this stimulation protocol induces a long-lasting form of plasticity that engages synaptic tagging mechanisms (Park et al., 2014) and that is itself resistant to the effects of sleep deprivation (SD) (Vecsey et al., 2009). We chose 1-train 100 Hz LTP as our weak stimulus because synapses experiencing that form of stimulation can be enhanced through synaptic tagging and capture (Huang et al., 2006, Park et al., 2014), and because 1-train LTP is unaffected by SD (Vecsey et al., 2009). By choosing these two SD-independent protocols, we hoped to observe whether the synaptic tagging process, in which a weak stimulus is enhanced by pairing it with a strong stimulus, was susceptible to disruption by SD.

In non-sleep-deprived (NSD) animals, tagging occurred effectively between massed 4-train through pathway S1 and 1-train through pathway S2 – both pathways experienced sustained potentiation (Fig. 1B and C). On the other hand, in SD animals, tagging was blocked – although plasticity induced by massed 4-train was long lasting, the paired 1-train stimulation returned to baseline (Fig. 1D and E). Statistical analysis found that there was an overall interaction between sleep deprivation, input pathway, and time point ($F(1, 24) = 4.01$, $p = 0.05$). *Post-hoc* analysis found that, during the first 20 min following tetanization, SD did not have a significant effect on either pathway S1 or S2. However, during the last 20 min of the recording, SD significantly reduced synaptic strength in the S2 pathway, but did not affect the S1 pathway.