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Serotonin dependent masking of hippocampal sharp wave ripples

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ABSTRACT

Sharp wave ripples (SPW-Rs) are thought to play an important role in memory consolidation. By rapid replay of previously stored information during slow wave sleep and consummatory behavior, they result from the formation of neural ensembles during a learning period. Serotonin (5-HT), suggested to be able to modify SPW-Rs, can affect many neurons simultaneously by volume transmission and alter network functions in an orchestrated fashion. In acute slices from dorsal hippocampus, SPW-Rs can be induced by repeated high frequency stimulation that induces long-lasting LTP. We used this model to study SPW-R appearance and modulation by 5-HT. Although stimulation in presence of 5-HT permitted LTP induction, SPW-Rs were "masked" – but appeared after 5-HT wash-out. This SPW-R masking was dose dependent with 100 nM 5-HT being sufficient – if the 5-HT re-uptake inhibitor citalopram was present. Fenfluramine, a serotonin releaser, could also mask SPW-Rs. Masking was due to 5-HT_{1A} and 5-$HT_{2A/C}$ receptor activation. Neither membrane potential nor membrane conductance changes in pyramidal cells caused SPW-R blockade since both remained unaffected by combining 5-HT and citalopram. Moreover, 10 and 30 μM 5-HT mediated SPW-R masking preceded neuronal hyperpolarization and involved reduced presynaptic transmitter release. 5-HT, as well as a 5-HT_{1A} agonist, augmented paired pulse facilitation and affected the coefficient of variance. Spontaneous SPW-Rs in mice hippocampal slices were also masked by 5-HT and fenfluramine. While neuronal ensembles can acquire long lasting LTP during higher 5-HT levels, lower 5-HT levels enable neural ensembles to replay previously stored information and thereby permit memory consolidation memory.

1. Introduction

In freely moving rodents, recordings of field potentials or local EEG activity in the hippocampus indicate theta activity with superimposed gamma oscillations during explorative behavior (Buzsaki, 1986; Vanderwolf, 1969; O'Keefe and Nadel, 1978). In contrast, during consummatory behavior, behavioral immobility, and slow wave sleep, hippocampal field potential activity is dominated by sharp wave ripples (SPW-Rs) (Buzsaki, 1986), during which stored information is thought to be replayed in form of sequentially activated pyramidal cells in a temporally compressed manner, thus allowing memory consolidation. GABAergic neurons play important roles in selecting those cells that are involved in the formation of a neuronal ensemble. Switching from theta rhythm activity to SPW-Rs and vice versa *in vivo* is rather abrupt. Variations in cholinergic tone (Hasselmo and McGaughy, 2004; Vandecasteele et al., 2014) are involved in these transitions, however, other neuromodulators such as serotonin (5-HT, 5-hydroxytryptamine) may also contribute.

5-HT, released from fibers which originate in the medial and lateral raphe nuclei (Dahlström and Fuxe, 1964; Aghajanian et al., 1967), can modify the behavior of many neurons simultaneously by volume transmission and thereby orchestrate neuronal interactions among many regions of the brain. Serotonergic mechanisms are activated during intracranial self-stimulation (Jacques, 1979) and are important in mood regulation (Heinz et al., 2001; Roth, 1994). They involve the activation of a wide variety of receptors, most of which are G protein coupled. One exception to the rule are ionotropic 5-HT_3 receptors (Barnes and Sharp, 1999; Kroeze et al., 2002), strongly expressed on interneurons in the

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hippocampus (McMahon and Kauer, 1997; Sudweeks et al., 2002). 5-HT affects postsynaptic excitability by activation of GIRK channels, thereby hyperpolarizing pyramidal cells (Andrade and Nicoll, 1987; Behr et al., 1997; Schmitz et al., 1998c; Segal, 1980). In addition, 5-HT reduces glutamate release in the entorhinal cortex, hippocampus and subiculum via activation of 5-HT_{1A} receptors at least in part due to reduced presynaptic Ca^{2+} influx (Schmitz et al., 1998b). Interestingly, 5-HT affects memory functions with a prominent role of 5-HT_{1A} receptors (Meneses and Liy-Salmeron, 2012; Cowen and Sherwood, 2013). It can be released from serotonergic fibers independent of neuronal activity by fenfluramine (Richter-Levin and Segal, 1990). In rat hippocampal slices, 5-HT and fenfluramine modulate both pharmacologically and stimulus induced gamma oscillations (Wojtowicz et al., 2009) and decrease SPW activity in the dentate gyrus (Richter-Levin and Segal, 1990; Kubota et al., 2003), potentially interfering with memory formation. Interestingly it was recently demonstrated that activation of serotonergic neurons in the raphe caused disappearance of ripple activity and interfered with memory consolidation (Wang et al., 2015). This, however, stands in contrast to another *in vivo* study (Ponomarenko et al., 2003) where blocking $5HT_{1A}$ receptors led to reduced ripple activity, perhaps pointing towards an augmenting effect of 5-HT on ripple oscillations *in vivo*. The molecular mechanisms behind these effects remain unknown. Their importance though lies not only in our understanding of the brains ability to quickly switch from one brain state to another. Patients who suffer from memory related problems as well as patients suffering from depression who are treated with serotonin re-uptake inhibitors might directly be influenced by this serotonergic modulation.

In ventral rodent hippocampal slices SPW-Rs occur spontaneously (Maier et al., 2003; Papatheodoropoulos and Kostopoulos, 2002). In addition, SPW-Rs can also be induced with stimulation protocols able to induce late LTP (Behrens et al., 2005). In this study we report that the appearance of SPW-Rs, evoked though high frequency stimulation, is masked by 5-HT in a dose dependent manner, presumably via activation of 5-HT_{1A} and 5-$HT_{2A/C}$ receptors. Neuromodulators may thereby regulate the activation probability of previously formed neural assemblies.

2. Methods

2.1. Slice preparation and solutions

Animal procedures were performed in accordance with the guidelines of the European Communities Council and approved by the regional Berlin animal ethics committee (LaGeSO Berlin: T0068/02). We decapitated adult Wistar rats (aged 6–8 weeks, >200 g, Charles River Laboratories, Sulzfeld, Germany) under deep ether anesthesia or under anesthesia induced by isoflurane and laughing gas (1% isoflurane in 70% N_2O and 30% O_2) and male C57Bl/6N mice aged 12–14 weeks. We prepared horizontal hippocampal slices (400 μm/at bregma - 4.7 to −7.3 mm) at an angle of ~12° in the fronto-occipital direction (with the frontal portion up) using a vibratome (752 M Vibroslice, Campden Instruments, Loughborough, England, or Leica VT1200 vibratome, Wetzlar, Germany).

Slices were prepared in cooled (~4 °C) artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl 129, $NaHCO_3$ 21, KCl 3, $CaCl_2$ 1.6, $MgSO_4$ 1.8, NaH_2PO_4 1.25, glucose 10, saturated with 95% O_2 – 5% CO_2. They were immediately transferred to an interface chamber perfused with aCSF at 36 ± 0.2 °C (flow rate: ~1.8 ml/min, pH 7.4, osmolarity: 297–303 mosmol/kg) and allowed to recover for 2–3 h before experiments were started. For each experimental condition, only one slice per animal was used with recording durations between 2 and 3 h. However, different protocols were often employed in 2–3 slices from the same animal in order to minimize the number of animals.

2.2. Recordings

Extracellular field potentials (FPs) were recorded under interface conditions from stratum pyramidale of area CA3 and CA1 with a custom-made amplifier using microelectrodes filled with 154 mM NaCl (5–10 MΩ). For intracellular recordings, sharp

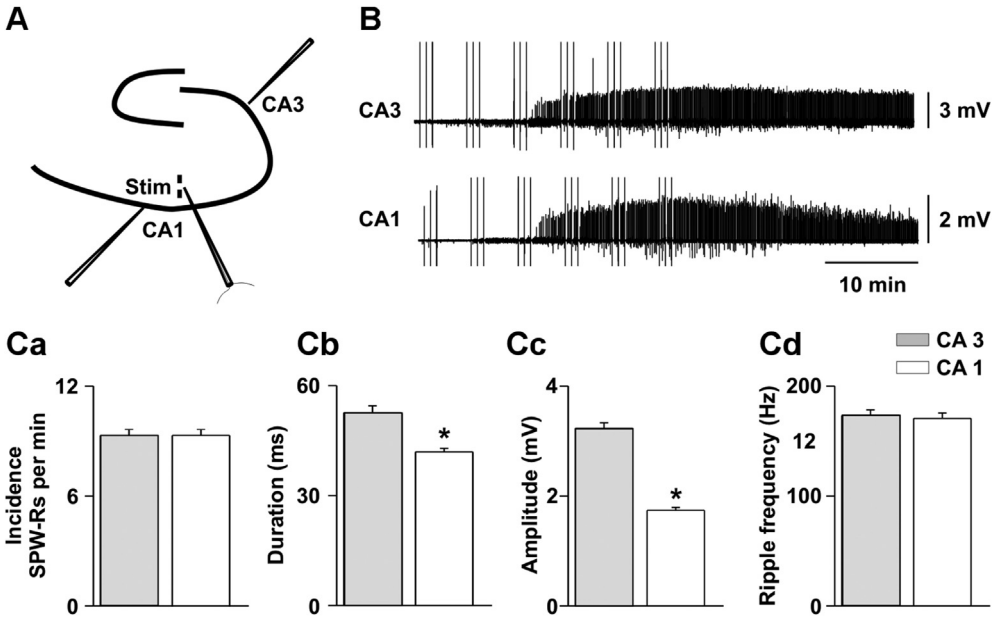

Fig. 1. Induction of SPW-Rs in rat hippocampal slices. A: Position of stimulation electrode in SR of area CA1 and recording electrodes in stratum pyramidale of area CA3 and CA1. B. Sample recording of stimulus-induced SPW-Rs in a condensed form. Note the appearance of SPW-Rs after 3 repetitions of the stimulation protocol. C. Average properties of induced SPW-Rs in area CA3 and CA1. Note that amplitude and duration of events in area CA3 are larger than in CA1.