Abstract

Sleep is pervasive throughout most of the animal kingdom—even jellyfish and honeybees do it. Although the precise function of sleep remains elusive, research increasingly suggests that sleep plays a key role in memory consolidation. Newly formed memories are highly labile and susceptible to interference, and the sleep period offers an optimal window in which memories can be strengthened or modified. Interestingly, a small but growing research area has begun to explore the ability of odors to modulate memories during sleep. The unique anatomical organization of the olfactory system, including its intimate overlap with limbic systems mediating emotion and memory, and the lack of a requisite thalamic intermediary between the nasal periphery and olfactory cortex, suggests that odors may have privileged access to the brain during sleep. Indeed, it has become clear that the long-held assumption that odors have no impact on the sleeping brain is no longer tenable. Here, we summarize recent studies in both animal and human models showing that odor stimuli experienced in the waking state modulate olfactory cortical responses in sleep-like states, that delivery of odor contextual cues during sleep can enhance declarative memory and extinguish fear memory, and that olfactory associative learning can even be achieved entirely within sleep. Data reviewed here spotlight the emergence of a new research area that should hold far-reaching implications for future neuroscientific investigations of sleep, learning and memory, and olfactory system function.

Keywords

sleep, olfaction, olfactory system, human brain, piriform cortex, olfactory bulb, associative learning, memory reactivation, emotion, cognition

1 INTRODUCTION

Behavioral states resembling sleep occur in the great majority of vertebrate and invertebrate species. Vertebrate sleep may have been present in the earliest jawed fishes 435 million years (My) ago, and based on fossil records, invertebrate sleep...
may date back to ancestors of the chambered nautilus 543 My ago (Kavanau, 2006). A brain is highly useful for going to sleep, but it is not necessary. For example, the highly lethal box jellyfish, *Chironex fleckeri*, which likely emerged a few million years ago, lacks a central nervous system but still takes the time to hover motionless over the Australian sea floor for up to 15 h a day (Kavanau, 2006; Seymour et al., 2004). The implication is that throughout evolution there has been a strong selective pressure to maintain sleep in an animal’s behavioral repertoire. Even animals that appear to have escaped the need for sleep, such as whales and dolphins that swim continuously, demonstrate unihemispheric sleep (Lyamin et al., 2002; Mukhametov et al., 1977). The next section of this chapter will consider some of the proposed behavioral, physiological, and cognitive benefits that sleep might provide.

We humans are utterly familiar with sleep, committing one-third of each day to the act, but we remain relatively ignorant about why we sleep. Throughout history, philosophers, artists, poets, and priests have attempted to make sense of sleep, long before psychoanalysis and neuroscience came into vogue. In ancient religion and mythology, spiritual embodiments of sleep were commonplace, and the gods and goddesses of sleep took many names: Ah Uaynih (Mayan), Caer Ibormeith (Irish/Celtic), Hypnos and his son Morpheus (Greek), Jum Sum (Chinese), Njörün (Norse), Somnus (Roman), Tutu (Egyptian), Urmya (Vedic/Hindu), and Zakar (Babylonian). The topic of sleep featured prominently in the Song of Songs (Song of Solomon) in the Old Testament, with passages describing a bride’s tormented dreams of her lover (see parts 3 and 5). During the fifteenth and sixtieth centuries, Renaissance painters portrayed the meaning of sleep in highly imaginative ways, providing religious, moral, mythical, and psychological depictions of sleep and dreaming: the divine and the damned, visions and transcendence, night and death, and the faintly erotic and the more erotic (Cecchi et al., 2013). Perhaps anticipating the future of olfactory sleep research (and the main theme of this chapter), the Venetian Renaissance painter Lorenzo Lotto (ca. 1480–1556/1557) depicted a young maiden napping against a tree, while a winged putto scatters aromatic (?) white flowers in a flowing stream down onto her head (Fig. 1), no doubt an early characterization of the potential for odor stimuli to influence the contents of sleep—in this case, the dream-like evocation of a pair of grotesque and debauched satyrs. A main goal of this chapter will be to present recent animal and human findings that extend Lotto’s incipient idea that odors have a direct influence on sleep physiology, memory, and behavior.

This chapter continues with a brief review of the phenomenology, physiology, and proposed functions of sleep, followed by an in-depth discussion of the interface between smells and sleep, with specific focus on both human and animal studies, and the concept of using olfactory-targeted memory reactivation (TMR) to influence memory consolidation in the sleeping brain. Because there is not enough space here to provide a detailed overview of the neuroscience of sleep or odor processing in the mammalian olfactory system, the reader may wish to consult many recent reviews on these topics (sleep: Brown et al., 2012; Fuller et al., 2006; Rasch and Born, 2013; Walker and Stickgold, 2006; and olfaction: Arzi and Sobel, 2011; Gottfried, 2010; Murthy, 2011; Wachowiak, 2011; Wilson and Sullivan, 2011).
FIGURE 1
Lorenzo Lotto (ca. 1480–1556/1557), *Allegory of Chastity*, oil on canvas, ca. 1505, Samuel H. Kress Collection, National Gallery of Art, Washington, DC.

SLEEP: A BRIEF OVERVIEW

Sleep can be loosely defined as a condition of reversible unconsciousness accompanied by relative inactivity and unresponsiveness. (This can be contrasted to pathological states of reversible unconsciousness that are accompanied by excessive activity, as in the case of sleepwalking or temporal lobe seizures.) It seems counterintuitive that such a behaviorally vulnerable state could be advantageous, but the preservation of sleep over the course of evolution, as well as its prevalence throughout the animal kingdom, suggests that sleep must have its uses (Cirelli and Tononi, 2008). Additional lines of evidence indicate that sleep is not only advantageous but also critical to survival. For instance, sleep is homeostatically regulated; that is, sleep loss results in longer and/or deeper sleep during the subsequent sleep period. After extended periods of sleep deprivation, sleep begins to intrude on wakefulness, and spectral analysis of electroencephalogram (EEG) recordings in rats reveals periods in which low-frequency oscillations characteristic of sleep invade bouts of wake (Franken et al., 1991; Friedman et al., 1979). Prolonged sleep deprivation also impairs cognitive function (Thomas et al., 2000; Van Dongen et al., 2003), and, in extreme cases, can be fatal (Rechtschaffen and Bergmann, 2002; Stephenson et al., 2007).

2.1 Sleep Phenomenology and Architecture

Sleep can be parsed into distinct electrophysiological stages, and sleep architecture refers to the pattern of those stages over the course of the night (Fig. 2). Originally classified by Rechtschaffen and Kales, each stage can be identified by its unique neuronal signature as recorded by EEG (Rechtschaffen and Kales, 1968). The characteristics of neuronal activity accompanying each stage are summarized below (Iber et al., 2007; Inostroza and Born, 2013).

During the wake state with eyes closed, brain activity is dominated by a posterior alpha rhythm (8.5–13 Hz). As drowsiness ensues, the alpha rhythm becomes increasingly attenuated, marking the onset of stage 1 sleep, which can include vertex sharp waves. As the subject slips further into stage 2 sleep, the EEG is typified by two features: biphasic negative/positive deflections (“K-complexes”) that stand out from background activity; and brief trains of 12–15 Hz oscillations (“spindles”) generated by thalamocortical loops. The duration of K-complexes and spindles must exceed 0.5 s to be classified as such. Stage 3 sleep, commonly referred to as slow-wave sleep (SWS), consists of low-frequency (0.5–4 Hz), high-amplitude (>75 μV) delta oscillations, upon which spindles are often superimposed (Fig. 2B, left). Recent research also shows that stage 3 sleep contains sharp-wave “ripples,” transient high-frequency bursts of activity (100–250 Hz). Ripples have been documented chiefly in the hippocampus and may play a key role in memory consolidation (Ego-Stengel and Wilson, 2010; Girardeau et al., 2009), but are extremely difficult to identify using surface EEG techniques. Rapid eye movement sleep (REM) consists of high-frequency, low-amplitude waves similar to those observed in the wake state (Fig. 2B, right), and may include 4–6 Hz trains of sharply-angled, serrated waves.
(“sawtooth waves”). As per its name, REM is associated with increased oculomotor activity on electrooculogram recordings, as well as muscle atonia interspersed with transient bursts of muscle activity on electromyogram (EMG) recordings.

Over the course of overnight sleep, these sleep stages progress in a cyclic manner, with each cycle lasting around 90 min. Humans complete four to five cycles each night (Fig. 2A). Of note, cycles in the first half of the night are predominated by SWS, whereas cycles in the second half consist chiefly of REM. One important implication for asymmetrical sleep architecture is that short naps contain mostly SWS, which holds potential relevance for sleep-based experimental designs in which
SWS is the main focus. Stages 1, 2, and 3 are cumulatively referred to as non-REM sleep (nREM), and in rodents, there is no distinction between individual nREM stages. In the context of rodent studies, SWS and nREM are synonymous.

2.2 The Proposed Functions of Sleep

Sleep likely serves many restorative and homeostatic functions. In the simplest formulation, physical activity and even mental effort are energy-demanding processes, and what better way to replenish one’s energy stores than to take a nap—though research intriguingly suggests that energy consumption is similar during wake and sleep states (Dworak et al., 2010). Sleep can exert restorative effects at the metabolic (Knutson et al., 2007; Morselli et al., 2012; Van Cauter et al., 2008) and immunological levels (Besedovsky et al., 2012; Lange et al., 2010). The observation that animals sleep more in the postnatal and juvenile periods than as adults has given rise to the interesting possibility that sleep is especially critical for brain development (Wang et al., 2011).

One influential hypothesis is that sleep is essential for synaptic homeostasis (Tononi and Cirelli, 2003, 2006). The basic idea is that over the course of a typical day, an animal experiences many new episodes and events (some meaningful, others irrelevant), all of which will induce some degree of synaptic plasticity, with molecular, physiological, and anatomical modifications in the brain. Sleep affords a window in which synaptic downscaling can take place, effectively cleaning up all of the synaptic noise that accrued in the waking state. Put differently, the physiological excesses of learning and plasticity that inevitably occur in the waking state are mitigated through mechanisms of synaptic homeostasis employed during sleep. This process would have important anatomical and functional consequences, ensuring efficient use of the finite space within the brain, and preserving only the most robust synaptic changes for subsequent consolidation.

Another important idea is that the function of sleep is to promote memory consolidation. In fact, this concept is not incompatible with the synaptic homeostasis hypothesis, insofar as pruning and downscaling of irrelevant synapses should help stabilize relevant memory traces for subsequent consolidation. The question of whether sleep can enhance memory has inspired countless research studies, and despite strong supporting data, the underlying mechanisms remain widely contested. In a landmark experiment, Jenkins and Dallenbach (1924) conducted a memory study that required subjects to memorize nonsense syllables and recall them after an interval of sleep or wake (Jenkins and Dallenbach, 1924). Notably, the sleep group demonstrated superior recall. The investigators speculated that sleep augments memory function simply by shielding memories from interference, a theory that garnered support for many years and continues to hold sway in the literature. In this scenario, sleep is viewed as a passive agent, enabling memory consolidation to take place without itself playing an active role.

However, recent behavioral and neurobiological studies provide compelling evidence that sleep may play a more active role in memory consolidation (Ellenbogen et al., 2006). Early support for this idea came from rodent studies, which
demonstrated that the same hippocampal place cells activated during a spatial exploration task were also activated during subsequent sleep (Pavlides and Winson, 1989), and particularly during SWS (Wilson and McNaughton, 1994). These findings were compatible with the idea that reactivation or replay of information processing during sleep is critical for memory consolidation. Since these seminal findings, and with many further contributions from both animal and human research (Deuker et al., 2013; Tambini and Davachi, 2013; Tambini et al., 2010), a working model of sleep-dependent memory consolidation has begun to emerge: memory traces are initially encoded in the hippocampus, where they are highly labile; during sleep, these memory traces are repeatedly reactivated, leading to stabilization and redistribution to extra-hippocampal cortical networks for long-term storage. It is thought that the slow oscillations that occur during SWS underlie reactivation of memories, whereas thalamocortical spindles and sharp-wave ripples may be involved in their redistribution (Rasch and Born, 2013). The recent implementation of TMR in human and animal studies (Antony et al., 2012; Bendor and Wilson, 2012; Diekelmann et al., 2011, 2012; Fuentemilla et al., 2013; Hauner et al., 2013; Oudiette et al., 2013a; Rasch et al., 2007; Rolls et al., 2013; Rudoy et al., 2009; van Dongen et al., 2012) has offered an exciting new method to test the active memory consolidation hypothesis and will be discussed in detail in Section 5.

3 THE INTERACTION BETWEEN SMELLS AND SLEEP: HUMAN STUDIES

For thousands of years, humans have used odors as fragrant sleeping draughts. In ancient Egypt, kyphi, an odorous resin of calamus, henna, spikenard, frankincense, myrrh, cinnamon, cypress, and terebinth (pistachio resin), was burned at the altar in Heliopolis to induce sleep and enhance dreams (Keville and Green, 1995). It seems quite probable that the smoke arising from many of these smoldering plants and herbs contained sedative-hypnotic or even hallucinogenic properties: scholars have suggested that at the Oracle of Delphi in ancient Greece, the fantastic visions and prophecies divined by the priestesses were due to the high concentrations of plant-derived fumes wafting into the inner sanctum (Pennacchio et al., 2010).

Empirical evidence for the ability of smells to promote sleep has been slow to emerge and modest in content. This research has mostly focused on the potential soporific role of lavender odor, likely motivated by long-standing anecdotal reports (including one from Queen Victoria herself!). Studies suggest that exposure to lavender before going to the sleep can enhance sleep quality in patients with mild insomnia (Lewith et al., 2005), extend the duration of the first cycle of SWS (Goel et al., 2005), improve vigor the following morning (Goel et al., 2005), and induce deeper sleep for napping infants (Field et al., 2008). The Goel group conducted a follow-up study in which they delivered peppermint odor prior to nighttime sleep, the prediction being that peppermint odor (which is considered to be stimulating) would disrupt sleep (Goel and Lao, 2006). Instead, exposure to peppermint odor increased the duration of SWS for those subjects who perceived the odor to be intense.
Further research is clearly necessary to establish the actual mechanisms by which certain odors influence sleep and arousal, but these data provide a scientific foundation for the use of “aromatherapy” in improving sleep.

Limited research has also examined the impact of sleep on odor perception. Killgore and McBride (2006) showed that after 24 h of sleep deprivation, odor identification on the University of Pennsylvania Smell Identification Test (Doty et al., 1984) was impaired. Intact performance on a test of sustained attention and executive function suggested that task difficulty or decreased vigilance per se could not explain the olfactory perceptual deficits. However, these results remain inconclusive, in that those participants reporting greater subjective sleepiness paradoxically performed better on the odor identification test. The group conducted a follow-up study confirming that 54-h sleep deprivation hindered odor identification to the same extent as 24-h sleep deprivation (McBride et al., 2006). Though far from definitive, the above studies illustrate the reciprocal influences between odors and sleep, and intimate that odors delivered during sleep may have a profound impact on the sleeper.

3.1 Response to Odors During Sleep

The experience of awakening to the sound of a quiet voice or a gentle tap on the shoulder is a common one, but it is unusual to be aroused by even the most pungent of odors. That said, the middle of the night is not usually punctuated by the sudden presence of an odor (with a few flatulent exceptions), so the question of whether olfactory stimuli can be detected during sleep has not really been put to the test. Indeed, compared to other sensory modalities, research investigating olfactory-evoked arousal has been scant (Velluti, 1997). However, based on the distinctive anatomical organization of the olfactory system—in which odor information processing in olfactory cortex does not need to be routed through the thalamus (Carmichael et al., 1994; Ray and Price, 1992; Russchen et al., 1987; Tanabe et al., 1975; Yarita et al., 1980)—one could plausibly expect that the sleeping brain should be responsive to odors.

A study by Badia and colleagues was the first to characterize olfactory sensitivity in humans during sleep (Badia et al., 1990). Either peppermint odor or clean air (control) was delivered in 3-min blocks over the course of stage 2 sleep, while odor-induced behavioral arousal (indexed via button-press), EEG and EMG activity, heart rate, and respiratory rate were concurrently recorded. Odor delivery (vs. control) was associated with a marginal increase in behavioral response, along with increased heart rate, decreased EMG activity, and increased EEG “speeding” (high-frequency EEG bursts lasting less than 10 s). Although somewhat primitive in its methodology, the Badia study offered evidence that olfactory processing is not obstructed during sleep in humans. That the trigeminal properties of the peppermint odor could have influenced these findings was not considered, but has since been addressed (Stuck et al., 2006, 2007).

More recent work in humans compared arousal thresholds of odors administered during sleep to arousal thresholds of auditory tones (Carskadon and Herz, 2004) (Fig. 3A and B). Two odors, peppermint and pyridine (fishy, pungent), were
FIGURE 3

Mixed physiological effects of odors on sleeping humans. (A) Delivery of peppermint odor at increasing concentration (one, most dilute; two, least dilute) had virtually no effect of self-reported behavioral arousals during stage 2, SWS, or REM. In contrast, delivery of auditory tones was highly successful at eliciting arousals in all sleep stages. (B) Delivery of pyridine odor (fishy, pungent) had a comparatively greater effect on arousals during lighter stages of sleep (stage 2 and REM), though arousals were inconsistent. Again, tones consistently provoked arousals. (C–E) In a separate study, four different odorants were delivered during sleep. Compared to a preodor baseline, the first postodor breath was associated with smaller inhalation volume (C) and larger exhalation volume (D), and across six consecutive breaths postodor (E), the inhale/exhale ratio was significantly different from baseline, progressively declining from the first to the sixth breath. Effects did not differ across odorants.

Data in (A) and (B) adapted and modified from Carskadon and Herz (2004) and data in (C–E) adapted and modified from Arzi et al. (2010).
delivered at varying concentrations during overnight sleep throughout all sleep stages, and subjects were instructed to press a button and give a verbal response if they smelled an odor. Odors were delivered for 15 s or until behavioral arousal. If odor delivery failed to arouse the sleeping subject, an auditory tone was then delivered for 5 s or until behavioral arousal, and subjects again pressed a button and gave a verbal response if they heard a tone. EEG activity was also monitored to determine sleep stage and arousal. Although subjects were aroused by 91% of odors delivered during stage 1 sleep, peppermint odor failed to evoke a behavioral response in any other sleep stage, and behavioral response to pyridine odor was sporadic. Occasionally, odor-associated EEG activation occurred in the absence of a behavioral response, though this too was infrequent. In contrast, subjects were aroused by 97% of tones across all sleep stages. Researchers concluded that, in stark contrast with audition, and also in contrast to the Badia study, olfactory responsiveness in all but the lightest stages of sleep is low to absent. One important limitation of this study was that data were based on only six participants.

Noting that the trigeminal properties of peppermint and pyridine odorants may have complicated interpretation of the Carskadon and Herz data, Stuck and colleagues used a pure olfactory stimulus (H₂S) and a pure trigeminal stimulus (CO₂), both at varying concentrations, to disentangle the abilities of olfactory and trigeminal stimuli to evoke arousal during sleep (Stuck et al., 2007). Arousal was assessed by monitoring EEG activity only, rather than employing additional behavioral measures. Results indicated that pure olfactory stimuli had no impact on the frequency of arousals when delivered during any stage of nREM (including stage 1) even at the highest concentrations, whereas trigeminal stimuli increased arousals during nREM in a dose-dependent manner. The same pattern of response to olfactory and trigeminal stimuli was observed in a follow-up study in which stimuli were delivered during REM (Grupp et al., 2008).

These studies pointed toward the idea that odors, especially pure olfactants, do not typically disrupt EEG sleep rhythms in the human brain. However, the inability of odors to arouse the sleeper does not exclude the possibility that more subtle responses to olfactory stimuli occur during sleep and might be identified using more precise methods and instruments. Moreover, it is important to note that surface EEG recordings are notoriously insensitive to signal sources emanating from deep brain regions within the medial temporal lobe, so it remains possible that electrophysiological arousals in olfactory cortical areas may have eluded detection. Interestingly, a much earlier study revealed that olfactory stimuli can exert a marked impact on sleep, whereby sardine odor (a classic feline-relevant smell) delivered during sleep desynchronized neocortical slow waves in cats (Hernández-Peón et al., 1960).

Research has shown that human subjects make deeper (shallower) sniffs in the presence of pleasant (unpleasant) odors, with similar respiratory profiles emerging when subjects merely imagine pleasant or unpleasant smells (Bensafi et al., 2003; Johnson et al., 2003). Extending this concept, Arzi and colleagues assessed the ability of odors to modify respiratory patterns when delivered during sleep (Arzi et al., 2010). In this study, four olfactory stimuli were used: two pure olfactory odors
vanillin, pleasant and ammonium sulfide, unpleasant) and two mildly trigeminal odors (lavender and vetiver oil). Subjects were exposed to one of the four odors during overnight sleep throughout all sleep stages except for stage 1. Inhalation, exhalation, and the ratio of inhalation to exhalation were measured for each of six breaths following odor onset, and these were compared to a baseline average of 30 breaths preceding odor onset. Results showed that onset of each odorant was associated with decreased inhalation and increased exhalation, with a significant change of the inhale/exhale ratio that was maximal for the first breath and persisted across all six breaths (Fig. 3C–E). Simultaneous surface EEG recordings suggested that odorants had no effect on arousals, in line with other studies (Arzi et al., 2012; Carskadon and Herz, 2004); if anything, odorants reduced the frequency of arousals compared to baseline. This study was critical in that it helped provide definitive support for the idea that the odors can modify physiology (in this case respiration) during sleep.

### 4 THE NEUROBIOLOGICAL INTERFACE BETWEEN SMELLS AND SLEEP: ANIMAL STUDIES

Animal research on the interaction between sleep and olfactory processing can be traced back to the French physiologist, Frédéric Bremer, whose pioneering studies in the 1930s had a far-reaching impact on the the field of neurobiology of sleep and wakefulness (Siegel, 2002). To elucidate the origin of sleep in the nervous system, Bremer developed an “isolated brain” (“cerveau isolé”) preparation in cats (Fig. 4), in which the cerebrum was transected from the rest of the neuraxis at the level of the rostral midbrain (Bremer, 1935, 1936). This procedure prevented most sensory inputs from reaching the brain, sparing only the olfactory and optic nerves. Remarkably, as long as the cat was fortified with artificial ventilation and a robust cerebrovascular blood supply, it (the cat) persisted in a functional and

![FIGURE 4](image)

This mid-sagittal drawing of the cat brain (left) depicts Bremer’s “cerveau isolé” preparation, with “S” denoting the surgical line of transection between the rostral midbrain and the lower thalamus, leaving the feline subject (right) in an irreversible sleep-like state and with pinpoint (miotic) pupils.

*Adapted from Bremer (1936).*
electrophysiological state closely resembling natural sleep. Inhalation of acetone (an odor with an admittedly strong trigeminal component) failed to rouse the animal from its sleep-like state. Bremer concluded that (a) ongoing sensory stimulation must be essential to maintain a waking state, (b) sleep was the consequence of blocking all sensory impulses to the cortex, and (c) olfactory and retinal stimulation was insufficient to restore wakefulness. From this work emerged the early and pervasive concept of sleep as a passive state, arising from functional deafferentation of sensory inputs—wholly incompatible with the idea that sleep might serve an active role in biological or behavioral processes, let alone that odor inputs might actively modulate those processes if delivered during sleep.

Actually, it took almost 20 years to show that Bremer’s conclusions about sleep were incorrect. As pointed out by Arduini and Moruzzi (1953), Bremer had not actually tested whether odors could disrupt the intrinsic EEG rhythms of the cerveau isolé cat. These investigators found that gomenol (camphorous odor) and terpineol (floral odor), and especially forcible introduction of air into the cat’s nostril, were capable of inducing fast, high-voltage activity in the olfactory bulb (OB), consistent with what Adrian had shown in urethane-anesthetized animals (Adrian, 1950). Moreover, odor or air stimulation caused a desynchronization of intrinsic EEG slow waves in sensorimotor cortex, temporal cortex, and thalamus, providing strong evidence that cortical arousal in the cerveau isolé cat could be achieved via external olfactory inputs. As discussed later in this section, the novel idea that odors had access to the cerebral cortex during sleep-like states conflicts with some (but not all) of the more recent published animal data.

4.1 Anesthetic Preparations as Models of Sleep-Like States

Apart from sporadic reports in cat (Hernández-Peón et al., 1960), dog (Domino and Ueki, 1959), rabbit (Faure and Vincent, 1964), and rat (Araki et al., 1980; Gervais and Pager, 1979, 1982), mechanistic studies linking smells and sleep have gained momentum only recently. An important set of studies by Fontanini, Bower, and colleagues has challenged the general notion that slow-wave cortical oscillations necessarily reflect a condition where the brain is shut down to external sensory inputs. These investigators demonstrated that in freely breathing rats anesthetized with a combination of ketamine–xylazine, slow (＜1.5 Hz) field potential oscillations within both piriform cortex and OB were synchronized with the inspiratory phase of respiration (Fig. 5), as well as with periodic fluctuations in the piriform cortical membrane potential (Fontanini et al., 2003). Moreover, in tracheotomized rats—in which nasal airflow is abolished while breathing persists—the entrainment between olfactory oscillatory activity and respiratory rhythm was disrupted, suggesting that the tactile sensation of air movement through the nose (rather than breathing per se) is critical for synchronizing brain and behavioral states (Fig. 5B–D). Such results imply that olfactory brain areas remain receptive to external afferent input during slow-wave states, unlike sensory neocortical areas in which sensory input appears to be blunted. An important take-home message (which will be a main focus of the next section) is that odor stimuli may have privileged access to the central nervous system during SWS.
In follow-up work, Fontanini and Bower (2005) also found that by varying the depth of anesthesia, they could modulate functional coupling within the rodent olfactory system. Under deep anesthesia, slow oscillatory responses (0.5–1.5 Hz) in OB and piriform cortex were strongly correlated to each other and to respiration, whereas under light anesthesia, fast oscillatory responses (>15 Hz) in these two regions became uncoupled, and only bulb activity remained in phase with the

FIGURE 5
Persistence of respiratory-driven activity during sleep-like states. (A) In freely breathing rats in a deep sleep-like state under ketamine–xylazine anesthesia, membrane potential oscillations in piriform cortex (Vm), and local field potentials (LFPs) in piriform cortex and OB are entrained to respiration (downward deflection represents inhalation). (B) In tracheotomized rats, the respiratory coupling to cortical and bulbar oscillations is disrupted, reflecting the importance of airflow through the nose to entrain these rhythms. (C) Cross-covariance analysis indicates that in intact rats (dashed line), there is a positive correlation between Vm and LFPs in OB that peaks near time 0, whereas in tracheotomized rats (solid line), this phase relationship is inverted. (D) The strong in-phase relationship between respiration and Vm observed in intact rats (dashed line, peaking at 225–315°) is virtually absent in tracheotomized rats (solid line). Start of inhalation is at 0°.

Adapted from Fontanini et al. (2003).
respiratory cycle. Insofar as these slow and fast electrical profiles correspond to states of SWS and REM/waking, respectively, the data further reinforce the idea that olfactory cortical regions are not isolated from the sensory periphery during sleep, but can be engaged with entry of air into the nose. Whether such effects are mediated by nonspecific activation of olfactory sensory neurons (Grosmaitre et al., 2009), by intranasal chemoreceptors that detect airflow-induced pressure changes (Grosmaitre et al., 2007), or by cortical (or subcortical) centrifugal projections remains unclear.

While the above studies examined olfactory sleep-like states in the absence of odor stimulation, Murakami and colleagues went one step further by testing whether odor-evoked responses in OB and cortex were state dependent (Murakami et al., 2005). To this end, rats were anesthetized with urethane, an agent that has the unique property of generating spontaneous alternations of slow-wave and fast-wave neocortical EEG patterns. Delivery of odor elicited significantly greater single-unit activity in anterior piriform cortex (APC) during fast-wave states than during slow-wave states (Fig. 6A), an effect that was seen during both natural and artificial respiration.

**FIGURE 6**
Sleep-dependent sensory “gating” of odor inputs between rodent OB and APC. (A) In urethane-anesthetized rats, odorant evoked significantly lower single-unit activity in the APC during slow-wave states (SWS, left) than during fast-wave states (FWS, right). (B) In contrast, odorant-evoked activity was similarly robust during both SWS and FWS in OB. Note that neither single-unit responses nor EEG oscillations appear to be in synchrony with artificially paced respiration of the animal. EEG recordings to monitor SWS and FWS were obtained from occipital neocortex.

Reproduced from Murakami et al. (2005), with permission from Elsevier, © 2005.
though spike responses were not coupled to respiration in either EEG state. In contrast to the dampening of odor-evoked responses in APC during the slow-wave state, odor-evoked activity in OB persisted during both fast-wave and slow-wave states (Fig. 6B), and electrical stimulation of OB elicited significantly fewer spike responses in APC during slow-wave (vs. fast-wave) states. These data were interpreted as evidence for sleep-dependent gating of odor processing at the level of piriform cortex. Given that there is no requisite thalamic intermediary between the olfactory periphery and olfactory cortex, the authors also conjectured that piriform cortex might in fact fulfill the type of gating function ascribed to the thalamus in other sensory systems. However, because single-unit recordings were confined to OB and olfactory cortex, it remains possible that gating is imposed via top-down projections from higher order regions, such as mediodorsal thalamus or orbitofrontal cortex.

4.2 Olfactory Cortical Processing During Natural Sleep States

The above findings in anesthetized preparations have been more recently extended to freely behaving rats during natural sleep (Manabe et al., 2011). Extracellular recordings from APC during SWS revealed spontaneous irregular slow oscillations (0.5–4 Hz) of local field potentials (LFPs), as well as olfactory cortical sharp waves accompanied by synchronized spike firing (Fig. 7A). Sharp waves were also identified in OB and were shown to be in synchrony with sharp waves in APC. The spontaneous manifestation of APC sharp waves was markedly suppressed during the wake state and during REM (Fig. 7B). Taking these results together with data from the Murakami study, the investigators conceived an olfactory framework of SWS in which the afferent flow of information is blunted (gated) at the level of OB, and the associative flow of information between higher order areas, olfactory cortex, and OB is heightened—effectively a “turning inward” of information processing to promote memory consolidation, with olfactory sharp waves mediating a replay of odor events experienced in the waking state. The general idea that SWS offers protection from external interference in order to promote memory replay and consolidation is a dominant theme in the current sleep literature (Rasch and Born, 2013) that will be further addressed in Section 5 of this chapter.

4.3 Impact of Odor Experience on Olfactory Processing and Behavior

Recent research from the Wilson lab has framed the question of smell and sleep in a different way: if SWS presents an optimal window in which memories can be reinforced for events (and odors) experienced during waking, then experimental manipulations designed to enhance odor experience should elicit neural changes in olfactory areas of the sleeping brain. In one of these studies, urethane anesthesia was used to evoke spontaneous fast-wave and slow-wave EEG states (as discussed earlier), during which single-unit activity in rodent piriform cortex was recorded (Wilson, 2010). First, spontaneous cortical activity was measured during slow-wave activity, establishing a baseline of spike firing in APC (Fig. 8A). Then, odor stimuli were delivered to the animal during fast-wave activity, mimicking odor experience...
that would occur in a wake-like state. Critically, a wide panel of odorants was tested during this period, enabling identification of odors that were able to drive activity of the neurons being recorded. These “adequate” odors were then delivered repeatedly during the fast-wave state. Finally, when the animal transitioned back to the slow-wave state, spontaneous firing in APC was again monitored, this time in the absence of odor.

By aligning single-unit activity to the peak of the LFP slow-wave, Wilson found that odor experience significantly modulated spike firing profiles for cells in APC, in comparison to a control group that did not receive odor (Wilson, 2010). Whereas

FIGURE 7
Spontaneous generation of olfactory sharp waves during natural sleep. (A) LFP recordings in rodent APC during SWS demonstrate the irregular emergence of large-amplitude sharp waves (downward deflections; arrows) that are aligned with phasic bursts of multiunit APC activity. (B) Both the frequency (top) and amplitude (middle) of the olfactory cortical sharp waves are increased during SWS and are virtually absent during other behavioral epochs, including exploration, grooming, and REM (bottom). The time-frequency power spectrum is based on EEG activity recorded from occipital cortex.

Adapted from Manabe et al. (2011).
spike firing remained tightly entrained to the phase of slow waves in the control group, the temporal structure of spike firing in the odor group became destabilized from pre- to postodor stimulation (Fig. 8B–D). There was also a significant change in the variability of the leading (early) edge of the slow-wave LFP from pre- to postodor, suggesting an impact on the population-level response. Based on these findings, it is tempting to conclude that the experience-dependent neural changes in the slow-wave state might reflect memory reactivation or replay of odor events.
occurring in the preceding fast-wave state, though the absence of corresponding behavioral data makes it difficult to substantiate these claims.

Complementary experiments from the Wilson lab have extended our understanding of the functional impact of sleep-like states on olfactory network plasticity. In rats under urethane anesthesia, the coherence of spontaneous spike firing activity between APC and OB (within delta and theta bands) decreased from the fast-wave state to the slow-wave state, whereas coherence between APC and hippocampus, and between APC and basolateral amygdala, significantly increased (Wilson and Yan, 2010) (Fig. 9). In addition, Granger causality analysis suggested that information flow from hippocampus to APC was stronger during slow-wave (vs. fast-wave) activity, and was also significantly stronger than in the reverse (APC to hippocampus) direction. In a separate study, functional magnetic resonance imaging (fMRI) resting-state connectivity (reflecting very low-frequency oscillations <0.1 Hz) was measured in urethane-anesthetized rats (Wilson et al., 2011). Because EEG measures could not be obtained during fMRI scanning, fast- and slow-wave states were estimated on the basis of differences in breathing rates. Using this indirect index, Wilson and colleagues identified

![FIGURE 9](image)

Sleep-induced modulation of olfactory network connectivity. Spontaneous LFP activity was recorded during urethane transitions between fast-wave activity (FWA) and slow-wave activity (SWA), with electrodes placed in rodent OB, layers I and III of anterior piriform cortex (PCX), and dorsal hippocampus (dHPC). Mean waveform coherence within both the delta (A) and theta (B) bands decreased between PCX III and OB from FWA to SWA, and increased between PCX III and dHPC from FWA to SWA.

Adapted from Wilson and Yan (2010).
significant state-dependent changes from fast-wave to slow-wave states, including enhanced functional connectivity between piriform cortex and dorsal hippocampus, piriform cortex and neocortex, and dorsal hippocampus and amygdala. Both this study and the Wilson and Yan study (Wilson and Yan, 2010) illustrate the dynamic effects of sleep-like state-dependent shifts in olfactory network interactions with both limbic and neocortical systems, which could help promote memory consolidation.

Given the uncertainties regarding the validity of anesthetized states to model natural sleep, Barnes and colleagues investigated odor-evoked piriform activity in una-nesthetized, chronically recorded rats (Barnes et al., 2011). Compared to wake and REM states, SWS was associated with reduced odor-evoked oscillatory activity in APC across theta, beta, and gamma bands, confirming a relative lack of olfactory responsiveness during SWS. Another goal of this study was to test the effect of experience—in this case, olfactory fear conditioning—on SWS. After learning to associate odors with foot shock in the awake state, rats spent more time in SWS compared to controls, and interestingly, on a subject-by-subject basis, the amount of time spent in SWS correlated with the strength of conditioned fear as indexed by freezing duration. Put differently, the extra time spent in SWS facilitated memory consolidation, possibly by extending the period of reduced external influence and enhancing cortical associative interactions.

5 OLFACTORY CUES CAN MODULATE COGNITIVE PROCESSES DURING SLEEP

Over the past 20 years, it has become increasingly clear that odors have a profound impact on the sleeper despite their inability to consistently evoke arousal. These qualities make odors ideal candidates to intervene during sleep without disrupting it. Notably, two distinguishing anatomical features of the olfactory system may indicate a privileged role for odors in memory modulation in the sleeping brain. First, the olfactory system shares considerable overlap with limbic and paralimbic brain areas that mediate emotion, memory, and behavior (Gottfried, 2006), likely reflecting the fact that, throughout much of the animal kingdom, the sense of smell has been paramount for survival. Second, olfactory sensory neurons in the nasal epithelium project to the OB, which in turn projects directly to piriform cortex, and from there to many downstream cortical regions. The important point is that odor information can be relayed to the cortex without having to pass through the thalamus, which in other sensory systems is thought to serve a “gating” function that impedes cortical transmission of sensory information during sleep.

5.1 Targeting Declarative Memories During Sleep

Over the past decade, researchers have employed olfactory cues in order to modulate memory consolidation, and even to demonstrate that learning can occur during sleep. In a pivotal study, Rasch and colleagues showed that odors could facilitate memory consolidation while human subjects were asleep (Rasch et al., 2007). Subjects performed a visuospatial object–location task in which they were required to learn the
unique locations of 15 visual stimuli, in the presence of either a rose odor or an odorless vehicle (control group) (Fig. 10A). During subsequent sleep, the rose odor was delivered in alternating on/off blocks of 30 s, to minimize odor habituation. Remarkably, when the odor was delivered during SWS, subjects in the odor group remembered the picture locations more accurately the following day than did subjects in the control group (Fig. 10B). This relative memory boost was not observed if the odor was delivered only during SWS, or if odor delivery following learning occurred during REM or during a wake state (Fig. 10C–E). In a complementary experiment, subjects learned the same visuospatial task in the presence of rose odor, then underwent fMRI scanning while the same odor was delivered during either SWS or wake. Odor-evoked hippocampal activity increased in the SWS group compared to the wake group, perhaps suggesting that presentation of a relevant odor context in sleep promoted reactivation of the associated hippocampus-dependent visuospatial memories, with consequent gains in memory performance the next day. This technique of using sensory cues to selectively modulate memory consolidation has also proven effective with auditory cues (Antony et al., 2012; Fuentemilla et al., 2013; Oudiette et al., 2013a; Rudoy et al., 2009) and has been termed targeted memory reactivation (TMR) (Oudiette and Paller, 2013).

In follow-up studies from the same group, subjects again took part in the aforementioned visuospatial memory task in the presence of a background odorant, isobutyraldehyde (“sweaty, sour” odor) (Diekelmann et al., 2011). The difference here was that after the sleep phase (or an awake control phase), subjects learned another set of object–location pairs, as a way to test whether sleep could shield the original (presleep) memories from the new interfering (postsleep) memories. Results showed that delivery of the olfactory cue during subsequent SWS stabilized visuospatial memories against interference, whereas the same cue delivered during the wake state rendered those memories more vulnerable to interference. A related fMRI experiment from this same study (Diekelmann et al., 2011) revealed that presentation of a sleep-associated odor during wake elicited activation in lateral prefrontal cortex, when compared to a control presentation of the same odor that had not been delivered during sleep. Again, significant odor responses in SWS were observed in hippocampus as well as retrosplenial cortex. These researchers have also found that delivering an olfactory cue during a 40-min nap facilitated memory consolidation to the same extent as did an uncued 90-min nap, whereas an uncued 40-min nap did not significantly benefit memory (Diekelmann et al., 2012). That memory performance following the 40-min odor-cued nap and the 90-min uncued nap were equivalent raises questions regarding the general robustness of the memory effects, and at a minimum illustrates the critical dependence of these effects on task parameters and timing.

### 5.2 Targeting Emotional Memories During Sleep

Though TMR has been employed most frequently to enhance declarative memories, the technique has also proven effective in modulating other types of memories. In recent work from our lab, Hauner and colleagues were able to extinguish fear
memories using olfactory TMR during SWS in human subjects (Hauner et al., 2013). In an initial fMRI conditioning session (Fig. 11A), subjects viewed two faces in the context of a “target” (tg) odor background. One face (the conditioned stimulus, or tgCS+) was paired on 50% of trials with a foot shock; the other face was not paired with shock (the control stimulus, or tgCS–). As a control for the tg odor context, in separate conditioning blocks, another two faces were presented in the presence of a “nontarget” (nt) odor context, resulting in ntCS+ and ntCS–.

Reproduced from Rasch et al. (2007), with permission from AAAS.

FIGURE 10
The first targeted memory reactivation study using olfactory cues to enhance declarative memory. (A) In a learning session taking place in the evening, subjects learned a visuospatial object–location task in the presence of rose odor. In subsequent sleep, rose odor or an odorless vehicle (control) was delivered during SWS. Memory for the object locations was assessed the following morning in the absence of odor. (B) Presentation of odor during both learning and SWS enhanced postsleep recall for the object (card) locations, compared to the control condition where odor was presented during learning only. (C) There was no effect of odor delivery on recall when odor was delivered during SWS but not during prior learning. There was also no effect on memory retrieval when odor was delivered during learning and again during subsequent REM (D) or subsequent wake (E).
FIGURE 11
Experimental paradigm of the fear conditioning task. (A) Olfactory contextual fear conditioning was completed in the MRI scanner. “Target” (blue) and “nontarget” (green) odorants served as background contexts in alternating trial blocks. Two conditioned stimuli (face images) were presented within the target context (tgCS+, tgCS−), and two within the nontarget context (ntCS+, ntCS−). Both tgCS+ and ntCS+ were paired with the US (mild electric shock) on 50% of trials (tgCS+p, ntCS+p) and were unpaired on the remaining 50% of trials (tgCS+u, ntCS+u). Control stimuli (tgCS−, ntCS−) were never paired with shock. Stimuli were presented in eight blocks (four target, four nontarget), in pseudo-randomized order. (B) Subjects in SWS underwent repeated reexposure to the target odorant (in 30-s on/off intervals), outside of the MRI scanner. The hypnogram illustrates sleep-staging data for a representative subject. (C) Upon waking, subjects completed a retrieval task in the MRI scanner. This task was identical to the presleep conditioning task, apart from a 12.5% partial-reinforcement schedule to prevent extinction.

Modified from Hauner et al. (2013).
stimuli. Skin conductance responses (SCRs) were measured as a physiological index of fear conditioning.

In a subsequent session outside the fMRI scanner, each subject took a 90-min nap during which the tg odor was delivered in alternating 30 s on/30 s off blocks during SWS (Fig. 11B). The goal here was to modulate fear memories selectively for the conditioned stimulus that had been previously encountered in the presence of the tg odor (i.e., tgCS+). During the first half of SWS, the tg odor evoked a robust SCR, compared to odor-off intervals, but during the second half of SWS, this response decreased significantly. These findings suggest that reexposure to the tg odor context in sleep induced within-session fear extinction. Finally, after waking, subjects returned to the fMRI scanner and took part in the same fear conditioning task (Fig. 11C), for direct comparison to the presleep session.

Analysis of the odor-evoked SCR data revealed a significant decrease in the “fear” response from pre- to postsleep for the tgCS+ face versus the ntCS+ face, and in comparison to the nontarget conditions (Fig. 12A). These findings suggest that delivery of the tg odor during SWS promoted the emergence of fear extinction in the

![A](image1.png)

**FIGURE 12**

Effects of TMR on conditioned fear response in humans. (A) SCR from pre- to postsleep decreased for tgCS+ compared to ntCS+. (B) Duration of odor delivery during SWS was correlated with degree of SCR reduction from pre- to postsleep. (C) Anterior hippocampal activity in response to tgCS+ decreased from pre- to postsleep compared to ntCS+.

(D) Voxel-wise ensemble maps of left amygdala activity in one subject (left) illustrate that, from pre- to postsleep, response patterns were more decorrelated when elicited by tgCS+ compared to ntCS+.

*Modified from Hauner et al. (2013).*
postsleep state. Interestingly, the degree to which the fear response decreased was correlated with the duration of tg odor delivery during SWS within individual subjects (Fig. 12B). These behavioral effects were also found to differ significantly from an independent group of wake subjects. The implication is that sleep constitutes a unique state in which targeted fear memories can be selectively extinguished, while nontargeted fear memories remain intact.

Concurrent analysis of the fMRI data showed that the cue-evoked mean activity in anterior hippocampus decreased from pre- to postsleep selectively for tgCS+ versus ntCS+ (adjusted for CS− baselines) (Fig. 12C). The reduction of activity in the hippocampus is consistent with other work suggesting that sleep accelerates consolidation of nonemotional memories, with reduced hippocampal dependence as learning proceeds (Wang et al., 2009). Interestingly, although there was no direct measure of memory for the tgCS+ and ntCS+ faces, we did find that reaction times (as an indirect measure of stimulus recognition) were significantly faster in response to tgCS+ than to ntCS+. Taken together, it is reasonable to speculate that delivery of the tg odor in SWS resulted in enhanced recognition memory for the tgCS+, reduced hippocampal engagement, and weakened behavioral expression of fear.

Finally, to the extent that presentation of the tg odor during SWS induced a fundamental shift in the “meaning” of the tgCS+ (i.e., from a conditioned fear memory in presleep to an extinguished fear memory in postsleep), we used fMRI multivariate techniques to test whether cue-evoked fMRI ensemble patterns in the amygdala significantly differed as a result of odor delivery during sleep. Data showed that the fMRI patterns evoked by tgCS+ significantly diverged (became more decorrelated) in the amygdala from pre- to postsleep, compared to the ntCS+ (Fig. 12D and E). This reorganization of amygdala ensemble activity suggests that odor contextual reexposure in SWS induces formation of a qualitatively unique memory trace in the amygdala (in line with findings in animal models; Herry et al., 2008), rather than weakening or “erasure” of the original trace per se.

Interestingly, a somewhat analogous experiment in mice reached an opposite conclusion (Rolls et al., 2013). Mice were conditioned to associate an odor with the delivery of a foot shock. After a 24-h delay, either the conditioned odor (CS) or a control odor was delivered during nREM. After another 24-h delay, the odor-evoked fear response was assessed by measuring the duration of freezing behavior following odor delivery (Fig. 13A). Although there were no significant differences in EEG delta power during delivery of CS odor versus control odor in sleep (Fig. 13B), in the postsleep behavioral assessment, mice in the conditioned odor group demonstrated increased fear response to the CS odor, compared to mice receiving the control odor (Fig. 13C). When a protein synthesis inhibitor was injected into the basolateral amygdala prior to odor reexposure, the opposite trend was observed. That is, the fear response following odor reexposure was attenuated rather than strengthened. Although these results seem to contradict those from the Hauner et al. study, there are several differences in the experimental designs that may account for the incongruous results (Oudiette et al., 2013b). Most notably, the 24-h delay period prior to odor reexposure allotted in the Rolls study likely led to more stable memory
storage than in the Hauner study where odor reexposure occurred within a couple of hours after conditioning. Another critical difference is that the odor served as a context cue in the Hauner study, as opposed to standing in for the CS itself in the Rolls study. Taken together, these studies illustrate the ability of olfactory cues to strengthen or attenuate emotional memories depending on experimental conditions. This is an important finding, as it may implicate TMR as a potential treatment for psychological disorders, such as posttraumatic stress disorder, which could prove to be a less stressful alternative to current reexposure treatments.

5.3 De Novo Olfactory Learning in Sleep

In spite of what pop culture might have taught us, placing a textbook underneath your pillow while asleep does not generally lead to higher test scores the next morning. More systematic efforts to demonstrate the ability to learn new material during sleep have been attempted. As early as 1942, Leshan tried to break a group of summer camp attendees of a nail-biting habit by delivering the message “my fingernails taste terribly bitter” via portable electric phonograph 300 times per night for 54 nights.
of 40 children, 8 of 20 in the experimental group stopped biting their nails (vs. 0 of 20 controls). As there was no physiological measure to ensure that the children were actually sleeping when the message was delivered (not to mention the likely lack of an IRB protocol), results from this study are far from convincing. However, Leshan’s efforts epitomize the long-standing interest in learning during sleep.

Given the apparent unique access of odor inputs to the sleeping brain, Arzi and colleagues set out to test this question using an associative conditioning paradigm (Arzi et al., 2012). This study was motivated by two prior findings: first, that odors delivered in sleep can in fact modulate breathing patterns (Arzi et al., 2010; see Section 3 above); and second, that subjects make larger inhalations in the presence of pleasant odors than unpleasant odors (Bensafi et al., 2003). The basic idea was to determine whether a tone paired with a pleasant (vs. unpleasant) odor would itself evoke larger (vs. smaller) inhalations in the absence of odor. While subjects were asleep, one of two distinct tones was delivered every 25–45 s. On two-thirds of trials, tones were followed by a unique odor after ~2.7 s; on the remaining third of trials, tones were unpaired (Fig. 14A). This paradigm conformed to a partial-reinforcement trace conditioning procedure and provided a way to dissociate tone effects from odor effects. Importantly, one tone was consistently paired with a pleasant odor, while the other was consistently paired with an unpleasant odor. Both EEG and respiration were monitored throughout. The next morning when subjects awoke, the two tones were presented again (without odor), alongside a third novel tone (Fig. 14B), to determine the strength of memory retention, again using respiratory measures.

While subjects slept in blithe ignorance during tone-odor conditioning, the volume of their odor-evoked “sniffs” was found to be larger for the pleasant odor than for the unpleasant odor (Fig. 14C, left), as expected based on prior work (Arzi et al., 2010). Critically, as conditioning proceeded during sleep, inhalation volumes became larger in response to the tone that had been paired with pleasant odor, compared to the tone that had been paired with unpleasant odor (Fig. 14C, middle). Moreover, these effects persisted into the next morning in the waking state: pleasant odor-associated tones evoked larger breaths than did unpleasant odor-associated tones (Fig. 14C, right). The effects observed during wake were less pronounced than those observed during preceding sleep, and when analysis was restricted to subjects that did have a single arousal within 30 s of tone onset during conditioning, responses to conditioned tones did not differ significantly from the response to the novel tone. Nonetheless, these exciting findings provide unique evidence that new information can be learned during sleep.

6 FORMS OF OLFACTORY HOMEOSTASIS DURING SLEEP

A handful of studies have considered the homeostatic effects of sleep on olfactory system processing at both the metabolic and cellular levels. Based on the observation that postprandial states are often followed by drowsiness and sleep, Gervais and
Pager (1979) reasoned that nutritional status and food odors might have a direct impact on sleep physiology. Multiunit recordings from the rodent OB showed that food odor increased mitral cell activity in awake hungry rats, compared to a nonfood odor, and compared to awake satiated rats. In contrast, during SWS, mitral cell activity increased in response to food versus nonfood odor, but there was no difference between hunger and satiety states. Moreover, while food odor delivered during SWS elicited an increased number of neocortical arousals (on EEG) in the hungry state compared to the satiated state, this effect was also evoked by nonfood odors, suggesting that hunger induces a nonspecific arousal response to olfactory stimuli during sleep. In subsequent work (Gervais and Pager, 1982), the ability of food odor to elicit a higher rate of neocortical desynchronization in the hungry (vs. satiated) state during

**FIGURE 14**

Effects of tone–odor trace conditioning during sleep on sniff response. (A) During sleep, one tone predicted delivery of pleasant odor and a second tone predicted delivery of unpleasant odor (67% partial reinforcement). Sniff volume evoked by tones alone was measured as an index of conditioning. (B) During wake, sniff volume evoked by conditioned tones and an additional control tone was measured to assess efficacy of conditioning. (C) Sniff volume increased in response to pleasant smells compared to unpleasant smells during sleep (left). Sniff volume increased in response to pleasant odor-associated tone compared to unpleasant odor-associated tone during sleep and during subsequent wake (middle and right).

*Adapted and modified from Arzi et al. (2012).*
SWS was abolished in rats with a lesion of the medial olfactory tract (i.e., anterior limb of the anterior commissure). The satiety-dependent effects were preserved in another group of rats with a lesion of the lateral olfactory tract. These results imply that behaviorally relevant olfactory information (in this case, food odor in food-deprived rats) can still access the brain during SWS, specifically via medial projections from OB that join the medial forebrain bundle en route to the reticular formation. Such mechanisms might ensure that in the case of starvation, a sleeping rat would not miss a meal should the smell of food waft into its bed chamber.

Curiously, interest in the effects of postprandial states on the olfactory system during sleep has been recently revived. Based on the idea that adult neurogenesis in OB is regulated by sensory experience, Yokoyama and colleagues tested whether food experience could influence the survival of adult-born granule cells (GCs) in the postprandial period (Yokoyama et al., 2011). When placed on a restricted feeding schedule, mice engaged in a range of behaviors following food consumption, including grooming, resting, and sleeping. In parallel, the number of caspase-3-activated GCs (where caspase-3 is a marker of apoptotic cell death) increased during this postprandial period, but only between 1 and 2 h after feeding. Of note, the duration of postprandial SWS correlated with the number of caspase-3-positive GCs, and if mice were disturbed or kept awake during this critical window, the effects on GC apoptosis were not identified. Somewhat oddly, these postprandial effects were not demonstrated in mice that were allowed to eat freely. Though this study raises many questions, the results point toward a role for SWS in reducing the number of adult-born neurons in OB, as a form of cellular homeostasis and structural reorganization. Given that ongoing olfactory sensory experience promotes an exuberant birth of many new GCs each day, a mechanism must be in place to eliminate those cells that are not incorporated effectively into the circuit. Sleep, perhaps with an instructive neuroendocrine or neuromodulatory signal, may help determine which of those GCs are ultimately destined for survival.

**7 CONCLUSIONS**

Smells and sleep have both held great relevance for survival since the earliest days of nervous system evolution. In contrast to some of the other senses, it seems that the olfactory system has successfully adapted to the behavioral necessities of sleep, providing a conduit between the external world and the internal world of the brain for an otherwise unresponsive animal. Certainly, there is strong evidence in animal models that neural oscillations in olfactory cortex remain in synchrony with respiratory rhythms during SWS (Fontanini and Bower, 2005; Fontanini et al., 2003). Because the delivery of odor information to the brain necessarily fluctuates with the ebb and flow of respiration, it makes sense that a cortical state of “readiness” in the olfactory system should be entrained to breathing. Indeed, as shown in hungry rats, delivery of a salient food odor during SWS is capable of generating physiological arousals (Gervais and Pager, 1982) that could improve the chances of securing a meal.
Compatible with the idea that there exists a direct line from the outside world to the olfactory system during sleep, research in sleeping human subjects convincingly demonstrates that odors delivered during SWS can have a significant impact, both behavioral and neural, on expression of declarative memory, fear memory, and associative learning (Arzi et al., 2012; Diekelmann et al., 2011, 2012; Hauner et al., 2013; Rasch et al., 2007).

In addressing the question of whether the olfactory system remains “open” during SWS, certain animal studies have arrived at opposite conclusions. Different research groups have found that odor-evoked single-unit activity in piriform cortex is considerably reduced during sleep-like slow-wave states, compared to sleep-like fast-wave states (Murakami et al., 2005; Wilson, 2010). These findings are taken to suggest that the olfactory system is “closed” to external odor stimulation during SWS, perhaps echoing the same functional organization that exists in the hippocampus where the “closed” state facilitates memory reactivation in the absence of outside interference (Wang et al., 2009). Although this is an attractive hypothesis, there are several reasons to adopt a more cautious perspective.

First, just because olfactory brain areas are less responsive to odors during SWS does not mean that they are unresponsive, a point actually made by Wilson and colleagues (Barnes et al., 2011). Even if one accepts that the flow of odor information from OB to piriform cortex is totally obstructed, there are still many other potential avenues by which odor information could engage the brain, since the OB also sends direct monosynaptic projections to the amygdala and entorhinal cortex, which themselves are linked with orbitofrontal cortex and other limbic and paralimbic regions.

Second, though anesthetics are often used to induce sleep-like states that resemble the behavior and neurophysiology of natural sleep, this does not mean that the anesthetized brain is a veridical model of the sleeping brain. Indeed, classic work by Domino and Ueki (1959) demonstrated that a myriad of different anesthetics each had unique effects on rhinencephalic and neocortical EEG rhythms. Thus, some of the reported differences in the animal studies described here might be attributable to this confounding factor. The need to examine natural sleep states in the absence of anesthesia will be critical for clarifying the functional organization of the olfactory system in sleep, and recent research has begun to move in this direction (e.g., Barnes et al., 2011; Manabe et al., 2011).

Finally, because respiration can substantially vary during wake, nREM, and REM, it is imperative to consider this parameter in sleep-based analyses of olfactory processing. For example, the demonstration of odor-evoked differences between sleep stages could simply reflect the fact that odor was delivered less efficiently in one of those stages, rather than reflecting intrinsic neurophysiological differences. Moreover, olfactory studies have long shown that the temporal relationship between odor onset and onset of inhalation (sniff) has a crucial impact on olfactory perception and odor coding (Bathellier et al., 2008; Buonviso et al., 2003; Carey and Wachowiak, 2011; Chaput, 1986; Shusterman et al., 2011), but in some of the sleep studies reported here, odor-evoked responses were characterized without regard to respiratory phase, possibly accounting for some of the observed variability.
Throughout history, sleep has been viewed as a gateway to knowing the unknown. It is the stuff of primal emotions: nightmares, night terrors, and nocturnal emissions. Sleep is the territory of one of the most classic psychological forays into the human mind (Freud, 1899), and of one of the most critically acclaimed comic book series of all time, *The Sandman* (Gaiman, 1991–1996). Sleep has emerged as an increasing focus of neuroscience research, serving as a powerful gateway to understanding brain function and behavior. Recent studies have only begun to probe the interface between sleep and smells, and the future holds great promise for the use of odor-based manipulations to clarify basic mechanisms of olfactory perception, and even to elucidate the ever-enigmatic function of sleep.

References


