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Directed Study 2013-14 Fall

At a basic level, memories may be shown through Hebbian learning. Donald Hebb found that repeated excitation of one cell (cell A) while the cell being exited was firing (cell B) increased synaptic strength between cells A and B. In other words, cells that fire together are likely to strengthen synaptic connections and improve cognitive abilities ("wire together"). This increase in synaptic strength can have long-lasting effects that can remain for several hours, a process known as long-term potentiation (LTP). Although the brain may perform this process naturally, studies have looked at using cognitive enhancers, drugs that may improve learning and memory, in order to boost the power of learning and memory and LTP effects in animal models and someday humans. Specifically, we are looking at how pregnenolone sulfate (PREGS) affects learning and memory and how it may be used as a cognitive enhancer. PREGS is an endogenous neurosteroid that is a derivative of pregnenolone, which is synthesized from cholesterol (Figures 2-4). Previous studies have suggested its benefits and we want to further the current research by seeing how PREGS changes responses to hippocampal place cells in the CA1 region of the hippocampus.

There is evidence that the effects of Hebbian learning and LTP can be observed in neural oscillation patterns, the rhythmic activity of neurons. These consistent patterns can be recorded and viewed as a summed waveform, the local field potential (LFP). In terms of LFP, the effects of Hebbian learning and LTP can be seen in short bursts of activity. It has been shown that short bursts of activity called hippocampal sharp waves (SPWs), also known as hippocampal ripples, are associated with a collective increase in pyramidal cell activity and interneuron activity (Buzsáki 1986). When hippocampal rhythmic slow activity (RSA/theta) is diminished, SPWs occur. They found that these SPWs occurred the most during slow-wave

sleep, suggesting that SPWs are connected to the consolidation of memories during sleep. They found that SPWs originated in CA3 and then affect the rest of the hippocampal formation due to connections to CA1 via Schaffer collaterals. So, they found that SPWs in CA1 seem to represent synchronous activation of pyramidal cells caused by activity in the Schaffer collaterals. This demonstration of synchronous activity suggests that SPWs present ideal conditions for long-term potentiation (LTP) to occur and provide the opportunity to use cognitive enhancers to increase this process, thereby increasing LTP and learning and memory.

Similar to the Buzáki paper, Siapas and Wilson (1998) published findings that supported that synchronous pyramidal activity in CA1 provides ideal conditions for LTP. They found that the CA1 region, known to be active when awake, becomes reactivated during sleep states, displaying high-frequency bursts of pyramidal cell activity called ripples. These ripples occur at about 200 Hz in sharp contrast to the slower cortical oscillations (7-14 Hz). In terms of local-field potentials (LFP), ripples show up as short, sharp pulses of high-frequency oscillations in the midst of low-amplitude cortical activity (Figure 1). The repeated presence of ripples may be able to modify neuronal circuitry, allowing for reorganization and consolidation. Thus, hippocampal ripples in CA1, the high-frequency pyramidal cell activity, may be important for memory processes. Following this study, a paper was published that provided the necessary link between hippocampal ripple activity and changes in LTP, laying the framework for experiments using cognitive enhancers.

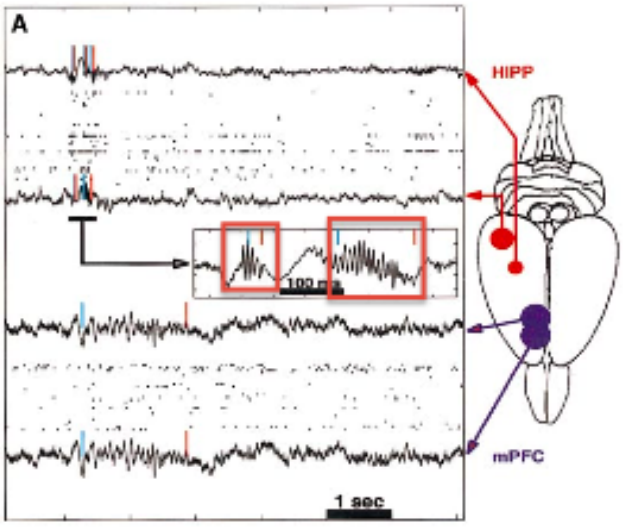

Figure1: Local Field Potential traces of hippocampal neurons (upper trace) and medial PFC neurons (Siapas 1998).

That paper, from King et al. (1999; Buzáki cited) looked at hippocampal cell activity in response to hippocampal sharp waves (SPWs). Earlier studies concerning LTP had shown that continuous stimulation of synaptic inputs might lead to LTP, but the authors wanted to look at how LTP may be induced using a less artificial stimulus. So, they looked at how SPWs affect synaptic strength in CA1 pyramidal cells. In essence, they wanted to see how CA3 activation, which traveled along Schaffer collaterals to CA1, affected synaptic efficiency. They found that the spontaneous CA3 SPWs could increase synaptic efficiency and affect LTP. So, a natural process in the brain is able to elicit responses known to provide long-term changes associated with improved learning and memory. Therefore, if a cognitive enhancer, such as PREGS, may be used to increase the instance of the SPWs, it could be able to improve learning and memory processes.

Pregnenolone sulfate (PREGS) is a steroid naturally found in the brain (Figures 2-4), formed from cholesterol, and known to affect memory processes in the hippocampus (Sliwinski et al. 2004). In a study from Sliwinski et al. (2004), they looked at the effect PREGS had on LTP-processes in rat hippocampal slices. They found that in nanomolar concentrations (300

nM), PREGS may enhance LTP in CA1 hippocampal neurons (Figure 5). They also found that PREGS enhanced the NMDA response, which increased intracellular calcium concentrations. PREGS does this by increasing NMDA current generation as well as increasing the length of NMDA-mediated responses. The increase in intracellular calcium caused by the NMDA response enhances LTP at the synaptic level. This increase was observed when the cells were continuously stimulated. However, King et al. showed that the stimulation could come from naturally occurring SPWs from the CA3 region via Schaffer collaterals. SPW activity could be increased due to synchronous activity in the pyramidal cells in CA1 and reactivation of place cells (Pangalos et al. 2012). So, if we record at CA1 and observe ripples and know that place cells are firing in response to SWRs, we should observe effects of LTP in CA1.

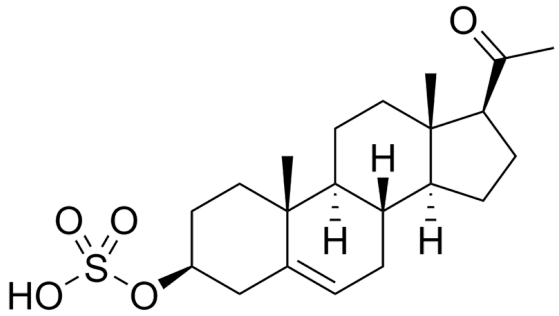

Figure 2: The chemical structure of pregnenolone, a steroid derivative of pregnenolone (Wikipedia 2013)

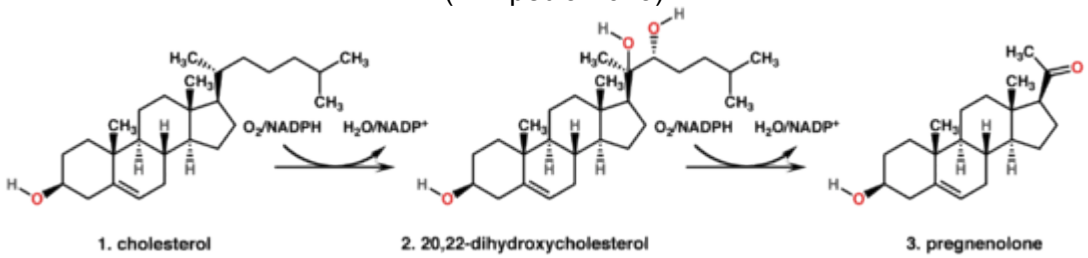

Figure 3: Biosynthesis pathway of pregnenolone from cholesterol (Wikipedia 2013).

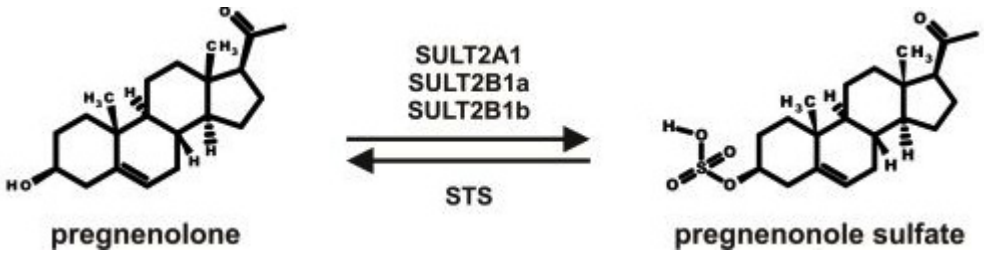

Figure 4: Biosynthesis of pregnenolone sulfate from pregnenolone (Harteneck 2013).

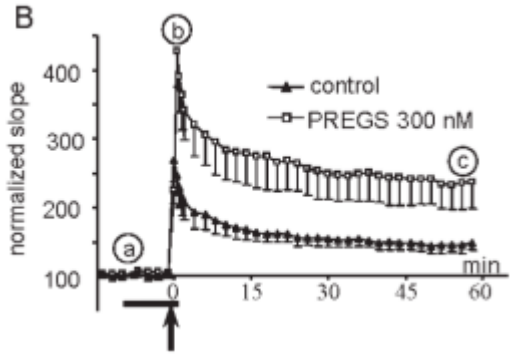

Figure 5: fEPSP slopes following stimulation for control and PREGS-treated slices. Note LTP is increased in the PREGS 300 nM slices (Sliwinski 2004).

Recently, a paper looked at the ability of PREGS to improve spatial orientation and object discrimination in rats, furthering the claim that PREGS might act as a cognitive enhancer, which provides us evidence to test how PREGS affects hippocampal place cell activity (Plescia 2014). It is known that PREGS facilitates excitatory neurotransmitter release at synapses. This study looked at two memory processes: acquisition and object discrimination and whether PREGS was able to improve these memory processes. They found that following PREGS administration, acquisition of spatial orientation improved. They also found that PREGS increased object-discrimination task performance. Specifically, they saw an increase in the CA3 region in terms of spontaneous firing events and mean neuronal firing, a phenomenon explained earlier as SPWs that affect LTP in CA1. So, PREGS increased the occurrence of SPWs in CA3 which through Schaffer collaterals stimulate CA1 pyramidal cells to fire in synchrony. Therefore, PREGS may cause improvements in memory through modulations to LTP processes, a finding which we wish to explore through recording of local field potentials and hippocampal place cell activity *in vivo*.

Our goal is to see how the cognitive enhancer pregnenolone sulfate affects hippocampal place cells in live rats in a novel-object recognition task. Using the previous findings, we expect PREGS to improve place cell remapping and act as a cognitive enhancer by improving learning and memory of the presented environments. This research can hopefully be applied to the

collective literature as explanation for PREGS's use as a cognitive enhancer and provide evidence for its beneficial effects in human memory.