CHAPTER 7

Phasic Pontine-Wave (P-Wave) Generation: Cellular-Molecular-Network Mechanism and Functional Significance

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INTRODUCTION

Sleep is a highly evolved global behavioral state in mammals. Sleep provides an exceptional opportunity to study the brain-based physical and physiological foundation of cognitive and homeostatic regulatory processes. The basic stages of sleep are introduced first in this chapter, followed by the basic elements of memory, and finally, a description of how these two fields are integrated.

Stages of sleep. Normally, when we first enter the sleep state, it is via quiet (non-rapid-eye-movement; NREM) sleep, which is a state that, behaviorally, is not very dramatic. We simply lie still, our eyes drift slowly back and forth, and at indeterminate intervals, we shift our sleep position. Upon first falling asleep, individuals may progressively lose awareness of the outside world and experience microhallucinations and illusions of movement of the body in space. During NREM sleep there are notable decreases in body temperature, blood pressure, heart rate, and respiratory rate. These decreases are accompanied by routine increases in the production of antibodies and pulsatile release of growth and sex hormones from the pituitary gland. NREM sleep is characterized by a change in the EEG from a low-amplitude, high-frequency pattern to one that is high amplitude, low frequency. The degree to which the EEG is progressively synchronized (that is, of high amplitude and low frequency) can be subdivided into four stages in humans: Stage one sleep (NREM-I) is characterized by relatively low-amplitude ($<50\,\mu V$), high-frequency (4–7 cycles per second; Hz) theta activity and vertex sharp waves in the EEG. Stage two sleep (NREM-II) is characterized by the appearance of distinctive sleep spindles (lasting between 0.5–1.0 sec, with peak amplitudes of $100\,\mu V$

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and composed of augmenting and decrementing waves at a frequency of 12–14 Hz) and K-complex (a negative sharp wave followed immediately by a slower positive component) waveforms in the EEG. Stage three sleep (NREM-III) is characterized by the addition of high-amplitude (>100 μV) slow waves (1–4 Hz), but with no more than 50% of the EEG record occupied by these slow waves. In stage four sleep (NREM-IV), the EEG record is dominated by these high-amplitude (150–250 μV) slow waves (1–4 Hz). The NREM-III and NREM-IV sleep stages are now considered to be a single sleep stage known as "slow-wave sleep" (SWS). Throughout the progression of NREM sleep (including SWS), as the EEG frequency decreases and the amplitude increases, muscle tone progressively declines and may be lost altogether in most of the somatic musculature. The slow rolling eye movements that first replaced the rapid saccadic eye movements of waking gradually subside, with the eyes finally assuming a divergent upward gaze. After varying amounts of time (depending upon the size of the animal and its brain), the progressive set of changes in the EEG reverses itself and the EEG resumes the low-amplitude, fast character previously seen in waking. Instead of waking, however, behavioral sleep persists, and this sleep phase is REM sleep. REM sleep is characterized by a constellation of events that includes the following: an activated pattern of cortical EEG activity; marked atonia of the postural muscles; rapid eye movements; a theta rhythm within the hippocampus; field potentials in the pons (P-wave); lateral geniculate nucleus and occipital cortex (ponto-geniculo-occipital [PGO]) spikes; myoclonic twitches, most apparent in the facial and distal limb musculature; pronounced fluctuations in cardiorespiratory rhythms and core body temperature; and finally, penile erection and clitoral tumescence. With a basic understanding of the stages of sleep now established, this chapter will move on to an introduction to memory processing, which is highly dependent on specific brain activity during sleep.

Stages of memory formation. The development and maturation of memory is a complex process that occurs in several distinct stages over time. The two major stages of memory formation are (1) acquisition of information (learning or encoding), and (2) consolidation of memory trace. Currently, there is a large body of evidence that has demonstrated that the different stages of memory development are influenced by specific stages of sleep. The first stage, acquisition, is triggered by engaging with an object or performing an action. The initial memory that then forms or becomes encoded leads to the formation of a representation of the object or action within the brain. This initial encoding of a memory is a relatively rapid process that requires only a few

milliseconds. At this stage, memory remains in a short-term store that has very limited capacity and, in the absence of rehearsal, persists for only minutes at most. However, if this encoded information persists in the form of reverberating activity in neuronal circuits, then another process transforms this short-term memory into an intermediate form. This intermediate form of memory is relatively more stable than the short-term memory and can last for several hours. However, at this stage, memory still remains sensitive to interference from competing or disrupting factors. This susceptibility is overcome through a process of consolidation. Memory consolidation was originally defined as a process whereby a memory trace, through the simple passage of time, becomes increasingly resistant to interference from competing or disrupting factors in the absence of further practice. However, the past 50 years of sleep and memory research have revealed that in addition to the passage of time, an adequate amount of sleep during this time is also required for memory to consolidate successfully. At the end of the consolidation stage, a memory has become stable and resistant to even extreme disruptions, such as electroconvulsive shock or application of neuronal gene and protein activation inhibitors. Memory consolidation itself is not a single-step process, but rather a multistep process that occurs exclusively during periods of sleep (Datta, 2010). Operationally, the cascading memory consolidation process can be divided into four stages: (1) search and read out of the intermediate form of memory, (2) elimination of unnecessary and/or redundant memory, (3) strengthening of cognitively relevant memory, and (4) transfer of stable memory to long-term storage. All of these processes occur over time, automatically, outside of awareness and without intent. Thus, they are distinct from the changes that result from conscious reminiscing or intentional rehearsal. Additionally, there is now a clear consensus that P-wave activity in REM sleep is critically involved in the last two steps of the memory consolidation process.

DESCRIPTION OF PGO/P-WAVES

Prominent phasic events of REM sleep include characteristic field potentials in the pontine tegmentum, which begin just prior to the onset of REM sleep and continue through its duration (Jouvet et al., 1959; Brooks and Bizzi, 1963; Datta & Hobson, 1994, 1995; Datta et al., 1998). These field potentials have been recorded in both the lateral geniculate body (LGB) and the occipital cortex of the cat (Mikiten et al., 1961; Mouret et al., 1963). Since, in the cat, these field potentials originate in the pons (P) and then propagate to the geniculate (G) and occipital cortex (O), they are called

PGO waves (Bizzi & Brooks, 1963; Brooks & Bizzi, 1963). Subsequent studies found that PGO waves in the cat could also be recorded at points throughout the extent of the thalamus and cortex. However, such PGO waves reach their highest amplitude in the LGB, primary visual cortex, and association visual cortex (reviewed in Datta, 1997). In addition to the pons, thalamus, and cortex, phasic potentials have been recorded in both the oculomotor nuclei (Brooks & Bizzi, 1963) and the cerebellum of the cat (Jouvet et al., 1965). Phasic potentials of pontine origin have also been recorded in the amygdala, cingulate gyrus, and hippocampus, which suggests that PGO waves also occur in the limbic system (Calvo & Fernandez-Guardiola, 1984). More importantly, all of these studies that have mapped PGO waves in the cat have demonstrated that the pons is the primary site of origin for PGO wave activity (reviewed in Datta, 1995, 1997). In addition to cats, PGO waves have also been documented and studied in other mammalian species including nonhuman primates, humans, and rodents. In nonhuman primates, PGO wave-like phasic field potentials have been recorded from the LGB and pons of macaques (Cohen & Feldman, 1968; Feldman & Cohen, 1968) and in the LGB of baboons (Vuillon-Cacciuttolo & Seri, 1978). In humans, phasic potentials have been recorded in the striate cortex during REM sleep (Salzarulo et al., 1975). Such striate field potentials are probably cortical components of state-specific phasic potentials of pontine origin. The observation of phasic scalp potentials associated with eye movements during REM sleep originally suggested that PGO wave-like activity may also be present in humans (McCarley et al., 1983; Miyauchi et al., 1987). Indeed, PGO waves have recently been recorded in the human pons, occurring immediately before and during REM sleep (Lim et al., 2007).

Based on recordings of PGO waves in the cat, initial attempts to record similar potentials in the LGB of the rat were unsuccessful (Gottesmann, 1969; Stern et al., 1974). Subsequent studies have recorded PGO-like waves in the pons of the rat that are equivalent to those in the pons of the cat (Gottesmann, 1969; Farber et al., 1980; Kaufman, 1983; Sanford et al., 1995; Datta et al., 1998, 1999). These initial failures led to the conclusion that state-specific pontine phasic waves in rats do not excite LGB neurons to produce the geniculate components of PGO waves (Datta, 1995). More recently, the absence of PGO wave-like activity in the rat LGB was shown to be due to a lack of afferent inputs from P-wave generating cells to the LGB (Datta et al., 1998). This field potential in the rat is therefore called a pontine-wave (P-wave), since it does not activate the geniculate nucleus (Datta et al., 1999, Datta 2000).

The waveform, amplitude, and frequency characteristics of PGO waves recorded from the pons, geniculate, and occipital cortex have been

examined most intensively in the cat (reviewed in Datta, 1997). PGO waves are biphasic in shape with a duration of 60–120 ms and an amplitude between 200–300 μV (Datta & Hobson, 1994). The P-wave in the rat is equivalent to the pontine component of the PGO wave in the cat (Datta & Hobson, 1994; Datta et al., 1998, 1999), with similar duration (see Fig. 7.1) (75–100 msec) and amplitude (100–150 μV) (Datta et al., 1998). PGO/P-waves can occur either as singlets or as clusters containing a variable number of waves (3–5 waves/burst) at a density range of 30–60 spikes/min during REM sleep (Datta & Hobson, 2000). Singlet PGO/P-waves, known as Type I waves, occur commonly in NREM sleep and are independent of eye movement; conversely, clusters of PGO waves, known as Type II waves, are associated with eye movement bursts and are typically indicative of REM sleep (Morrison & Pompeiano, 1966). In fact, Type II PGO wave activity accounts for 55–65% of the total number of PGO waves recorded during REM sleep (Datta et al., 1992; Datta & Hobson, 2000).

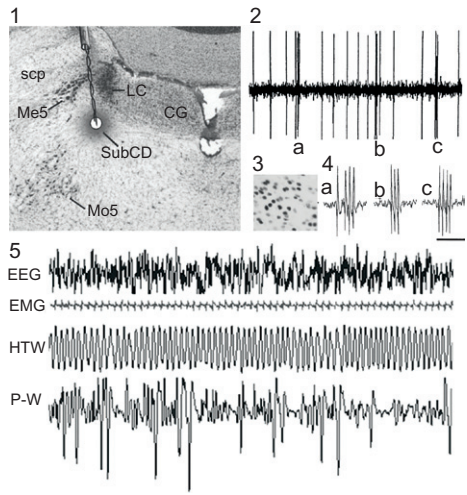

Figure 7.1 The location and activity of the pontine-wave (P-wave) generator. (1) Coronal section through the pons showing the P-wave generator (SubCD) of the rat in relation to neighboring areas of the brain. The figure also shows a bipolar recording electrode targeted directly into the P-wave generator to record P-wave activity. (2) A train of action potentials from a P-wave generating neuron showing recurrent high-frequency bursts in the background of tonic action potentials. (3) Photomicrograph of the P-wave generator of the rat showing pCREB-ir nuclei after cholinergic activation. (4) The three high-frequency bursts (a, b, and c) seen in (2), displayed on an expanded time scale (time scale = 10 ms). (5) Sample polygraphic appearance of REM sleep (trace duration = 10 sec) in rats showing low-voltage, high-frequency waves recorded from the frontal cortex (EEG); neck muscle atonia (EMG); theta-waves in the hippocampal EEG (HTW); and P-waves in the pontine EEG (P-W). Unpublished results of S. Datta.

CELLULAR AND MOLECULAR CHARACTERISTICS OF THE PGO/P-WAVE GENERATOR

Early transection and PGO wave recording studies in the cat indicated that the PGO wave generator is located within the pons (Bizzi & Brooks, 1963; Jouvet et al., 1965; Gottesmann, 1969; Datta, 1997). Subsequently, a number of single cell activity recordings in and around the PPT and laterodorsal tegmentum (LDT) observed a small population of neurons (about 3–5%) that discharged in bursts (3–5 spikes/burst) immediately preceding individual LGB PGO waves (McCarley et al., 1978; Steriade et al., 1990a,b). Based on this observation, these cells were originally believed to be PGO-wave generating neurons (McCarley et al., 1978; Steriade et al., 1990b). Recent studies in the cat, however, clearly indicate that the burst cells in the PPT/LDT are not PGO-wave generating neurons (reviewed in Datta, 1995). Instead, these cells, called transferring neurons, are responsible for conveying information from the pontine PGO wave generator to the forebrain (Datta, 1997). In the rat, because P-wave generating cells transmit P-wave information directly to the forebrain (Datta et al., 1998), these transferring neurons are absent (Datta & Siwek, 2002).

Utilizing chemical microstimulation, cell-specific lesions, and single cell recording techniques, the P-wave generator in the cat was localized within the caudolateral peribrachial (C-PBL) area (Datta et al., 1992; Datta & Hobson, 1994, 1995). Subsequently, using similar experimental techniques, the P-wave generator in the rat was localized within the dorsal subcoeruleus nucleus (SubCD) (Datta et al., 1998; 1999). In humans, as in the cat, the PGO wave generator is located in the C-PBL (Lim et al., 2007). Immunohistochemical identification of cholinergic and glutamatergic cells in the brainstem indicates that PGO-wave generating cells in the cat are capable of synthesizing both acetylcholine and glutamate (Quattrochi et al., 1998), thus these cells could be labeled as both cholinergic and glutamatergic. However, P-wave generating cells in the rat have been identified by specific monoclonal antibodies as glutamatergic, but not cholinergic (Datta, 2006).

Since the P-wave generator is also involved in sensorimotor integration (Morrison & Bowker, 1975), the differences in its anatomical location and neurotransmitter identity between the rat and cat may provide species-specific advantages. Specifically, in prey animals (i.e., the rat), the P-wave generator is anatomically closer to the locus coeruleus (LC). This shorter distance is advantageous during REM sleep (when animals are naturally paralyzed due to muscle atonia) because it permits quick communication

with the LC for a flight response, which facilitates escape from predators. This rapid flight response is vital for the survival of prey animals. In contrast, the predatory mammalian (i.e., the cat) PGO wave generator is farther from the LC, and instead, closer to the PPT. Since predators rarely face the threat of predation, there is no advantage to having a quick arousal response to any nonthreatening type of noise during REM sleep. In fact, frequent interruptions could actually harm a predatory animal by preventing the necessary functions (i.e., cognitive) of REM sleep. Thus, for these types of interruptions, the P-wave generator signals the cholinergic PPT to intensify REM sleep, rather than wake up the animal, by activating the LC.

Single cell recording studies have shown that P-wave generating neurons discharge high-frequency spike bursts (>500 Hz, 3–5 spikes/burst) in the background of tonically increased firing rates (30–40 Hz) during the P-wave related transitional state between SWS and REM sleep (tS-R) and REM sleep (Datta & Hobson, 1994; Datta, 1997). Normally, the glutamatergic P-wave generating cells remain silent during wake (W) and SWS (Datta & Hobson, 1994). A neuroanatomical pathway tracing study demonstrated that functionally identified P-wave generator cells in the rat project to the dorsal hippocampus (DH), amygdala, entorhinal cortex, visual cortex, and many other regions of the brain involved in cognitive functions (see Fig 7.2) (Datta et al., 1998). Similar studies have also demonstrated that the P-wave generator in both the cat and rat receives afferent projections from the raphe nuclei (RN) and locus coeruleus (LC) nuclei (Quattrochi et al., 1998; Datta et al., 1999). It has been demonstrated that cholinergic activation of the P-wave generator increases glutamate release in the DH (Datta, 2006). In addition, P-wave activity has a positive influence on hippocampal theta-wave activity in the DH (Karashima et al., 2002, 2005). Most recently, we have demonstrated that activation of the P-wave generator increases (1) phosphorylation of cAMP response element-binding protein (CREB), (2) activity-regulated cytoskeletal-associated protein (Arc), and (3) brain-derived neurotrophic factor (BDNF), as well as the messenger ribonucleic acids (mRNAs) of Arc, BDNF, and early growth response-1 (Egr-1) in the DH and amygdala (see Fig. 7.2) (Saha & Datta, 2005; Ulloor & Datta, 2005; Datta et al., 2008, 2009).

MECHANISMS OF P-WAVE ACTIVITY GENERATION

As mentioned in the previous section, experimental evidence has demonstrated that P-wave activity of REM sleep is generated by the activation of a distinct cell group, located in the SubCD in the rat and C-PBL in the cat (reviewed in Datta, 1995; Datta & McLean, 2007). It should be emphasized

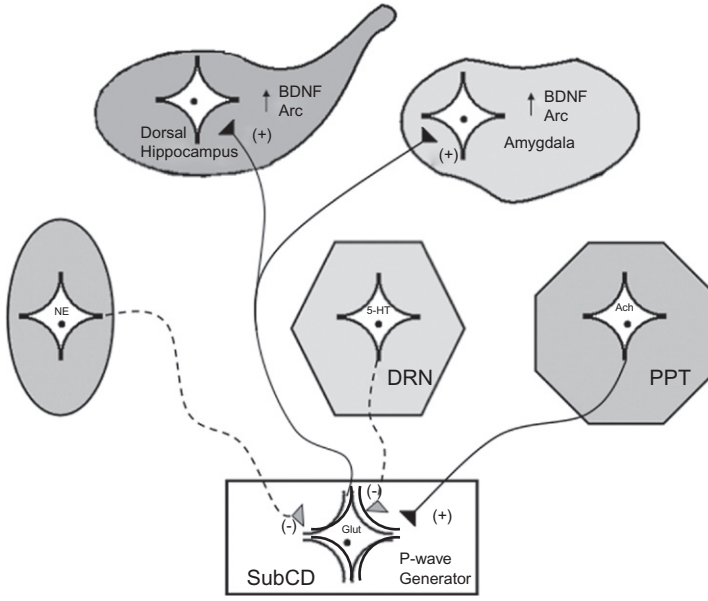

Figure 7.2 A summary diagram illustrating some important elements of the circuit regulating pontine-wave (P-wave) generator activity and targets of P-wave generating cells in the forebrain. The P-wave generator in the rat is located in the dorsal part of the subcoeruleus nucleus (SubCD), and the neurochemical phenotype of the P-wave generating neurons is glutamatergic (Glut). These P-wave generating cells receive afferent inputs from the cholinergic cells (Ach) in the pedunculopontine tegmentum (PPT), norepinephrinergic cells (NE) in the locus coeruleus (LC), and serotonergic cells (5-HT) in the dorsal raphe nucleus (DRN). Activation of PPT Ach cells excites (+) P-wave generating cells by releasing acetylcholine into the P-wave generator. Conversely, activation of LC NE cells and DRN 5-HT cells inhibits (-) P-wave generating cells by releasing NE and 5-HT into the P-wave generator. Thus, P-wave generating cells are activated in the presence of high levels of Ach and low levels of NE and 5-HT in the P-wave generator. Also, activation of P-wave generating cells increases glutamate release in the dorsal hippocampus and amygdala. Increased glutamate release activates dorsal hippocampus and amygdala cells via NMDA receptors, which ultimately increases expression of plasticity-related genes, BDNF and Arc.

here that this particular cell group simply represents the executive neurons for the P-wave. Turn-on or turn-off conditions of P-wave generating executive neurons are regulated by the ratios of available aminergic and cholinergic neurotransmitters within the P-wave generator. The source of aminergic neurotransmitters is the LC and DRN, while cholinergic neurotransmitters originate from the PPT (Fig. 7.2). The activity of both aminergic and cholinergic cells is approximately equal during wakefulness, and the onset of SWS results in an equal reduction in activity. Therefore,

the ratio of aminergic to cholinergic neurotransmitters in the P-wave generator is proportionate during wakefulness and through SWS. During REM sleep, however, aminergic cell activities are markedly reduced or absent and cholinergic cell activities are comparatively high (Datta et al., 2009b). The level of cholinergic cell activity during REM sleep is roughly 35% less than that of wakefulness. Thus, when a hypothetical ratio of aminergic and cholinergic neurotransmitters is 1:1, the P-wave generator remains in turned-off condition; however, when this ratio is 0:0.65, the generator is turned on to express P-wave activity (Datta & Siwek, 2002). Besides cholinergic and aminergic neurotransmitters, the inhibitory neurotransmitter GABA is also involved in the regulation of P-wave generating cells' activity, especially in the expression of high-frequency bursts.

EVIDENCE TO LINK THE P-WAVE GENERATOR WITH MEMORY CONSOLIDATION

Physiological evidence. Long-term potentiation (LTP) of synaptic transmission is widely considered to be a model of activity-dependent synaptic plasticity that could be involved in certain forms of learning and memory (Bliss & Collingridge 1993; Datta, 2006). It is well documented that REM sleep increases following learning trials and that deprivation of REM sleep soon after learning trials causes a subsequent decrease in performance of the learned task (Karni et al. 1994; Datta & Patterson, 2003; Datta et al., 2004). Associated with these changes in REM sleep are changes in the efficacy of synaptic transmission in the brain, manifested as LTP (for references see Datta, 2006). LTP is significant in that it is thought to be the physiological substrate of learning and memory at the level of the hippocampus and amygdala (Bliss & Collingridge, 1993). The standard protocols used by most researchers to induce LTP in the hippocampus, amygdala, neocortex, and many other areas of the brain are (1) high-frequency stimulation in which several hundred pulses at frequencies of 250–400 Hz are given; and (2) short high-frequency (>200 Hz) bursts of stimuli with an interburst interval of ~200 msec, called theta-patterned stimulation (for references see Datta & Patterson, 2003; Datta, 2006). In an experimental situation, the high-frequency electrical stimulation of an afferent pathway is key for induction of LTP. However, during REM sleep, the physiological source of this presynaptic high-frequency stimulation is unclear. Therefore, the identification of this source would be a significant contribution to the current body of knowledge about the physiological substrates of learning and memory.

For REM sleep-dependent memory processing and learning, the source of the LTP-inducing high-frequency stimulus must come from the REM sleep sign generating structures of the brainstem. Over the past 25 years, a number of laboratories have recorded the single cell activity patterns of several different REM sleep sign generating structures in rats, cats, and nonhuman primates (for reviews see Datta, 1995, 1997; Datta & MacLean, 2007). Depending on the specific REM sleep sign generating structure, the neuronal activity patterns of these singular cells are classified as tonic single-spike type, bursting type, or both tonic and bursting type. The only type of cell within the REM sleep sign generating structures that fires as a high-frequency burst, similar to the high-frequency stimulus required for the generation of LTP, is located within the P-wave generator (Datta, 1997). These P-wave generating neurons discharge high-frequency (>500 Hz) spike bursts (3–5 spikes/burst) on the background of tonically increased firing rates (30–40 Hz) during the P-wave related states of tS-R and REM sleep (Datta & Hobson, 1994; Datta, 1997). High-frequency bursting patterns of these P-wave generating cells support the idea that the P-wave generator may be the source of electrical stimulus for the induction of physiological LTP. Experimental evidence suggests that the activation of P-wave generating cells is capable of inducing LTP. Microinjection of the cholinergic agonist carbachol into the P-wave generator activates P-wave generating cells (Datta et al., 1991, 1998). Cholinergic activation of the P-wave generator in the cat markedly increases P-wave activity and REM sleep (Datta et al., 1991, 1992). This cholinergic stimulation-induced potentiation of P-wave density and REM sleep lasts for about 7–10 days. This long-lasting increase in P-wave density and REM sleep is a physiological sign of synaptic, as well as intracellular, plasticity. Activation of the P-wave generator facilitates hippocampal theta activity (Karashima et al., 2002, 2005; Datta, 2006). Physiological evidence suggests that the hippocampal theta rhythm favors induction of LTP in the hippocampus, as well as in many different parts of the cerebral cortex (for references see Poe et al., 2000; Pavlides and Ribeiro, 2003; Booth and Poe, 2006). Thus, the collection of P-wave generating cells is not only capable of inducing physiological LTP, but also represents the only group of cells in the REM sleep generating network that is capable of inducing this type of physiological plasticity.

Anatomical evidence. If the P-wave generator is a presynaptic input for the induction of synaptic plasticity, a prerequisite for learning and memory processing, P-wave generating cells would be expected to send anatomical

connections to the forebrain structures involved in memory processing. To test this hypothesis, the anterograde tracer biotinylated dextran amine (BDA) was microinjected into the physiologically identified cholinoceptive pontine P-wave generating site of rats to identify brain structures receiving efferent projections from those P-wave generating sites (Datta et al., 1998). In all cases, small volume injections of BDA in the cholinoceptive P-wave generating sites resulted in anterograde labeling of fibers and terminals in many regions of the brain. The most important output structures of those P-wave generating cells were the occipital cortex, entorhinal cortex, piriform cortex, amygdala, hippocampus, and many other thalamic, hypothalamic, and brainstem nuclei that participate in the generation of REM sleep (Datta 1995, 1997; Datta et al., 1998). All of these forebrain structures are also well known to be involved in memory processing (for references see Datta & Patterson, 2003). More recently, it has been demonstrated that these functionally identified P-wave generating cells are glutamatergic and stimulation of these cells releases glutamate in the DH (Datta, 2006). These monosynaptic axonal connections between P-wave generating glutamatergic cells and forebrain structures provide anatomical evidence that P-wave generating cells have the necessary anatomical substrate to be the presynaptic input for the induction of synaptic plasticity, a required process for learning and memory processing.

Behavioral evidence. Several studies indicate that rapid eye movements may represent the element of REM sleep that is crucial for memory consolidation (Verschoor & Holdstock 1984; Mandai et al., 1989; Smith & Weeden 1990; Smith & Lapp, 1991). For example, when a background clicking noise was presented during acquisition of a learned skill, presentation of the same auditory stimulus during subsequent eye movements during REM sleep (cueing) was correlated with a 23% improvement on retest performance one week later. The same cueing applied during non-eye-movement REM sleep episodes correlated with only an 8.8% retest improvement. It has been hypothesized, therefore, that the eye movements (or at least that segment of REM sleep in which they occur) are selectively important in REM sleep-dependent memory consolidation (Smith & Weeden, 1990). Visual learning tests in human volunteers showed that in addition to increases in percentage of REM sleep, the percentage of eye bursts during post-training REM sleep increased (Verschoor & Holdstock, 1984). Researchers hypothesize that these augmented eye bursts represent the scanning of visual stimuli encountered during the learning task, as part of the process of sorting, organizing, and consolidating daily input

(Verschoor & Holdstock, 1984). A study of Morse language learning in humans provides further evidence for an eye movement role in learning and memory processing during REM sleep. After a 90-minute Morse language learning session immediately prior to bedtime, subjects who had the greatest success had the densest rapid eye movements (Mandai et al., 1989). It is well established that the occurrence and direction of rapid eye movements during REM sleep depends exclusively on the excitation of P-wave generating cells (Datta & Hobson, 1994). Therefore, the studies described above indirectly suggest that the excitation of P-wave generating cells may be involved in REM sleep-dependent memory consolidation. The following paragraph describes some of the behavioral studies that have tested directly the relationship between P-wave generator activity and memory consolidation.

Using two different types of learning paradigms—two-way active avoidance (TWAA) and the Morris water maze (spatial learning)—studies have shown that learning training increases REM sleep and P-wave activity (Datta, 2000, 2006). More importantly, the results of such studies have shown that the increase in P-wave density during post-training REM sleep episodes is positively correlated with the effective consolidation, retention, and recall of the learning task. Together, the results of these studies indicate that P-wave generator activation may have a positive influence in the REM sleep-dependent memory processing of TWAA and spatial navigational learning.

In another behavioral study, we have demonstrated that supplemental activation of the P-wave generator, to a level greater than the normal post-training increased level, boosts retention of TWAA learning (Mavanji & Datta, 2003). The evidence from this study suggests that P-wave generator activation during REM sleep may enhance consolidation and integration of memories, resulting in improved performance on a recently learned task. Subsequently, another study has shown that activation of the P-wave generator prevents the memory-impairing effects of post-training REM sleep deprivation (Datta et al., 2004). The results of this study further substantiate the idea activation of the P-wave generator during REM sleep enhances the physiological process of memory processing, which naturally occurs during post-training REM sleep. Finally, another study has shown that selective elimination of cell bodies from the P-wave generator prevents retention of TWAA learning memory (Mavanji et al., 2004). The results of this study also have shown that lesions in the P-wave generator eliminate P-waves during REM sleep without changing the

amount of time spent in W, SWS, or REM sleep. These findings provide direct evidence that P-wave generating cells are crucial for normal REM sleep-dependent memory processing.

Biochemical/molecular evidence. A number of studies have shown that the afferent path for DH reactivation-dependent LTP and/or memory formation is glutamatergic, and transmission at these synapses involves NMDA receptors on the postsynaptic side (Morris et al., 1986; Zanatta et al., 1996; Packard & Teather, 1997; Steward & Worley, 2001). As mentioned earlier, the P-wave generator directly projects to the DH, amygdala, and many other forebrain structures that are involved in memory processing (Datta et al., 1998). More importantly, we have shown that P-wave generating cells are glutamatergic and activation of P-wave generating cells increases glutamate release in the DH (Datta, 2006). Additionally, our behavioral studies have shown that learning training increases P-wave activity and activation of the P-wave generator during a post-training period improves memory (Datta, 2000, 2006; Mavanji & Datta, 2003; Datta et al., 2004). We have also demonstrated that the elimination of P-wave generating cells prevents retention of memory (Mavanji et al., 2004). Collectively, the results of these studies suggest that P-wave generating cells are one of the major sources of glutamate for postsynaptic NMDA receptor activation–mediated memory processing in the DH.

A number of studies have suggested that neuronal activation-induced stimulation of the cAMP and/or Ca^{++}-PKA-CREB pathway is involved in the induction of a variety of gene expressions and ultimately in the protein synthesis of long-term memory formation (Kandel & Schwartz, 1982; Abel et al., 1997; Datta et al., 2009a). Using molecular and cellular techniques, we have shown that TWAA learning training causes P-wave generator activation and spatiotemporal phosphorylation of CREB (pCREB) in the DH and amygdala (Saha & Datta, 2005). Similarly, we have also demonstrated that TWAA learning training increases pCREB, BDNF, and Arc proteins in the DH and amygdala (Ulloor & Datta, 2005). The results of this study show that the increase in P-wave activity during the post-training 3-hour recording session is positively correlated with the increased levels of pCREB, BDNF, and Arc in the DH. This suggests that memory processing of TWAA learning involves excitation of P-wave generating cells and increased expression of pCREB, Arc, and BDNF proteins in the DH and amygdala. Finally, using a combination of cell-specific localized lesions and molecular techniques, we have shown that elimination of P-wave-generating cells abolishes P-wave activity and TWAA learning training trials-induced expression of

pCREB and Arc proteins and Arc, BDNF, and Egr-1 mRNAs in the DH and amygdala (Datta et al., 2008). More recently, it has been demonstrated that P-wave generator activation-dependent TWAA memory processing involves the intracellular PKA signaling system in the DH (Datta et al., 2009a). This study has shown that P-wave generator activation-mediated PKA activation is necessary for the expression of TWAA learning training-induced BDNF gene expression in the DH. Collectively, these cellular and molecular studies have shown that TWAA memory processing-specific gene activation and protein synthesis in the DH and amygdala are initiated by the activation of the P-wave generator. These studies also suggest that the P-wave generator activation is the primary mechanism for the REM sleep-dependent memory consolidation process.

CONCLUSIONS

In this chapter, I have discussed some of the compelling evidence that I believe to be significant for our understanding of the functional significance of P-wave generator activity in both REM sleep and REM sleep-dependent memory processing. These findings are the following: (1) Both TWAA and Morris water maze spatial navigation learning training increase REM sleep and P-wave activity during the subsequent sleep period. Improved performance in both TWAA and Morris water maze spatial navigation learning is proportional to the increase in P-wave density during the REM sleep episodes following these training trials (Datta, 2000, 2006). (2) After TWAA training trials, immediate supplemental activation of the P-wave generator, to a level above the normal post-training increased level, significantly increases retention of learning in later testing (Mavanji & Datta, 2003). (3) Activation of the P-wave generator prevents the TWAA memory-impairing effects of post-training REM sleep deprivation (Datta et al., 2004). (4) Elimination of P-waves by selective elimination of P-wave generating cells prevents retention of TWAA learning in the test trials (Mavanji et al., 2004). (5) We have shown that P-wave generating cells are glutamatergic, which project directly to a number of forebrain regions, including the DH and amygdala (Datta et al., 1998; Datta, 2006). Efferents from the P-wave generator project to areas that are directly involved in learning and memory processing. Activation of the P-wave generator increases glutamate release and theta wave frequency in the DH; both of these conditions have a positive influence on memory processing (Datta, 2006). (6) REM sleep-dependent TWAA memory processing depends on the P-wave generator

activation-mediated interaction with the DH-CA3 region (Datta et al., 2005). (7) Chemical activation of the P-wave generator and/or TWAA learning training increases the phosphorylation of transcription factor CREB and expression of immediate early genes Arc, BDNF, and Egr-1 in the DH, amygdala, and cerebral cortex (Saha & Datta, 2005; Ulloor & Datta, 2005; Datta et al., 2008).(8) P-wave generator activation-mediated TWAA memory processing involves PKA activation and PKA activation-mediated BDNF expression in the DH-CA3 (Datta et al., 2009a). These findings substantiate the idea that P-wave generator activation during post-training REM sleep may be critical for REM sleep-dependent memory processing of two-way active avoidance and spatial learning.

At present, our understanding of sleep-dependent memory processing mechanisms remains incomplete. Nevertheless, based on the existing findings, we suggest that training paradigms cause an increase in homeostatic demand for the activation of P-wave generating cells in the brainstem, which ultimately increases the total duration of P-wave related states, tS-R and REM sleep. Activation of P-wave generating cells during post-learning-training tS-R and REM sleep provides a glutamatergic-activating stimulus to the hippocampus and amygdala, which leads to the physiological reactivation and neuronal activation-dependent gene expression and protein synthesis processes that are necessary for long-term neuronal plasticity and memory formation.

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