

# THE PROCESSING OF SOUNDS IN AUDITORY CORTEX DURING SLEEP

by

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## Abstract

To what degree the brain still processes sounds during sleep even though they do not reach conscious awareness remains unclear. Unlike the visual system, which has the eyelids, the auditory system possesses no external physical mechanism to block incoming signals during sleep. Yet, our intuition is that our auditory experience is very different, if not non-existent during sleep. We chose to explore this problem at the neural level in a non-human primate (common marmoset) hoping to derive direct insights into the perceptual consequences of sleep on hearing that we as humans experience every night.

We found that neurons in primary auditory cortex are responsive to sounds almost as strongly during sleep as when the animal is awake. Moreover, downstream neurons in secondary auditory cortex also had acoustically driven responses. We estimate that responses only drop 10% on average during slow-wave sleep (SWS) and rapid eye movement sleep (REM).

Although we did not observe a dramatic reduction in activity during sleep, we found evidence that this activity could not support the same processing performed in awake. Neurons during SWS had limited responses to quiet sounds, but for loud sounds, SWS responses could actually exceed those during wakefulness. These observations suggested a model with reduced excitation and inhibition in SWS. The conclusion we reach is that the dynamic range (excitation+inhibition) of processing is limited even if extracellular activity (excitation-inhibition) is preserved in SWS. REM proved to have more awake-like levels of dynamic range.

Finally, we asked how sleep affects the population activities of neurons. Nearby neurons were modulated randomly, but the same pattern of modulation reappeared in subsequent sleep cycles. In other words, the network can reach at least three different stationary states, awake, SWS, and REM. Stronger joint firing was present in SWS locally between neurons and globally with field potentials, so network activities are more correlated in deep sleep than in awake or REM. The findings described herein provide a valuable neural basis for future investigations into the mechanistic and perceptual effects of sleep and for an eventual understanding of how the brain operates in this mysterious but essential state.

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## Chapter 1: Introduction

*'The psyche isolates itself during sleep (...) nevertheless we are not always awakened by the mere sensory force of the impression, but by the psychic relation of the same; an indifferent word does not arouse the sleeper, but if called by name he awakens...The mother awakens to the faintest sound from her child...hence the psyche differentiates sensations during sleep...we may be awakened by the lack of a sensory stimulus if it relates to the presentation of an important thing...the miller wakes when the mill stops.'*

-K. F. Burdach (1830) (excerpted from Oswald et al., 1960)

Most of what happens in sleep is outside of an individual's conscious access or control, making sleep mysterious even to the individual himself. This may be what fuels human curiosity to learn more about sleep. Unfortunately, our scientific understanding of sleep is still quite limited. Only a few basics are known, that sleep is essential and that the brain is critically involved. These are reasons enough to explore what happens during sleep. Especially interesting is what happens to sounds once they reach the brain. It is difficult to ask someone who is sleeping what they hear, but neurophysiological studies can probe neurons directly to extract out the representation of sounds in the sleeping brain.

## 1.1 Basics of sleep

### *1.1.1 Physiology of sleep*

From an evolutionary perspective, sleep seems to have diverged early on before the split between mammals and birds, so birds, cats, rodents, primates, and humans all share similar sleep patterns (Siegel, 2005). Fruit flies can even show signatures of mammalian sleep (Hendricks et al., 2000; Shaw et al., 2000). Cross-species similarity is important from a comparative perspective if one is to use experimental animals.

Typically, sleep is divided into five stages (Carskadon and Rechtschaffen, 2000). The most important of these states are light and deep slow-wave sleep (SWS) (stage 3 and stage 4 sleep) and rapid eye-movement sleep (REM) (stage 5). SWS is considered the deepest stage of sleep when subjects are least easily aroused. REM sleep is associated with dreaming (Siegel, 2001), and all mammals and birds go through REM (Siegel, 2005). So even animals can dream in the physiological sense. Stage 1 and stage 2 sleep are the earliest and lightest stages of sleep. Although stage 2 occupies the largest fraction of the sleep cycle (~50%) and manifests with signature sleep spindles, it is not as commonly studied as SWS and REM.

A human sleep cycle averages 90 minutes. In the beginning of the night, sleep cycles are short (60 minutes) and get longer as the night progresses. An episode of SWS always precedes an episode of REM. The duration of SWS and REM periods trade off over the course of a night. Initially, episodes of SWS are very long and slow-waves very large in amplitude while REM is very brief. But by the early morning, REM episodes

become very prolonged and dominate the sleep cycle. SWS is then minimal. Another point to make is that at the end of every cycle the sleeping subject wakes up, no matter how briefly, before returning to sleep. A small fraction (5-10%) of every sleep cycle is spent awake (Carskadon and Rechtschaffen, 2000).

Most animals used in prior sleep physiology experiments have been nocturnal such as rats, cats, and guinea pigs. The convenience of doing experiments during the day, low cost, and ready availability of nocturnal animals has made them the dominant animal model. However, if strides are to be made in understanding the phenomenology of human sleep, nonhuman primate models have to be more widely adapted in experiments. The common marmoset (*Callithrix jacchus*), which was used in the experiments of this thesis, has very similar patterns of sleep to humans except that the marmoset sleep cycle is slightly shorter (~60 minutes) (Crofts et al., 2001). Importantly, marmosets are diurnal, so they sleep during the dark hours of the day.

### *1.1.2 Theories of sleep*

What purpose sleep serves has long been a subject of speculation. Certainly, sleep serves restorative functions for the body (Siegel, 2005) but what about for the brain? Original ideas from the likes of Pavlov and Sherrington held that sleep serves no role in the brain, that the brain has no activity during sleep (Hobson, 2005). The discovery of EEG rhythms and regular alterations in brain activity along with the fact that brain metabolism only reduces by 20% during sleep overturned notions that the brain shuts down during sleep (Hobson, 2005). This realization may have created a problem of plenty as now a variety of equally plausible but unproven theories exist about the

function of sleep in the brain. One theory is that sleep is important for the learning and consolidation of the waking day's events (Stickgold, 2005). Alternatively, it has been argued on at least a couple occasions that sleep serves a housecleaning role, taking out the unwanted information experienced during the day (Crick and Mitchison, 1983; Tononi and Cirelli, 2006). One of these theories holds that long-term potentiation (LTP) of synapses during wakefulness upregulates cortical activity and that only through strong long-term depression (LTD) during SWS can synaptic homeostasis be achieved (Tononi and Cirelli, 2006). Some experimental evidence has been found in support of this theory (Vyazovskiy et al., 2008). Crick and Mitchison (1983) argued that the illogical nature of most dreams during REM sleep is for the purpose of removing spurious associations in the network. The difficulty with assessing these theories is how does one determine which correlations are functionally unnecessary and need to be removed. Most sleep theories tend to make high level claims that are conceptually and technically difficult to assess in an experimental setting. Perhaps the most exciting evidence of brain activities during sleep is the appearance of bursts of neuronal replay of sequences from awake experience (Wilson and McNaughton, 1994). But it is difficult to bridge this circumstantial evidence to a true understanding of sleep. The replay may confer a direct advantage to the network in a manner that would support learning or memory consolidation or it may be too sparse to link to any functional changes. It could be an epiphenomenon related to spurious correlations in the network (Tononi and Cirelli, 2006). This last possibility is seeming more and more unlikely given different patterns of replay such as forward replay that are related to specific events (Foster and Wilson,



2006). Nonetheless, since the days of Sherrington, it is not much clearer how sleep works, just that it does.

### *1.1.3 Functional role of sleep in the brain*

Research on sleep's role in learning and memory has seen a revived interest recently (Hobson, 2005; Vertes, 2004). A major finding has been that if subjects trained on a given task experience a night of sleep their performance improves the next day (Karni et al., 1994). No enhancement is seen if the subjects simply stay awake for the same amount of time. This finding has been replicated in a number of task settings whether in declarative (semantic) or non-declarative (procedural) tasks and across modalities, auditory, visual, and motor (Mandai et al., 1989; Walker et al., 2002; Walker et al., 2003; Fischer et al., 2003). Some hold that the data are not conclusive because of the confounding drawbacks of sleep deprivation. Benefits in the experimental sleep group may not be the benefits of sleep *per se* but of the adverse physiological effects of sleep deprivation in the control group (Vertes, 2004). Such issues have been mostly addressed (Stickgold, 2005).

A second line of evidence for a functional role of sleep has come from studies in the primary visual cortex (V1). Taking advantage of the robust phenomenon of critical period ocular dominance plasticity after monocular deprivation, Frank and colleagues have shown that sleep is critical to shifts in ocular dominance from the deprived eye to the nondeprived eye (Frank et al., 2001). They used the technique of intrinsic imaging to visualize patterns of activity on the cortical surface related to each eye. Normally, if one eye is shut, the maps shift so that the other eye becomes dominant. Sleep deprivation,

however, freezes any plastic processes. Going one step further they silenced activity in V1 during sleep with lidocaine. They found that ocular dominance plasticity did not take place even though the animals slept normally (Jha et al., 2005). In an independent and nearly simultaneous study, this time using tetrodotoxin to silence activity, Krahe et al. also found that neural activity in V1 during sleep is necessary for plasticity (Krahe et al., 2005). These results are among the first to establish a causal relationship between activity in sleep and post-sleep functional effects. Interestingly, Krahe et al. (2005) found that mRNA translation, an indicator of protein synthesis, did not seem to be involved. This observation suggests that plastic processes specifically depend on neural activity not protein synthesis during sleep. Patterned neural activity may lead to later changes in gene expression or protein synthesis as some have suggested (Ribeiro et al., 2004).

## **1.2 Hearing during sleep**

### *1.2.1 Auditory performance during sleep*

The ideas and experiments mentioned to this point have been concerned with internal activations of the brain. The focus of this thesis, however, is how the brain is activated by external events, namely sounds, during sleep. This may seem like a futile cause. After all, a hallmark of deep sleep is general behavioral unresponsiveness to events in the environment (Bonnet, 1982). Before the adaptation of the EEG, SWS was characterized by elevated acoustic arousal thresholds (Carskadon and Rechtschaffen,

2000). Other behavioral work, though, suggests that sounds can enter the brain during sleep, albeit subliminally (Oswald et al., 1960).

The studies that have measured arousal thresholds in humans have found that sounds are blocked quite impressively during sleep. In stage 2, REM, and SWS, 70, 83, and 92 dB SPL sounds, respectively, are required to awaken a subject (Bonnet et al., 1978). Arousal thresholds are usually highest in SWS (Bonnet, 1982). In some cases REM thresholds are considered to be near those of SWS and in others lower than even stage 2. Regardless, thresholds in SWS and REM are much higher than sound levels of conversational listening (50 dB) and border on those of a jackhammer or traffic from a major road driving through your bedroom.

Although most sounds seem to be ignored by a sleeping subject, a well-known fact is that subjects will selectively awaken to their own name. The study by Oswald, Taylor, and Treisman (1960) was the first to show this. They played 65 different names to sleeping subjects. One of the names was the subject's own. The authors found a tendency for subjects to react to their own name and not to others. Reaction was measured by the galvanic skin potential on the finger. Subjects could also awaken or squeeze the hand of the experimenter. In a control, forward names but not names played in reverse were found to elicit a skin potential (Oswald et al., 1960; Langford et al., 1974). This experiment helps prove that it is the semantic quality of the sound and not low-level differences in loudness or other acoustic properties that causes the subjects to respond. Somehow sleeping subjects are able to perform high-level discrimination of the sounds even though they are not aware of them. Other studies have replicated this finding by measuring blood flow to the finger or changes in heart rate when a sleeping

subject's name is played (Beh and Barrat, 1965; Langford et al. 1974; McDonald et al., 1975; Voss and Harsh, 1998). These findings raise the possibility that detection of certain, important sounds during sleep may be important for survival. For example, mothers need to awaken to the cries of their babies (Formby, 1967; Poitras et al., 1973). One has to consider that humans generally sleep in safe quarters. For other animals, safe sleep is rarely ensured (Zepelin, 2000). Quite possibly, hearing may serve a functional role, to alert the vulnerable sleeper of potential threats. A few studies have shown that animals will awaken to meaningful stimuli or stimuli that had gained meaning as opposed to insignificant stimuli (Halperin and Iorio, 1981; Rowland, 1957; Siegel & Langley, 1965; Van Twyver and Garrett, 1972; Maho and Hennevin, 1999).

### *1.2.2 Incorporation of sounds into dreams*

Subjects will sometimes report hearing external sounds in their dreams (Ramsey, 1953; Berger, 1963; Burton et al., 1988). A common occurrence is when one sleeps through their alarm clock but hears the alarm clock in their dreams, feeling all the while that the sound is strangely familiar. Dream reports are generally subjective limiting their scientific value. Some groups have indirectly probed the effects of external stimuli during REM by associating the sound with an unrelated task (Hars et al., 1985; Hennevin et al., 1995). In a second order conditioning paradigm, sound (conditioned stimulus) is paired with a shock (unconditioned stimulus) by playing the sound along with a prior conditioned tactile stimulus during sleep. In other words, the animal learns to associate a tactile stimulus with foot shock during the day. Then at night, a sound is paired with the tactile stimulus. The next day, the animal starts avoiding foot shock upon hearing the

auditory stimulus even though that sound had not been heard before by the animal when awake. Other studies in humans have shown that auditory stimulation during REM improves memory task performance (Guerrien et al., 1989) and logic task performance (Smith and Weeden, 1990). All of these studies have depended on associating the sensory stimulus with a behavioral meaning, so effects of nighttime stimulation are indirectly causing measurable changes through non-sensory pathways. The idea still remains though that sounds can enter into the brain and be processed during sleep.

### **1.3 State dependence of neural measures**

#### *1.3.1 Sleep modulation of single neurons in the visual and somatosensory system: thalamus and cortex*

The thalamus serves as an obligatory relay of signals from the sensory periphery to cortex (Guillery and Sherman, 2002). As a relay, the thalamus may be a good place to gate signals from reaching cortex. There is strong evidence that this may be the case in sleep. Intracellular and extracellular studies in the somatosensory and visual thalamus have consistently found that responses to external stimuli are depressed (Mukhametov and Rizzolatti, 1970; Coenen and Vendrik, 1972; Hirsch et al., 1983; Livingstone and Hubel, 1981; Mariotti et al., 1989; Edeline et al., 2000). During stage 2 sleep, 7-14Hz spindles inhibit thalamic neurons (Steriade, 2003). The transfer ratio in somatosensory thalamus (output/input) reduces to 0.5 not because of the lowering of input but because of a baseline shift making the input less effective (Coenen and Vendrik, 1972). An onset

response can be seen in somatosensory thalamus, but later portions of the response are quickly and strongly suppressed (Mariotti et al., 1989). In the visual thalamus, a baseline polarization of membrane potential is also seen during SWS (Hirsch et al., 1983), and extracellular recordings found weakened visually-evoked responses in 14 neurons during sleep (Livingstone and Hubel 1981).

When recordings are made downstream in primary sensory cortex, depressed responses are seen. Gucer (1979) found a 64% reduction in SWS responses in primary somatosensory cortex while Hubel and Livingstone (1981) found over one-third of the 130 cells recorded decreased their response in sleep and none increased their response. A peculiar observation, however, was that even nearby V1 cells showed very different effects of sleep. Hubel and Livingstone (1981) noted that this differed from their recordings of the thalamus where cells showed consistent behavior. Although no V1 cells increased firing in sleep, they seemed more active than those in thalamus which was counterintuitive. In the Gucer (1979) study, a puzzling observation was that responses were nearly abolished (9% of their awake strength) in REM sleep. Such a dramatic reduction in REM in addition to the reduction in SWS is difficult to explain since SWS is usually considered the deepest sleep. Regardless, the emergent theme from studies of sleep in the visual and somatosensory systems is that neural responses to external stimuli during sleep are strongly gated by the thalamus before they reach cortex. Based on these data, one would predict that a similar reduction of responses should occur in auditory cortex. The shutting down of the senses is interpreted as serving a “sleep-protecting” role (Hennevin et al. 2007). Blocking out sensory inputs as early as stage 1 sleep allows subjects to more easily enter deep sleep (Czich et al., 2002).

### *1.3.2 Imaging studies*

Imaging studies can provide a valuable bird's-eye view of activity during sleep. Since they are non-invasive, human subjects can be used. In a landmark PET imaging study of auditory responsiveness during sleep, Portas et al. (2000) made two interesting observations. The first was that activation in the temporal lobe was similar in non-REM (NREM, includes SWS) and awake whether insignificant (tones) or significant (subject's name) stimuli are used. This occurred despite lowered activation in thalamus and prefrontal cortex during NREM. Second, there was a large difference signal (name minus tone) in prefrontal cortex during sleep as if an alarm was triggered. Their study suggests that auditory cortical areas can be activated during sleep and that activity in higher areas during sleep depends on the complexity and/or significance of the stimulus.

In later fMRI work, Czich et al. (2002 and 2004) found a different result. They found that the BOLD signal response to sounds in the temporal lobe is weakened during sleep. Unfortunately, intersubject variability prevented Czich et al. from measuring the effect of sleep on thalamic responses. It is possible that thalamus is even more depressed than cortex which would then agree with Portas et al.'s (2000) findings of a more active auditory cortex. Another detail is that Czich et al. found the strongest depressive effects in stage 2 sleep not in SWS. Negative effects have been found as early as stage 1 (Tanaka et al., 2003). Finally, and most importantly, Czich et al. admit that scanner noise made interpretation of responses in primary auditory cortex difficult to interpret. For one thing, no positive BOLD signal was seen possibly because the background noise washed out effects of stimulation. Also, when the subjects went into stage 2 sleep, a BOLD

signal decrease (BSD) was seen. Negative BOLD signals have been seen in the primary visual cortex in response to acoustic stimulation during REM (Czich et al., 2002). Why the signal should decrease during stimulation is unclear (Harel et al., 2002). These caveats underscore the limits of functional imaging especially in interpreting results. If sleep has complex effects on neural responses, functional imaging may be too gross of a measure to capture changes in the network. Single-neuron physiology can be a valuable complement as spatial and temporal resolution are much finer. Imaging signals tend to correspond with the inputs to an area (Logothetis et al., 2001; Viswanathan and Freeman, 2007) since those require the most energy. Little can be said by imaging studies about the outputs which are carried in the spiking responses of neurons and represent the information that is sent forth.

Two other partly relevant imaging studies should be mentioned at least in passing. One study tested auditory responses in vegetative patients in hopes of better diagnosing the vegetative state (Laureys et al., 2000). They found that auditory activation in these patients was as strong as controls. Only in higher association areas was a difference apparent. In another study, this time in mildly sedated patients, it was again found that auditory areas in the human temporal lobe were weakly affected by sedation (Davis et al., 2007). Rather, activation to complex sounds (words as opposed to matched noise) was diminished in the frontal lobe. The biggest difference between awake and sedated conditions was found in the difference signal for high versus low ambiguity sentences. This signal was present in the frontal lobe only in awake subjects suggesting that a higher processing capacity was lost upon sedation. What this study (Davis et al., 2007) and the study of vegetative patients (Laureys et al., 2000) share with sleep studies is that they



involve subjects who are not consciously aware of the sounds being played. Caution should temper any direct comparisons between vegetative, sedated, and sleep states, but a consistent theme in all three is that higher faculties such as processing of complex sounds and corresponding responses in higher areas are affected more than primary auditory areas of the temporal lobe.

### *1.3.3 Evoked potential studies*

Much like functional imaging studies, evoked potential studies are a non-invasive means of recording correlates of neural activity in humans. The evoked potential is a grand-average waveform recorded from the scalp. Because it is heavily averaged, it contains components arising from multiple sources. The early component (<15ms) is thought to reflect low-level sensory activation while the late component (>50ms) reflects the higher processing of cortex such as assigning personal affect to the stimulus or determining its semantic consistency (Bastuji et al., 2002; Hennevin et al., 2002). Components can be as late as 300 or 400ms after the stimulus (called the P300 and P400 respectively). Three basic experiments have been used to assess how well sounds are processed in sleep: an oddball paradigm, own name versus others, and semantic congruency.

In an oddball paradigm, a stimulus is repeated, establishing an expectation that the stimulus will be played again. Then a novel, unexpected stimulus is played and a strong negative component called the mismatch negativity (MMN) is seen in the evoked potential. The MMN presumably arises in cortex and represents some additional quality of surprise assigned to the stimulus beyond its physical properties. Subjects in REM

sleep still manifest a weak MMN suggesting that the brain is keeping track of stimulus probabilities during this stage of sleep (Loewy et al., 1996; Nashida et al., 2000; Atienza et al., 2000). The MMN is not present in SWS (Sabri et al., 2003; Sabri and Campbell, 2005). A variation has been observed (Nielsen-Bohlman et al., 1991) but may be confounded by K-complexes (Sallinen et al., 1994; Cote, 2002).

The late P300 signal for own name presented in the context of other names is found in SWS and REM, albeit with different response latency and strength (Berlad and Pratt, 1995; Pratt et al., 1999). In one study, names were not presented with equal probability possibly confounding name recognition effects with MMN effects. A later study controlled for presentation probability and found specificity for the subject's name in REM sleep but did not test SWS (Perrin et al., 1999). The difficulty with using names to test for semantic processing is that they also have affective significance so that any specialized processing over non-names may not be semantic *per se*. When semantically congruent or incongruent pairs of words are used, an evoked potential signal can still be found in stage 2 and REM sleep (Brualla, 1998). However, when a third condition is added, that of non-words which are composed of syllables sharing the acoustic properties of words but having no meaning, a separate signal was elicited in waking, but no differential signal was found in stage 2 sleep or REM (Perrin et al., 2002). SWS was not tested. This result suggests that not all semantic processing may be possible during sleep. But the glass can be viewed as half-full rather than half-empty. The imaging and evoked potential studies discussed so far tend to find evidence that much of basic sensory processing is possible in sleep. This is a very different picture than single-neuron studies suggesting stimuli are blocked by the thalamus during sleep.

#### *1.3.4 Anesthetic effects in the auditory system*

Before discussing the effects of sleep on auditory neurons, it is important to note the more well-studied effects of anesthetics. Although anesthetics represent an artificial state and sleep a natural state, the effects of anesthetics have received a disproportionate amount of attention in auditory physiology studies. This is because anesthetics are central to the experimental preparations of many labs. Unfortunately for audition, anesthetics seem to have large effects on responses even early in the auditory pathway. In the dorsal cochlear nucleus, experiments using anesthetics essentially missed the critical type IV cell type (Evans and Nelson, 1973; Young and Brownell, 1976). Further downstream, studies have repeatedly shown the effects of anesthetics (Ter-Mikaelian et al., 2007; Moshitch et al., 2006). For example, in anesthetized auditory cortex, sustained responses to acoustic stimuli are rarely observed (Phillips, 1985; DeWeese et al., 2003). Most neurons respond with an onset to sound and then go silent. Awake preparations commonly encounter sustained responses and rarely find onset responses (Brugge and Merzenich, 1973; Wang et al., 2005; Hromadka et al., 2008). Recently, a push has been made to use different types of anesthetics that have milder effects on neural responses. Various gas anesthetics or ketamine have been used with some success (Moshitch et al., 2006).

Like sleep, the anesthetized state involves a lack of conscious awareness. A synchronized UP-state with low frequency oscillations exists under anesthesia (Worgotter et al., 1998). Such an EEG pattern is similar to that in SWS. The non-synchronized DOWN-state under anesthesia involves higher frequency  $\beta$  much like the awake state (Lydic and Baghdoyan, 2005). Despite these similarities, the argument has been made

that sleep should not be compared with anesthesia (Hennevin et al., 2007). Studies directly comparing responses during sleep and anesthesia have not found similarity between the two (Kishikawa et al., 1995; Torterolo et al., 2002; Cotillon-Williams and Edeline 2003). Another simple fact that immediately discounts comparison between sleep and anesthesia is that REM is a unique state with no anesthetic equivalent.

#### *1.3.5 Attentional modulation of neural responses: visual and somatosensory system*

The attentional state may be more comparable to sleep. Sleep could be viewed as the withdrawal of attention. If this is the case, then we would expect the exact opposite effects of attention during sleep. This hypothesis may be too simple as it assumes sleep is nothing but a gradual loss of arousal and assumes that the same top-down circuits attentions uses are also involved in sleep. In reality, sleep may involve more states, local circuits, and different neuromodulatory influences than attention.

A rich literature exists on the effects of attention in the visual system. Attention affects neural responses throughout the visual pathway from V1 to MT and IT (Ito and Gilbert, 1999; McAdams and Maunsell, 1999; Reynolds et al., 2000). The visual thalamus (LGN) can be modulated by attention (O'Connor et al., 2002) although it is unclear whether this is the result of feedback from V1. Attention effects are greater in higher visual areas like V4 than they are in V1 (Pessoa et al., 2003). Effects tend to be in one direction, that is enhancing, changing responses in a majority of cells (>50% up to 80%) (McAdams and Maunsell, 1999). A simple model for attention is a multiplicative gain change rather than a simple additive boost. However, the finding that attention effects manifest more strongly at low stimulus contrasts has modified this model to

include saturation. And this has led some to propose that attention is more like an enhancement of stimulus contrast (Reynolds and Chelazzi, 2004).

Another recurring idea in the attention literature is that attention is meant to operate in challenging situations where there are multiple stimuli or in binding of features across space (Desimone and Duncan, 1995). The idea of an attentional bottleneck posits that only a few stimuli can be attended to, perceived, and thus acted on. Studies in V2 and V4 have shown how this may operate at the neural level (Reynolds et al., 1999). When two stimuli are in a cell's receptive field, attention plays a role in biasing the competition toward the attended stimulus and filtering out competing interactions of a second stimulus. In work by Gilbert and colleagues, it was shown that although attention effects in V1 are weaker than in higher areas, attention strongly modulates contextual influences outside the classical receptive field (Ito and Gilbert, 1999). Attention seems to aid the difficult integration required in collinear interactions of disparate line elements. Integrating such elements is necessary for grouping and for forming contours which later go on to be an important part of visual form processing.

Some insights can be drawn from the large body of attention literature. For one thing, an emphasis should be placed on the notion of processing. Attention is not simply a volume knob on activity. Attention aids processing for near threshold image contrasts (Reynolds et al., 2000), for multiple competing objects (Desimone and Duncan, 1995; Reynolds et al., 1999), and for forming complex feature combinations (Ito and Gilbert, 1999; Motter, 1994). In studying auditory processing during sleep, it will be important to consider that processing beyond simple changes in activity may be affected. Also, the attention work seems to suggest that higher areas are more affected and that the effect

trickles down to lower areas. The closer an area is to top-down feedback the more immediate and strong the impact is. This theme has been repeated in the somatosensory system (Hsiao et al., 1993) and in studies exploring perceptual learning effects on neurons (Yang and Maunsell, 2004). For sleep, if a top-down effect is at play then it will be important to examine higher areas and compare responses to lower areas.

#### *1.3.6 Sleep modulation in the auditory system: brainstem*

The auditory signal passes through an extensive subcortical pathway before reaching cortex. The signal could be attenuated at the cochlear nucleus, olivary complex, or the inferior colliculus. The middle ear muscles could also attenuate the sound even before it is transduced in the cochlea. However, it appears that the middle ear muscles do not operate in this capacity during sleep (Baust et al., 1964; Dewson et al., 1965). In the cochlear nucleus, responses during both SWS and REM are somewhat greater than those in awake (Pena et al., 1992). Downstream neurons in the lateral superior olive vary with some increasing and some decreasing their response (Pedemonte et al., 1994). Finally, in the inferior colliculus, the requisite last station before the auditory thalamus, responses are as strong in SWS and REM as in awake (Tortero et al., 2002). All indications are that neurons in the auditory brainstem are active during sleep, and the influence of sleep probably arises at the level of thalamus or beyond.

#### *1.3.7 Sleep modulation in the auditory system: thalamus and cortex*

One might expect that sounds reach the auditory part of the thalamus where they would get blocked and go no further. This appears to be the case for the visual and

somatosensory systems based on physiology data. But given the imaging and evoked potential data in the auditory system, one might expect some sounds to reach auditory cortex. Murata and Kameda (1963) provided the first report of the effects of sleep on neural responses in auditory cortex. In the 20 neurons that they studied in unanesthetized cats, they observed 11 whose responses decreased in SWS and none whose responses increased. Brugge and Merzenich (1973) in unanesthetized primates mentioned in passing having observed cells decrease their responses when the animal fell asleep. The exact number of cells is not reported. Over 20 years elapsed before a more complete study was performed by Pena et al. (1999) in the guinea pig. Their results were the first surprise in neural studies of sensory processing during sleep. They found that most neurons did not change their responses in SWS or REM, and the ones that did could either increase or decrease their responses. This led them to conclude that activity at the neural level was mostly preserved in sleep. Following this study, Edeline and colleagues reported their results from the auditory thalamus and cortex of guinea pigs. The thalamus data showed clear suppression of responses. 70% of cells in SWS and 68% in REM were significantly suppressed (Edeline et al., 2000). Average evoked activity in awake was  $15.6 \text{ spikes s}^{-1}$  and dropped to nearly half in sleep (SWS=7.5 and REM=8.8  $\text{spikes s}^{-1}$ ). When the authors measured responses in A1, they found that cells were usually depressed in their responses. But, to the authors' surprise, their cortical population of cells showed statistically more activity than the thalamic cells that they recorded (Edeline et al., 2001). Mean evoked activity in SWS ( $12 \text{ spikes s}^{-1}$ ) was not much lower than mean activity in awake ( $15 \text{ spikes s}^{-1}$ ). Furthermore, there was more diversity in modulation so that some

cells actually increased their response in SWS. This study also found that receptive field size as measured by frequency tuning width did not change in SWS.

## 1.4 Outline

### *1.4.1 Hypotheses*

Having summarized the literature, it may be important to list the hypotheses that have emerged in this review. These hypotheses are by no means mutually exclusive, and no one study could decide between them. But taken together they represent the ideas entertained in the remainder of this thesis.

**Attention Hypothesis.** Sleep is a withdrawal of attention. Specific predictions: (1) Neurons will show a gradual reduction of activity as sleep becomes deeper and deeper. (2) Little difference should exist between responses in SWS and REM since both are states of inattention. (3) Neurons strongly modulated in an attention task when the animal is awake should have a tendency to be the most modulated neurons when the animal falls asleep. (4) Effects of sleep should be strongest at quiet sound levels such that responses to quiet sounds are most suppressed during sleep; effects at louder levels should not be as strongly suppressed. (5) Secondary cortical areas should be more suppressed than primary areas. (6) Multiplicative as opposed to additive changes in firing rate should be observed. (7) Neurons should be uniformly depressed but may display local variations similar to those observed in attention studies; rarely should enhanced responses in sleep be observed.



**Thalamic Gate Hypothesis.** Thalamus prevents sensory signals from reaching cortex. Specific predictions: (1) Responses in cortex should be suppressed as much or more than those in thalamus. (2) Because of common inputs from thalamus, nearby cells should behave very similarly when the animal falls asleep. (3) Latency of sleep effects should be immediate and not depend on later cortical processing (i.e. observable in early and late components of response). (4) Responses in secondary cortical areas should not differ much from those in primary areas since thalamus is the bottleneck.

**Mentation Hypothesis.** Internal activations during sleep control responses to external stimuli. Specific predictions: (1) Cortex should be quite active during sleep whether in internal activity or responses to external sounds. (2) Responses to sounds in cortex should correlate with the presence of different brain rhythms in the EEG. (3) Responses should vary on the same timescale that internal activities vary. This could be in the switch from SWS to REM or could be over the course of the night from one cycle to the next or from one night to the next. (4) High spontaneous activity may predict periods of high stimulus-driven activity. (5) Local patterns may be observed such that nearby cells are activated or deactivated together. (6) Overall activity during sleep should be similar in primary and secondary areas. (7) Effects should be independent of the type of stimulus used.

**Vigilance Hypothesis.** Hearing operates during sleep as an alerting mechanism. Specific predictions: (1) Some auditory processing capacity and its associated pathway should be preserved or even enhanced during sleep. (2) Specific sounds should be more effectively processed than others. (3) Animal vocalizations may activate the network more efficiently than artificial sounds such as pure tones. (4) Responses in higher areas

may become increasingly sparse so that information about a select few sounds is passed further downstream. (5) Selectivity or tuning along certain dimensions and not others should be modified to meet the functional goal of the system. (6) Responses should be stable from one cycle to the next and one night to the next. In other words, a certain set of neurons performs the alerting function. (7) The signal may be processed up through the decision making areas of the frontal lobe. Rather than conserve energy by attenuating the signal early, the auditory system under this hypothesis derives maximal information about acoustic events in the environment for the sleeper's protection.

#### *1.4.2 Specific aims*

The work in this thesis divides into 3 parts. The first part simply measures how responsive neurons in auditory cortex are during sleep. The second part delves into the tuning properties of these neurons during sleep. And the third part examines network behaviors of neurons in sleep.

**Aim 1.** Only a handful of studies have examined sensory processing during sleep. In fact, almost 30 years have elapsed since the last neurophysiology study in primates. From the literature described above, even the most basic facts are unclear. Do cortical neurons respond to sounds during sleep? We decided to thoroughly explore this question in this aim by extending on previous studies in three ways. First, we recorded in a non-primary cortical area. To our knowledge, no other study to date has quantitatively measured sensory responses outside of a primary cortical area during sleep. Second, we attempted to measure awake responses under different levels of arousal. One confound of all sleep studies is that the 'awake' condition is taken simply as the period when

animals passively awaken between sleep cycles. This may not constitute sufficient arousal, confounding estimates of how much stronger awake responses really are. Finally, we performed a quantitative characterization of temporal discharge patterns in sleep. Prior studies have mainly focused on changes in discharge rate; however, the temporal code could change in sleep without affecting firing rates.

**Aim 2.** In aim 2, we ask more specific questions about sensory processing. What processing are neurons capable of in sleep? Only one study to date has attempted to approach this question by measuring frequency tuning in awake and sleep states finding little difference. Here, we extend on previous work in three ways. First, we measured the effects of sleep on driven and suppressed responses in order to characterize the balance of excitation and inhibition in the network. Second, we measured the effects of sleep at different sound levels from very quiet to very loud. By varying sound level, we could engage a spectrum of excitatory and inhibitory processing and determine sleep's effects. Third, we measured temporal processing in neurons during sleep through modulation tuning and phase-locking for modulated stimuli.

**Aim 3.** Most studies of sensory processing during sleep have focused on single neurons, but sleep presumably changes network-level properties. We made two basic measurements to gain insight into the organization of the network during sleep. First, we measured activity from local pairs of neurons ( $<200\mu$  apart), often recorded simultaneously. We examined whether any organization of sleep modulation exists at this fine scale. We measured rate correlations between neurons in awake, SWS, and REM to see if functional connectivity breaks down or is enhanced in sleep. Second, we measured the stimulus-driven local field potential (LFP) during sleep. The LFP measures

locally organized synaptic activity and may be a window to coordinated changes among neurons. We measured the effects of sleep on the LFP alone and on the correlation between the LFP and single neurons across different LFP frequency bands.

## **Chapter 2: General Methods**

### **2.1 Animal preparation**

Naïve marmosets were adapted to sit quietly in a primate restraint chair over a period of two weeks. A surgery was then performed under sterile conditions to implant two stainless steel headposts held by dental acrylic to the skull. Details of this standard implant surgery can be found elsewhere (Lu et al., 2001). Post-surgery, a two-week recovery period ensued. During the first week, animals were put on a cautionary antibiotic regime to prevent risk of infection. The animal's wounds were dressed on a daily basis and monitored for signs of healing, and the animal's diet was supplemented until appetite returned. Approximately one month after surgery, animals were fully recovered.

Neural recordings were performed during the daytime in the first month of experiments. Animals quickly adapted to this daily routine and sat quietly in the chair for a period of up to 6 hours. A flat-tipped 1mm stainless steel screw was implanted into the caudal aspect of the temporal lobe near the junction of the temporal, parietal, and occipital lobes for measuring the electroencephalogram (EEG). This placement is optimized for detecting delta (.7-4.2 Hz) and alpha (7-12 Hz) activity but not for sleep spindles. The signal was referenced to a ground screw located in frontal cortex (implanted during surgery). The EEG screw was implanted by performing a 1 mm

diameter craniotomy and accurately lowering the screw so that it just impinges on the dural surface, providing good signal contact.

We then switched recordings to the night hours (8pm to 4am). This was done because we had little success with marmosets falling asleep during daytime sessions. Although animals would close their eyes, they only became drowsy during the day. Signatures of SWS or REM rarely would appear, only the alpha rhythms of relaxed inattentiveness. Taking after Gucer (1979), who performed sleep experiments with macaque monkeys from 6pm to 6am, we found that marmosets slept much more naturally at night. Sleep was deeper, more consistent, and had continuity during night recordings. Animals slept in the chair while sounds were played, passing through 5-10 sleep cycles in a predictable rhythmic fashion. Much like Gucer (1979), we found that animals adapted to sleeping in the chair within a few sessions (< 1 week).

## **2.2 Physiological recordings**

### *2.2.1 Neural recordings*

Extracellular action potentials were recorded using 2-5 M $\Omega$  tungsten microelectrodes (A-M Systems). An online spike-sorting algorithm used template-matching to sort spikes into single units (MSD, Alpha-Omega Engineering) (Worgotter et al., 1986). Sorted single-units were generally well-isolated. In practice, signal-to-noise ratios ( $20 \cdot \log_{10}(V_{\text{peak-to-peak}}/\sigma_{\text{noise}})$ ) where units could be comfortably sorted online were >10dB. However, the majority of units had SNRs >20dB since this made them much

easier to work with especially over the long periods of time that sleep recordings required (Fig. 2.1A). An oil-based hydraulic microdrive (Trent-Wells) was used to advance the electrode slowly forward stopping every 50 microns for at least a few minutes. Units were searched for solely based on their spontaneous firing so as not to bias our sample of neurons toward those that are only driven in sleep (animals were asleep during a majority of the time that we recorded). Since we did not pass up on any units encountered, most recordings are likely from the supragranular layers, but, using our methods, we cannot be certain of laminar location.

Units used in our data set were held for an average of 90 minutes (median=76 minutes) (Fig. 2.1B). The lack of movement when animals were sleeping contributed to our stable recordings. The period during which many units were lost was right after REM when the animal awakened and regained muscle tone. Because our testing required that we wait for the animal to cycle through different sleep stages, many units could not be held long enough. In fact, we recorded 1218 total units from 5 hemispheres of 4 animals but could use data from only 493 units. Of the remainder, some could not be driven, but most were simply not held long enough (Fig. 2.1B).

### *2.2.2 EEG and LFP recordings*

This study was the first in our lab to combine electroencephalogram (EEG) and local field potential (LFP) recordings with neural recordings. The EEG was measured from the EEG screw and was filtered from 1-500Hz and notch filtered at 60Hz using an A-M Systems model 1800 differential amplifier. The LFP was simply the low frequency components taken from the microelectrode signal. It was filtered from 1-300Hz using a

programmable filter. Both the EEG and LFP were amplified 10,000x and digitized at 1kHz sampling rate. The EEG was recorded in all four animals (2p, 41o, 43q, and 16s), but the LFP was only adopted for the last two animals used (43q and 16s). The EEG is usually considered in four frequency bands: delta (.7-4.2 Hz), theta (4.2-7.5 Hz), alpha (7.5-12 Hz), and beta (12-20 Hz). The EEG was monitored on a digital oscilloscope with grid spacing along the abscissa (time) of 500ms and grid spacing of 1V on the ordinate (amplitude). Grids were roughly 1 cm tall and wide so that we were monitoring 2 cycles/cm. Standard EEG paper uses 1 cycle/cm. In general, EEG amplitude was contained within two boxes (2V peak-to-peak). During SWS, amplitude would increase beyond two boxes and up to four (2-4V peak-to-peak, generally 3V). Later, we found that recording the EEG on a separate screw may not be necessary as the EEG in the electrode signal was sufficiently similar to the EEG measured from the screw. The EEG screw did provide a better solution though for our specialized needs since it was stable from day to day and was less noisy than the EEG measured using our high-impedance microelectrodes.

## **2.3 Behavioral assessment**

### *2.3.1 Video monitoring*

In lieu of an electromyogram (EMG), we used video monitoring of the animals to assess their movements. Low-light CCD cameras fitted with high magnification zoom lenses and sensitive in the infrared range were used to monitor details of the face and tail.



By closely monitoring the face, we could monitor eye openings, twitches of face muscles, and smooth movements of face muscles. The tail camera was only used in the last two monkeys after we realized that the tail uncurls and drops when muscle tone is lost in REM (marmosets will curl their tail in the sleeping position). Originally, we had planned on measuring the EMG to monitor muscle tone, but information from the face and tail cameras in combination with the EEG was sufficient to accurately classify different behavioral states.

### *2.3.2 Behavioral measures*

A number of behavioral measures were manually noted according to the following code on each repetition of a stimulus set:

**0 – ignore** (trial to be ignored because the unit was suddenly lost, transient noise entered the signal, or quality of recording compromised in some way)

**1 - awake eo** (animal is awake with eyes open)

**2 - awake ec** (animal is awake but eyes are closed)

**3 - light SWS**

**4 – SWS**

**5 - REM**

**6 - A to SWS** (transition from awake to SWS, later replaced by 21)

**7 - A to REM** (animal woke up before completing REM and is going back into REM)

**8 - SWS to REM** (period when animal comes out of deep sleep passing through stage 2 and stage 1 before entering REM)

**9 - SWS to awake** (animal starts moving in SWS or shows signs of interrupted sleep)

- 10 – transition** (period during which difficult to tell what state animal is in or is going to)
- 11 - opened door** (experimenter opened the door to arouse animal before a repetition)
- 12 - opened door + loud sound** (experimenter opened the door and made loud sounds, generally by clapping or jingling keys)
- 13 - entered chamber** (experimenter enters chamber and walks in front of animal so that animal sees experimenter)
- 14 - entered chamber + loud sound** (experimenter goes in chamber in front of animal and makes loud sounds to get the animal's full attention)
- 15 – airpuff** (an airpuff is delivered to the face through a plastic tube running into the chamber; this form of arousal was not often used)
- 16 – lights** (lights turned on to arouse the animal)
- 17 - touched tail** (experimenter goes in chamber and touches the animal's tail which usually elicits a quick withdrawal response)
- 18 – fed** (experimenter goes in chamber and feeds animal liquid reward)
- 19 - loud sound** (experimenter plays a sound louder than normally heard through the speaker)
- 20 - not monitoring** (in some cases, the experimenter could not monitor the animal or was not scoring the animal's state for that session)
- 21 - stage 1 sleep**
- 22 - stage 2 sleep**
- 23 - awake transition** (animals would often awaken briefly and then become relaxed and inattentive; this code was used to describe this passively awake condition which was later combined with codes 1 and 2 as part of the awake state)

**31 - large theta** (not used, was supposed to denote presence of large amplitude theta activity)

**32 – spindles** (not used, was supposed to denote the seconds long 7-14Hz spindles that occur during stage 2 sleep)

**33 - K-complex** (not used, was supposed to denote the negative K-complexes observed during stage 2-4 sleep)

**34 – motion/motion artifact** (animal movements that were detected on the video cameras or that generated myogenic potentials on the EEG trace were noted using this code; these included lifting of the brow, chewing, sticking out the tongue, movements of the ear, and scrunching/stretching of the face)

**35 - face/tail twitch** (twitches of the face and tail characteristic of REM)

**36 - eye/eyelid twitch** (twitches of the eyelid or underlying eye presumably from rapid eye movements)

**37 - tail drop** (uncurling and dropping of the tail during onset of REM)

**38 - tail up** (picking up of tail back off the platform at REM offset when the animal wakes up, often followed by face movements)

**39 - tail lowered** (tail slowly lowering as muscle tone is gradually lost across multiple trials before entering REM)

**41 - interrupted sleep** (usually we allowed animals to awaken on their own; in rare instances, often as a control, we would awaken the animal in the middle of a sleep cycle)

**51-60 - awake episodes #'s 1-10**

**61-70 - stage1/2 episode #'s 1-10**

**71-80 - SWS episode #'s 1-10**

## 81-90 - REM episode #'s 1-10

### 2.3.3 Sleep scoring

Both EEG and video monitoring were used to assess an animal's behavioral state. In modern day sleep research, automated methods are increasingly adopted. We considered automating our sleep scoring process and started with a semi-automated process that could be verified offline. Eventually we found that classification of the sequence of SWS and REM was relatively straightforward using visual monitoring. So all scoring was done manually online. Three major behavioral states were readily identified using standard human sleep scoring techniques (Carskadon and Rechtschaffen, 2000): **Awake** - eye openings, myogenic potentials in the EEG during facial movements, and energy in all EEG frequency bands including the  $\beta$  band; **SWS** - large amplitude, low frequency activity in the  $\delta$  range, and no energy in  $\beta$  band; and **REM** - low amplitude, almost sinusoidal, high frequency ( $\alpha$  and  $\beta$ ) EEG, and twitching of face muscles but no myogenic potentials on EEG trace. In two of the animals, upon loss of muscle tone at REM onset the tail uncurled and dropped, and REM offset coincided with lifting of the tail back into a tucked position, presumably from recovery of muscle tone. For forced awakenings, we actively awakened the animal by playing loud sounds, turning the lights on, and entering the chamber to increase awake arousal. As a note, we found that REM was salient and easy to classify although we had initially not planned on studying REM since it occupies such a brief part of sleep. Technically, the REM EEG is similar to that in awake and should not be used to distinguish REM from awake according to standard practice. But we found that one could appreciate the difference with some experience.

The EEG in REM was devoid of muscle artifacts, not as low frequency in nature, and occasionally reached periods where the waveform became low amplitude and was sinusoidal because of its clean narrowband frequency content. The REM EEG was not always reliable, but combined with observable face twitches and tail drops and the sudden onsets and offsets typical of REM, REM was most clearly defined unlike SWS which has smoother transitions or awake which involves an unclear mixture of arousal and drowsiness.

## **2.4 Event Windowing Algorithm**

A novel methodological contribution of this thesis is the development of an automated algorithm that detects events in the spike record. This algorithm is widely applicable to any spiking data, and its simplicity and ease-of-use make it adoptable by others. The reason I decided to develop the algorithm was that choosing a time window for calculating mean discharge rate should not be arbitrary. During experiments, I found myself constantly dragging the analysis window around to capture driven neural firing. Sometimes the neuron had an onset response and then sometimes an offset. The only solution was to use a window wide enough to capture all responses, onsets, sustained, offset, and persistent, but how long is long enough and what if there is intervening inhibition or you average out any significant firing by making the window too long? How do you compare across stimuli that elicit different response types? On the conceptual side, I was also interested in what a ‘good’ cortical response is. Certainly, it

is nice to achieve a sustained response, but these can be weak and scattered compared to tight response bursts. Given the rapid temporal nature of auditory processing, sharp but strong responses can be useful. Using a standard window size does not allow separation of these response types and penalizes short events by averaging over a long window.

It seemed to me from visual inspection that a simple algorithm should be able to capture the multifarious events in the record from short to long, strong to weak. The events seemed discrete, and it might be important to capture them since they might arise from different sources or for different mechanistic reasons. It was harder than it appeared. Before presenting the method that worked, I present some sketches of ideas that I tried.

#### *2.4.1 Algorithms attempted*

**Edge detection.** In vision, a bounded object is defined by its edges. This is no different for 1-D PSTH's. In principle, an event lies between a high positive slope followed by a high negative slope. In practice, edge detection is hard because it is a multiscale problem and is susceptible to noise (derivatives are highpass and magnify noise which tends to contain high frequencies). I ran into typical problems edge detection algorithms face when processing images. How do you distinguish rapid, high frequency noise from an edge? How do you tell extended, slow rising contours of a histogram using only local derivatives?

**Triangle (Geometric) Method.** I thought rather than use the edges I could use the peaks. The rise and fall of an event should be centered about a peak. This could be captured using triangles. The idea was to draw triangle from every peak (values crossing

a pre-determined threshold) down to the nearest time when baseline was reached. After all such triangles were found, I would choose from them in an intelligent way to know where the events were. If triangles overlapped, this was the result of the same event or local maxima, and they were combined. This method makes the most sense but ran into two problems. The first was how you determine the peaks. A peak is best determined in the context of slopes, and throwing that information away made peak detection arbitrary. The second problem was in determining baseline points. If two events are slightly merged, then firing need not return to zero. Still, these were practical not theoretical obstacles. Where to set the peak and baseline thresholds could be empirically determined. The triangle method was closest in spirit to the method that was finally adopted which used a more iterative approach.

**Wavelet.** If there are discrete events in the spike record, and they are local in time then a wavelet transform may be the appropriate way to find independent events. A Fourier transform would extract components of different frequencies but most events are finite in time and have edges that lead to a broad splatter of frequency. The problem with wavelet methods was really in choosing the appropriate scale. There is a scale by number trade-off. At a long time scale, two events are treated as one. Certainly, two events would also be returned at a shorter timescale, but then arbitrary criteria would have to be used to prune the tree and determine what was the appropriate number and scale of events. By being a complete basis agnostic to these issues, the wavelet was returning everything but missing something.

**Window growing.** In edge detection methods, the window is determined from the outside-in (find edges then fill in interior). The opposite is to build inside-out (find

the center and then build out until no more mass accumulates). In this method, a significant bin is found then pieces are added to the left or right until the overall window stops being strongly significant. This method is simple but powerful. In this recursive method, the important timescales emerge on their own. As an event builds, if it is a long event, it will keep accumulating small pieces. If it is a fast strong event, then it will end quickly after the seed window. Evidence accumulates in favor of one type of window or another. By taking care of time scale, the number of windows that need to be detected falls out since all windows are individually found and are considered non-overlapping.

**Re-inventing the wheel.** As with many things in science, somebody has probably already done it before. The algorithm I settled on was basically a modification of the one developed by Legendy and Salcman (1985) and used by others (Hanes et al., 1995; Sheinberg and Logothetis, 2001). The only difference was that they applied their algorithm to single trial responses whereas mine applied to responses averaged from multiple trials. The advantage is that with multiple trials events are more statistically significant. Legendy and Salcman were trying to detect bursts in single trials, so they used Poisson statistics for spike counts. We used Gaussian statistics since we could not only derive a spike count but derive the standard deviation about the mean count (Poisson assumes that the standard deviation always equals the mean). Considering individual trials in this way is more statistically powerful since the estimate of mean rate improves as  $\sqrt{n}$ . Simply summing spike counts across all the trials and assuming a Poisson process would not allow for improving estimation accuracy with the number of trials. Later, we adapted our algorithm to detect suppression in the spike record. This is a novel



extension of the methods of Legendy and Salcman and proved to be a difficult problem in and of itself. The main difficulty with suppression is that cortical spontaneous rates are low. Statistical distributions of firing rates near spontaneous are highly non-Gaussian in that they have long tails (more Poisson-like) and many trials falling in the 0 spikes bin (all-or-nothing nature of spiking non-linearity is binary-like). We found that it was necessary to return to a Poisson assumption of spike counts, to lengthen our windows to include more possible spikes, and to perform permutation tests to supplement the Poisson assumption. In general, the algorithm would not detect windows unless spontaneous rates were high enough to reach significant values of suppression. This biases the algorithm in favor of higher spontaneous rate neurons. Overall, the excitatory and inhibitory algorithms were successful, working under a variety of conditions, for neurons with low or high driven and background firing rates, for small or large numbers of trials, and for long-lasting or brief events. Some example windows detected by the algorithm are shown in Figure 2.2, and the specific algorithm is given below.

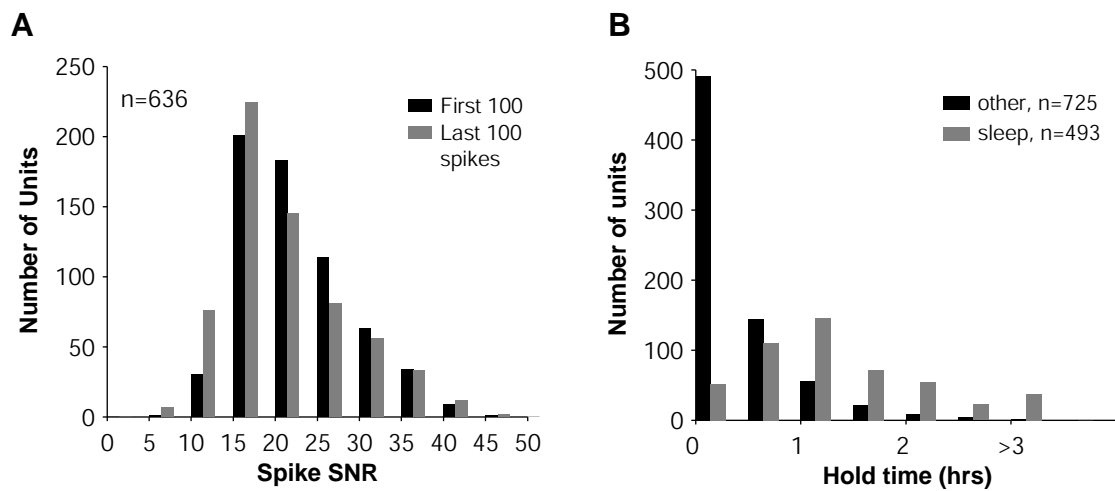
#### *2.4.2 Algorithm: driven responses*

The algorithm starts with a seed window of 30ms that grows in either the left or right direction in 30ms segments. To determine when to stop expanding the window, the algorithm uses thresholding with hysteresis. Window segments are added as long as they meet a soft criterion ( $p_{\text{soft}} < 0.1$ ) and the whole window meets a hard criterion ( $p_{\text{hard}} < 0.01$ ,  $3 \times \text{sem}$  above spontaneous rate). Gaussian statistics are assumed for estimates of mean firing rate in a particular analysis window. The mean and standard error of the mean are computed and compared to spontaneous firing to determine a p-value for a given

window. The windows turned up by the algorithm were only used as guides in data analysis. For the final excitatory window firing rate is required to be 4\*sem above spontaneous rate ( $p < 10^{-4}$ ).

#### *2.4.3 Algorithm: suppressed responses*

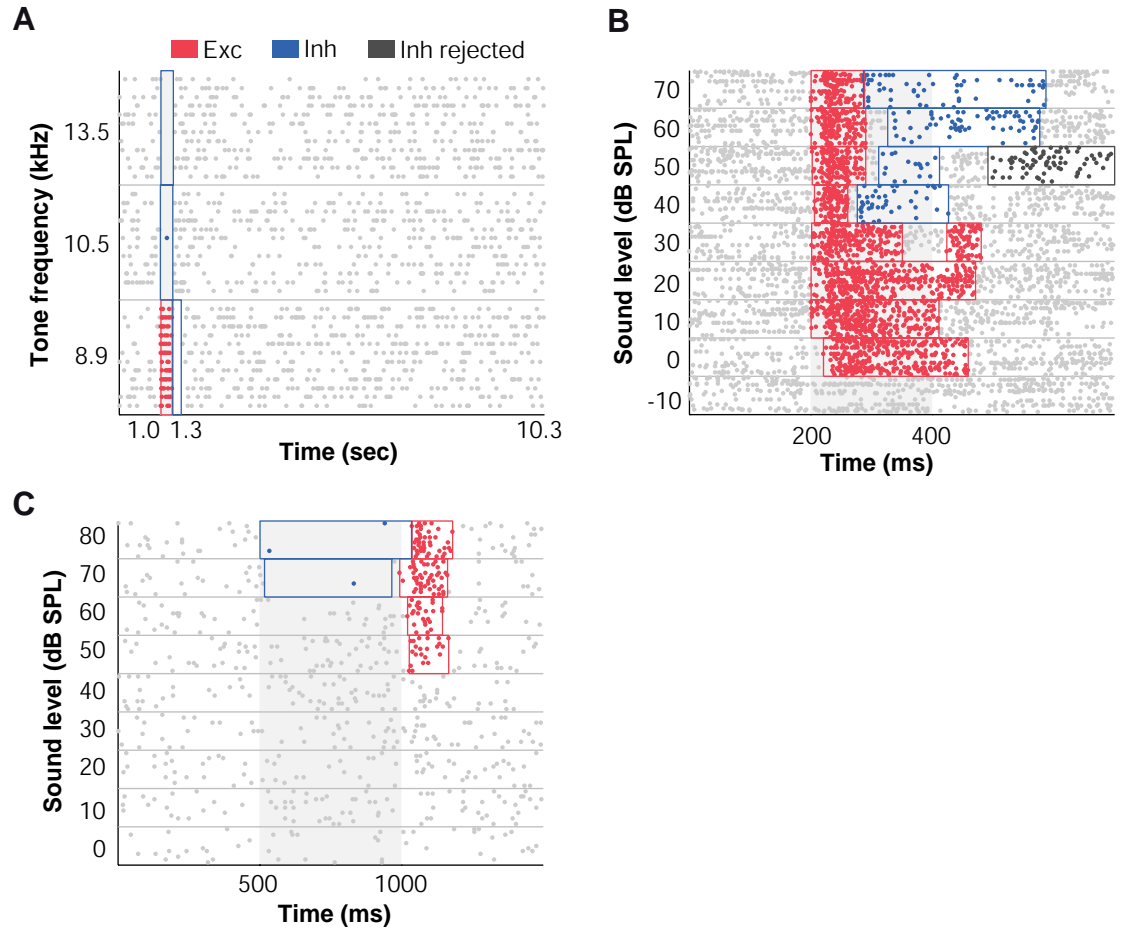
For detecting inhibitory windows, we required spontaneous firing to be  $> 1.5$  spikes  $s^{-1}$  and used: seed window=100ms, chunk size=50ms,  $p_{\text{soft}}=.02$ , and  $p_{\text{hard}}=.00005$ . Like in the excitatory algorithm, chunks were added to the inhibitory window as long as  $p_{\text{soft}}$  and  $p_{\text{hard}}$  were met, or, as was often the case since p-values rarely started below the hard criterion, we allowed the algorithm to continue as long as the p-value decreased toward the hard criterion. We assumed Poisson statistics to compute p-values from spike counts since low firing rates resulted in non-Gaussian distributions. Even so, responses often deviated from our Poisson assumption because of an unusual number of 0 spike trials, so we performed a post-hoc permutation test for the means at the  $p < .01$  level (Rice, 1995). Although the excitatory and inhibitory algorithms were run independently, they usually yielded non-overlapping events, which is critical to the assumption that these events represent different processes.



**Figure 2.1.** Single-unit SNR and hold time statistics.

(a) Signal-to-noise ratios ( $SNR = 20 \cdot \log_{10}(V_{pp}/s)$ ) for units ( $n=636$ ) where spike waveform was digitized and stored. One concern may be that isolation quality decreased with time since units were often held for long periods of time. SNRs of the last 100 spikes recorded from a unit (gray) compared favorably to the SNRs when units were first encountered (black) (median first 100 spikes=22dB, last 100 spikes=20dB).

(b) Hold times in all recorded units. In general, hold times for units used in the sleep analyses (gray) were much longer than hold times for other units that were not used (black) (median sleep=76 minutes, other=15 minutes).



**Figure 2.2.** Example driven and suppressed events detected by windowing algorithm.

(a) Well-defined periods of excitation (red) and inhibition (blue) were detected among a background of high spontaneous firing. No false windows were detected in the 9 second long spontaneous period following the stimulus.

(b) The varying durations of excitatory responses are well captured by the algorithm. At higher sound levels, excitation is shorter in duration and is followed by inhibition. In some cases, an inhibitory window was detected by the algorithm but then thrown out (dark gray) because it did not pass a post-hoc permutation test for the means when compared to spontaneous firing.

(c) An example where inhibition precedes excitation at high sound levels. Note that windows are non-overlapping even though excitatory and inhibitory algorithms were run independently.

## **Chapter 3: Responses Properties of Single Neurons**

### **3.1 Summary**

Most sensory stimuli do not reach conscious awareness during sleep. It has been thought that the thalamus prevents the relay of stimuli to cortex. In order to test this hypothesis, we recorded two cortical areas downstream of the thalamus in naturally sleeping marmoset monkeys. Surprisingly, in primary auditory cortex (A1), responses to sounds during sleep were quite strong. We supposed that responses would be more depressed in a higher sensory area. However, in lateral belt (LB), responses were far from diminished. We estimate that stimulus-driven responses dropped ~10% in A1 and LB during sleep. This estimate was consistent across recovery and arousal controls. When we closely examined the temporal pattern of responses, we found little difference between awake and SWS responses. Sustained responses, which are rare in anesthetized preparations, were commonly encountered in sleep. Finally, we found that REM behaved differently than SWS providing evidence for at least two functional states during sleep. Our results suggest that sounds are not exclusively gated by the thalamus and can reach up to secondary sensory cortex. They contradict prevailing notions of depressed sensory responses, leaving open the possibility of sensory processing during sleep.

### **3.2 Introduction**

The effects of arousal, attention, and anesthetics have been well-documented in a variety of sensory brain areas and animal species (Hubel et al., 1959; Desimone and

Duncan, 1995; Ter-Mikaelian et al., 2007). However, the role of sleep in sensory processing has not received much interest even though this natural behavioral state is critical to an animal's survival. Only a handful of studies in the somatosensory, auditory, and visual system have documented sleep effects on neural responses to external stimuli (see Hennevin et al., 2007 for review). Perhaps this is because many assume the brain is shut off from the external world during sleep (Steriade, 2003). Studies in the thalamus have supported this notion (Mukhametov and Rizzolatti, 1970; Coenen and Vendrik 1972; Hirsch et al., 1983; Livingstone and Hubel, 1981; Mariotti et al., 1989; Edeline et al., 2000), leading some to propose that the thalamus serves as a sensory gate. In line with this hypothesis, studies in primary visual cortex (Evarts, 1963, Livingstone and Hubel 1981), primary somatosensory cortex (Gucer, 1979), and primary auditory cortex (Murata and Kameda, 1963; Brugge and Merzenich 1973) found that most cortical cells respond more weakly in sleep and found almost no cells that responded more strongly.

But two recent studies in auditory cortex found some neurons that responded more strongly in sleep (Pena et al., 1999; Edeline et al., 2001). Complementing the physiology studies, a PET imaging study in humans found that auditory cortex was identically activated in sleep and awake for both simple (tone) and complex (subject's name) sounds (Portas et al., 2000). On the other hand, fMRI studies have found that activation in the auditory regions of the temporal lobe decreases during sleep (Czich et al., 2002; Czich et al., 2004).

Given the conflicting imaging and physiology results, it remains unclear whether cortex is responsive to sounds during sleep. The prevailing model of a disconnected auditory cortex may be too simplified. Sleeping subjects can still tell difference between

their name and those of others (Oswald et al., 1960), and evoked potential studies have suggested that the signal is processed to the point of deriving semantic meaning (Bastuji et al., 2002). It may be just as important to process some stimuli as it is to suppress them. Furthermore, to our knowledge no physiology study has tested higher sensory areas to determine whether responses there are strongly depressed as would be predicted in the simple model.

Here, we recorded in primary auditory cortex (A1) and then secondary auditory cortex, lateral belt (LB). We measured general response properties such as spontaneous and driven rates much as earlier studies had done. We controlled for the arousal of the animal and tested the repeatability of sleep effects. We also examined temporal discharge patterns in sleep to see if they retained the character of awake responses. What we found is that primary and secondary auditory cortex are quite active during sleep, preserving many of the response properties of wakefulness.

### **3.3 Results**

Data were collected in a total of 1218 units of which 493 units, 399 units in primary auditory cortex (A1) of 3 animals and 94 units in lateral belt (LB) of 2 animals, are reported here. These units were held for an average of 1.5 hours so that responses could be measured across at least one marmoset sleep cycle (~1 hour). Two unique stages of sleep, slow-wave sleep (SWS) and rapid eye movement sleep (REM), were differentiated in addition to the awake state (see Chapter 2: General Methods). 77% of

A1 units (645/838) and 65% of LB units (160/245) were driven by at least one stimulus ( $\geq 4 \times \text{sem}$  above spontaneous rate). Median spontaneous rate in A1 was  $1.0 \text{ spikes s}^{-1}$  and was  $1.4 \text{ spikes s}^{-1}$  in LB. In each A1 unit, awake responses were compared to SWS responses for 7.7 stimuli on average and to REM responses for 6.4 stimuli on average. LB units were tested with an average of 3.9 stimuli in SWS and 3.9 stimuli in REM.

### *3.3.1 Example units*

The three example units from A1 illustrate the range of effects observed in sleep (Fig. 3.1). The unit in Figure 3.1A responded with high driven rates during awake trials (dark gray) when EEG amplitude (dark bars) was low. When EEG amplitude went up during SWS (light gray), firing rate dropped to almost zero ( $\text{Gain}_{\text{SWS}} = -96\%$ ). In other cases, we were surprised to find the opposite scenario, that firing often increased during sleep (Fig. 3.1B,  $\text{Gain}_{\text{SWS}} = 83\%$ ). Finally, the unit in Figure 3.1C had a nearly identical response in awake, SWS, and REM (orange) ( $\text{Gain}_{\text{SWS}} = -10\%$ ,  $\text{Gain}_{\text{REM}} = -1\%$ ). In all 3 units, similar response modulations were recovered in later sleep episodes spanning the course of hours. Units were well-isolated throughout the recordings. The signal-to-noise ratios of the spike waveforms at the beginning and end of the sessions were  $>20\text{dB}$  (see spike waveform insets in Fig. 3.1). The stable spike waveforms combined with consistent recovery of responses suggest that modulation of responses during sleep for these 3 cells cannot be accounted for by non-specific factors like adaptation to the stimulus over the course of the session, declining cell health, poor unit isolation, or unreliable classification of behavioral state.



### 3.3.2 SWS modulation (A1)

Similar to these example units, strong but variable modulations were encountered across the population of A1 units tested ( $n=340$ ). Units were modulated an average magnitude of 38% in both directions. Although 44% of units decreased their response significantly when the animal fell asleep ( $>3 \times \text{sem}$  difference between awake and SWS firing rates), 34% of units increased their response significantly when the animal fell asleep. As a result of the offsetting bidirectional effects of sleep, overall activity dropped by only 5% (mean gain of all units) over the A1 population ( $n=340$  units) (Fig. 3.2A). If we examined gains for each stimulus tested instead of averaging the stimulus gains for each unit, similar results were found. Mean stimulus gain was -6%, and the magnitude of modulation averaged 46% for individual stimuli ( $n=2378$ ) (Fig. 3.2B). Distributions of best driven rates (Fig. 3.2C) and spontaneous rates (Fig. 3.2D) were not significantly different between awake and SWS ( $p_{\text{best}}=.17$ ,  $p_{\text{spont}}=.75$ , Wilcoxon rank sum,  $n=384$ ). The mixed effects in SWS are unlike those of attention and anesthetics which tend to be more systematic and unidirectional (Reynolds and Chelazzi, 2004).

Modulation of responses in sleep may differ depending on where in the cell's operating regime measurements are made (near response threshold vs. optimally driven). For example, at high firing rates, responses may saturate leaving little room for modulation (Reynolds and Chelazzi, 2004). In general, gain did not depend on firing rate ( $r^2=3 \times 10^{-6}$ ,  $p=.94$ ,  $n=2378$ ); gains only became more variable (more extreme in magnitude) at low rates (Fig. 3.2E). This could be the result of operating closer to a unit's firing threshold where small effects on membrane potential can lead to large changes in firing rate. Alternatively, this may be the result of increasingly variable

estimates of firing at low driven rates where only a few spikes are elicited. Gain also did not depend strongly on spontaneous rate ( $r^2=.01$ ,  $p=2*10^{-8}$ ,  $n=2378$ ) (Fig. 3.2F).

Regardless of the spontaneous activity of a neuron or how strongly it was driven, mean gain remained near -5% for the population.

### 3.3.3 SWS modulation (LB)

Given the prevalence of activity in A1, we reasoned that higher cortical areas may be more influenced by a change in behavioral state and possible loss of top-down processing. After physiologically defining the primary area A1, we recorded from lateral belt (LB), a secondary sensory area (Kaas and Hackett, 2000). Recordings were made far lateral of A1 so as to avoid the A1/belt border where areal identity is ambiguous (see Methods). Minimal tonotopic tuning was seen in these recordings (Fig. 3.3A). Unlike A1 units, units in LB were hard to drive with simple stimuli and were weakly driven in general (Fig. 3.3B). Whereas A1 responses tapered off at high sound levels, population LB responses continued to grow (Fig. 3.3C). These physiological properties gave us confidence that our recordings were outside of the core areas.

Figure 3.4A shows the effects of SWS on 84 LB units. The distribution is not significantly different than the gain distribution in A1 ( $p=.93$ , Wilcoxon rank sum,  $n_{A1}=340$ ,  $n_{LB}=84$ ). Over the population, mean spiking rate dropped little in LB (mean=-6%), and a majority (63%) of units were active at least as strongly in SWS as in awake (36% up, 37% down, and 27% no change in SWS) so that the effects of sleep were again bidirectional. Units were modulated by a magnitude of 37% in either direction on average.

One concern may be that LB units were not driven as strongly as A1 units (median driven rate A1=16.2 and LB=8.8 spikes s<sup>-1</sup>,  $p=2.6 \times 10^{-8}$ , Wilcoxon rank sum,  $n_{A1}=340$ ,  $n_{LB}=84$ ) (see Fig. 3.3B). Hence, they may have been tested in a different firing regime or with non-preferred stimuli during sleep. As mentioned earlier, gains in A1 did not depend on firing rate. To illustrate this, we plotted the gain distributions in A1 for different firing rate ranges. The ranges 0-5, 5-20, and >20 spikes s<sup>-1</sup> roughly divide the data into the bottom quartile, middle half, and top quartile respectively. The gain distribution is tightest for high firing rates and very broad at low firing rates, but the mean of the distribution does not vary (Fig. 3.4B). When the LB gain distribution is overlaid, it falls on the gain distribution for intermediate (5-20 Hz firing rate) firing rates in A1 (Fig. 3.4B). This is consistent with 93% of LB driven rates falling below 20 Hz. Therefore, the fact that LB units were driven to different rates than A1 units should not affect the conclusion that the mean gain in LB is similar to A1 during SWS.

### 3.3.4 REM modulation

Responses were also measured in REM for 332 units in A1 and 74 units in LB (Fig. 3.4, C and D). Similar to SWS, units in REM underwent both positive and negative modulations. 33% of A1 units increased and 39% decreased their driven response in REM. The magnitude of REM modulation averaged 37%, but mean gain in A1 during REM was only -6%, representing a small drop of driven activity compared to awake responses (Fig. 3.4C). One key difference between SWS and REM is that spontaneous firing rates were often elevated when the animal entered REM (mean awake=4.0, REM=5.4 spikes s<sup>-1</sup>). This difference did not reach significance ( $p=.29$ , Wilcoxon rank

sum,  $n=332$ ) possibly because of the number of units without appreciable spontaneous rates but is apparent when spontaneous rates are plotted on a logarithmic axis (Fig. 3.4, E and F).

A possibly interesting feature is that mean modulation was -12% in LB responses during REM, the result of many LB units that shut down in REM (Fig. 3.4D). On second look, the SWS distribution in LB displays a similar pattern, extreme negative modulations offset by a high number of weak positive modulations (Fig. 3.4A). But our sample was too small to establish a pattern. REM distributions in A1 and LB did not significantly differ ( $p=.33$ , Wilcoxon rank sum) (compare Fig. 3.4C and 3.4D), and the REM distribution in LB did not significantly differ from that in SWS ( $p=.52$ , Wilcoxon rank sum) (compare Fig. 3.4A and 3.4D). We caution that LB units were driven at lower firing rates than in A1. Earlier we showed that at low driven rates, extreme gains are encountered more often, even in A1 (Fig. 3.4B). It will be interesting to see in future studies if in a higher area more cells are completely shut down in sleep, but our data do not allow us to speculate further.

### *3.3.5 Correlation between SWS and REM*

A general process such as a loss in arousal would have predicted that all responses would be depressed and does not seem to explain the bidirectional effects of sleep. Further negating the idea of a simple loss of arousal, we found that modulation of responses in REM was independent of SWS. For example, the unit in Figure 3.5A had a weak response in SWS (Gain=-69%) but a strong response in REM, as strong as in awake (Gain=+8%). On a unit-by-unit basis, gains in SWS were poor predictors of gains in

REM: out of 382 units tested in all 3 states, roughly one-third (n=135) showed opposite changes in SWS and REM. This is the fraction expected if SWS and REM are uncorrelated (see Methods). In fact, there was a slight tendency for REM and SWS firing rates to differ more from each other than from awake rates (median  $|SWS-REM| = 4.6$  and  $|awake-(SWS+REM)/2| = 3.8 \text{ spikes s}^{-1}$ ,  $p=.005$ , Wilcoxon rank sum,  $n=382$ ) (Fig. 3.5B). The differing SWS and REM modulations appear to be the result of independent ongoing sleep processes. If so, changes in ongoing spontaneous activity may be an indicator of changing excitability. We observed little such correlation between modulation of spontaneous and stimulus-driven firing ( $r^2=.009$ ,  $p=.18$ ,  $n=195$ ) in units having appreciably different awake and SWS spontaneous rates ( $>2 \times \text{sem}$  difference) (Fig. 3.5C). This suggests the need to test driven responses directly in order to assess neural excitability with respect to feedforward sensory input which may very well differ from the mixture of internal activity.

### *3.3.6 Arousal controls*

A key difficulty is that animals may have never reached full awake arousal during the night. Awake firing rates could have been underestimated. To address this issue, we tested two awake conditions, one in which the animal naturally awakens in a darkened chamber and falls back asleep within minutes as in a typical experiment and another condition in which the experimenter turns the lights on and actively keeps the animal awake (see Methods). The sleep gains between the two conditions were similar (mean natural awakening=-22% vs. forced awakening=-22%,  $p=.95$ , Wilcoxon rank sum,  $n=20$ ) (Fig. 3.6A). Another possibility is that awake arousal was highly variable, sometimes

reaching full arousal and sometimes only reaching drowsy levels. We used the eyes being open as an indicator of full arousal or drowsiness. Eyes open trials gave a 9% improvement in gain compared to eyes closed trials (mean eyes closed=-5% vs. eyes open=-14%) suggesting some additional arousal effect although not reaching significance ( $p=.15$ , Wilcoxon rank sum,  $n=84$ ) (Fig. 3.6A). Finally, we compared the activity in the first five trials to the remaining trials the animal was awake. Activity immediately upon arousal, though, was no greater than in the remaining awake period before the animal fell back asleep ( $p=.69$ , Wilcoxon rank sum,  $n=298$ ) (Fig. 3.6B). In summary, although animals spent very little time awake, these controls suggest that a sufficient and stable awake arousal was reached for measuring firing rates without underestimating them.

### *3.3.7 Multiple sleep cycles*

It is highly possible that responses are dynamic, varying from one sleep cycle to the next. As a control, we tested the repeatability of SWS and REM modulation by re-testing approximately half of the units in a later sleep cycle (Fig. 3.7, A and B). Gains computed in later episodes of SWS correlated with gains measured in previous cycles ( $r^2=.74$ ,  $p=1*10^{-38}$ ,  $n=127$ ) (Fig. 3.7A). This was also the case for REM modulations measured in two separate episodes ( $r^2=.57$ ,  $p=1*10^{-22}$ ,  $n=116$ ) (Fig. 3.7B). These recovery controls suggest that the effects of sleep can be repeated each cycle and were not dominated by non-specific factors (noise in our measurements or non-stationarity of a neuron's response).

We also tested whether the activity of the population changed over the course of the night from the evening to the early morning. Activity could conceivably change

because the amplitude of slow-waves increases as animals sleep more deeply in the middle of the night and because at the end of the night REM periods become more dominant. Also, awake levels may progressively decrease during the night. No trend, however, was present in awake or SWS firing rates with sleep episode (Fig. 3.7C, gray curves). The relative closeness of SWS and awake responses (small gain) was consistently present throughout the night. The distributions of maximum unit driven rates early in the night (cycles 1-2) did not differ from those late in the night (cycles 7-10, ~5 hours later) whether in the awake state ( $p=.67$ , Wilcoxon rank sum,  $n=34$ ) or SWS ( $p=.56$ , Wilcoxon rank sum,  $n=34$ ). Correspondingly, mean gain in the early night (-3%) was similar to mean gain at the end of the night (-9%) ( $p=.93$ , Wilcoxon rank sum,  $n=34$ ). Responses in REM displayed similar consistency (Fig. 3.7D). Combined with the reliable recovery of responses within units (Fig. 3.7, A and B), these data suggest that sensory responses were state-specific but otherwise stationary in the population across the night.

### *3.3.8 Temporal discharge patterns in SWS and REM*

Units in A1 and LB during the awake state often demonstrate sustained firing in addition to an initial onset response (Brugge and Merzenich, 1973). Some anesthetics are known to diminish the later, sustained component (Phillips, 1985). This was not the case in sleep though. In units whose response started within 60ms of stimulus onset, sustained responses (response after 100ms) were only somewhat more diminished than onset responses in SWS (mean gain sustained=-12% vs. onset=-5%,  $p=.07$ , Wilcoxon rank sum,  $n=348$ ) (Fig. 3.8A) or in REM (mean gain sustained=-16% vs. onset=-8%,  $p=.05$ ,

Wilcoxon rank sum,  $n=311$ ) (Fig. 3.8B), and sustained responses were still the dominant form of response (Fig. 3.8C); onsets and offsets were a minority even in REM. The tendency for slightly stronger sustained responses in awake led to longer average response windows detected by our algorithm (mean awake=118, SWS=104, REM=102 ms,  $n=3516$  stimuli). This difference is reflected in the population response histogram (Fig. 3.8D). The onset is similar in all states, but the awake response diverges slightly in the sustained portion. Nonetheless, responses are long-lasting in awake, SWS, and REM.

Interestingly, the pattern flips in the offset portion of the population response (Fig. 3.8D, right side). SWS responses persist longer than awake and REM responses especially in the late portion of the offset ( $>100$ ms post-stimulus). In general, sustained responses gave negative gains (awake  $>$  sleep) while onsets and offsets gave near zero or slightly positive gains (sleep  $\geq$  awake) (Fig. 3.8, E and F). There was a negative correlation between the duration of the response and sleep modulation with gain plateauing at -10% for responses lasting longer than 150ms (thick black curves in Fig. 3.8, E and F). For short responses such as onsets and offsets and short sustained, effects were above 0% (red and blue colored triangles in Fig. 3.8, E and F).

Breaking down responses by duration and whether they are onset, offset, or sustained is a crude measure of temporal properties. To confirm that responses retained their temporal character in SWS and REM, we computed a vector similarity with awake PSTH patterns. Median correlation was 0.78 ( $n=1068$ ) between awake and SWS responses (Fig. 3.9A) and 0.79 ( $n=709$ ) between awake and REM responses (Fig. 3.9B). Correlation rarely went below 0.5. These correlation values are noteworthy given that estimated within-state similarity (see Methods) was only slightly better (median=0.85 for



awake/SWS and median=.87 for awake/REM data used in between-state comparisons). So correlations of PSTHs between awake and asleep (~.78) approached the limit of similarity possible given noise in PSTH estimates (~.85). This high similarity suggests that in principle SWS and REM response patterns could support the same temporal code awake patterns can.

### *3.3.9 Bursting*

Averaged PSTHs can be identical but have different underlying spiking statistics. An important property of SWS responses reported in the literature is their bursting pattern (Edeline et al., 2000; Edeline et al., 2001). Using a simple measure (fraction of spikes occurring within 5ms of each other), we found that SWS responses were burstier than awake responses (mean burst fraction in awake=.09 vs. SWS=.11,  $p=3*10^{-9}$ , Wilcoxon rank sum,  $n=1848$ ), and SWS responses were burstier than REM responses (mean REM=.09 vs. SWS=.11,  $p=3*10^{-8}$ , Wilcoxon rank sum,  $n=1848$ ) with no difference between REM and awake ( $p=.8$ , Wilcoxon rank sum,  $n=1848$ ). This pattern was true regardless of which minimum ISI criteria (4, 5, 8, 10ms) was used to identify bursts (Fig. 3.9C).

## **3.4 Discussion**

In this chapter, we measured general response properties of neurons in auditory cortex during sleep. Our measurements reveal that sleep depresses stimulus-driven

activity by only ~10%. This was the case in both A1 and LB. Given that this is the first study to explore responses in a non-primary area, it will be interesting to see results from future studies especially imaging studies which are beginning to distinguish more and more auditory cortical fields (Petkov et al., 2006).

#### *3.4.1 Comparison to previous studies*

Our results appear to differ markedly from most previous sleep studies in cortex. In the visual, somatosensory, and auditory systems, neurons were found to be less responsive during sleep (Evarts, 1963; Gucer, 1979; Livingstone and Hubel, 1981; Murata and Kameda, 1963; Brugge and Merzenich, 1973). One possible difference is that our recordings tended to be in the upper lamina while other studies may have tended toward middle lamina (granular layers). Layer 4 cells receive direct thalamic input, and thalamic responses are already known to be depressed (Edeline et al., 2000). Recurrent intracortical connections between upper layer neurons or feedback connections from other areas may re-amplify the response received from input layers (Liu et al., 2007). A species difference may also explain the departure in our results. We recorded from a New World primate, the common marmoset, which is diurnal. Most previous studies were done in nocturnal animals and not primates. However, we point out that from an evolutionary perspective, sleep seems to have diverged early on before the split between mammals and birds (Siegel, 2005), and all of the above studies used mammals which display common sleep patterns.

Some observations from earlier studies are consistent with our findings. Previous data did seem to suggest that neurons could be more active in cortex than subcortically.

In a study comparing neurons in thalamus and V1, the authors noted that cortical neurons displayed more mixed effects of sleep than those in thalamus (Livingstone and Hubel 1981). A second group studying auditory thalamus and A1 also observed that cortical neurons (Edeline et al. 2001) were modulated much more heterogeneously compared to thalamic neurons (Edeline et al. 2000). Another study in A1 found that sleep most often (>50% of neurons) did not affect firing rates (Pena et al., 1999).

Human imaging studies in sleeping and lightly sedated patients found activation to external sounds in the temporal lobe (Portas et al., 2000; Davis et al., 2007). However, a second group found a contradictory result (Czich et al., 2002; Czich et al., 2004). This ambiguity may be because the BOLD signal measures synaptic activity (Logothetis et al., 2001) which could be a mixture of the depressed thalamic input and enhanced cortical processing. Spiking activity, corresponding to the output of an area, is not directly reflected in imaging signals (Viswanathan and Freeman, 2007) making comparison to extracellular physiology difficult.

A possibility that one hopes to avoid is having confounded data. But with the mixed results in the literature, the question arises as to whether everyone is right. Here, we took great care to ensure that our measurements were accurate and as unconfounded as possible. We showed that responses were stable from cycle to cycle and over the course of the night. Also, this study attempted to quantitatively assess the animal's level of awake arousal. Arousal controls were performed to determine whether awake firing rates were underestimated. We determined that arousal only affects our estimates by <10%. Our controls were simple (forcing full arousal, comparing eyes open to eyes closed, comparing early versus late night, and looking at the first 5 trials immediately

after waking). Future studies using more powerful techniques could elaborate on our findings. For one thing, a comparison of firing rates when the animal is awake during the day versus during the night would help control for circadian influences. Furthermore, engaging the animal in a task upon waking may give best results. However, it would be very difficult to engage the animal without upsetting the smooth rhythms of their sleep cycle.

No study exists for comparing effects of sleep on response patterns. The large variety of stimuli we used elicited a range of firing patterns. We found that temporal patterns were very stable across behavioral states. An interesting observation was that onsets and offsets were less affected. In fact, long duration offsets were enhanced not depressed in SWS. We currently lack an explanation for this puzzling result. In line with previous work, we found an increase in burstiness strictly during SWS. The 2-5% increase we observed (depending on max ISI allowed) is similar to that observed by Edeline et al. (2001) in guinea pig auditory cortex. Bursting could improve information transfer to the next stage of cortical processing or may reflect burstier thalamic inputs (Edeline et al., 2000).

The observed effects of sleep should be distinguished from those of attention and anesthetics. Attention effects are usually unidirectional unlike the mixture of up and down modulations of responses observed in sleep (Reynolds and Chelazzi, 2004). Anesthetics may induce cortical oscillations and behavioral unresponsiveness similar to SWS (Lydic and Baghdoyan, 2005), but unlike neural firing under anesthesia (Phillips, 1985), SWS firing patterns were mainly sustained responses. Many neurons actually improved their response in SWS. These findings and the fact that REM behaved

independently from SWS suggest that the sleep state is different than the anesthetized state at the neural level as has been shown by others (Tortorolo et al., 2002; Cotillon-Williams et al., 2003; Kishikawa et al., 1995; see review by Hennevin et al., 2007).

### *3.4.2 Functional significance*

Our findings are consistent with studies of auditory performance during sleep. Although acoustic arousal thresholds are much higher in both SWS and REM (Bonnet, 1982), an interesting finding has been that sleeping subjects retain the capability to differentially respond to their own name over others and to discriminate forward from reversed names (Oswald et al., 1960). Activity in higher auditory areas such as LB may be necessary for such detection to take place. From an ecological standpoint, sleep is a vulnerable but necessary period (Zepelin, 2000). As the only distance sense remaining, hearing may be important for detecting sounds at night to help protect the animal from predators or awaken a mother to its baby's cries. It is notable that studies in the auditory modality have tended to show the most activity in sleep (Pena et al., 1999; Edeline et al., 2001; Portas et al., 2000). Studies in vision (Livingstone and Hubel, 1981) and somatosensation (Gucer, 1979) have not seen strong responses in cortex. A comparison of two or modalities in the same experimental animal and setting could help determine the generality of sleep effects.

### *3.4.3 A revised view of hearing during sleep*

Original theories of sleep had assumed that the brain shuts off to the external world (Steriade, 2003), but our results oppose such notions and are in line with current

hypotheses that cortex is quite active during sleep. While this activity is usually assumed to relate to internal processes involved in learning and memory consolidation (Jha et al., 2005; Siegel, 2001; Stickgold, 2005), cortex appears to be capable of external activation. A simple effect like a drop in arousal level cannot account for the bidirectional modulations within SWS and between SWS and REM. Feedforward thalamic input, local inhibitory circuits, recurrent connections, and top-down modulations may play a role in setting a neuron's response. Sleep likely modifies all of these leading to complex patterns of modulation. The idea of a simple thalamic gate that shuts down responses seems unlikely. We found no evidence for such a gate even at a secondary cortical station. Although many other auditory fields (Kaas and Hackett, 2000) cannot be ruled out because they were not tested, the gate may lie even higher up in association areas involved in integrating sensory percepts or frontal areas involved in decision making as suggested by limited evidence from imaging studies in sleeping, vegetative, and lightly sedated subjects (Portas et al., 2000; Laureys et al., 2000; Davis et al., 2007). For now, the results presented here establish the validity of our preparation for studying sensory processing during sleep. In the next chapter, we go beyond general properties and ask how auditory processing changes at the neural level during sleep.

### **3.5 Methods**

For details of physiological recordings, sleep scoring, event detection algorithm, and stimuli used, see Chapter 2: General Methods. We note here that for forced

awakenings animals were actively awakened by playing loud sounds, turning the lights on, and entering the chamber to increase and maintain awake arousal. In the first two animals (M2p and M41o), we did not keep track of absolute sleep episode number only relative sleep cycle, so their data were not used in the early versus late night comparison of activity (Fig. 3.7, C and D) but could be used in the recovery controls comparing one cycle to the next (Fig. 3.7, A and B). In M16s and M43q, we kept accurate track of the absolute episode number of each cycle starting from when the animal first fell asleep.

### *3.5.1 Identification of A1 and LB*

Primary auditory cortex was identified based on its proximity to the lateral sulcus (LS) marked during surgery, responsiveness to pure tones, and presence of a clear rostral-to-caudal (low-to-high) tonotopic gradient (Fig. 3.3A). After identifying A1, we recorded from LB by moving further lateral (~4mm away from the LS) (Kaas and Hackett, 2000) in two animals (M43q and M16s). LB neurons often responded to noise stimuli, were not very responsive to tones, and were poorly frequency tuned if at all (Rauschecker et al., 1995). We caution here that although our recordings are from medial lateral belt (as opposed to antero- or caudo- lateral belt), no effort was made to distinguish LB from parabelt as no physiological criteria currently exist to differentiate these two fields. Recordings may well have encroached on parabelt judging by distance from sulcus, the increasing presence of visual responses, and post-mortem histology in one animal (the other animal is still in use).

### 3.5.2 Data analysis

For the results reported here, we do not distinguish between different stimulus types. We used the data for any stimulus that elicited a significant driven response ( $>4$  sem above spontaneous rate) in at least one of the behavioral conditions (awake, SWS, or REM). Mean discharge rate  $r$  was determined using the analysis windows found by our algorithm. The algorithm returned multiple windows per response corresponding to different components (onset, offset, sustained). For general analyses, an overall window starting at the earliest detected window and ending with the latest detected window was used. For specific analyses, individual windows were used. A window was classified as onset if it started and ended within 100ms of the stimulus being turned on. A window was considered a sustained response if it started at least 30ms before the stimulus was turned off and continued beyond the first 100ms of stimulation. An offset window started and ended after the stimulus was turned off.

We computed percent modulation in sleep by comparing stimulus driven firing rates in two different states according to the formulas:

$$\% \text{Gain}_{\text{SWS-awake}} = 100 * \frac{r_{\text{SWS}} - r_{\text{awake}}}{\max(|r_{\text{SWS}}|, |r_{\text{awake}}|)}, \quad \% \text{Gain}_{\text{REM-awake}} = 100 * \frac{r_{\text{REM}} - r_{\text{awake}}}{\max(|r_{\text{REM}}|, |r_{\text{awake}}|)}$$

The overall gain for a unit was the mean of all the individual stimulus gains. A unit was considered significantly modulated if sleep firing rate differed by  $3 * \text{sem}$  from awake firing rate for any stimulus ( $p < .01$ ). We did not correct for multiple comparisons since the number of stimuli tested for each unit was not large (mean=7), and we were already using a strict significance criterion. Usually, the number of stimuli traded off



with the number of trials that could be run so that there was a reduced advantage to performing multiple comparisons across stimuli, but the effect of multiple comparisons did persist (80% of units reached significance but only 47% of stimuli).

For correlating PSTH's, we first subtracted spontaneous rate, smoothed PSTH's by a 20 ms moving average filter, subtracted out the mean, and performed a dot product according to:

$$\rho_{SWS,awake} = \frac{(PSTH_{awake} - \mu_{awake}) \bullet (PSTH_{SWS} - \mu_{SWS})}{\|PSTH_{awake} - \mu_{awake}\| \|PSTH_{SWS} - \mu_{SWS}\|},$$

$$\rho_{REM,awake} = \frac{(PSTH_{awake} - \mu_{awake}) \bullet (PSTH_{REM} - \mu_{REM})}{\|PSTH_{awake} - \mu_{awake}\| \|PSTH_{REM} - \mu_{REM}\|}$$

To estimate an upper bound on the similarity that is possible given the noise in PSTH measurements, two new PSTHs were simulated from Gaussian draws using the mean and standard deviation of each 1 ms bin of the PSTH in a given state. Similarity between these two PSTHs from the same state was compared to correlation obtained between PSTHs from different states.

For bursting analysis, inter-spike intervals (ISI) were computed during stimulus presentation for all repetitions. Percent bursting was the number of ISI's < x (where x=4, 5, 8, or 10 ms) divided by the total number of ISI's.

**Correlation between SWS and REM.** To perform an unbiased, non-parametric 3-way comparison between Awake (A), SWS (S), REM (R), we ordered firing rates in the three states for each unit. We then counted the frequency of certain patterns. If REM and SWS are correlated, then the pattern XXA or AXX (where X = REM or SWS) should

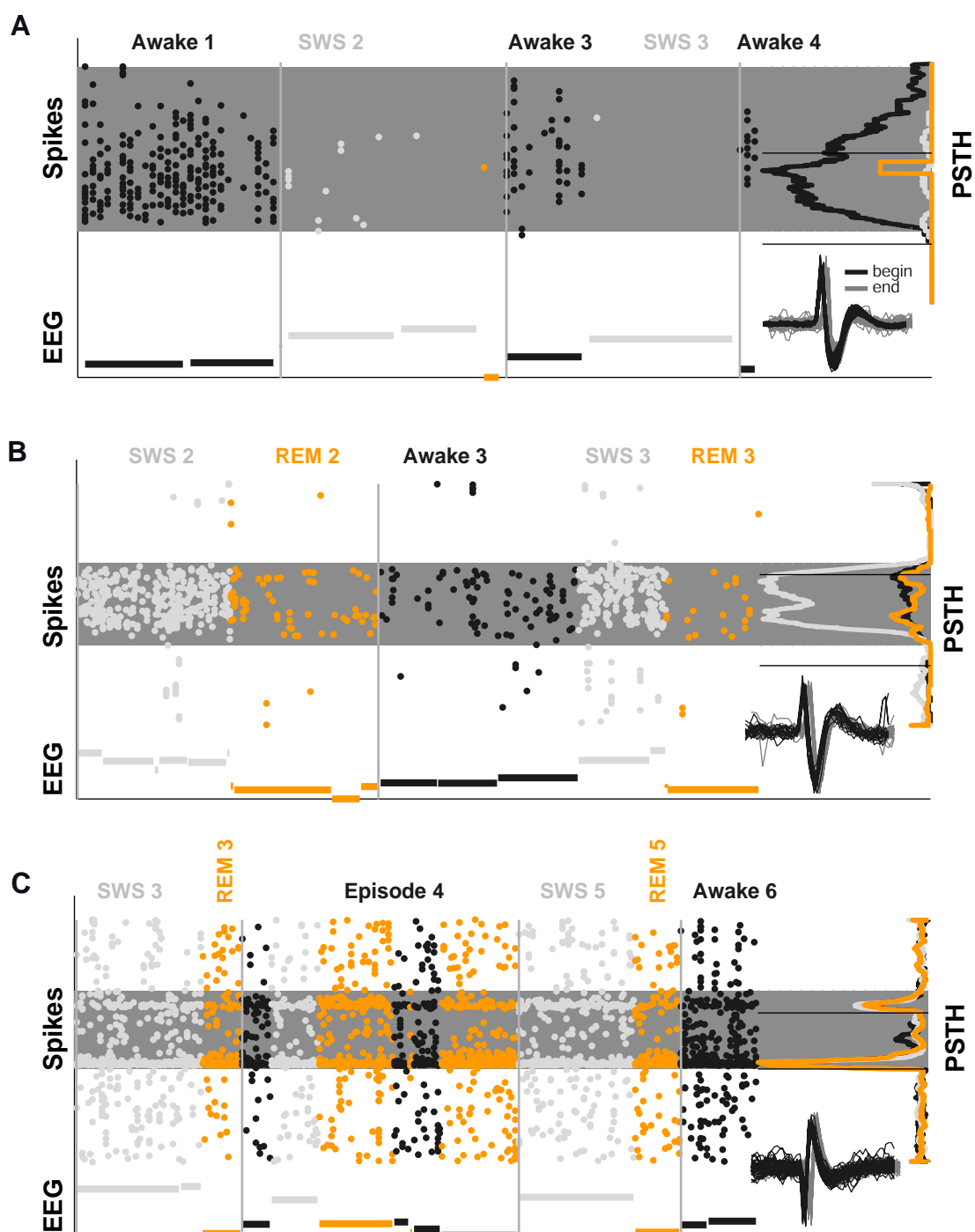
occur more often than chance, and the pattern XAX should occur less often than chance  
(chance levels:  $p_{XAX}=1/3$ ,  $p_{XXA|AXX}=2/3$ ).

**Figure 3.1.** Example units modulated during sleep.

(a) Spiking activity and EEG during different time points while the animal slept (data was not collected continuously). This unit's response consistently went down when the unit was tested in sleep (gain=-96% in SWS) (dark gray=awake, light gray=SWS, and orange=REM). The spike raster (oriented vertically, each column represents a single trial) shows a clear loss of spiking upon entering into SWS in episode 2, a recovery of response upon awakening in episode 3, and a loss of response in SWS of episode 4. Far right, the PSTH averages the activity across all trials for each state and is plotted on its side. The clear response in awake is absent in SWS and REM. Gray shaded strip is the analysis window determined by our windowing algorithm. Dashed lines demarcate stimulus playing. Bottom, the normalized EEG amplitude for each epoch tested is shown (breaks between EEG bars denote non-continuous measurements). When driven spiking is high in awake periods, EEG amplitude is low. In SWS periods, high EEG amplitude accompanies diminished firing. Inset, first 50 (black) and last 50 (gray) spike waveforms collected during the 2.5 hour long session. Horizontal offset is for display purposes only.

(b) Same format as (a). This unit's response went up in sleep (gain=83% in SWS). Firing rate increased when EEG amplitude (SWS) increased. Also, despite the large drop in awake and REM firing rates, they retained similar response profiles to SWS as seen in the PSTHs. Session spanned 2 hours.

(c) Same format as (a). Unit's response was similar in awake, SWS, and REM (gain=-10% in SWS). Regardless of multiple changes in EEG amplitude and behavioral state over the 3 hour long session, the unit remained consistently well-driven by the stimulus, and the PSTH retained its distinct onset and offset pattern in all 3 states.



**Figure 3.2.** Stimulus-driven and spontaneous activity in A1 during awake and SWS.

(a) Histogram of %Gain(SWS-A) for cells recorded in A1 (n=340). Negative gains are units whose responses went down in SWS. Positive gains represent units whose responses went up in SWS. Lightly shaded portions of bars represent responses that did not significantly differ between awake and SWS ( $<3 \times \text{sem}$  difference). Mean gain = -5% (vertical dotted line).

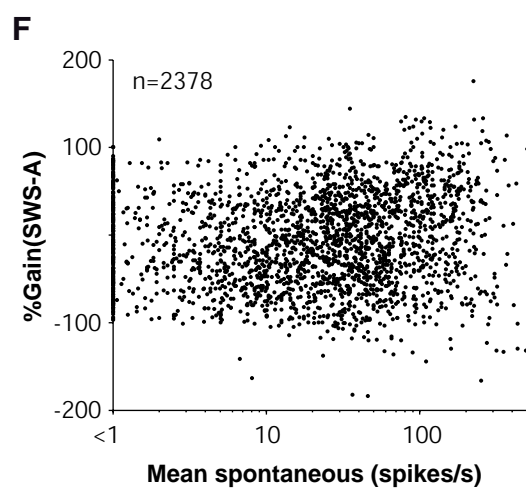
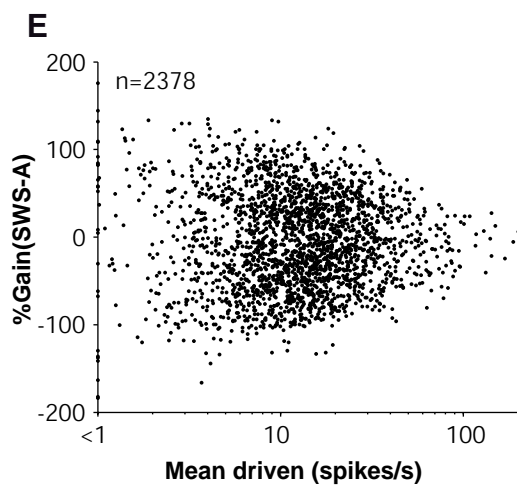
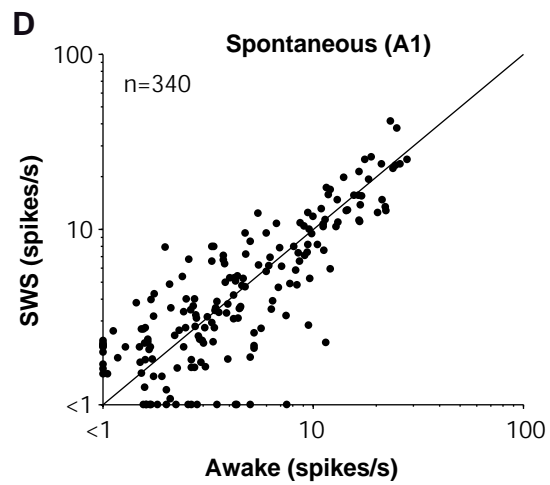
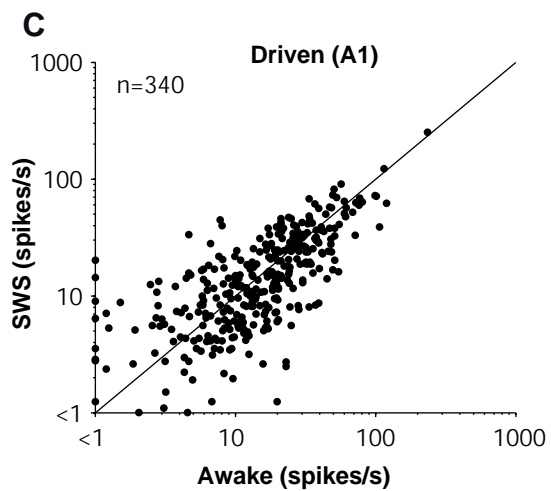
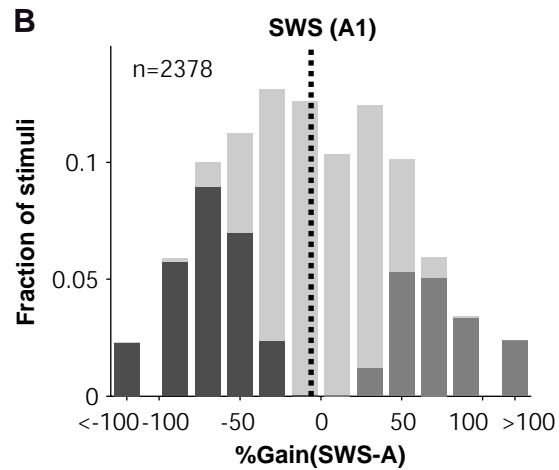
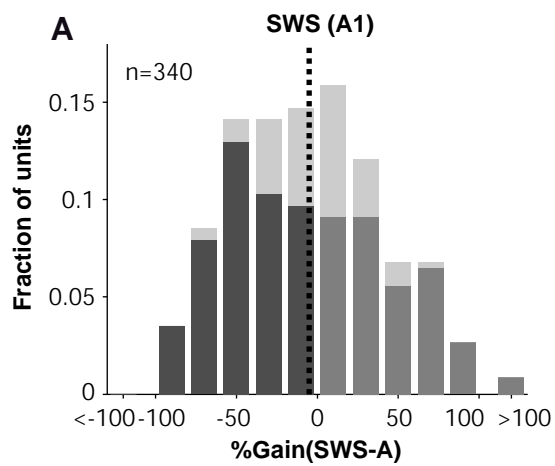
(b) Histogram of %Gain(SWS-A) for all stimuli tested in A1 (n=2378). Same format as (a). Mean gain = -6%.

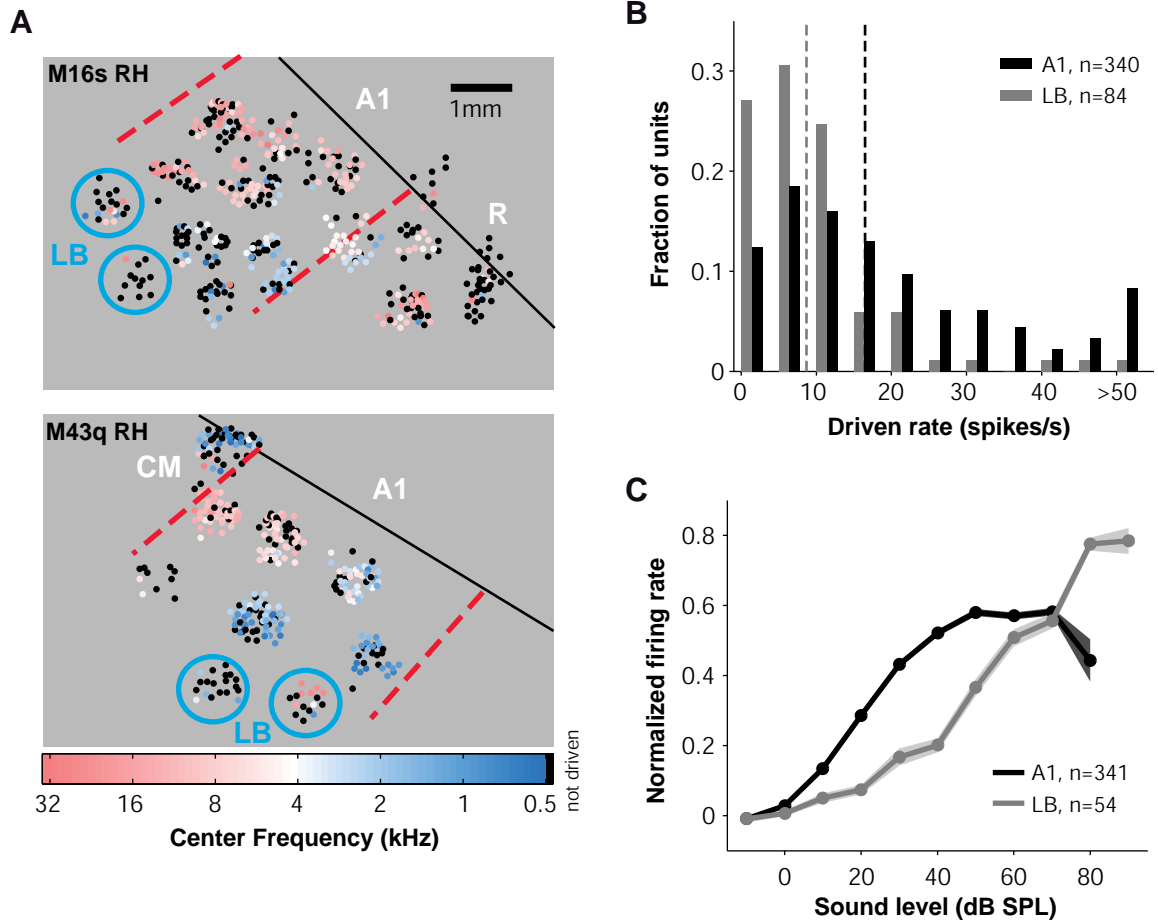
(c) Scatter plot of stimulus-evoked firing rates in awake and SWS for the best stimulus of a unit. Maximum driven rates did not significantly differ ( $p=.17$ , Wilcoxon rank sum, n=340).

(d) Scatter plot of unit spontaneous firing rates in awake and SWS. Spontaneous rates did not significantly differ ( $p=.75$ , Wilcoxon rank sum, n=340).

(e) %Gain as a function of driven rate for all stimuli tested in A1. No trend is present with driven rate ( $r^2=3 \times 10^{-6}$ ,  $p=.94$ , n=2378).

(f) %Gain as a function of spontaneous rate for all stimuli tested in A1. A weak trend is present with driven rate ( $r^2=.01$ ,  $p=2 \times 10^{-8}$ , n=2378).





**Figure 3.3.** Location and properties of lateral belt recordings.

(a) Tonotopic maps in the 2 animals where LB recordings were obtained. Before recording in LB, A1 was mapped out. The tonotopic progression can be seen in the maps of both animals. In M16s (top), A1 was bounded rostrally by R (rostral field). In M43q (bottom), A1 was bounded caudally by CM (caudomedial field). In both cases a clear frequency reversal is seen at the borders (red dashed lines). Colored dots indicate units whose center frequencies were determined. The dots are slightly dispersed for display purposes. Black dots represent track locations where center frequencies were not determined because units were unresponsive to tones or narrowband noise. Belt recordings (blue circles) were made far from the lateral sulcus (diagonal black line). These regions had weak overall responses and poor tonotopic gradients as evidenced by the lack of colored dots. Both maps are from the right hemispheres of the animals.

(b) Units in LB were not driven as strongly as units in A1 (median best response LB=8.8 vs. A1=16.2 spikes  $s^{-1}$ ,  $p=3 \times 10^{-8}$ , Wilcoxon rank sum,  $n_{LB}=86$ ,  $n_{A1}=340$ ). Vertical dashed lines represent medians.

(c) Population rate-levels in A1 ( $n=341$ ) and LB ( $n=54$ ). Curves from either awake or SWS were normalized by peak response then summed. Activity in LB is more monotonic (grows with sound level) than in A1. Shaded regions represent  $\pm 5$  sem.

**Figure 3.4.** Stimulus-driven and spontaneous activity in LB during SWS and REM.

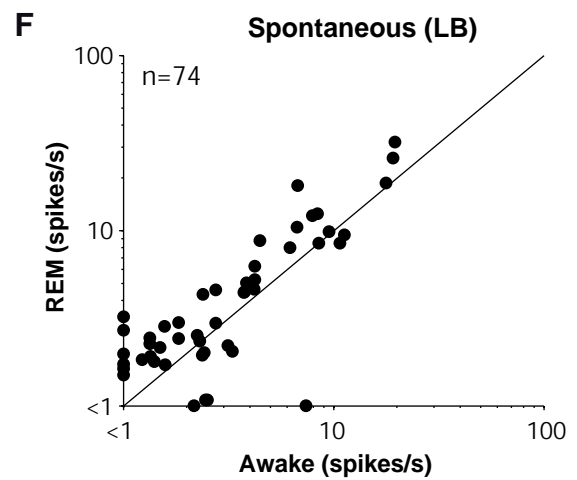
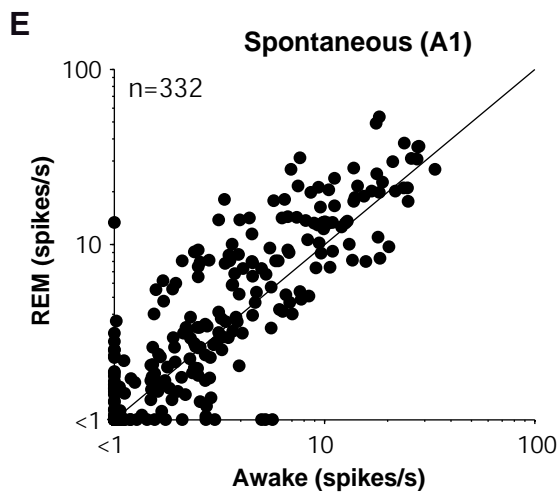
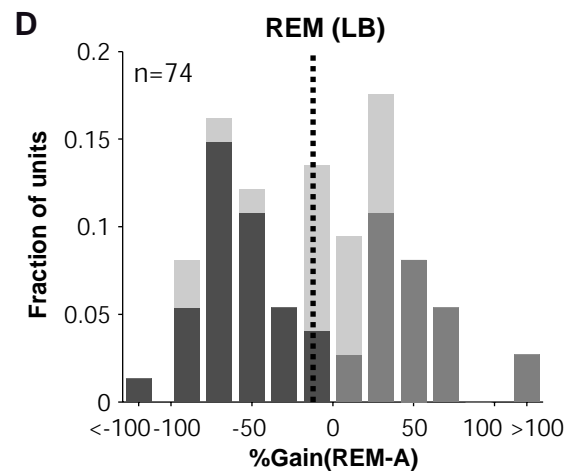
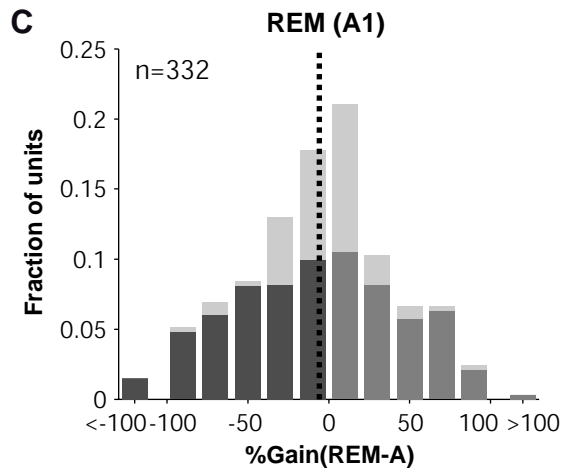
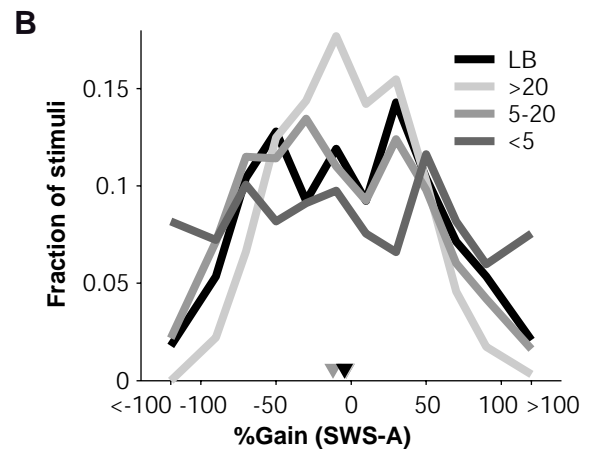
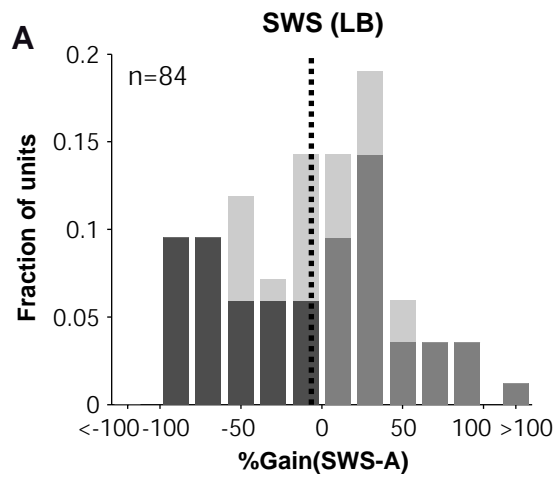
(a) Histogram of %Gain(SWS-A) for cells recorded in LB (n=84). Same format as Figure 3.2a. Mean gain = -6% (vertical dotted line).

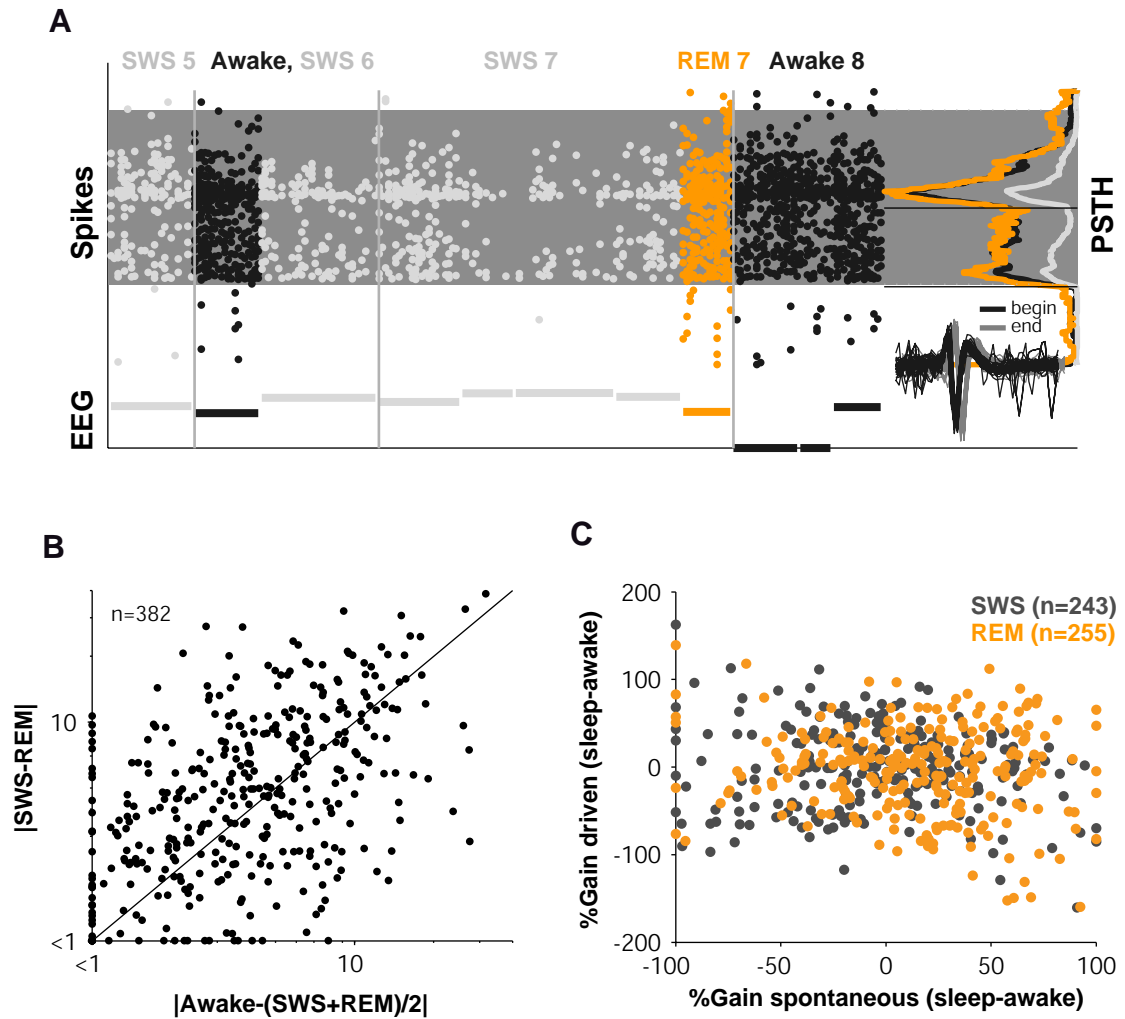
(b) Control for effects of firing rate in comparing A1 and LB. Dividing the A1 data into low (0-5 Hz), middle (5-20 Hz), and high (>20 Hz) firing rates, yields three gain distributions of near identical medians (-6%, -13%, and -3% respectively) (inverted triangles) but increasing variance (standard deviation=80%, 55%, 43%). The gain distribution is tightest for high firing rates. The distribution for LB (black) falls on the A1 distribution for intermediate firing rates (5-20Hz) consistent with LB rates averaging  $8 \text{ Hz} \pm 7\text{Hz}$ .

(c-d) Gain histograms in REM for A1 (c) and LB (d). Same format as (a). Mean gain=-6% (A1) and -12% (LB) (vertical dashed lines).

(e-f) Scatter plots of spontaneous firing rates in A1 (e) and LB (f). Although a trend toward higher REM spontaneous rates appears to be present, spontaneous rates were not significantly different from those in awake whether in A1 or LB ( $p_{A1}=.29$ ,  $p_{LB}=.31$ , Wilcoxon rank sum,  $n_{A1}=332$ ,  $n_{LB}=74$ ).





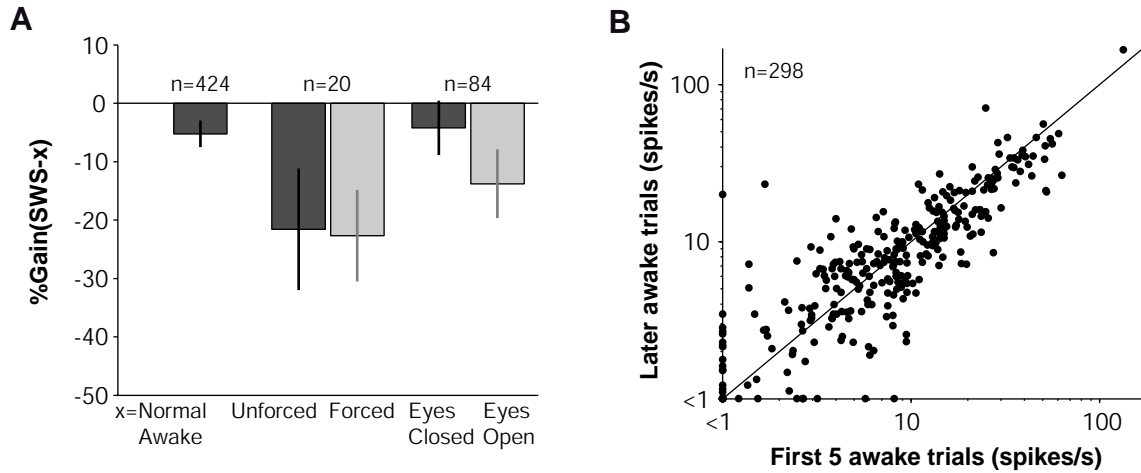


**Figure 3.5.** Lack of similarity between SWS and REM responses.

(a) Example unit that responds strongly in REM (gain=+8%) but not in SWS (gain=-69%). Same format as Figure 3.2. The cell's response in REM is similar to awake, but in three different occurrences of SWS, the response is much weaker. Windowing algorithm captures the significant 250 ms portion of the response which extends well beyond stimulus offset (gray shaded region). Unit was well-isolated at beginning and end of the ~4 hour long recording (see inset spike waveforms).

(b) Difference between SWS and REM firing rate plotted against the difference from awake firing rate. Firing rates in SWS and REM are no closer to each other (median difference=4.6 spikes  $s^{-1}$ ) than they are to awake (median difference=3.8 spikes  $s^{-1}$ ) ( $p=.005$ , Wilcoxon rank sum,  $n=382$ ).

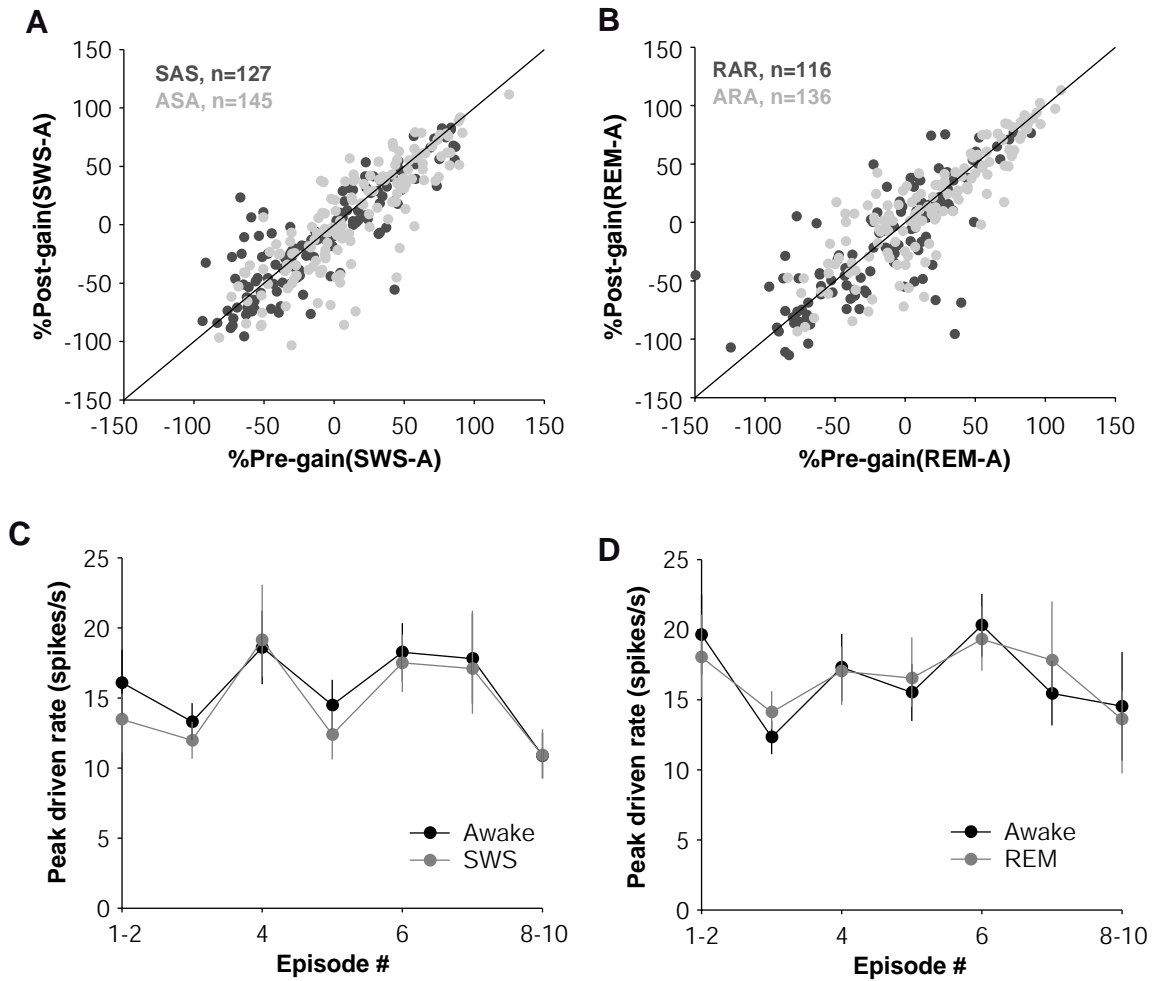
(c) In units having spontaneous rates that were >2 Hz and significantly modulated in sleep, gain of stimulus-driven responses (spontaneous rate subtracted) did not positively correlate with the modulations of spontaneous rate whether in SWS ( $r^2=.01$ ,  $p=.18$ ,  $n=195$ ) or REM ( $r^2=.01$ ,  $p=.26$ ,  $n=202$ ).



**Figure 3.6.** Arousal controls.

(a) %Gain(SWS-A) recomputed under different arousal conditions. Far left bar represents mean $\pm$ sem of SWS gain distribution for all awake conditions. Middle bars compare gain for passive versus active (experimenter induced) awakenings in units tested in both conditions (n=20). Right bars compare gain for eyes open versus eyes closed trials in units where at least 4 trials were collected in both conditions (n=84).

(b) Comparison of awake firing rates for the first 5 trials after awakening and the remaining trials the animal was awake before falling back asleep. One might expect that firing would be strongest immediately when the animal awakens, but firing rates did not significantly differ from those before the animal became drowsy again (p=.69, Wilcoxon rank sum, n=298).



**Figure 3.7.** Stability of responses from cycle to cycle and throughout the night.

(a) In some units, a recovery control was performed. Modulation of firing was re-tested in a later sleep cycle. Order of testing was either Awake-SWS-Awake (ASA) or SWS-Awake-SWS (SAS). There was a high correlation between pre-gains and post-gains suggesting similar modulation of a neuron in two separate sleep cycles ( $r^2=.73$ ,  $p=7*10^{-78}$ ,  $n=272$ ).

(b) Recovery controls for REM. Order of testing was either Awake-REM-Awake (ARA) or REM-Awake-REM (RAR). There was a high correlation between pre-gains and post-gains suggesting similar modulation of a neuron in two separate sleep cycles ( $r^2=.69$ ,  $p=4*10^{-65}$ ,  $n=252$ ).

(c) Trend in population activity over the course of the night (error bars represent  $\pm$ sem). Recordings were performed throughout the night starting from when the animal first fell asleep until the early morning. No clear trend in either awake or SWS responses is present, and the two curves follow each other, representing the idiosyncrasies specific to the population of neurons sampled during that episode.

(d) Same format as in (c) except for REM responses in comparison to awake responses.

**Figure 3.8.** Onset, offset, and sustained firing patterns observed in awake and asleep.

(a) Comparison of unit gains in SWS for onset (first 100ms of stimulus) and sustained portions of response (remainder of stimulus). SWS gains are similar for both portions of the response with a slight tendency toward more depressed sustained responses (mean onset =-5% vs. sustained gain=-12%,  $p=.07$ , Wilcoxon rank sum,  $n=348$ ).

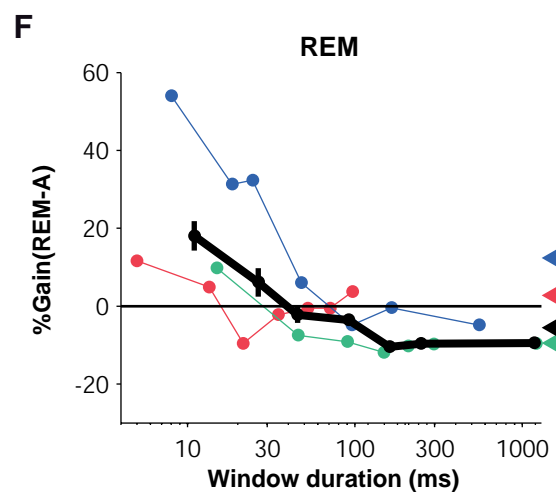
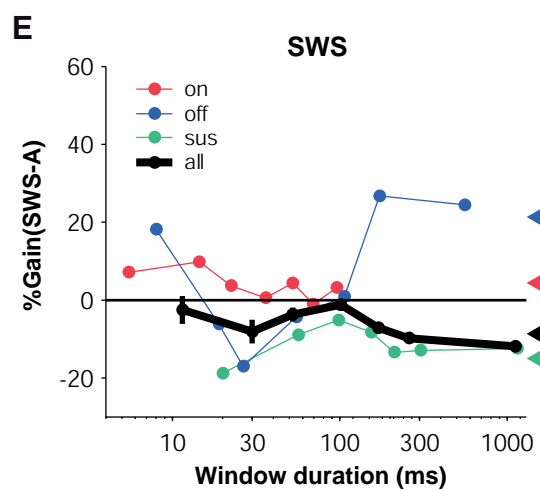
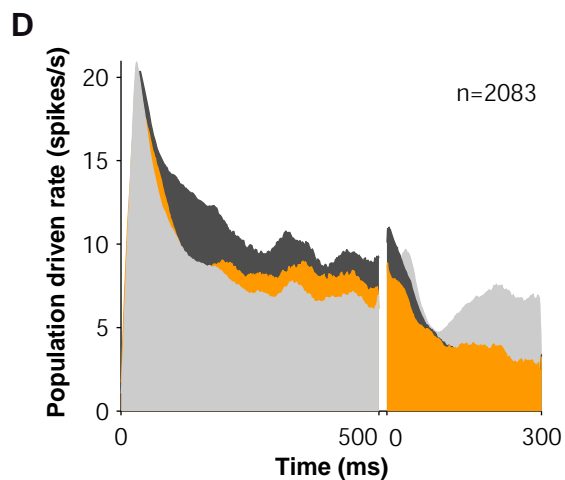
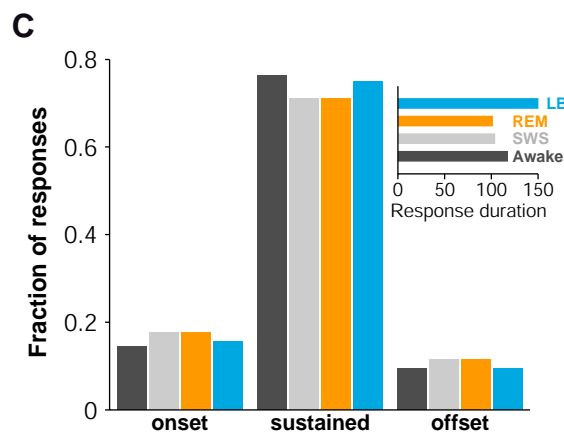
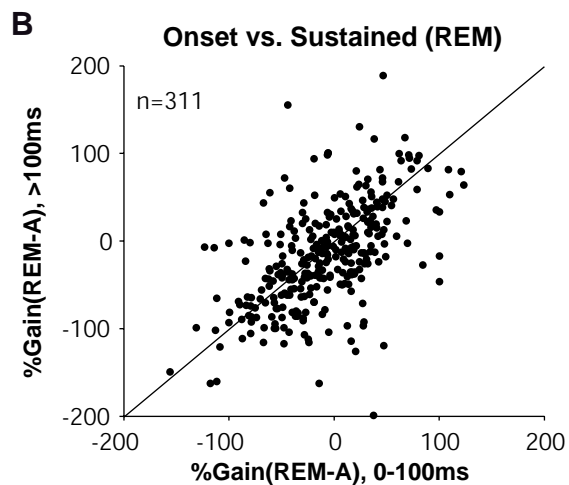
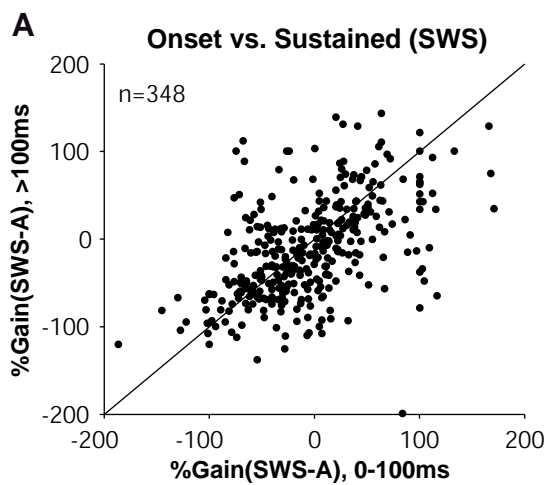
(b) Same as (a) except for REM. A tendency toward more depressed sustained responses was present (mean onset=-8% vs. sustained gain=-16%,  $p=.05$ , Wilcoxon rank sum,  $n=311$ ).

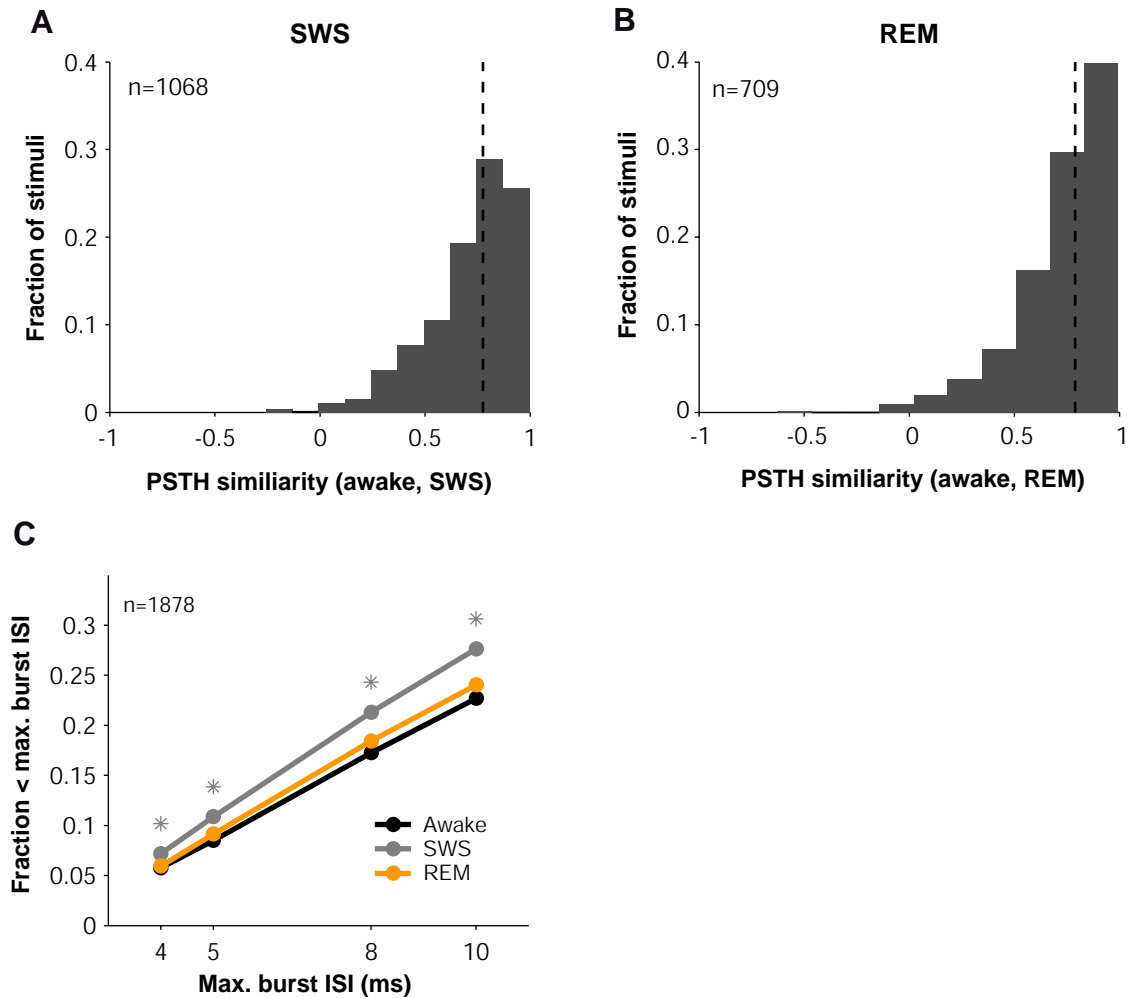
(c) Windows detected by our algorithm (see Chapter 2: General Methods) were classified as onset, offset, or sustained. The number of sustained responses dropped slightly in sleep (SWS or REM) as reflected in the shorter average duration of detected responses (inset). Windows detected in LB (blue) are plotted for comparison. Even in LB sustained responses were prominent.

(d) Population response profiles for all stimuli tested in awake, SWS, and REM. Population PSTH was obtained by summing, without normalizing, all responses (spontaneous rate subtracted) during their respective driven windows. Responses were long-lasting in all 3 states. Offset portion (after stimulus ends) of responses is indicated by the break on the abscissa. SWS responses demonstrated a strong late offset component.

(e) %Gain in SWS as a function of the length of the response for different response types. Long offsets (blue) were stronger in SWS than awake. Sustained responses (green), however, were stronger in awake (negative gains). The sampled points represent the 0-5<sup>th</sup>, 5-10<sup>th</sup>, 10-20<sup>th</sup>, 20-40<sup>th</sup>, 40-60<sup>th</sup>, 60-80<sup>th</sup>, and 80-100<sup>th</sup> percentiles of all window durations. Colored triangles on right hand side represent medians for each response type. Error bars represent  $\pm$ sem and are only shown for the curve that uses all windows (black).

(f) %Gain in REM as a function of the length of the response. Same format as (e). Like SWS, REM onset and offset gains are more positive than sustained response gains. For longer windows, gain tends to become more negative.





**Figure 3.9.** Measure of PSTH similarity and burst fraction.

(a) The distribution of similarity values (Pearson's product-moment correlation coefficient) between PSTH profiles in awake and SWS. Response patterns were highly correlated (median=0.78, vertical dashed line).

(b) The distribution of similarity values between PSTH profiles in awake and REM. Response patterns were highly correlated (median=0.79, vertical dashed line).

(c) Neurons tended to be burstier (fraction of inter-spoke intervals < maximum ISI) in SWS. Awake and REM spike trains had similar burst fractions. Asterisks represent significance at the  $p < .001$  level in comparison of SWS to awake (or REM) (Wilcoxon rank sum,  $n=1848$  stimuli).

## **Chapter 4: Tuning Properties of Single Neurons**

### **4.1 Summary**

During sleep, hearing is dulled. Yet our results and those of others using both single-neuron physiology and functional imaging reveal strong activation to sounds in auditory cortex. Here, we asked how hearing can be diminished in sleep despite the presence of sound-evoked activity in cortex. By closely examining neural responses across a wide range of stimuli, we found that the systematic loss of both excitation and inhibition reduced the dynamic range of SWS responses. Importantly, this occurs without changing overall activity since the loss of excitation appears to be compensated by less inhibition. This phenomenon was observable in both A1 and LB and resulted in weak but predictable modifications of sound level threshold, intensity tuning, frequency tuning, and phase-locking. In REM, dynamic range was not as strongly weakened as in SWS. A simple model was able to capture these phenomena. We conclude by suggesting that the availability of dynamic range in wakefulness may be important for coding the dynamics of sounds or for matching responses to the statistics of the environment. This novel perspective may provide a neural basis for the perceptual consequences of SWS.

### **4.2 Introduction**

Humans in deep sleep only awaken to the loudest sounds (Bonnet, 1982). But they are still capable of discriminating between their own name and those of others



during sleep (Oswald et al., 1960). The brain seems to still process sounds during sleep even though they do not reach conscious awareness (Bastuji et al., 2002). It is important to study how this processing during sleep differs from awake processing. In the previous chapter we demonstrated that processing can occur because of the presence of neural activity in primary and secondary auditory cortex. The question of how auditory information is processed is the topic of this chapter. One limitation of previous studies is that they relied on measurements of gross activity which belie possibly complex modifications during sleep. The seemingly random effects of sleep on single neurons (Livingstone and Hubel, 1981; Pena et al., 1999; Edeline et al., 2001) suggest that peak responses are a poor indicator of underlying neural mechanisms. Here, we tested neural responses across the dynamic range of sound intensities using a variety of sounds and found a systematic pattern of effects in sleep.

## **4.3 Results**

### *4.3.1 Driven responses in SWS*

As detailed in Chapter 3, we recorded from 340 neurons in the primary auditory cortex (A1) of naturally sleeping marmoset monkeys during at least one episode of slow-wave sleep (SWS) and one episode of wakefulness. Similar to recent studies finding little depression in SWS (Pena et al., 1999; Edeline et al., 2001), the average gain change of all sampled neurons was only -6% in SWS (Fig. 4.1 and see Chapter 3). However, it is important to point out that the modulations individual neurons underwent for a given

stimulus averaged 46% (average of the magnitude of gain in Fig. 4.1 ignoring sign) suggesting that some aspects of auditory processing are changed during SWS.

#### *4.3.2 Dependence of SWS modulation on sound level*

To test for the presence of more selective changes across the range of hearing, we divided the data according to sound level. The effects of SWS are illustrated in the example neuron in Figure 4.2. The neuron's response to quiet sounds (20-30dB) disappears in SWS (Fig. 4.2 middle plot, black arrows) even though the response to loud sounds is preserved. Out of the 159 neurons where we obtained intensity tuning curves, we observed 64 neurons in A1 like the example unit of Figure 4.2 that responded to both quiet (0-30dB) and loud (50-90dB) sounds. These neurons showed a small mean drop in firing rate for loud sounds (-4%) during sleep but a large drop for quiet sounds (-22%) (Fig. 4.3A). Their gain distribution for loud sounds is not significantly shifted from 0% modulation ( $p=.43$ , t-test,  $n=64$ ) (Fig. 4.3A, black curve). For quiet sound levels in the same neurons, however, the distribution is significantly shifted toward negative gains ( $p=.0003$ , t-test,  $n=64$ ) (Fig. 4.3A, green curve). Unlike diminishing attentional enhancement at high image contrasts in the visual system (Reynolds and Chelazzi, 2004), the effects of sleep cannot be accounted for by a saturation of firing rates since mean firing rates for quiet sounds were similar to those for loud sounds (quiet 16.4, loud 19.0 spikes  $s^{-1}$ ,  $p=.54$ , Wilcoxon rank sum,  $n=64$ ). Also, as detailed in the previous chapter, we found that mean gain does not increase for low, near threshold driven rates (mean for low firing rates=-1%, for high firing rates=-2%) but the spread of the distribution increases (standard deviation low rates = 70%, high rates = 42%) (Fig. 4.3B). So a shift

in mean gain seems to be a phenomenon specific to quiet sounds and not to low firing rates.

Across all neurons, activity in A1 was not much different between the awake and SWS conditions for loud sounds (Fig. 4.3C, right bars) (awake 13.9 vs. SWS 14.7 spikes  $s^{-1}$ ,  $p=.35$ , Wilcoxon rank sum,  $n=166$ ). In contrast, for the quietest stimuli near the threshold of hearing (0-20dB SPL), the average firing rate during SWS was ~30% less than that during awake (awake 15.2 vs. SWS 10.6 spikes  $s^{-1}$ ,  $p=2*10^{-5}$ , Wilcoxon rank sum,  $n=147$ ) (Fig. 4.3C, left bars).

We also tested whether these results generalized to a separate cortical field. In lateral belt (LB), a higher stage of auditory processing (Rauschecker et al., 1995), activity for quiet and loud sounds showed a similar pattern to that in A1 (compare Fig. 4.3C & 4.3D). SWS responses to quiet sounds were 37% weaker than awake responses (Fig. 4.3D, left bars) even though SWS activity matched awake activity at high sound levels in LB (Fig. 4.3D right bars). In contrast to A1, however, not many LB neurons responded to both quiet and loud sounds as most LB neurons preferred loud sounds (mean preferred A1=49 dB, LB=71 dB,  $p=6*10^{-10}$ , Wilcoxon rank sum,  $n_{A1}=275$ ,  $n_{LB}=36$ ) (Fig. 4.3E). In the small sample of neurons responsive at both low and high sound levels ( $n=9$ ), the same differential effect across sound level was observed as in A1. SWS suppression at quiet sound levels (-34%) was greater than suppression at loud sound levels (-12%) for these neurons. Thus, whether in A1 or LB, we consistently observed stronger effects of sleep on neural responses to quiet sounds.

### 4.3.3 Suppressed responses in SWS

An important property of A1 cells is that responses do not simply saturate at high sound levels. They exhibit non-monotonic intensity tuning (Brugge and Merzenich, 1973) (super-saturation, see Fig. 4.2, right panel for an example), the result of the increasing involvement of inhibition (Wu et al., 2006; Tan et al., 2007). Given the possibly important role of inhibition in auditory processing, we sought to identify the effects of sleep on inhibition. Inhibition is hard to detect extracellularly, but we used suppressed spiking responses as an indirect measure of inhibition. By modifying the algorithm we had used previously to detect driven responses (see Chapter 2: General Methods), we were able to isolate a number of events where firing was suppressed below spontaneous levels ( $n=314$ ) although these were still far fewer than the large number of driven events ( $n=2714$ ). Consistent with the notion that inhibition increases with level, these suppressed responses occurred mostly at high sound levels (75% of events at  $\geq 50$  dB) (Fig. 4.4A). Figure 4.4B shows inhibited responses in awake and SWS for an example neuron. The inhibitory response in SWS is 24% weaker and does not last as long as in awake. Over the population in SWS, the strength of suppression decreased by an average of 23% (Fig. 4.4C). This drop was significant ( $p < 10^{-5}$ , t-test,  $n=314$ ) and notably stronger than the -6% gain shift seen for driven events ( $p = 4 \times 10^{-4}$ , Wilcoxon rank sum,  $n_{\text{driven}}=2714$ ,  $n_{\text{suppressed}}=314$ ) (compare Fig. 4.1 & 4.4C).

### 4.3.4 Dependence of suppressed responses on spontaneous rates

We note a couple difficulties, however, with estimating inhibitory strength. For one thing, levels of spontaneous activity may limit how much inhibition is observed

extracellularly. A higher spontaneous rate allows more suppression to be measured. There was a high correlation between spontaneous and suppressed rates in our data ( $r^2=.43$ ,  $p=4*10^{-79}$ ,  $n=628$ ) (Fig. 4.5A). Because of this dependence on spontaneous rate, changes in suppression during SWS tended to be highly correlated with changes in spontaneous rate ( $r^2=.27$ ,  $p=3*10^{-23}$ ,  $n=314$ ) (Fig. 4.5B). Instead of measuring absolute changes in firing rate, we normalized each response by the spontaneous rate to measure relative inhibition. This removed a large part of the trend of suppression with spontaneous rate ( $r^2=.002$ ,  $p=.29$ ,  $n=628$ ) (Fig. 4.5C). It also reduced a large part of the dependence of change in SWS suppression on changes in spontaneous rate (compare 5B to 5D). However, gains for our normalized measure were still correlated with gain of spontaneous rate ( $r^2=.20$ ,  $p=5*10^{-17}$ ,  $n=314$ ) (Fig. 4.5D). This nonlinearity may be related to the fact that at very low spontaneous rates, a neuron is much closer to the flat part of its input-output (membrane potential-to-spiking) function. Strong modulation of firing rates during a suppressive event may only occur at high spontaneous rates. Fortunately, the neurons included in Figure 4.4C generally had high spontaneous firing rates that were similar between the awake and SWS states (mean spontaneous rates: awake 11.7, SWS 10.9 spikes  $s^{-1}$ ,  $p=0.22$ , Wilcoxon rank sum). A differential in spontaneous rates was more of a concern in the comparison of awake and REM data (see below). In our sample, spontaneous rates rarely saturated (<5% of responses reached 0 spikes  $s^{-1}$ ), and using relative changes in firing rates proved unnecessary as absolute changes in spiking gave similar results (mean  $\text{Gain}_{\text{SWS}}=-24\%$ ).

#### *4.3.5 Dynamic range of responses in SWS*

When plotted against sound level, the effects of sleep on driven and suppressed responses became apparent. The loss of driven responses to quiet sounds and of suppressed responses to loud sounds combined to give a loss of ~40% (Fig. 4.6A). This limits the range of possible responses in sleep at any given sound level, and dynamic range is narrower across the duration of the response as shown in the population PSTH (Fig. 4.6B). This implies that from moment to moment neurons cannot respond as dynamically in SWS as in awake at any given intensity.

The sound-level specificity of the losses observed should not be overlooked. Driven (excitatory) responses are only weaker at quiet sound levels, and suppressed responses (inhibitory) are reduced at loud sound levels in a complementary fashion (Fig. 4.6A). This pattern suggests that two independent processes contribute to the dynamic range of responses. Suppressive events are not simply the result of a withdrawal of excitation as no opposing change in driven (excitatory) strength was observed at high sound levels. Presumably, by being careful to only select extreme events (highly driven or highly suppressed), we isolated events dominated by only one process (excitation or inhibition).

#### *4.3.6 Simple model*

The possibility of two processes prompted us to think of a simple model for generating driven and suppressed responses. With two processes, we could possibly account for the range of sleep effects we had encountered: 1-no change in driven responses to louder sounds, 2-decrease of driven response to quiet sounds, and 3-decrease

of suppression. As mentioned, a simple model involving saturation could not account for the differences between loud and quiet sounds since in auditory cortex similar firing rates are elicited across sound levels (in visual cortex, responses are invariably stronger at high than low contrasts; Henrie and Shapley, 2005). The effects of inhibition probably contributed to super-saturation of rates at high sound levels, the same inhibition observed in suppressed responses. We reasoned that incorporating inhibition would provide the flexibility of responses that was needed.

Based on results from recent intracellular studies (Wehr and Zador, 2003; Wu et al., 2006; Tan et al., 2007), we modeled excitation as a quadratic, increasing to a preferred sound level and falling off at loud sound levels (Fig. 4.7A, red curve). We modeled inhibition as a linear function that rose with sound level (Fig. 4.7A, blue curve). These two underlying inputs gave rise to the nonmonotonic spiking output observed in rate-level curves (Fig. 4.7A, black curve). If SWS reduces the strength of both inputs as inferred from our data, then little change is seen in firing rate since the drop in excitation is offset by the loss of inhibition. Excitation and inhibition, however, are not perfectly balanced. At low sound levels where excitation is dominant, the reduction of excitation in SWS leads to a reduction in driven activity. While at high sound levels where inhibition is prominent, a reduction in inhibition actually leads to stronger activity in SWS than awake. This was the first surprising prediction made by the model, that responses in SWS should not saturate as much as awake. We then extended the model to frequency tuning by assuming the canonical form of a difference of two Gaussians (Mexican hat) (Rodieck, 1965) (Fig. 4.7B). Broadly tuned inhibition leads to inhibitory sidebands. If we apply the same reductions to excitation and inhibition as before, then

two predictions are made about frequency tuning in SWS. First, tuning bandwidth does not change. Although less excitation may lead to narrower tuning, the drop in inhibition raises responses back above threshold. Second, responses to frequencies far away from the preferred frequency should increase in SWS because these responses are normally suppressed by inhibition in awake. The predictions the model makes are very specific to features of cortical tuning. Note that at peak intensity (Fig. 4.7A) and peak frequencies (Fig. 4.7B) the model predicts little change.

#### *4.3.7 Testing model predictions*

The frequency and intensity tuning data collected confirmed the predictions of our conceptual model (Fig. 4.7, C and D). Neural thresholds in deep sleep were elevated (mean shift=10dB,  $p=.002$ , Wilcoxon rank sum,  $n=150$ ) suggesting a lack of excitatory sensitivity at quiet sound levels (Fig. 4.8A). Also, neurons tended to be more monotonic in SWS as measured using a non-monotonicity index ( $NMI = 1 - \text{rate}_{\text{loudest}} / \text{rate}_{\text{peak}}$ ). The change was slight (mean=5%) but significant ( $p=.01$ , sign test,  $n=145$ ) (Fig. 4.8B) and is more clearly seen when the population intensity tuning curves are plotted (Fig. 4.7C). Not only does the population response not saturate in SWS, it actually exceeds the awake response at high sound levels. Had we tested levels louder than 70 dB, SWS responses might have been even stronger (sound levels were usually limited to 70 dB SPL to avoid awakening the animals). So depending on sound intensity, activity in SWS could either be stronger (loud sound levels) or weaker (quiet sound levels) than activity in awake. This dual effect of sleep was unlikely to occur by random chance ( $p=.0004$ , bootstrap,  $n=159$ ) (see Methods) and was present individually in all 3 animals tested ( $p=.14$ , .05,



.002; n=37, 37, 85) (Fig. 4.9, A-C) and in LB ( $p=.15$ ; n=17) (Fig. 4.9D) although not always reaching statistical significance.

Along the frequency axis, center frequencies were consistent between awake and SWS (Fig. 4.8C), and tuning bandwidth did not change in SWS (Fig. 4.8D). Auditory filter widths were just as sharp as those in awake consistent with findings of a previous study (Edeline et al., 2001). However, for non-preferred frequencies far away from the main excitatory peak in the population tuning data, SWS responses surprisingly exceeded those of awake (Fig. 4.7D). This suggests a release of these flanking frequencies from suppression during SWS as predicted by the model.

The dual effects of sleep on firing rates confirmed predictions of the model but present a difficulty in interpreting whether sleep is a depressed or enhanced state. Generally a change in behavioral state is thought to have a singular effect on responses. The model suggests that this difficulty can be resolved if underlying excitation and inhibition are considered instead. The loss of both means dynamic range (excitation+inhibition) is lost in SWS, and SWS can be interpreted as a clearly depressed state. Overall firing rates (excitation-inhibition) are mostly preserved.

#### *4.3.8 Dynamic range in REM*

When we examined REM responses, we did not find clear evidence for a change in dynamic range. Much like in SWS, REM driven rates were unchanged on average (mean=-16%, n=2046) even though neurons underwent large modulations (mean magnitude=44%) (Fig. 4.10A). Activity at quiet sound levels was selectively depressed (mean awake=14.2, SWS=11.9 spikes  $s^{-1}$ ,  $p=.19$ , Wilcoxon rank sum, n=138) compared

to activity at loud levels (mean awake=16.9, SWS=16.4,  $p=.86$ , Wilcoxon rank sum,  $n=121$ ) in A1 (Fig. 4.10B), and this was also the case in LB (Fig. 4.10C). For the most part, suppressed responses were just as strong in REM as awake (mean=3%,  $n=241$ ) (Fig. 4.10D) which is a departure from what was observed in SWS (mean=-23%,  $n=314$ ) (Fig. 4.4C). If anything, we detected a slight trend in that inhibition was strengthened in REM and excitation was weakened which is more easily seen when both are plotted as a function of sound level (Fig. 4.10E). This can be seen in the population response histogram where the driven portion is stronger in awake but the suppressed portion is stronger in REM (Fig. 4.10F).

Unfortunately, the difference in the degree of suppression between awake and REM may be confounded by the fact that spontaneous rates were not similar for both states (mean awake=11.8, REM=14.3,  $p=.01$ , Wilcoxon rank sum,  $n=241$ ). REM had higher spontaneous rates which allows for larger absolute suppression. The population PSTH in Fig. 4.10F is just such a measure of absolute spiking suppression since it is not normalized. Using our normalized measure for suppression proved critical. Otherwise, the mean gain for suppression would have been +17% instead of +3% as in Fig. 4.10D. However, 3% may still be an underestimate as we cannot be sure that suppression is not actually stronger in awake (negative gain) but that the nonlinear effect of high REM spontaneous rates is making suppression seem equal to that in awake. From our data, we could only draw the limited conclusion that in either direction (slightly positive or negative change), suppression in REM and awake are not very different.

#### 4.3.9 Model and predictions for REM

Setting the model parameters to reflect this lack of change in inhibition coupled with a small drop in excitation (Fig. 4.11, A and B) predicted the REM intensity tuning curve derived from the data (Fig. 4.11C). Essentially, REM intensity tuning remains below the awake curve for all sound levels because of limited excitation at quiet sound levels followed by slightly stronger inhibition at loud sound levels. The shape of the REM curve predicts higher thresholds which was confirmed by our data (mean threshold shift=12 dB,  $p=4*10^{-4}$ , Wilcoxon rank sum,  $n=139$ ) (Fig. 4.11D). The model REM curve maintains the same non-monotonicity as the awake curve which was also confirmed by the data in individual units (mean shift of NMI=-2%,  $p=.14$ , sign test,  $n=119$ ) (Fig. 4.11E). The model predicts little change in frequency tuning (Fig. 4.11B). We could not test this prediction because of the limited number of complete frequency tuning curves collected during the brief REM episodes, but tuning bandwidths in our sample were highly correlated between awake and REM (Fig. 4.11F). In summary, SWS and REM display similar extracellular firing rates, but we suggest that this happens for different reasons. In the case of SWS, large changes in excitation and inhibition offset each other so that  $(E-I)_{\text{awake}}=(E-I)_{\text{SWS}}$  (despite  $E_{\text{awake}}>E_{\text{SWS}}$  and  $I_{\text{awake}}>I_{\text{SWS}}$ ). In REM, however, only small changes in excitation and inhibition occur so that  $E_{\text{awake}}\approx E_{\text{REM}}$  and  $I_{\text{awake}}\approx I_{\text{REM}}$ , and changes need not be offsetting (i.e. excitation drops and inhibition rises) since they are slight overall.

#### *4.3.10 Effects of stimulus type*

We now turn to the effects of sleep on different stimulus types. As described in Chapter 2: General Methods, we used a battery of stimuli to test units during sleep. These stimuli were selected to test a variety of hypotheses. For example, we used vocalizations to test whether more behaviorally relevant stimuli elicit stronger responses in sleep. And we used amplitude-modulated stimuli to test temporal processing to determine whether stimulus following responses become more sluggish during sleep. With regard to spectral processing, we tested many units with noise stimuli to see if responses to broadband stimuli are as strong as responses to narrowband stimuli. Any differential effect may give an insight into spectral integration during sleep. The different stimulus types also served as a valuable test of the results from simple tone or narrowband noise stimuli of different sound levels. Do the changes we found in excitation and inhibition during SWS and REM generalize to stimuli with more complex temporal or spectral character? For example, we have suggested that the interaction of excitation and inhibition could be important in the temporal aspects of the response. If inhibition is weaker in SWS, we might expect this to lead to poorer phase-locking while stronger inhibition such as in REM could lead to better phase-locking.

#### *4.3.11 Temporally modulated stimuli*

When tested with sinusoidal amplitude modulated stimuli, units in SWS demonstrated similar phase-locking to those in awake. This was the case whether measured by the upper limit of phase-locking (mean  $f_{\max}$  awake=14.0, SWS=11.6 Hz,

$p=.37$ , Wilcoxon rank sum,  $n=58$ ) (Fig. 4.12A) or by the average vector strength (mean VS awake=.45, SWS=.43,  $p=.11$ , Wilcoxon rank sum,  $n=212$ ) (Fig. 4.12B). It bears mentioning that  $f_{\max}$  and VS were slightly improved in awake over SWS even though the effect was not strong. So this does not negate the possibility that there is less inhibition in SWS that leads to less precise timing of responses.

In REM, responses to temporally modulated stimuli were more comparable to those in awake. Phase-locking in REM occurred at nearly the same maximum rates as in awake (mean  $f_{\max}$  awake=12.7, REM=10.2 Hz,  $p=.46$ , Wilcoxon rank sum,  $n=40$ ) (Fig. 4.12C). And the strength of phase-locking was well-preserved if not slightly enhanced (mean VS awake=.44, REM=.49,  $p=.07$ , Wilcoxon rank sum,  $n=147$ ) (Fig. 4.12D).

Another popular measure of temporal processing is tuning of firing rate to modulation frequency (Krishna and Semple, 2000; Liang et al., 2002). Preferred modulation frequency and bandwidth of modulation tuning were not different in awake and SWS ( $p_{\text{rBMF}}=.30$  and  $p_{\text{BW}}=.45$ , Wilcoxon rank sum,  $n=59$ ) (Fig. 4.13, A and B). If anything, there was a slight tendency toward larger modulation tuning bandwidths in SWS ( $p=.04$ , sign test,  $n=59$ ) (Fig. 4.13B). The overlap of the population averaged modulation tuning curves in awake and SWS was consistent with the similarities of tuning properties at the single neuron level (Fig. 4.13C). In REM, the center and bandwidth of modulation tuning were also relatively unchanged ( $p_{\text{rBMF}}=.73$ ,  $p_{\text{BW}}=.64$ , Wilcoxon rank sum,  $n=33$ ) (Fig. 4.13, D and E) although the population modulation tuning curve in REM displayed less of a response at the low frequency end (Fig. 4.13F). Responses in REM lose the weak low-pass character of awake responses. This preference for high modulation frequencies may reflect slightly increased inhibition in

REM. When we followed this difference in population activity up further, we did not find the trend to be significant at the single-unit level. For low modulation frequencies ( $f_{\text{mod}} < 32$  Hz), firing rates in REM and awake were not different ( $p = .38$ , Wilcoxon rank sum,  $n = 86$ ). They also did not differ at high modulation frequencies ( $f_{\text{mod}} > 64$  Hz) ( $p = .71$ , Wilcoxon rank sum,  $n = 52$ ).

#### *4.3.12 Narrowband and broadband noise*

Neurons were tested with broadband stimuli in two different settings. First, stimuli of increasing bandwidth were presented to see how integration across frequencies varies for broadband sounds. Second, we played a broadband sound as a masker of a narrowband sound. Our hypothesis was that sleep responses may severely degrade in the presence of any competing sound. The largest bandwidths eliciting responses in awake, SWS, and REM were similar suggesting that neurons are capable of responding to these stimuli in all three states (Fig. 4.14, A and B). When noise stimuli of increasing bandwidth were tested in SWS, mean gain appeared to decrease over the population (Fig. 4.14C); SWS responses were especially weaker for broadband stimuli. This observation appears to violate our model. If less suppression is present in sideband frequencies in SWS, increasing the bandwidth of the noise should give SWS responses a decided advantage over awake responses because more energy is placed in the flanking frequencies. However, the opposite is seen. Responses became stronger in awake relative to SWS except when wideband noise is used. The main difficulty with interpreting this trend is the confound of sound level. Because noise stimuli are normalized by their peak value in our experiments, the energy level in a frequency band

decreases as bandwidth is made broader. In other words, larger bandwidths lead to perceptually quieter sounds using this method for normalization. Given that awake responses are stronger than SWS responses at quieter sound levels in our model, it is possible that responses to broader bandwidth but perceptually quieter stimuli are actually stronger in awake not SWS. The trend with bandwidth is not as strong in REM and appears to reverse in one bin (Fig. 4.14D).

Limited data on the effects of masking were collected in SWS and REM (only in one animal, 43q). At negative masker levels (masker quieter than probe), responses were strongest (Fig. 4.14, E and F). When the masker was >30dB above the probe level, responses dropped off. This drop-off (masking effect) was not noticeably different in SWS or REM when compared to awake given our small sample.

#### *4.3.13 Sleep modulation across stimulus classes*

Figure 4.15 summarizes the average gains for the stimulus types that were most often tested (tone, bandpass noise, sAM, vocalizations, environmental sounds, and wideband noise). Regardless of stimulus type, mean %Gain in SWS was usually near 0% or slightly negative. Only the gain distribution for environmental sounds was significantly different from chance ( $p_{\text{SWS}}=.008$ , bootstrap,  $n=30$ ;  $p_{\text{REM}}=.03$ , bootstrap,  $n=20$ ) (Fig. 4.15, A and B). These included sounds from our marmoset colony such as cage clanks as well as sounds of the ocean and river. The use of these complex sounds and the small sample size make it difficult to interpret why SWS and REM responses were different from awake responses. For one thing, the reason we used these sounds was as a last resort to drive cells which did not respond to simple stimuli. Possibly, such

highly selective cells are more prevalent in the awake state. As a note, the environmental sound stimulus set was added toward the end of the study (coinciding with LB recordings) which is why the sample is small. It is quite possible though that hearing during sleep is adapted to ignore background nighttime sounds. Our earlier result that quiet sounds are suppressed in SWS is in line with this notion. Other statistical properties of background sounds may also be used for their selective suppression.

Within cell comparisons were possible for a few combinations of stimulus types (Fig. 4.15, C and D). Whether for modulated versus unmodulated, narrowband versus broadband, or meaningful (vocalizations) versus non-meaningful stimuli, no clear difference in gain was seen, and gains averaged near 0%. This suggests that at the level of A1 and maybe LB the effects of sleep generalize across stimulus classes. To demonstrate the homogeneity of the effect of sleep across stimulus type, we used the gain for tones to predict the gain for all other stimuli and found a good correspondence (SWS:  $r^2=.22$ ,  $p=4*10^{-6}$ ,  $n=88$ ; REM:  $r^2=.15$ ,  $p=5*10^{-4}$ ,  $n=75$ ) (Fig. 4.16, A and B). When we computed the vector similarity of tuning curves between SWS and awake (see Methods) much as we did for PSTH profiles in Chapter 3, we found that median similarity was again quite high ( $\rho=.62$ ,  $n=352$ ) (Fig. 4.16C) although not as high as median similarity between awake and SWS PSTH's ( $\rho=.78$ ,  $n=1068$ ). Essentially, a simple multiplicative gain change can account for a substantial (43%) (Fig. 4.16C) amount of the variance between awake and SWS tuning curves and between awake and REM tuning curves (46%) (Fig. 4.16D) across all stimuli. These numbers are noteworthy given that the variance explained by using firing rates on odd trials to predict rates on even trials only improved values by 15% (56% for awake/SWS,  $n=702$ ; 61% for awake/REM,  $n=636$ ).



Ideally, odd trials should match even trials in firing rate and explain 100% of variance. The fact that values between odd and even trials fall far short highlights that variance left unexplained between awake and sleep firing rates is mostly the result of noise in our measurements (random error  $\approx 40\% = 100-60$ ) and not the result of additional structure not captured by the model (systematic error  $\approx 15\% = 100-40-45$ ). The high error from noise may reflect the variability of behavioral states over time and between sleep cycles. The residual error that cannot be attributed to noise probably reflects some of the changes in sound level and frequency tuning mentioned earlier or other likewise subtle changes.

#### **4.4 Discussion**

In this chapter, we found that in certain sound level regimes SWS responses could not be driven or suppressed as strongly as in awake. Mean effects were around 20-40%. These values are stronger than any observed in the previous chapter ( $<10\%$ ). The narrowing of response range coupled with the sound level specificity of the phenomena observed led us to propose a conceptual model where underlying excitation and inhibition are both reduced in SWS. This is a radical departure from the idea of a simple drop of average activity (Steriade, 2003). Instead, the offsetting effects of excitation and inhibition result in minimal changes to average activity. A pattern emerges where awake responses are strong for quiet sounds, but SWS responses are stronger for loud sounds and non-preferred frequencies. We termed this combined loss of excitation and inhibition during SWS a loss in dynamic range (excitation+inhibition).

Changes in cerebral metabolism during sleep may be a parsimonious explanation of our findings. Cerebral blood flow decreases by ~20% during SWS (Hobson, 2005). Synaptic activity is known to correlate strongly with local blood delivery (Malonek and Grinvald, 1996; Viswanathan and Freeman, 2007). It is plausible that SWS can only support weak excitatory and inhibitory synaptic activity because of lowered cerebral metabolism. In REM, cerebral blood has been found to be at or above awake levels (Franzini, 2000). This correlates well with the finding of preserved dynamic range of activity in REM.

#### *4.4.1 Comparison to previous intracellular study*

One intracellular study during sleep performed by Timofeev et al. (2001) is directly relevant to the results reported here (although they electrically stimulated the thalamus whereas we used external auditory stimulation). The study reported that disfacilitation occurs during the low frequency oscillation (<1Hz, lower than delta band) in SWS. In other words neurons become less responsive not because of increased inhibition but because of the loss of excitation. However, this is followed by strongly excitable periods during the peak of the slow oscillation (Steriade et al., 1993). Such disfacilitation may correlate with the drop of excitation we observed in SWS. But our data suggests that there is also an independent change in inhibition which should actually enhance responses at loud enough sound levels. The periodic nature of the slow-oscillation phenomenon observed by Timofeev et al. (2001) is also different than the time averaged nature of our data collection. Perhaps, more relevant is the finding by Timofeev et al. (2001) of active inhibition in the form of short, low-amplitude IPSP's during REM

and awake. This compares favourably with our finding that in REM inhibition is as strong or stronger than in awake and adds support to the notion that REM is an awake-like state. Interestingly, inhibition was strongest during the rapid eye movements in REM and eye movements in awake. We did not collect data on when eye movements occurred in REM, but future studies could investigate whether there are specifically strong periods of inhibition during REM.

#### *4.4.2 Comparison to attention studies*

Comparisons can be drawn between this study and extracellular studies of visual attention. Reynolds et al. (2000) examined attentional enhancement across the range of possible image contrasts and found that the effects of attention were strongest at low image contrasts. We observed such a change in sensitivity between awake and asleep. Neural thresholds were elevated by ~10dB in SWS (Fig. 4.8A) and REM (Fig. 4.11D). One difference between the auditory and visual systems, though, is that auditory cortical neurons exhibit non-monotonic behaviour at loud sound levels. Firing rates can super-saturate. An additional inhibitory component was used to account for this phenomenon and was critical in explaining why SWS responses can overtake those in awake. It is possible, however, to model the effect of SWS as an effective shift of the awake intensity tuning curve rightward (Fig. 4.7C) (visual attention is modelled as a shift in stimulus contrast). Such a shift would not account for the decrease of saturation in SWS. Our choice of including inhibition was grounded in intracellular findings in the auditory system and proved essential to capturing the effects of sleep on suppression.

#### *4.4.3 Functional implications*

Elevated acoustic arousal thresholds are a hallmark of deep sleep in humans (Bonnet, 1982). Our finding of elevated neural thresholds in SWS provides a neural correlate for this reduced sensitivity to the environment. On the other hand, REM responses were similar to awake responses. This is consistent with behavioural data suggesting that REM is lighter sleep (Bonnet, 1982) and may be a substrate for external sounds penetrating into dreams (Ramsey, 1953; Berger, 1963; Burton et al., 1988).

In a broader sense, the loss of inhibition in SWS may have important consequences for gain control. Gain control mechanisms are desirable computationally because they match the dynamic range of neural coding to the statistics of the environment (Laughlin, 1981; Schwartz and Simoncelli, 2001). A recent study showed that inhibition that grew with stimulus intensity was important in setting the gain of a leech behavioral circuit (Baca et al., 2008), and inhibition appears to play a critical role in setting neural properties during attention (Mitchell et al., 2007). We suggest that such adaptive mechanisms are weakened in SWS but present in awake.

We did not observe strong changes in frequency tuning even though inhibition is often implicated in sharpening tuning (Rodieck, 1965; Suga, 1995). Our data were in line with the prediction of our model since the loss of excitation narrows tuning in opposition to the broadening of tuning from less inhibition in SWS. A change in dynamic range may play a larger role in temporal processing. Rapid modulations of excitation and inhibition are probably necessary for precise coding of the time-varying acoustic signal. Unfortunately, temporal processing is poorly understood in the auditory cortex, and no good means of measuring what is meant by higher-level processing of the temporal

aspects of the signal exists. Measures adopted from studies in the periphery include those of phase-locking and modulation tuning. Cortical responses are not strongly phase-locked and often demonstrate non-synchronized firing (Lu, et al., 2001). Phase-locking may not be a critical aspect of cortical responses. Furthermore, modulation tuning is often broad and not robust to variations in stimulus parameters as early as the inferior colliculus (Krishna and Semple, 2000). We did not see much difference in awake and SWS responses to amplitude modulation. Any prediction that we could generate about responses should be tempered by the fact that we approximated long-lasting not fast inhibition when we measured suppressed responses. Because inhibition is difficult to detect extracellularly, we were forced to use very long detection windows in our algorithm ( $\geq 100\text{ms}$ ). Measuring the strength of suppression at these long timescales may have little to do with inhibition at faster timescales more relevant to phase-locking (Markram et al., 2004). It is likely that the extra inhibition in REM may be of the fast kind and therefore not comparable to that in SWS (Timofeev et al., 2001). This suggestion underscores an assumption of our model. We assumed one global inhibitory process that generalizes across frequency and interacts linearly with excitation. This neglects ideas of shunting, fast, and slow inhibition, sideband inhibition, and interactions with nonlinear thresholds. One could imagine doing away with inhibition entirely and giving the excitatory input a more complex shape to explain the effects we observed. A number of models could account for our observations, but we tried to choose the most parsimonious model. In our model, two processes are simply linearly scaled without changing the shapes of curves in SWS. Future studies could test these assumptions and determine if a more complex model is necessary.

#### *4.4.4 Comparison to extracellular sleep studies*

Only two neural studies of sleep went beyond measuring general responses and directly tested tuning. In primary visual cortex (V1), Hubel and Livingstone (1981) found that tuning to orientation and motion improved slightly when the animal woke up. In A1, Edeline et al. (2001) reported little change in frequency tuning bandwidths with a slight trend toward sharper tuning in awake. This result is consistent with the lack of a change in frequency tuning that we observed. If anything, we would predict slightly sharper tuning because of a gain of inhibition in awake consistent with what Edeline et al. (2001) observed. Even so, prior studies had relied on tones or noise bursts (Brugge and Merzenich, 1973; Pena et al., 1999; Edeline et al., 2001). No published neural study to our knowledge has used more complex stimuli such as we did.

#### *4.4.5 Multiplicative model of modulation*

The lack of a strong effect of stimulus type in our experiments leads us to propose that a multiplicative gain change accounts for a large part of modulation in sleep possibly similar to gain changes reported in the attention literature (McAdams and Maunsell, 1999). A simple linear scaling could account for ~40% of the variance of sleep modulation across stimulus types. This suggests to us that the assumption of the model of a linear scaling of excitation and inhibition rather than a change in their shape may be valid to some extent.

#### *4.4.6 Limitations of testing different stimulus types*

In general, we found no clear systematic effect of stimulus type. This lack of a result could be taken as going against the idea of reduced dynamic range in SWS. We note that a major limitation was that stimuli were not controlled tightly enough to directly test our model. One key parameter, sound level, is critical to setting the balance of excitation and inhibition. All our stimuli were normalized by peak amplitude leading to lower average power in any given frequency for non-tonal stimuli. This means that these stimuli were perceptually quieter than a tone played at the same sound level. Another parameter that is important is the tuning bandwidth of a neuron at a given sound level. Only when controlling for tuning width can effects under the peak of tuning versus those outside the peak in the inhibitory sidebands be truly characterized and quantified. Unfortunately, time did not permit us to derive specific properties of each neuron's receptive field and then tailor the parameters of complex stimuli to explore further. Instead, we simply hand-tuned neurons for best frequency and level and then played standard stimulus sets. Future studies with a more formal quantitative model to guide the design of stimulus sets may be able to explore the subtle changes that occur on a unit by unit basis.

#### *4.4.7 Comparison to imaging and evoked potential studies*

The best comparison to our data may come from human imaging studies using subjects' names and speech as stimuli in addition to tones (Portas et al., 2000; Davis et al., 2007). We would predict that discrimination performance for complex stimuli should

be worse in SWS because of the loss of both excitation and inhibition. Indeed, imaging studies in sleeping (Portas et al., 2000) or mildly sedated (Davis et al., 2007) humans have found a loss of activity in higher frontal areas for the discrimination of complex sounds despite finding strong activity in the temporal lobe (indicating that sounds are still detected in the brain). Along the same lines, studies have found that the evoked potentials for higher discriminations such as name recognition (Pratt et al., 1999), oddball detection (Nashida et al., 2000; Loewy et al., 1996), and semantic discrimination (Perrin et al., 2002) are not preserved in the same form in SWS even though evoked potentials are still elicited (for review, see Bastuji et al., 2002). REM evoked potentials tend to retain patterns similar to those in awake (Atienza et al., 2000). Our data provide a neural basis for the results of these studies in humans by suggesting that limited dynamic range of processing in A1 during SWS is responsible for poor read-out in higher areas involved in complex discriminations.

Such deficits may be similar to the shortcomings of hearing aids; they deliver the same volume of sound but cannot match the dynamic range of normal listening leading to severe reductions in speech intelligibility (Moore, 1995). In demonstrating the specific changes that limit the dynamic range of auditory processing in SWS, our findings put forward a new model where auditory cortex can still perform the general detection of sounds in sleep but processes the fine details of sounds only in awake. This result represents a significant step toward resolving the paradox of how hearing during sleep can be vastly different despite similar overall cortical activity.



## 4.5 Methods

See Chapter 2: General Methods for details of electrophysiological recordings, sleep scoring, detection algorithm for driven responses, and detection algorithm for suppressed responses. See Methods in Chapter 3 for details on identification of A1 and LB.

### 4.5.1 Gain measures

Average discharge rate in awake and SWS was measured during the driven and suppressed periods detected by our algorithm and spontaneous rate subtracted. We used

$$\%Gain_{driven} = 100 \frac{SWS - awake}{Max[|SWS|, |awake|]} \text{ to compare driven responses between awake and}$$

$$SWS \text{ and } \%Gain_{suppressed} = 100 * \left( \frac{awake}{awake_{spont}} - \frac{SWS}{SWS_{spont}} \right) \text{ to compare suppressed}$$

responses (the ratios in the expression measure the fraction of spontaneous firing that was suppressed). We also re-calculated gains of suppressed responses using absolute firing rates and the gain formula for driven responses. Although we mainly report gains for individual stimuli, comparable results were obtained when we computed a single gain for each neuron by averaging all stimulus gains.

### 4.5.2 Model

For simplicity, excitation was modelled as a quadratic and inhibition as a linear function:

$$E(i) = 1 - \left( \frac{i - i_{peak}}{i_{peak}} \right)^2, \quad I(i) = a * \frac{i}{100}, \text{ where } i \text{ is sound intensity.}$$

These forms of excitation and inhibition underlying intensity tuning are based on recent findings from intracellular studies (Wu et al., 2006; Tan et al., 2007). These studies support a critical assumption of the model that excitation and inhibition are not balanced, but this has not been the case in all studies (Wehr and Zador, 2003). For frequency tuning, we assume a difference of Gaussians (Rodieck, 1965):

$$E(f) = b * e^{-(f/\sigma_E)^2}, \quad I(f) = c * e^{-(f/\sigma_I)^2}, \text{ where } f \text{ is sound frequency.}$$

And resulting firing rates are:

$$R_{awake} = E - I, \quad R_{SWS} = g_E E - g_I I$$

The model does not incorporate a spiking nonlinearity and assumes a linear relation between membrane potential and firing rate. Peak sound level  $i_{peak}$  was set as 50dB SPL. Relative inhibitory strength  $a$  was .67 (inhibitory input reaches two-thirds the level of excitatory input). The weights  $b$  and  $c$  of the inputs used to derive frequency tuning were chosen based on the strengths of the inputs at 50dB SPL such that  $b = E(i=50) = 1.0$  and  $c = I(i=50) = .34$ . Excitatory input was set to be more sharply tuned than inhibitory input ( $\sigma_E = .5, \sigma_I = 2$ ). Finally, we chose excitatory modulation  $g_E$  in SWS to be 80%, and inhibitory modulation  $g_I$  to be 50%. These values are based on our data. We assume that our data overestimated excitatory modulation since at quiet sound levels near threshold only small changes in membrane potentials are required to give large changes in extracellular firing rates (spiking nonlinearity). Likewise, we may have underestimated modulation of inhibition since the relationship between membrane

potential and spiking is very flat when a neuron is suppressed far below threshold. For REM,  $g_E$  was set to 90% and  $g_I$  to 110% (slightly lower excitation and higher inhibition compared to awake).

#### *4.5.3 Statistical tests*

Wherever two distributions are statistically compared throughout the text and figures, a Wilcoxon rank sum test was used although occasionally a sign test was used for very tightly distributed data. A Student's t-test was used to determine if a distribution was significantly different from 0. Correlation between two variables was quantified using the Pearson's product-moment correlation coefficient. All p-values are reported in the text and figure legends.

To test for the dual modulation pattern of sleep on intensity tuning curves, we generated random population intensity tuning curves ( $n=10,000$  simulations) from our data by randomly assigning individual tuning curves for each neuron to awake or SWS before averaging. We then computed the difference between SWS modulation at low sound levels and at high sound levels. The strict asymmetry we observed in that SWS responses were only weaker for sounds below 40dB and stronger for sounds louder than 40dB was not often observed by chance.

#### *4.5.4 Various measures derived from tuning curves*

**Threshold:** quietest sound level eliciting a response  $> 4 \times \text{sem}$  above spontaneous rate.

**Nonmonotonicity Index (NMI):**  $1 - \frac{rate_{loudest}}{rate_{peak}}$ , NMI=0 implies strongest response was to

loudest sound tested, and unit was monotonic.

**Center Frequency:** The frequency eliciting the maximum response in a frequency tuning curve.

**Frequency Tuning Bandwidth:**  $BW = \sum_i r_i |f_i - f_{centroid}|$ , where  $f_{centroid} = \sum_i r_i f_i$  and  $r_i$  is

the firing rate at each frequency  $f_i$  tested. This method computes the first moment of the tuning curve about the centroid. It captures the central width of the tuning curve without making assumption about its shape.

**Vector Strength (VS):**  $VS = \frac{1}{n} \sqrt{\left( \sum_i \cos(2\pi t_i / T) \right)^2 + \left( \sum_i \sin(2\pi t_i / T) \right)^2}$ , where  $t_i$  is the

time of the  $i$ th of  $n$  spikes and  $T$  is the period of the modulation equivalent to the interclick interval of a click train (Lu et al., 2001; Liang et al., 2002).

**Rayleigh Statistic:**  $2 * n * VS^2$ ,  $RS > 13.8$  indicates a significantly phase locked response (Lu et al., 2001).

**Center of Modulation tuning:**  $mf_{centroid} = \sum_i r_i * mf_i$ , where  $mf_i$  is the given modulation

frequency tested. We used this approach over simply taking the modulation yielding peak response because modulation tuning curves were sampled coarsely and had little curvature. We found that applying a *max* operation to such tuning curves is noisy. The centroid takes advantage of averaging to get rid of noise and locate the center of mass of the curve.

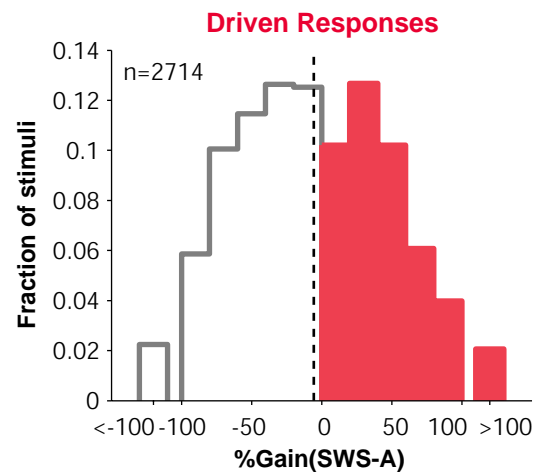
**Modulation Tuning Bandwidth:**  $BW = \sum_i r_i |mf_i - mf_{centroid}|$ . This is the same measure

as the one used to derive frequency tuning bandwidth.

**Tuning Similarity:**  $\rho_{SWS,awake} = \frac{(rates_{awake} - \mu_{awake}) \bullet (rates_{SWS} - \mu_{SWS})}{|rates_{awake} - \mu_{awake}| |rates_{SWS} - \mu_{SWS}|}$ ,

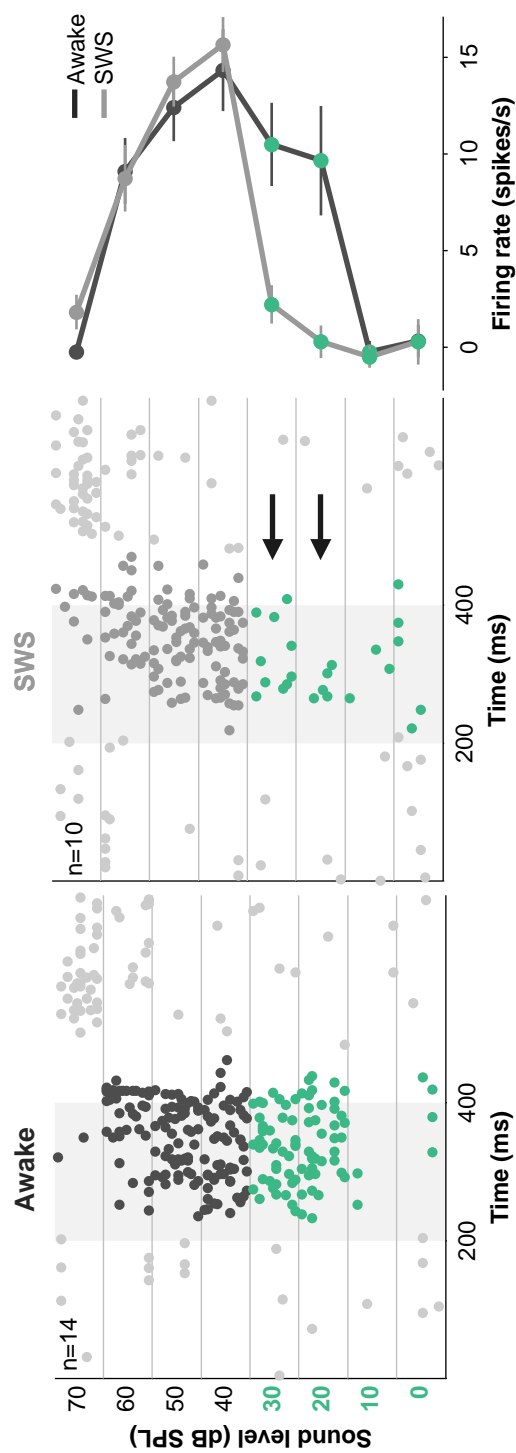
$\rho_{REM,awake} = \frac{(rates_{awake} - \mu_{awake}) \bullet (rates_{REM} - \mu_{REM})}{|rates_{awake} - \mu_{awake}| |rates_{REM} - \mu_{REM}|}$ , where *rates* are the driven rates

(spontaneous rate subtracted) in each state ordered by stimulus number. This dot product is simply a correlation measure between firing rates and is similar to the one used to measure PSTH similarity in Chapter 3.



**Figure 4.1.** Driven responses in awake and SWS.

Summary of percent change of all driven responses during SWS. Positive gains indicate that responses were more strongly driven by sounds in SWS than awake. Mean gain is -6% (vertical dashed line).



**Figure 4.2.** Example cell showing loss of responses to quiet sounds in SWS.

An example neuron whose response to quiet sounds (left panel, green highlight) disappears in SWS (black arrows, middle panel) even though a robust response to loud sounds remains. This neuron responded to both quiet and loud sounds because of its non-monotonic tuning (right panel) which is often observed in A1 (error bars represent  $\pm 1$  sem).

**Figure 4.3.** Selective loss of responses to quiet sounds in SWS.

(a) For all neurons that could be driven by both quiet and loud sounds, SWS modulation of responses was not different from zero for 50-90dB sounds (median=-2%, black inverted triangle) ( $p=.43$ , t-test,  $n=64$ ). But responses were negatively modulated at the low end (0-30dB) of their sound level tuning curve (median=-30%, green inverted triangle) ( $p=.0003$ , t-test,  $n=64$ ).

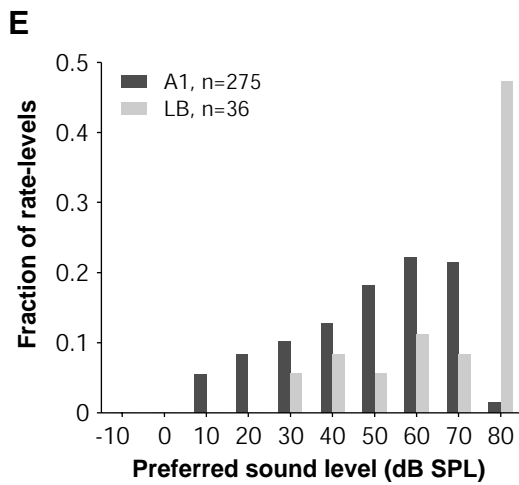
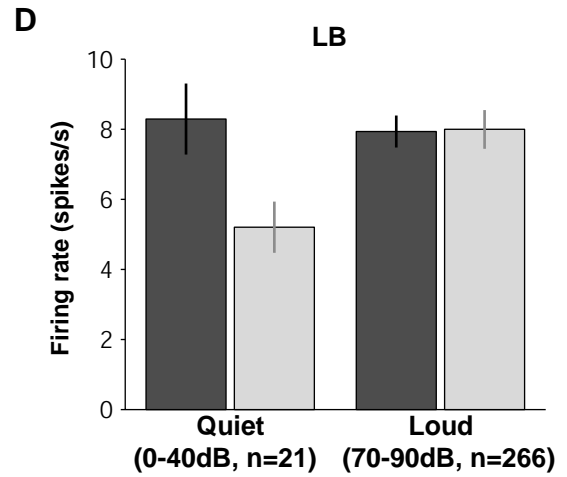
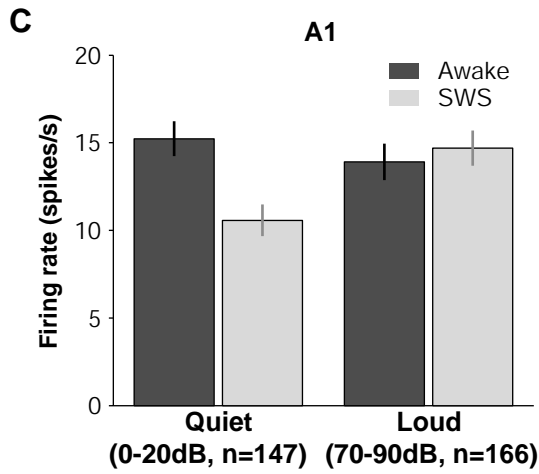
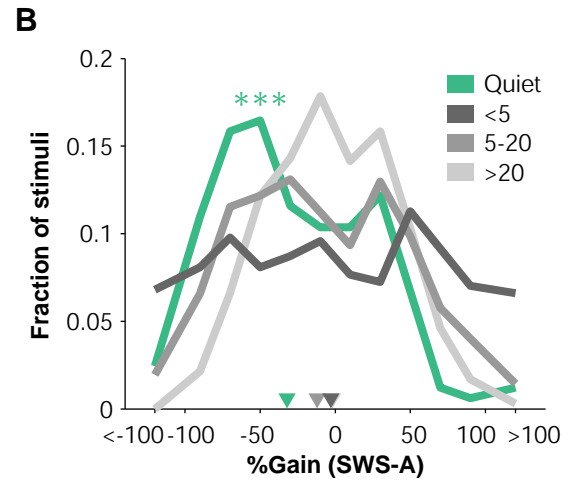
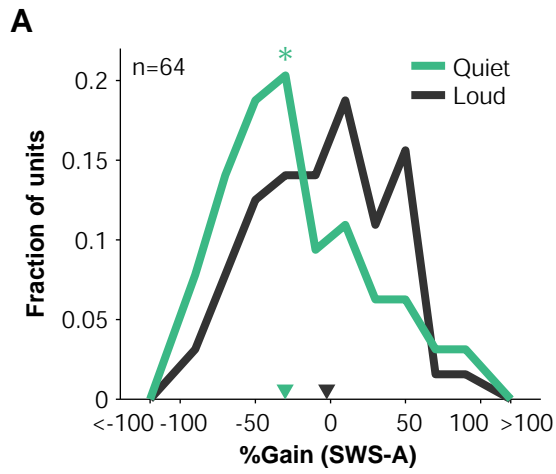
(b) The negative gain for quiet sounds (green curve) cannot be explained by firing rate. Gain is near 0 for all firing rate ranges (gray curves). Three firing rate ranges (0-5, 5-20, and >20 spikes/s) were used for all stimuli tested in A1 and LB ( $n=2714$ ). Rate was taken as the mean of awake and SWS firing for that stimulus. The ranges roughly divided the data into a lower fourth ( $n=470$ , dark gray), middle half ( $n=1594$ , gray), and upper fourth ( $n=650$ , light gray). No trend exists in median gain with rate (medians=-2%, -12%, -3%, respectively) (inverted triangles). Rather, a trend exists in the spread of the distributions. Distributions tighten with increasing rate range (standard deviation=70%, 53%, 42%). For comparison, the distribution for quiet sounds (0-20dB) (green curve,  $n=164$ ) (median=-32%, standard deviation=49%) shows a clear shift in median away from 0 and significantly differed from the other 3 distributions (Wilcoxon rank sum,  $p=3*10^{-5}$ ,  $2*10^{-4}$ ,  $2*10^{-8}$  respectively) as indicated by \*\*\*. Mean rate for quiet sounds was 19.3 (15.2) spikes/s in awake (SWS).

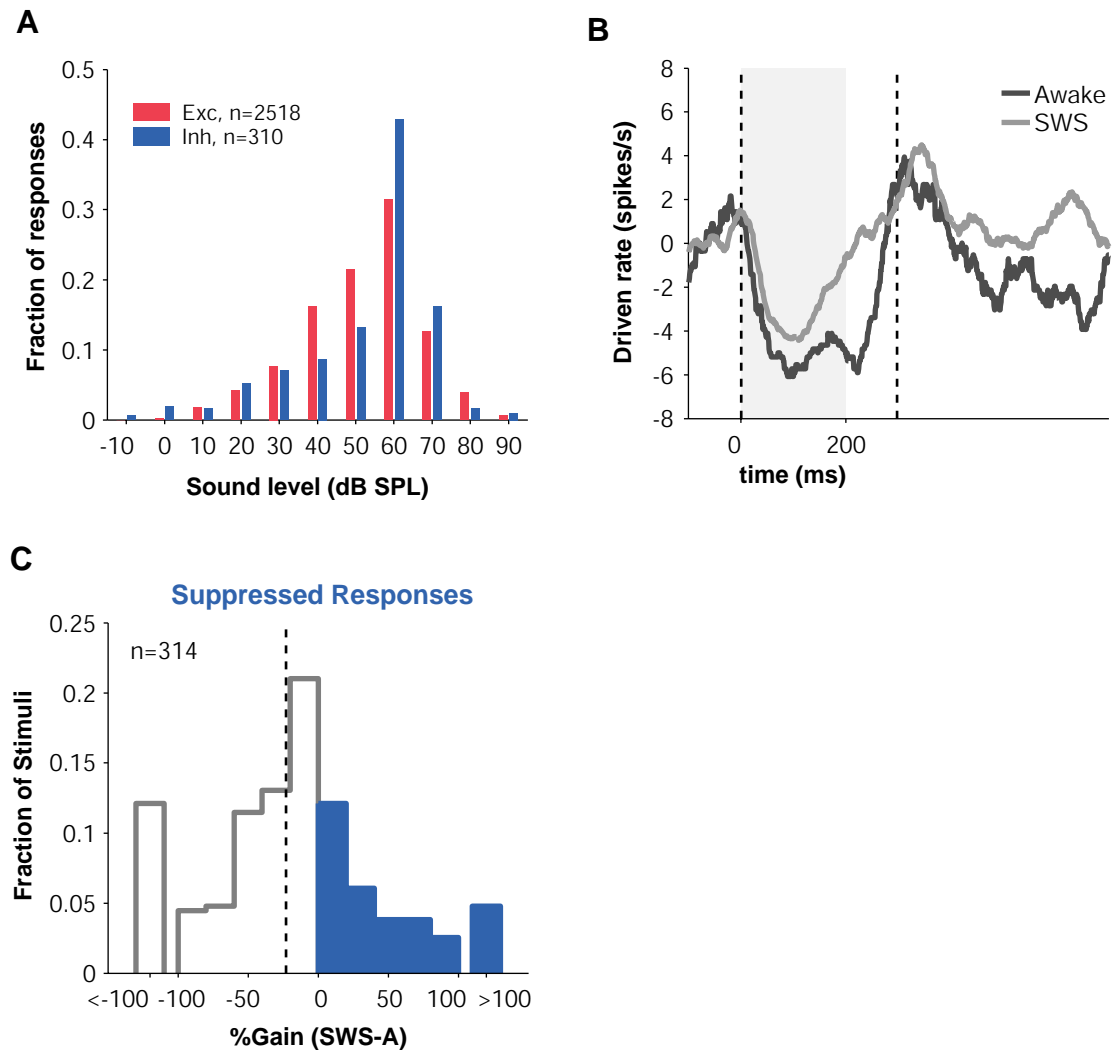
(c) Activity to quiet sounds across the population of A1 neurons in SWS averaged significantly less than activity in awake (mean awake=15.2, SWS=10.6 spikes/s,  $p=2*10^{-5}$ , Wilcoxon rank sum,  $n=147$ ) (error bars represent  $\pm$ sem).

(d) Activity to quiet sounds was also reduced in the population of LB neurons (mean awake=8.3, SWS=5.2 spikes/s,  $p=.01$ , Wilcoxon rank sum,  $n=21$ ) (error bars represent  $\pm$ sem).

(e) Preferred sound level distributions in A1 and LB. LB neurons often preferred loud sounds as evidenced by the height of the 80 dB SPL bin (light gray). A1 units had a more even preference across sound level (dark gray). This difference between A1 and LB (mean preferred A1=49 dB, LB=71 dB,  $p=6*10^{-10}$ , Wilcoxon rank sum,  $n_{A1}=275$ ,  $n_{LB}=36$ ) may be partially explained by the more complex stimuli we sometimes had to use to drive LB neurons.





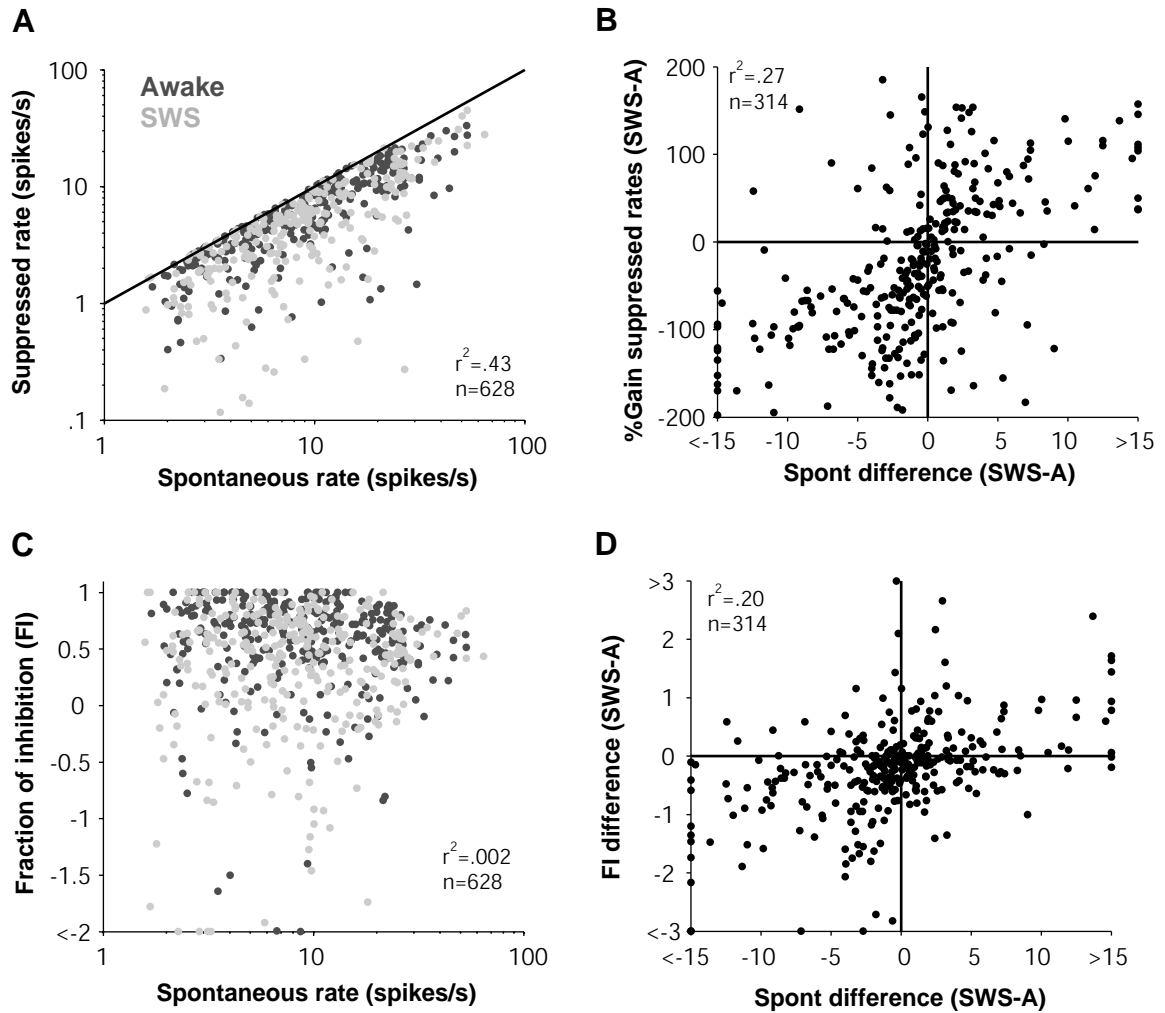


**Figure 4.4.** Suppressed responses.

(a) Suppressed responses tended to occur at louder sound levels than driven responses (median driven=50 dB, suppressed=60 dB,  $p=.03$ , Wilcoxon rank sum,  $n_{\text{driven}}=2518$ ,  $n_{\text{suppressed}}=310$ ).

(b) An example unit whose response was suppressed during the stimulus (gray shaded region) and for a short time following (vertical dashed lines represent analysis window returned by our windowing algorithm). Inhibition was weaker and shorter-lasting in SWS (light gray) than awake (dark gray) (gain=-24%). Curves were generated by first subtracting spontaneous rates then smoothing with a 50ms moving average window.

(c) Percent change in SWS of responses that were suppressed below spontaneous activity. The distribution is shifted toward negative gains (mean=-23%, vertical dashed line) indicating that the strength of suppression was weaker in SWS.



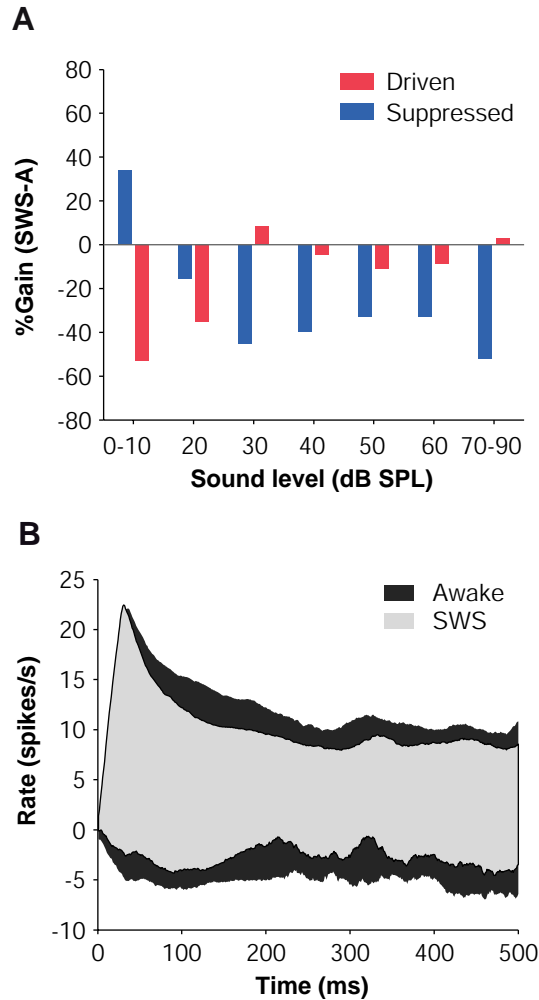
**Figure 4.5.** Comparison of two different measures of suppression.

(a) Absolute changes in spiking for suppressed events depend strongly on spontaneous rate ( $r^2=.43$ ,  $p=4*10^{-79}$ ,  $n=628$ ).

(b) When suppression is compared between awake and SWS using a similar formula to that for driven responses ( $\%Gain = 100*(A-SWS)/\max(|SWS|,|A|)$ ), the gain depends heavily on how different spontaneous rates were in the two states ( $r^2=.27$ ,  $p=3*10^{-23}$ ,  $n=314$ ). This is because a higher spontaneous rate allows a larger change in absolute spiking to be observed.

(c) To obtain a more invariant measure of suppression, we measured relative suppression or fraction of inhibition ( $FI = (\text{spontaneous-rate})/\text{spontaneous}$ ) instead of absolute suppression ( $\text{spontaneous-rate}$ ). This removed the dependence of suppression on spontaneous rates ( $r^2=.002$ ,  $p=.29$ ,  $n=628$ ).

(d) When relative suppression is compared between awake and SWS ( $FI_{SWS}-FI_A$ ), the gain still depends somewhat on the difference in spontaneous rates ( $r^2=.20$ ,  $p=5*10^{-17}$ ,  $n=314$ ), but this dependence is not as severe as when absolute spiking rates are used (see (b)).



**Figure 4.6.** Loss of the range of responses (driven+suppressed) in SWS.

(a) Changes in population averaged driven and suppressed responses as a function of sound level. Suppressed responses were weakened in SWS across a wide range (negative blue bars). However, driven responses were weakened only at extremely quiet levels (negative red bars). Population averages of activity were taken by summing firing rates of all detected driven or suppressed responses at each sound level without normalizing and then computing a percent gain at each sound level to estimate the change in the population response between awake and SWS.

(b) Population averaged histogram of all driven (positive) and suppressed (negative) responses showing their time course. Because responses are not driven or suppressed as strongly in SWS as in awake, the dynamic range of responses is limited (light shaded area is encompassed by dark area). Population post-stimulus time histograms were obtained by averaging all detected driven ( $n=3133$ ) and suppressed responses ( $n=272$ ) during the time window in which they occurred. Responses were smoothed by a 30ms moving average window.

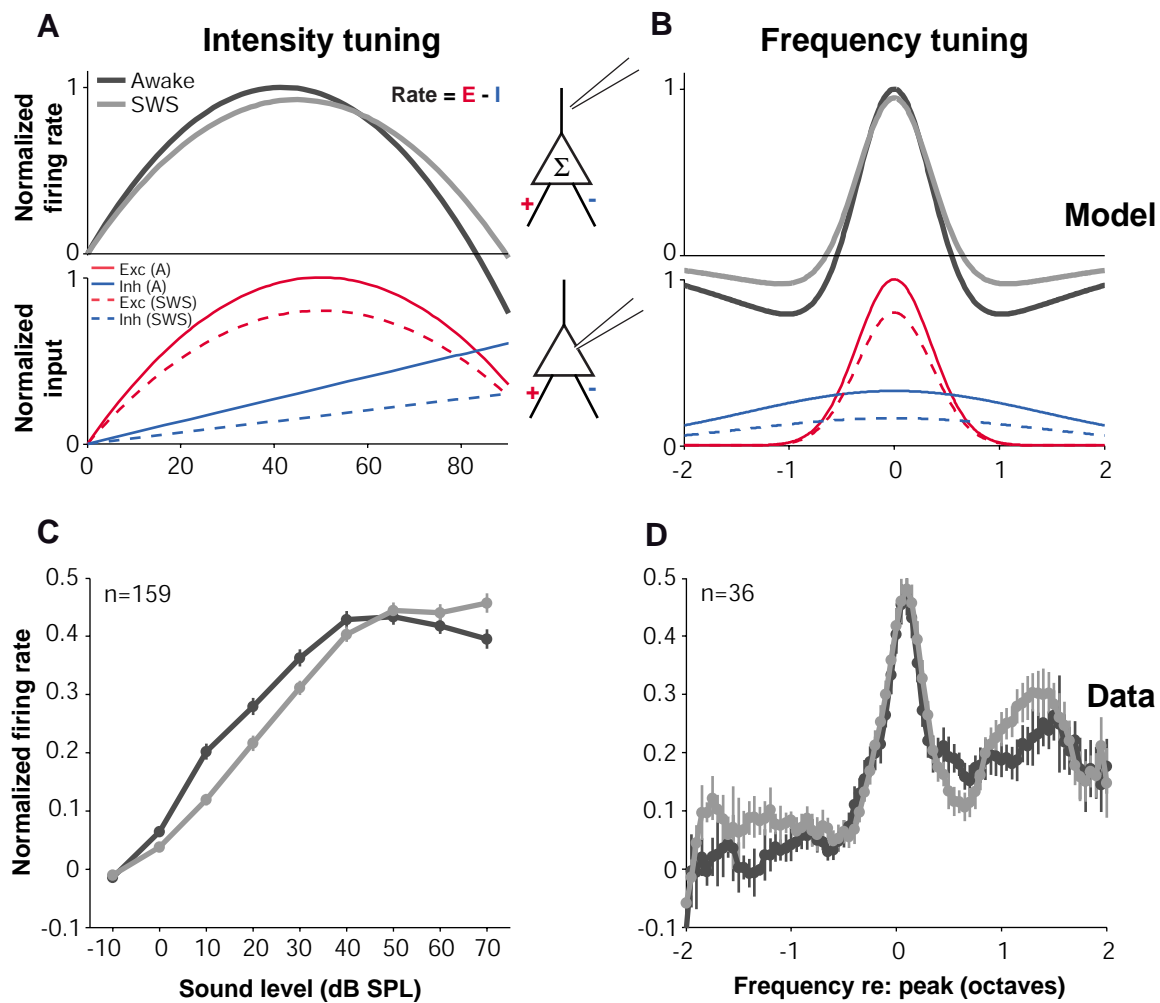
**Figure 4.7.** Conceptual model of the pattern of effects in SWS.

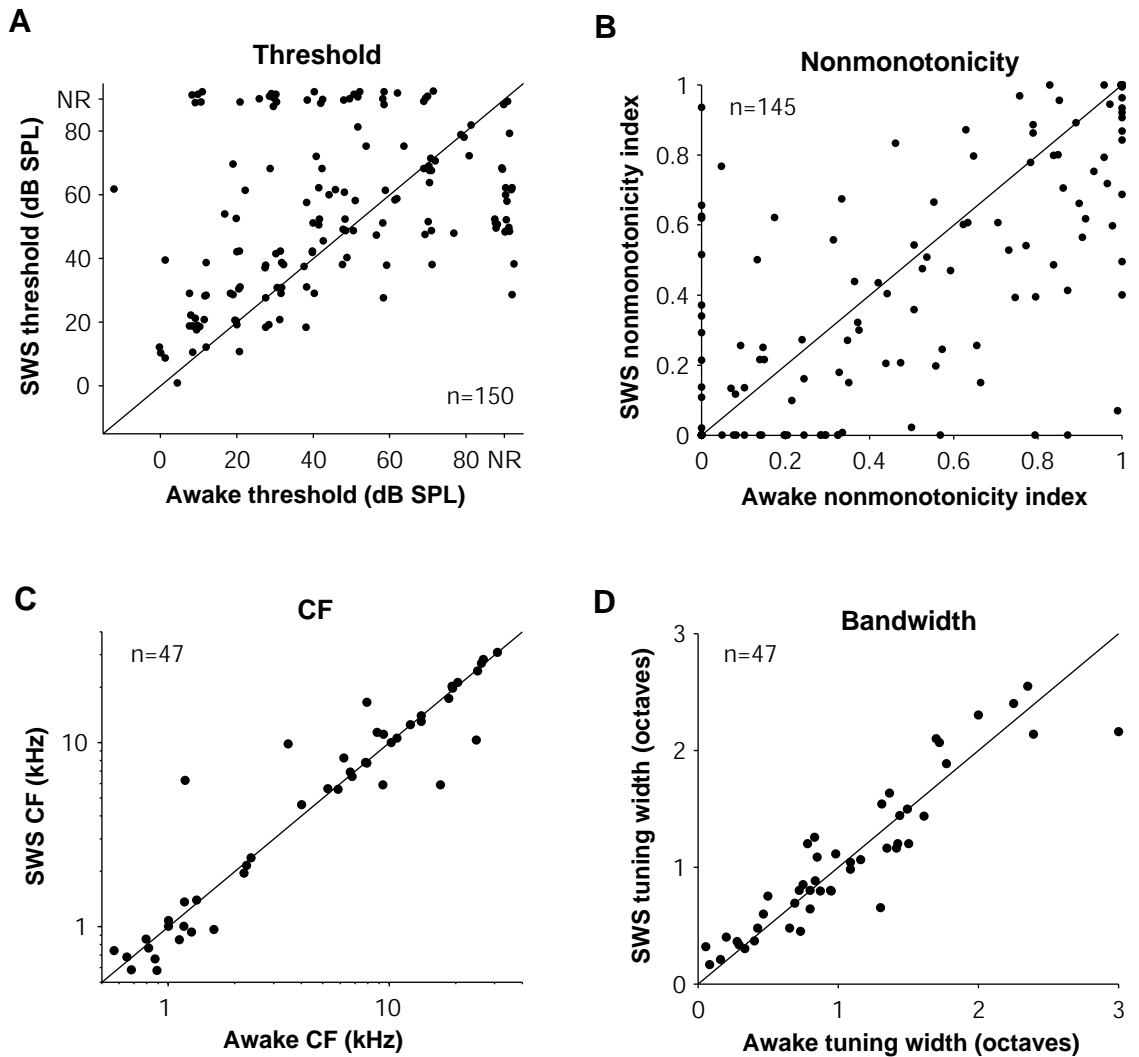
(a) Intensity tuning in A1 can be modelled as the combination of non-monotonic excitation (red) and monotonic inhibition (blue) which are summed to generate output firing rates (black) (see Methods). The inputs can be measured directly using intracellular recordings (illustrated by lower cartoon neuron). However, extracellular recordings such as those performed in the present study can only measure the signal after the inputs have been summed (upper cartoon). If sleep reduces excitation and inhibition (dashed lines), the resulting SWS intensity tuning curve (gray) will be weakened at quiet sound levels and strengthened at loud sound levels compared to the awake curve (black).

(b) In the frequency domain, inhibitory sidebands can be modelled using inhibition (blue) that is more broadly tuned than excitation (red). The loss of inhibition in SWS (dashed lines) is predicted to elevate the responses to non-preferred frequencies.

(c) Population intensity tuning data in awake (black) and SWS (gray) showing the pattern of effects predicted by the model (error bars represent  $\pm 5$  sem). Individual tuning curves for each neuron were first normalized by their peak value in either awake or SWS before being averaged.

(d) Population frequency tuning data in awake (black) and SWS (gray) show evidence of SWS enhancement of responses to non-preferred frequencies (error bars represent  $\pm 5$  sem). To ensure that peaks in awake and SWS matched, awake and SWS curves for each neuron were normalized separately by the peak value in each state rather than by an overall peak value. For this comparison, only neurons whose center frequencies in awake and SWS were within 0.4 octaves of each other were selected. Population curves were smoothed by a 6-point moving average filter.





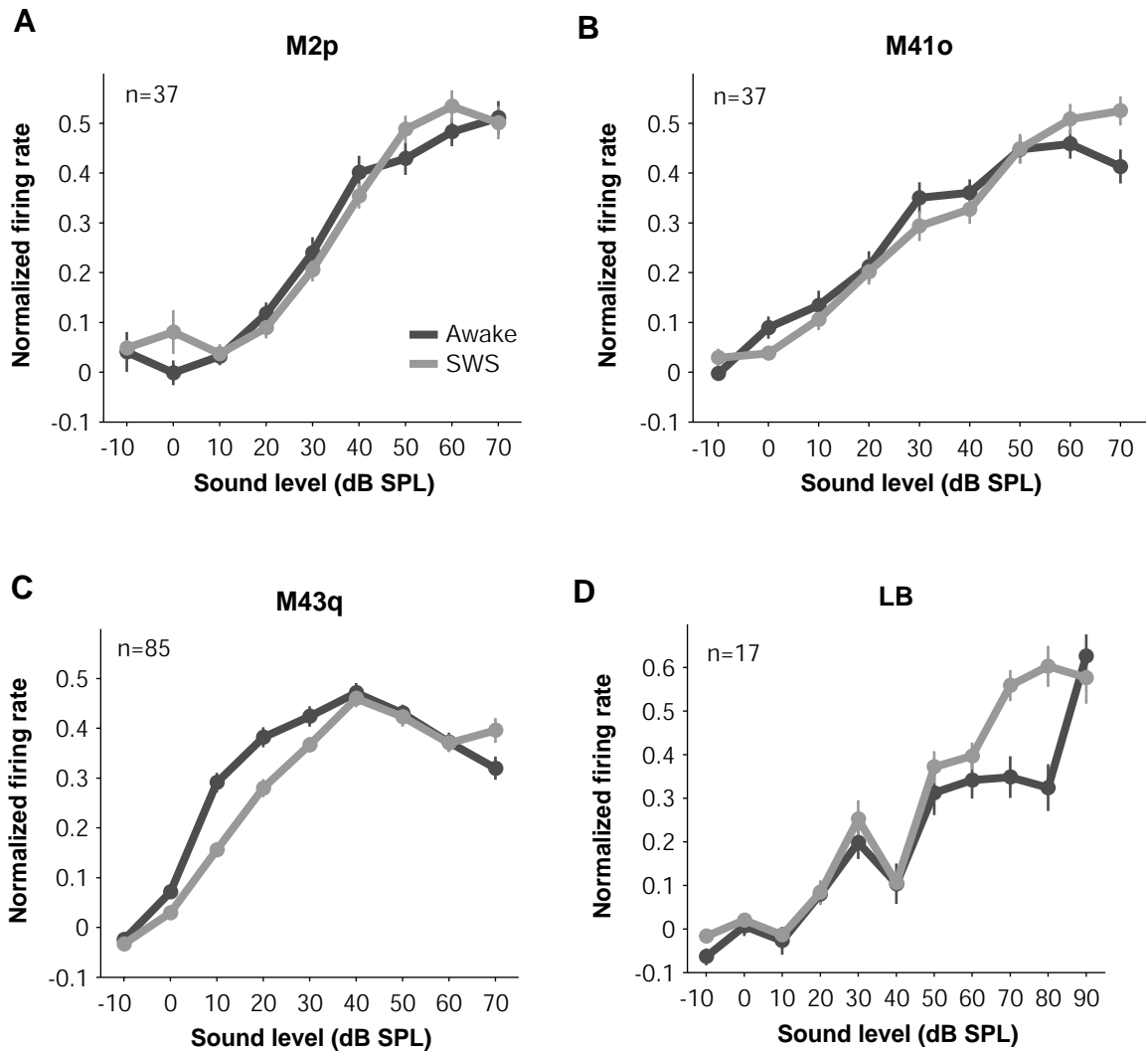
**Figure 4.8.** Intensity and frequency tuning properties.

(a) Sound level thresholds (quietest sound level eliciting a response  $4 \times \text{sem}$  above spontaneous) were elevated in SWS ( $p=.002$ , Wilcoxon rank sum,  $n=150$ ) as predicted by the model in Fig. 4.7a (dots are randomly jittered for display purposes; NR=no response at any sound level).

(b) The non-monotonicity index ( $\text{NMI} = 1 - r_{\text{loudest}}/r_{\text{peak}}$ ) measures how much responses fall off at loud levels.  $\text{NMI}=1$  indicates that responses completely go away at the loudest levels. In SWS, neurons were slightly more monotonic than in awake ( $p=.24$ , Wilcoxon rank sum; but  $p=.01$ , sign test,  $n=145$ ) as predicted in Fig. 4.7a.

(c) Preferred frequencies (frequency eliciting peak response) in awake and SWS were tightly correlated ( $r^2=.85$ ,  $p=3 \times 10^{-20}$ ,  $n=47$ ).

(d) Frequency tuning bandwidth (the first moment about the centroid of the tuning curve, see Methods) in awake and SWS were tightly correlated ( $r^2=.82$ ,  $p=1 \times 10^{-18}$ ,  $n=47$ ) as predicted in Fig. 4.7b.



**Figure 4.9.** Population intensity tuning curves for A1 in 3 animals and for LB.

(a-c) SWS curves in all three animals remained below awake curves before eventually crossing over at high sound levels.

(d) In LB, activity was rarely observed at quiet sound levels ( $< 40$ db) (Fig. 4.3e), but at louder sound levels, SWS responses were stronger than awake responses. This pattern was present in A1 (a-c), but appears more pronounced here. Unfortunately, the sample in LB was small ( $n=17$ ) making it difficult to draw any conclusions.



**Figure 4.10.** Driven and suppressed responses in REM.

(a) Summary of percent change of all driven responses during REM. Overall mean gain is -6% (vertical dashed line) (compare to Fig. 4.1 for SWS).

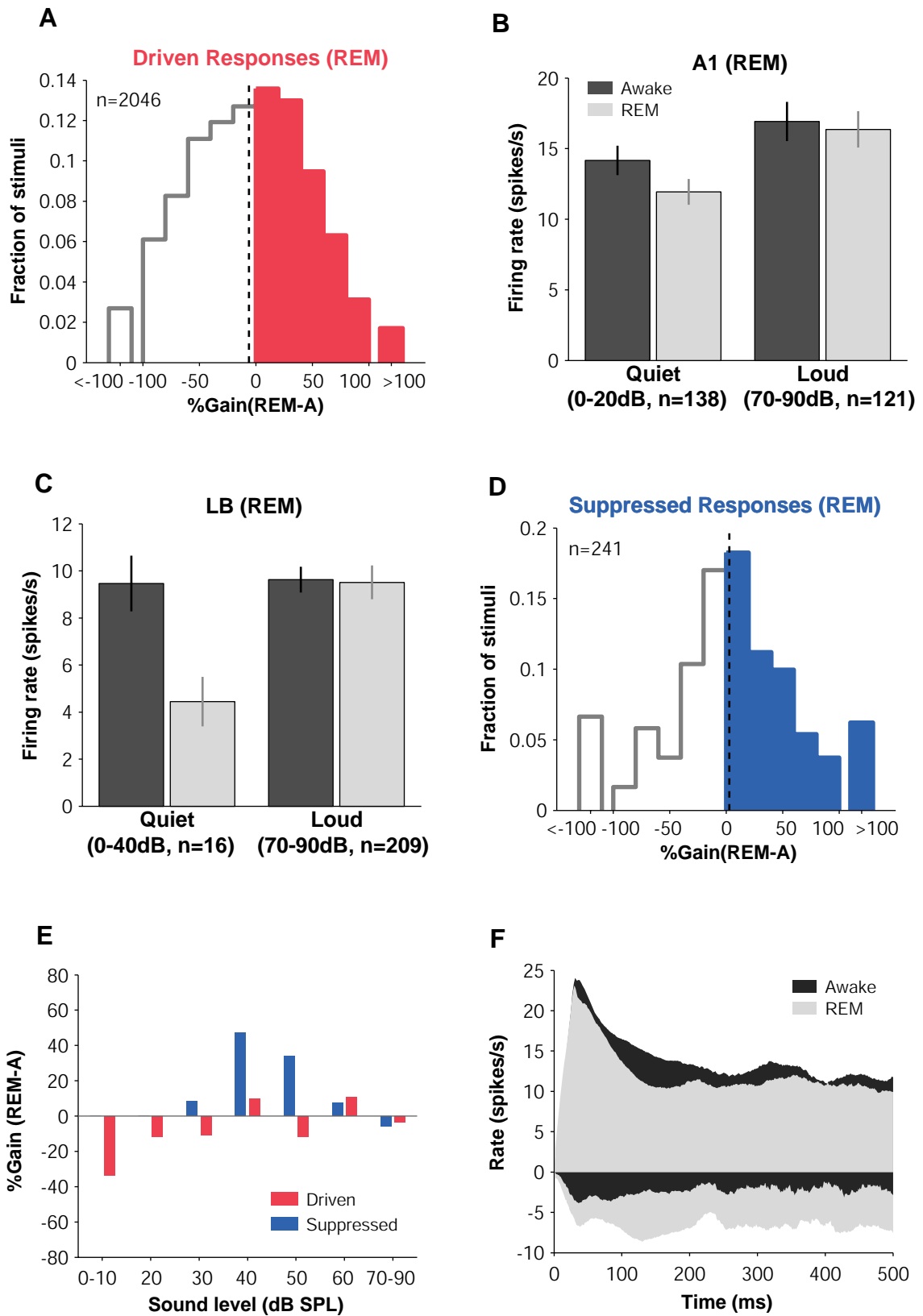
(b) Unlike in SWS, activity to quiet sounds across the population of A1 neurons in REM did not average much less than activity in awake (mean awake=14.2, REM=11.9 spikes/s,  $p=.19$ , Wilcoxon rank sum,  $n=138$ ) (error bars represent  $\pm$ sem; compare to Fig. 4.3c for SWS).

(c) Activity to quiet sounds was more clearly reduced in REM for the small sample of LB neurons (mean awake=9.5, REM=4.4 spikes/s,  $p=.01$ , Wilcoxon rank sum,  $n=16$ ) (error bars represent  $\pm$ sem; compare to Fig. 4.3d for SWS).

(d) Percent change in REM of responses that were suppressed below spontaneous activity. The distribution is near 0 (mean=+3%, vertical dashed line) indicating that the strength of suppression was similar in awake and REM. (compare to Fig. 4.4c for SWS)

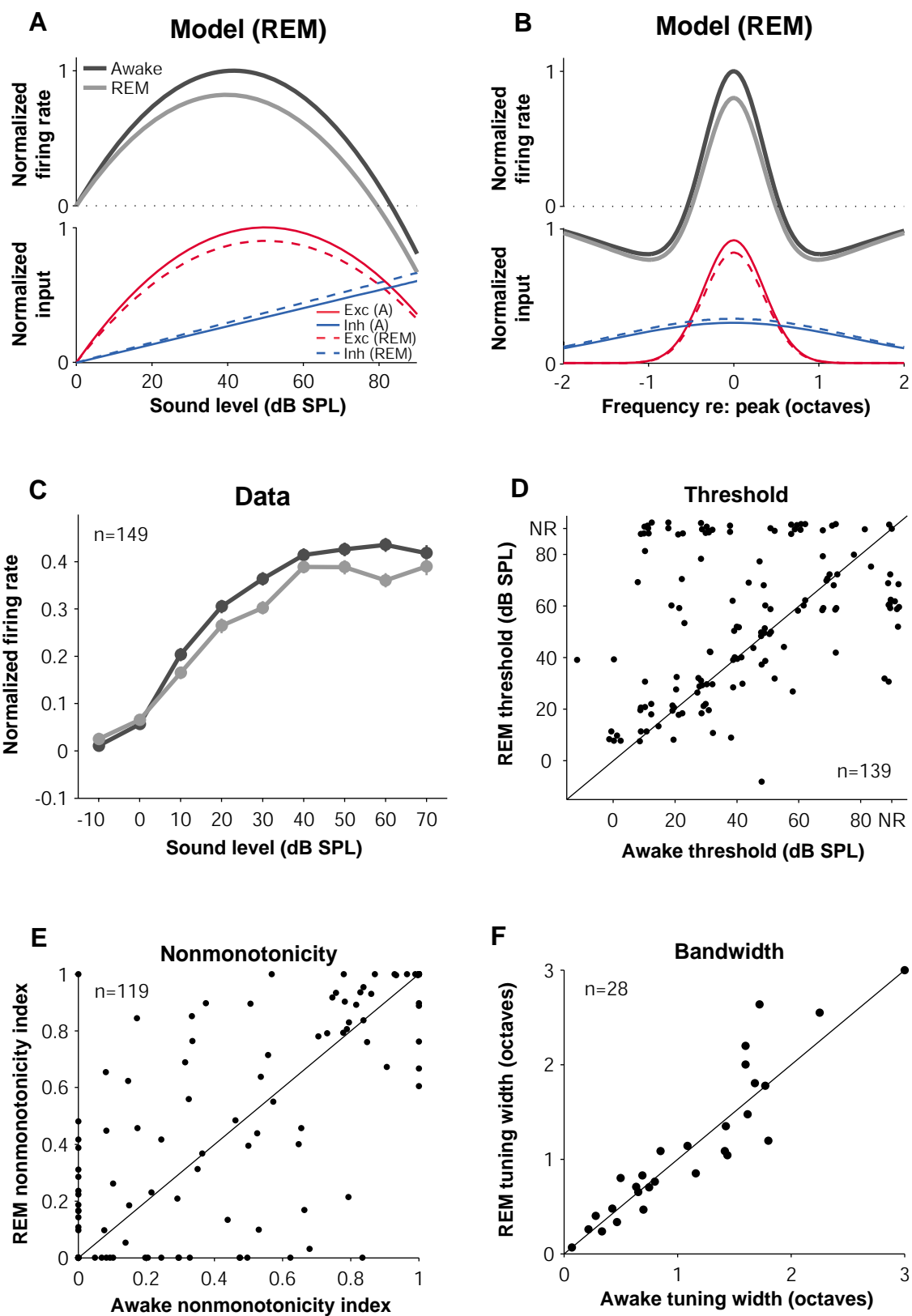
(e) Changes in population averaged driven and suppressed responses as a function of sound level. It was difficult to discern a trend in REM activity with sound level (not as clear as trends in SWS, Fig. 4.6a). Driven responses appear to be attenuated 20-30% for very quiet sounds, and suppressed responses appear to be enhanced at some sound levels. A clear difference between SWS and REM is that gains for suppressed responses in REM are not negative whereas in SWS there was a tendency for weak suppression (compare to Fig. 4.6a for SWS).

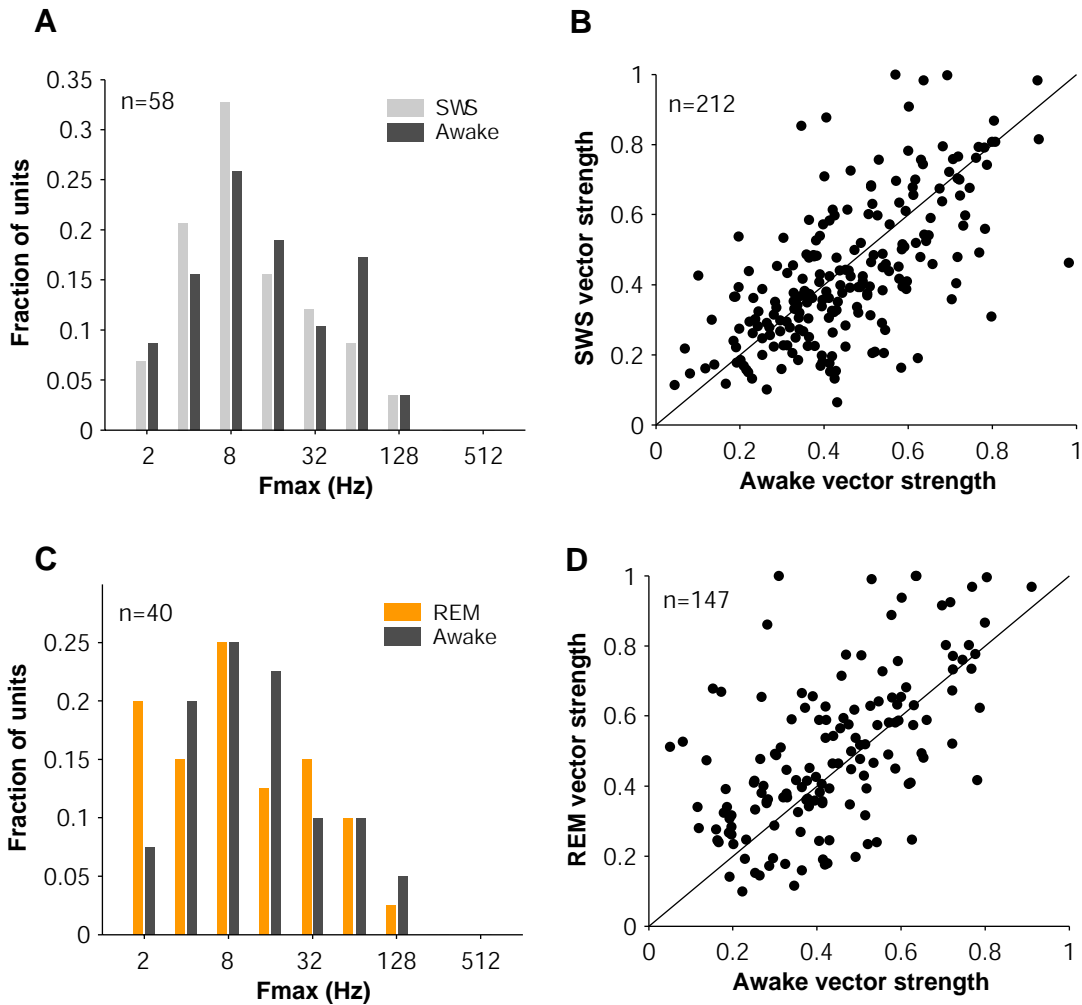
(f) Population averaged histogram of all driven (positive) and suppressed (negative) responses showing their time course. Because responses are driven less strongly and suppressed more strongly in REM, it is hard to say whether dynamic range changes (REM=light shaded and awake=dark shaded area are almost equal). Population post-stimulus time histograms were obtained by averaging all detected driven ( $n=2746$ ) and suppressed responses ( $n=267$ ) during the time window in which they occurred. Responses were smoothed by a 30ms moving average window (compare to Fig. 4.6b for SWS).



**Figure 4.11.** Conceptual model and data for REM tuning.

- (a) REM appears to modify excitation and inhibition to smaller degrees than SWS. Adjusting excitation down (dashed red line) and raising inhibition slightly (dashed blue line) in our model from Fig. 4.7 yields a REM intensity tuning curve (light gray curve) that remains below the awake curve (dark gray curve) (does not cross-over like in SWS; compare to Fig. 4.7a for SWS).
- (b) In the frequency domain, the subtle changes of excitation and inhibition in REM do not modify tuning except for causing suppression at the peak. Tuning width and inhibitory troughs are similar in awake and REM (compare to Fig. 4.7b for SWS).
- (c) Population intensity tuning data in awake (black) and REM (gray) showing the pattern of effects predicted by the model (error bars represent  $\pm 5$  sem) (compare to Fig. 4.7c for SWS).
- (d) Sound level thresholds were elevated in REM ( $p=4 \times 10^{-4}$ , Wilcoxon rank sum,  $n=139$ ) as predicted in (a) (compare to Fig. 4.8a for SWS).
- (e) In REM, neurons were not any more non-monotonic than in awake ( $p=.77$ , Wilcoxon rank sum;  $p=.14$ , sign test,  $n=119$ ) as predicted in (a) (compare to Fig. 4.8b for SWS).
- (f) Frequency tuning bandwidths in awake and REM were tightly correlated ( $r^2=.88$ ,  $p=1 \times 10^{-13}$ ,  $n=28$ ) as predicted in (b) (compare to Fig. 4.8d for SWS).





**Figure 4.12.** Phase-locking in SWS and REM.

(a) Comparison of upper limit of phase-locking (Fmax) in awake (dark gray) and SWS (light gray). In general, neurons were able to follow similar repetition frequencies of periodic stimuli in both states (mean Fmax awake=14.0, SWS=11.6 Hz,  $p=.37$ , Wilcoxon rank sum,  $n=58$ ).

(b) Vector strengths in awake and SWS were comparable. Although there appears to be a slight tendency toward weaker phase-locking in SWS, it was not significant (mean VS awake=.45, SWS=.43,  $p=.11$ , Wilcoxon rank sum,  $n=212$ ).

(c) Fmax distributions were similar in awake and REM (mean awake=12.7, REM=10.2 Hz,  $p=.46$ , Wilcoxon rank sum,  $n=40$ ).

(d) There appears to be a slight tendency toward stronger phase-locking in REM over awake (mean VS awake=.44, REM=.49), but this difference did not reach significance ( $p=.07$ , Wilcoxon rank sum,  $n=147$ ).

**Figure 4.13.** Modulation tuning in SWS and REM.

(a) Preferred modulation frequency (centroid of rate modulation tuning curve, see Methods) was highly correlated in SWS and awake ( $r^2=.86$ ,  $p=6*10^{-26}$ ,  $n=59$ ).

(b) Modulation tuning bandwidth (first moment about the centroid, see Methods) was also highly correlated in SWS and awake ( $r^2=.62$ ,  $p=2*10^{-13}$ ,  $n=59$ ).

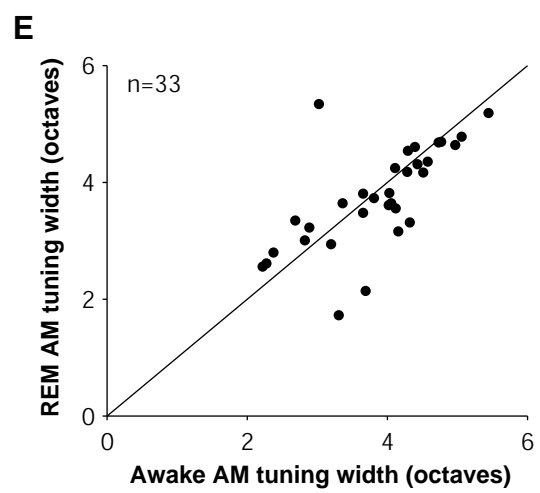
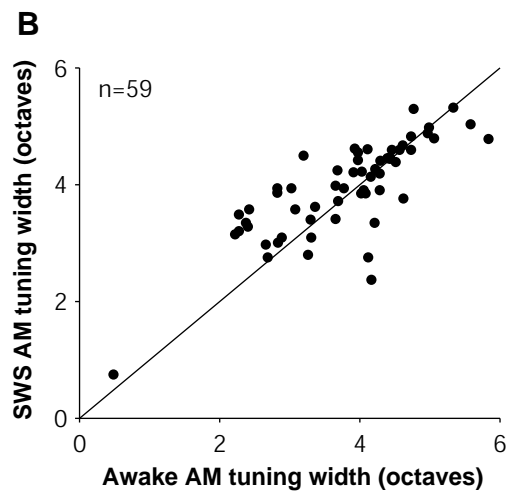
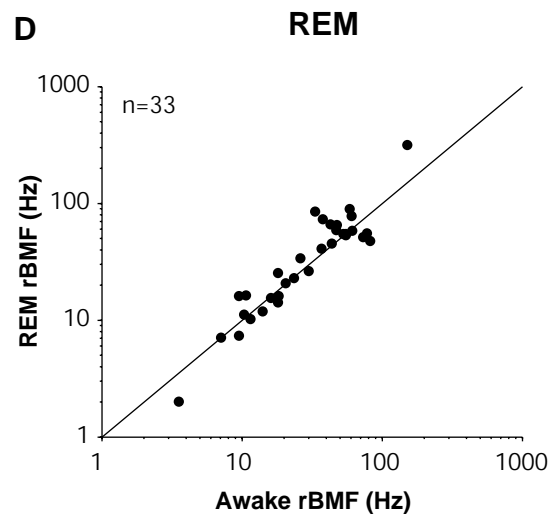
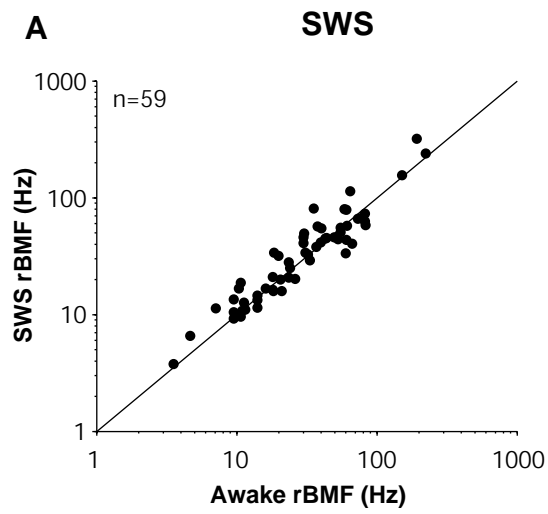
(c) Population modulation tuning curve in awake (dark gray) and SWS (light gray). Individual tuning curves for each neuron were first normalized by their peak value in either awake or SWS before being averaged (error bars represent  $\pm 5$  sem). The two curves have a similar pattern.

(d-f) Same as (a-c) except for REM instead of SWS in comparison to awake.

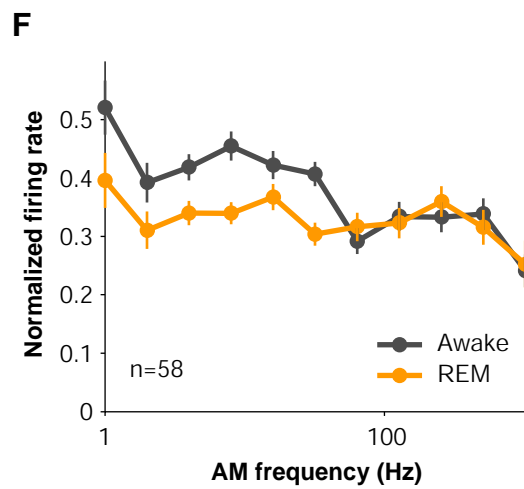
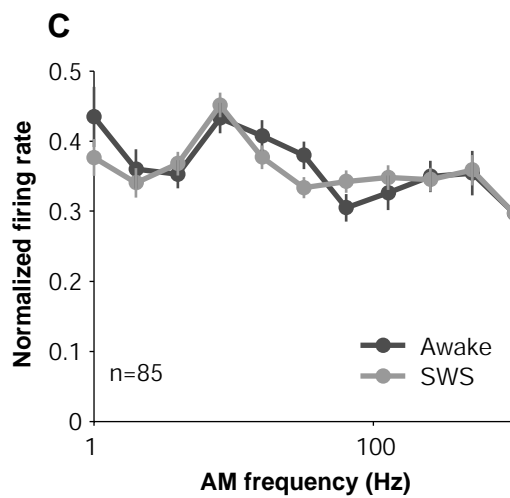
(d) Preferred modulation frequencies were highly correlated in REM and awake ( $r^2=.74$ ,  $p=1*10^{-10}$ ,  $n=33$ ).

(e) Modulation tuning bandwidth was also highly correlated in REM and awake ( $r^2=.51$ ,  $p=3*10^{-6}$ ,  $n=33$ ).

(f) The population modulation tuning curve in REM (orange) did not have the lowpass character of the awake curve (dark gray).



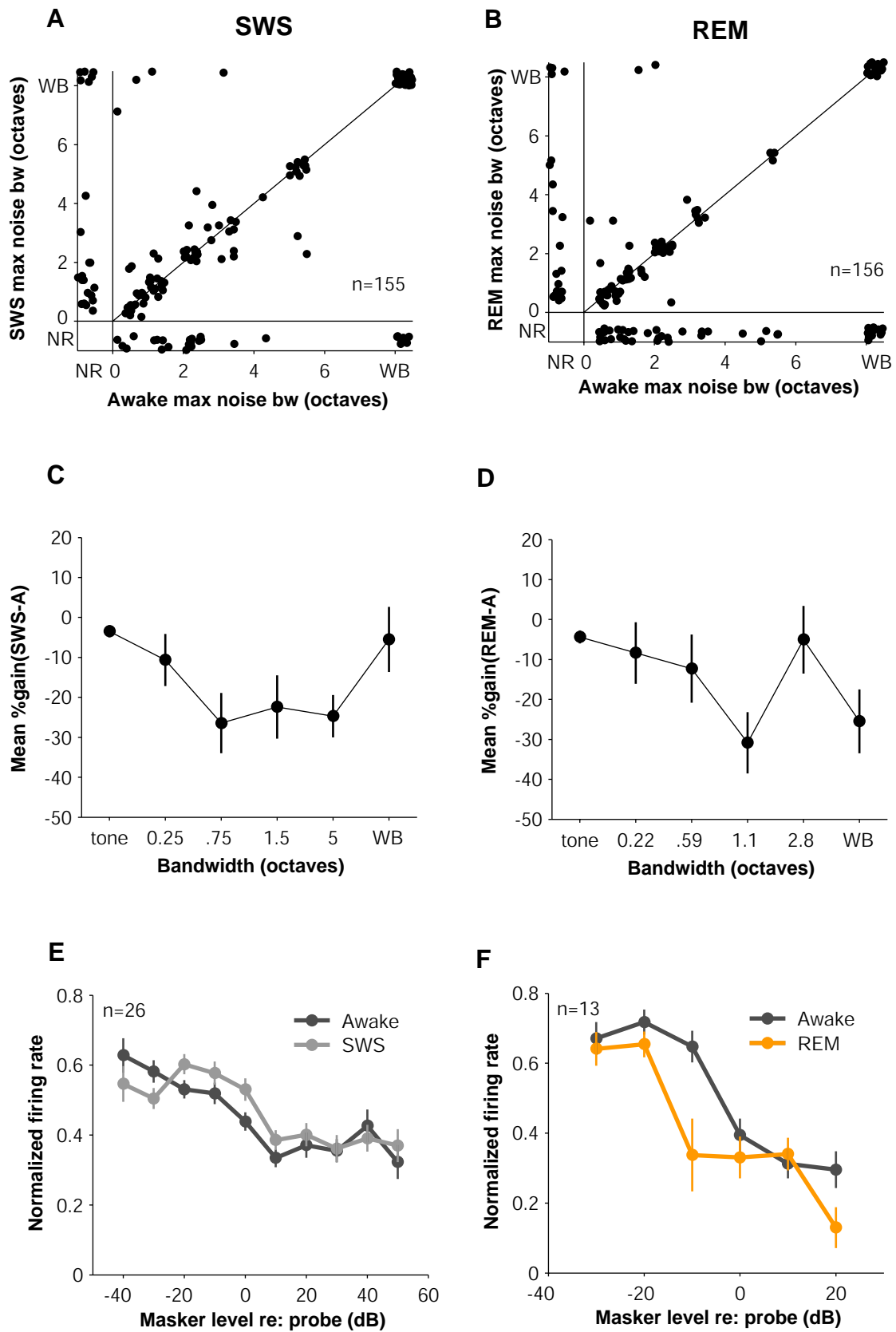
### Population Tuning

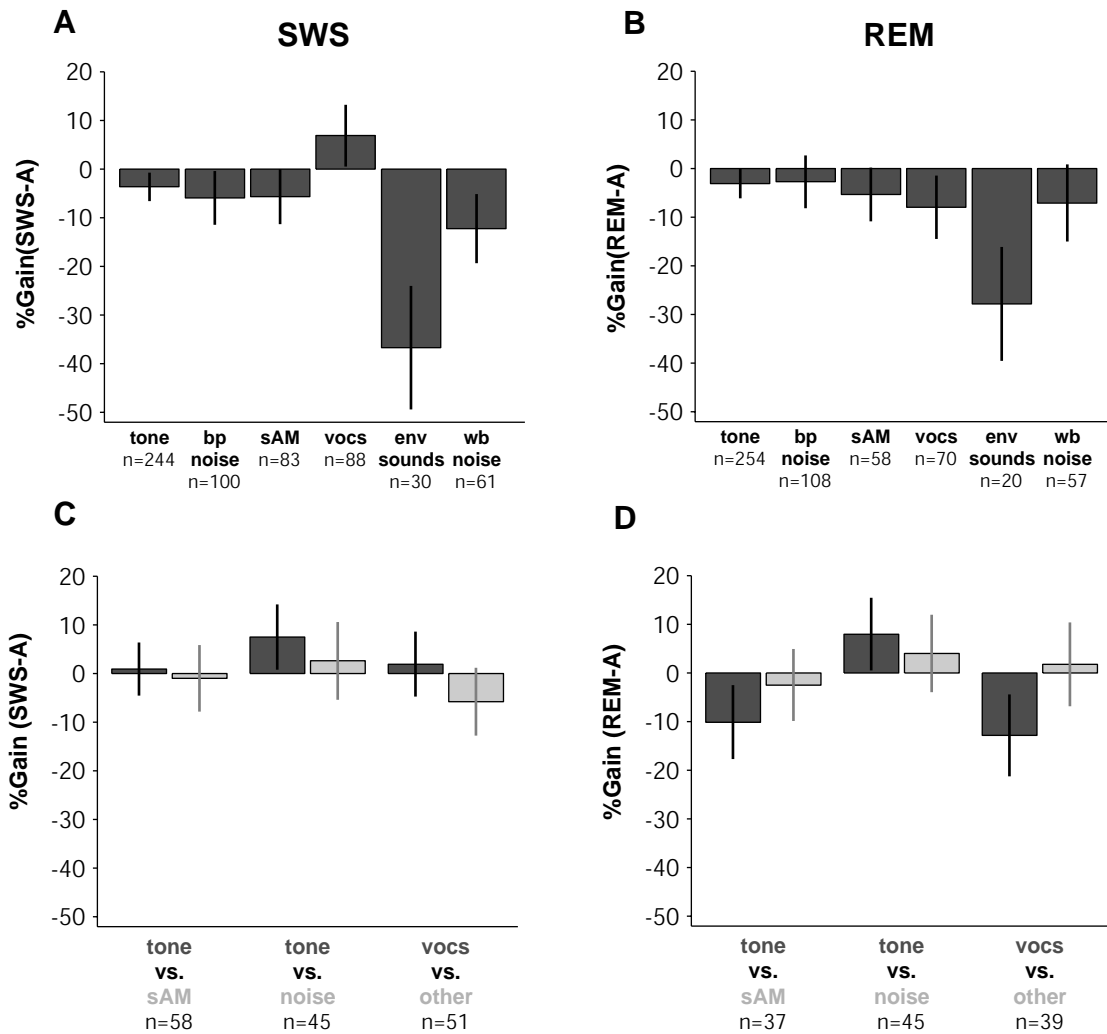


**Figure 4.14.** Responses to broadband sounds in SWS and REM.

- (a) Noise stimuli of different bandwidths drove neurons in both awake and SWS (NR=no response to any bandwidth, WB=wideband noise). The maximum bandwidth eliciting a response could vary greatly between states, but no systematic bias was present.
- (b) Plot of maximum noise bandwidths eliciting a response in REM versus awake.
- (c) Mean gain over the population seemed to drop as bandwidth increased (stronger responses in awake to broadband sounds), but this was followed by a near 0 gain between awake and SWS for wideband noise ( $n_{\text{total}}=346$  stimuli) (error bars represent  $\pm\text{sem}$ ).
- (d) In REM, there was no consistent trend of gain with increasing bandwidth of the stimulus ( $n_{\text{total}}=262$  stimuli) (error bars represent  $\pm\text{sem}$ ).
- (e) Population averaged masking curves. Awake and SWS curves were first normalized by the overall peak before averaging. In the presence of a wideband noise masker, responses to narrowband target sounds were degraded in a similar manner in awake and SWS (error bars represent  $\pm.5*\text{sem}$ ).
- (f) Broadband masker effects may be somewhat stronger in REM (orange curve drops more rapidly), but the sample is too small to draw any conclusions (error bars represent  $\pm.5*\text{sem}$ ).







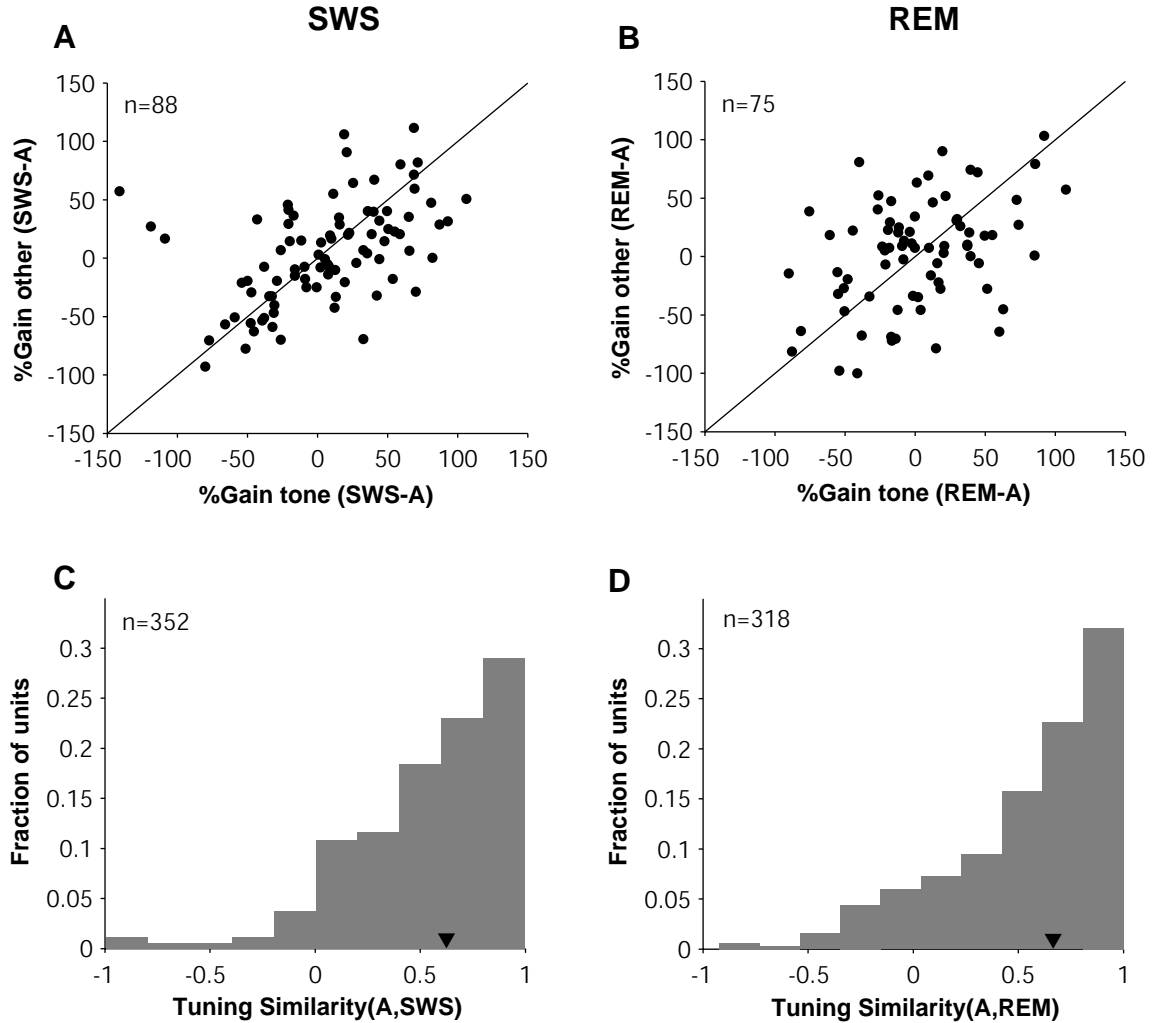
**Figure 4.15.** Sleep gains for different stimulus types.

(a) We used 6 main stimulus types to probe neural responses (tones, bandpass noise, sinusoidal amplitude modulated tones or noise, vocalizations, environmental sounds, and wideband noise). In general, gains were somewhat negative but not different from 0% except for environmental sounds which were significantly negative ( $p=.008$ , bootstrap,  $n=30$ ).

(b) Like in SWS, gains in REM for the 6 main stimulus types were not different from 0% except for environmental sounds ( $p=.03$ , bootstrap,  $n=20$ ).

(c) In many cases, more than one stimulus type was tested on a neuron, and direct comparisons could be made within a neuron. No difference was found in SWS gain for unmodulated versus modulated tones ( $p=.68$ ,  $n=58$ ), for tones versus narrowband stimuli ( $p=.51$ ,  $n=45$ ), and for vocalizations versus non-vocalization stimuli ( $p=.32$ ,  $n=51$ ) (Wilcoxon rank sum). Gain remained near 0% in all cases.

(d) Within unit comparisons of the effects of REM on various stimulus types did not reveal any difference in sleep gains for modulated ( $p=.54$ ,  $n=37$ ), larger bandwidth ( $p=.80$ ,  $n=45$ ), or semantically meaningful ( $p=.24$ ,  $n=39$ ) (Wilcoxon rank sum) stimuli.



**Figure 4.16.** Evidence for a multiplicative effect of sleep.

(a) Plot of SWS gain for tones versus gains to other stimuli for each unit tested with at least two stimulus types. Tone gains are the average of gains for all tones that drove a unit. Gains to other stimuli are taken as the average across those stimuli that were not tones but drove a unit. There was reasonable correlation in gains ( $r^2=.22$ ,  $p=4*10^{-6}$ ,  $n=88$ ).

(b) REM modulation of tone responses was partially predictive of modulation of responses to other stimuli ( $r^2=.15$ ,  $p=5*10^{-4}$ ,  $n=75$ ).

(c) The vector similarity between awake and SWS firing rate profiles (see Methods) across all stimuli was relatively high (median  $\rho=.62$ , black inverted triangle).

(d) Awake and REM firing rate profiles across all stimuli also tended to be highly correlated (median  $\rho=.67$ , black inverted triangle).

	A	SWS	p	A	REM	p
<b>NMI</b>	.47	.42	<b>.006</b>	.46	.47	.14
<b>Thresh</b>	45	55	<b>.001</b>	42	54	<b>.0004</b>
<b>CF</b>	8.6	8.5	.93	9.1	8.0	.64
<b>BW</b>	1.1	1.1	.98	1.1	1.2	.93
<b>Fmax</b>	14.0	11.6	.37	12.7	10.2	.46
<b>VS</b>	.45	.43	.12	.44	.49	.07
<b>rBMF</b>	28.9	31.2	.30	28.1	30.6	.73
<b>BW<sub>AM</sub></b>	3.8	3.9	.45	3.9	3.8	.64

**Table 4.1.** Summary of basic tuning properties.

Comparison of tuning properties between awake (A) and SWS and between awake (A) and REM. Values represent means. Wilcoxon rank sum tests used to derive p-values. NMI=non-monotonicity index, Thresh=sound level threshold (dB), CF=center frequency (kHz), BW=frequency tuning bandwidth (octaves), Fmax=fastest phase-locked modulation frequency (Hz), VS=vector strength, rBMF=rate best modulation frequency (Hz), and BW<sub>AM</sub>=bandwidth of modulation tuning (octaves).

## Chapter 5: Population Properties of Neurons and LFP

### 5.1 Summary

Instead of being in a dormant state during sleep, the brain is active. Whether these activations are patterned or serve a functional purpose is a topic of intense research. In the auditory cortex, sleep has been shown to strongly modulate sensory responses of single neurons, but it is unclear if these modulations are patterned. We recorded from pairs of nearby neurons and found that average firing rates were independently modulated by sleep. Slow-wave sleep did enhance functional connectivity between neurons but at the cost of disrupting the pattern previously present in awake. Connection patterns like those of awake re-emerged in rapid eye-movement sleep. We recorded local field potentials (LFP) concurrently with single-units as an index of local activity. LFP energy in low frequency bands ( $<40\text{Hz}$ ) was modulated in a consistent fashion across multiple sleep cycles, but effects of sleep on LFPs were not correlated with those in single-units. These results suggest only weak organization of neurons at a larger spatial scale during sleep and that overall, single neuron responses across the cortical mantle are disorganized. This is in contradistinction to what would be predicted from down regulation of a common thalamic input during sleep. Any population coding of sounds may be disrupted leading to reductions in the fidelity of acoustic representations during sleep.

## 5.2 Introduction

Recordings of neural populations in a variety of settings have demonstrated the ability of the brain to organize. During sleep in the hippocampus, coordinated replay events involving many neurons take place (Wilson and McNaughton, 1994). These events tend to correlate with ripples ( $>100\text{Hz}$ ) in the local-field potential (Foster and Wilson, 2006). Also, neurons in the hippocampus will lock to certain phases of the theta oscillation so that populations of cells fire in a precise order (Harris et al., 2002). In visual area V4, attention has been shown to induce coordination of neural firing with the gamma ( $40\text{-}100\text{Hz}$ ) oscillations in the LFP (Fries et al., 2001). This locking may give rise to improvements in neural synchrony such as those seen in the somatosensory cortex with attention (Steinmetz et al., 2000). Finally, neural recordings in middle temporal cortex (MT) have found that neuron pairs exhibit correlated trial-by-trial fluctuations in their firing rates (Zohary et al., 1994; Bair et al., 2001). These correlations arise partly because of common inputs from thalamus or V1. Such neuron-neuron or neuron-LFP recordings are critical because they reveal network relationships that would have otherwise not been observable at the single-unit level. Any patterns that are found can be windows into functional connectivity. This is especially important in a state change such as sleep where functional organization may change. Novel brain rhythms and neuromodulatory influences during sleep may change the state of the network (Steriade et al., 1993a; Steriade et al., 2001). One study found that cortical slow oscillations ( $<1\text{ Hz}$ ) that are prominent in SWS lead to prolonged periods of hyperpolarization (Steriade et al., 1993a; Steriade et al., 1993b; Steriade et al., 1993c). During these periods, spiking is

decreased. The same group also found that SWS leads to greater spatial coherence of LFPs (Destexhe et al., 1999). In REM, coherence returns to awake levels so that nearby LFP sites are not in phase, similar to wakefulness (Destexhe et al., 1999).

Most studies of network properties during sleep have relied on observations of internal activity (Pavrides and Winson, 1989). But spontaneous firing can be quite low in cortex, limiting the number of neurons that can be studied. Recently, robust externally driven responses have been demonstrated in auditory cortex during both SWS and REM (Pena et al., 1999; Edeline et al., 2001). However, responses display quite diverse patterns of modulation. Excitability of neurons in SWS can be either up or down regulated and is inconsistent with responsiveness in awake or even REM (see Chapter 3). The question arises as to whether these diverse responses are organized with respect to the network during sleep. For one thing, sleep may up or down regulate specific sets of neurons or may induce synchronous behaviors between neurons through various brain rhythms. Also, if sleep acts through the thalamus to achieve sensory gating as some have suggested (Steriade, 2003), then nearby cortical neurons receiving common thalamic input should show similar sleep behaviors.

These questions were addressed by recording from pairs of neurons and recording the LFP in conjunction with single neurons under conditions of external stimulation during sleep. Analyses were performed to determine whether correlations between neurons or between neurons and the LFP existed at three time scales: cycle-by-cycle, trial-by-trial, spike-by-spike (locking). Using these methods, a number of properties of network organization during sleep were revealed by natural stimulation with sounds.

## 5.3 Results

Single neuron recordings were performed in 5 hemispheres of 4 animals. Activity was compared between awake, slow-wave sleep (SWS), and rapid eye movement sleep (REM) in 493 units. Often multiple neurons were encountered on a single track, so that we recorded 325 pairs of neurons sequentially of which 71 were recorded simultaneously. An exemplary recording where three units were recorded simultaneously is shown in Figure 5.1. These three nearby units showed three different behaviors during sleep. The top unit only responded strongly in SWS ( $\text{Gain}_{\text{SWS}}=+83\%$ ). The middle unit only responded strongly in awake ( $\text{Gain}_{\text{SWS}}=-73\%$ ). And the third unit responded strongly in all states ( $\text{Gain}_{\text{SWS}}=+15\%$ ,  $\text{Gain}_{\text{REM}}=+18\%$ ). In the next sleep cycle, this pattern of responses was again encountered (episodes 3 of SWS and REM in Fig. 5.1). So sleep modulation was consistent from cycle to cycle as was shown in Chapter 3 for the population of single units we recorded. The inconsistency between neurons, though, suggests a complex spatial effect of sleep. Random behavior between nearby neurons during sleep has been noted previously in the primary visual (Livingstone and Hubel, 1981) and auditory cortices (Pena et al., 1999; Edeline et al., 2001).

### 5.3.1 Sleep modulation of neighboring neurons

In Figure 5.2, we quantified this heterogeneity in an analysis of all neurons recorded on the same electrode track. Neurons were encountered at a variety of spacings, and neurons very close to each other were often tested (199 pairs at  $<200\mu$  separation).



Despite their proximity, SWS modulation of firing rates in neuron pairs was not correlated ( $r^2=.02$ ,  $p=.08$ ,  $n=199$ ) (Fig. 5.2A). This was also the case for the subset of simultaneously recorded neurons ( $r^2=.07$ ,  $p=.08$ ,  $n=46$ ). In REM, the lack of organization persisted ( $r^2=.001$ ,  $p=.67$ ,  $n=201$ ) (Fig. 5.2B). In almost half of the pairs (SWS=46%; REM=49%), neurons were modulated in opposite directions. Whether neurons were modulated in the same or opposite direction did not depend on the distance between the pair or whether they were recorded simultaneously (Fig. 5.2, C and D). The fraction of pairs changing in opposite directions remained above 40%. Finally, there was no trend in sleep modulation with absolute depth (Fig. 5.2, E and F). This analysis could be confounded, though, because our estimates of depth are inexact as electrode positioning varies daily. But even at the extremes of the depth distribution (very superficial or very deep recordings), no clear bias in gain was seen (Fig. 5.2, E and F).

The lack of correlation between nearby units implies that neurons recorded on the same night were not modulated in a similar fashion (Fig. 5.3A). This random spatial pattern can be quite disruptive to any population map especially given the large average magnitude of sleep modulation (~40%, see Chapter 3). Figure 5.3B shows what the map of activity might look like across the cortical surface if the disorganization we observed orthogonal to the surface (in depth) is also present across the surface. Figure 5.3C shows actual data across the cortical surface of the most extensively tested hemisphere (43q, right hemisphere). This map shows neurons recorded from both A1 and lateral belt (LB). In general, the pattern of modulation (up or down) seems to be dispersed and not localized to any patch of cortex. Read-out of relative firing rates would be affected unless sufficient averaging is employed.

### 5.3.2 Rate correlations in simultaneously recorded neurons during sleep

Another important aspect of a population code is the joint firing statistics of nearby neurons. In about half of simultaneously recorded neuron pairs, both neurons could be driven with the same stimulus. This allowed us to study trial-by-trial correlations in firing. Neighboring neurons in primary sensory areas are known to exhibit correlated firing which arises from common inputs. We wondered if sleep might disrupt these functional connections and hence affect population codes that depend on the covariation of firing rates across neurons. In the awake state, mean rate correlation was .13 which is consistent with findings from previous studies (Zohary et al., 1994; Bair et al., 2001). This value was highly significant (mean chance=-.002,  $p=2*10^{-12}$ , Wilcoxon rank sum,  $n=268$  stimuli) (Fig. 5.4A). In SWS, correlation rose to even higher levels than awake (SWS=.21, awake=.13,  $p=.01$ , Wilcoxon rank sum,  $n_{SWS}=378$ ,  $n_{awake}=268$ ). In REM, firing correlations returned to awake levels (REM=.12, awake=.13,  $p=.53$ , Wilcoxon rank sum,  $n_{REM}=144$ ,  $n_{awake}=268$ ) (Fig. 5.4A).

An interesting observation was that correlation strengths did not simply scale in SWS. The pattern of strongly and weakly connected neurons in SWS was not correlated to that in awake ( $r^2=.004$ ,  $p=.54$ ,  $n=104$ ) (Fig. 5.4B). The increase in overall correlation strengths during SWS appears to override existing patterns. In REM, the pattern of neuron correlations was restored to resemble those in awake ( $r^2=.16$ ,  $p=.001$ ,  $n=64$ ) (Fig. 5.4C) and did not share properties with that in SWS ( $r^2=.01$ ,  $p=.41$ ,  $n=65$ ) (Fig. 5.4D). An increase in functional connectivity during SWS is consistent with spatial coherence imposed by large amplitude slow-waves (Destexhe et al., 1999). In REM and awake, the relative independence of neurons is maintained. Finally, we mention here that we were

unable to compare spike firing synchrony between neurons. Spike train cross-correlations (see Methods) were low in most of our small sample not allowing for a proper comparison between states.

### *5.3.3 LFP examples*

The local field potential (LFP) was recorded simultaneously with single units at 299 sites in 2 animals (43q and 16s). The LFP is a gross measure of synaptic activity in a 1-3mm region of cortex (Kreiman et al., 2006) that can give a more stable picture than single neurons. Especially since single neurons demonstrated diverse responses during sleep, we hoped to use the LFP to gain a better view of how sleep locally modulates activity on average. Example LFP recordings from A1 and LB are shown in Figure 5.5. A few general observations can be made. LFPs on single trials can be quite noisy, but if the stimulus-triggered average of the LFP is taken, a clear stimulus-locked waveform can be seen (thick black curves in Fig. 5.5, A-E). The earliest observed waveform is a negative deflection that occurs at 17 ms in A1 and is followed by slower timescale oscillations (Fig. 5.5, A and B) and an occasional offset potential (Fig. 5.5, A-C). The initial peak is thought to reflect the thalamic volley which reaches cortex first. Later waveforms are less well-studied but probably correspond to cortical processing. In LB, the initial peak is usually reduced to a small wiggle followed by a stronger, wider second peak (Fig. 5.5, D and E). LFPs in LB were usually broad whereas LFPs in A1 could display narrow (Fig. 5.5, A and B) or broad (Fig. 5.5C) peaks. The power spectrum of the LFP displays lowpass behavior. Most of the power is concentrated below 40Hz (Fig. 5.5F).

#### 5.3.4 Sleep modulation of the LFP

Five measures were derived from LFP recordings: peak-to-peak amplitude (PTP) and absolute power in the 1-10, 10-40, 40-120 (high gamma), and 100-300 (ultra-high gamma) Hz bands (Liu and Newsome, 2006; Kreiman et al., 2006; Kayser et al., 2007). Gains in LFP power during SWS and REM were computed in a similar manner to gains computed for single neuron firing rates in Chapter 3 ( $\%Gain = 100 * (\text{sleep-awake}) / \max(\text{sleep}, \text{awake})$ ). Consistent with the idea that low frequency activity increases in SWS, gain in the two lowest frequency bands (1-10 and 10-40 Hz) and gain of peak-to-peak amplitude were significantly positive (mean  $Gain_{1-10} = 37\%$ ,  $Gain_{10-40} = 28\%$ ,  $Gain_{PTP} = 10\%$ ;  $p_{1-10} = 0$ ,  $p_{10-40} = 0$ ,  $p_{PTP} = 8 * 10^{-11}$ ;  $n_{1-10} = 205$ ,  $n_{10-40} = 248$ ,  $n_{PTP} = 278$ , t-test) (Fig. 5.6A, left three bars). On the other hand, REM activity demonstrated a consistent decrease of power in the low frequencies which is what would be expected during this stage of sleep (mean  $Gain_{1-10} = -19\%$ ,  $Gain_{10-40} = -11\%$ ;  $p = 5 * 10^{-14}$ ,  $p = 4 * 10^{-10}$ ;  $n_{1-10} = 210$ ,  $n_{10-40} = 233$ , t-test) (Fig. 5.6B). PTP response amplitude in REM was unaffected compared to awake ( $-2\%$ ,  $p = .13$ ,  $n = 309$ , t-test).

In the higher frequency bands (high and ultra-high gamma), effects were small and not as systematic as in the low frequency bands (Fig. 5.6, A and B). High gamma activity could either increase or decrease when the animal fell into SWS (Fig. 5.6C) or REM (Fig. 5.6D). This behavior resembles that of single units which could also be modulated up or down. The difference is that modulation was much weaker for LFP gamma. Average sleep modulation of single-unit firing rates ( $\sim 40\%$ ) was twice that of LFP high-gamma (SWS=18%, REM=17%). This is reflected in the relatively narrow  $\%Gain$  distributions for high gamma power in SWS (Fig. 5.6C) and REM (Fig. 5.6D).

### 5.3.5 Comparison of LFP in A1 and LB

A comparison of LFP properties between A1 and LB revealed stronger overall LFP amplitude (Fig. 5.7, A and B) and energy (Fig. 5.7, C and D) in A1. Although the LFP was stronger in A1, the pattern of sleep gains observed in A1 was also observed in LB. Positive SWS gains and negative REM gains in low frequency power were found in both A1 and LB. Gains in the power of higher frequency bands were near 0% in A1 and LB (Fig. 5.7, E and F). Given the similarity in sleep modulation in the two regions, we grouped their data together in the remaining LFP analyses.

### 5.3.6 LFP modulation in later sleep cycles

To be certain that changes in the LFP were systematic and not just random fluctuations, we re-measured LFP modulation in a later sleep cycle. LFP gains in different cycles of SWS were correlated but only weakly. Best correlations were observed for gains in PTP amplitudes ( $r^2=.15$ ,  $p=1*10^{-8}$ ,  $n=196$ ) (Fig. 5.8A). Low frequencies (0-10 Hz) had significant correlations ( $r^2=.13$ ,  $p=5*10^{-7}$ ,  $n=183$ ) (Fig. 5.8B) which were reduced to marginally significant levels in the high gamma band ( $r^2=.06$ ,  $p=.0003$ ,  $n=206$ ) (Fig. 5.8C) and absent in the ultra-high gamma band ( $r^2=.01$ ,  $p=.21$ ,  $n=208$ ). A similar pattern was observed in REM which is summarized along with SWS in Figure 5.8D. Modulation of low frequencies was in opposite directions between SWS (strong 0-10 Hz) and REM (weak 0-10 Hz), but the strength and direction of this change re-occurs in a later episode of SWS or REM given the high correlation values between cycles. This may not be surprising since the strength and frequency of slow-waves is known to be correlated between cycles. Slow-waves start weak and become strong

toward the middle of the night before becoming weak again. Comparing the LFP in the (0-10 Hz) band between episodes might reflect local consistencies during these trends. We were more interested in the correlations in the high frequency bands. These may correlate with local activities of neurons (Liu and Newsome, 2006; Kreiman et al., 2006; Kayser et al., 2007). Unfortunately, correlations in the gamma bands were weak ( $r^2 < .08$ ). That any significant correlation exists between sleep cycles may reflect some organization in LFP fluctuations.

### *5.3.7 Sleep modulation of single units and LFP*

Up to this point, we have analyzed single-unit and LFP data separately. In the remainder of the analyses, we will examine correlations between single-units and LFPs at three time scales: episode (sleep modulation), trial (rate modulation), and spike (locking of spikes to LFP). When we compared the sleep gain for the LFP and a single-unit recorded at the same site, we found highly significant correlations in the ultra-high gamma band in SWS ( $r^2 = .06$ ,  $p = 2 \times 10^{-5}$ ,  $n = 315$ ) (Fig. 5.9A) and REM ( $r^2 = .08$ ,  $p = 1 \times 10^{-7}$ ,  $n = 328$ ) (Fig. 5.9B). Correlations were higher than in any other frequency band. This was surprising and suspicious at once. It was a surprise because it suggested that modulations in the LFP may be an index of how nearby neurons will modulate in sleep. This was suspicious because we knew from our single-unit data, that even nearby units behave very differently in sleep. How could the LFP correlate with the randomly modulated single-units? Also, this correlation was very specific to the ultra-high gamma band. The answer came when we divided our data into units with low signal-to-noise ratio (SNR) spike waveforms ( $< 20$  dB) and those with high SNRs ( $> 23$  dB). The

correspondence between unit gains and LFP gains was only present in the high SNR population of units ( $r^2=.15$ ,  $p=.0005$ ,  $n=80$ ) and not the low SNR population ( $r^2=.003$ ,  $p=.65$ ,  $n=73$ ) (Fig. 5.9C). This was also the case in REM (Fig. 5.9D).

#### *5.3.8 Influence of high SNR spikes on the LFP*

The fact that high SNR units were well-correlated with the LFP can be explained by leakage of energy from the spike waveform into the LFP waveform. In many cases, this overlap in signal energy artificially introduced a correspondence in the upper LFP band with single-unit firing. Figure 5.10 illustrates this confound in a particularly well-isolated single-unit (SNR=35 dB). In many cases, LFP and spikes are co-tuned making it difficult to determine whether an increase in LFP power is independent of an increase in spike rate. In the case of non-monotonic units like the one in Figure 5.10, a dissociation between firing rate and LFP amplitude can be made. In Figure 5.10A, LFP amplitude rises with the loudness of the stimulus. The single-unit response, however, is non-monotonically tuned to quiet sounds, particularly 10 dB SPL (LFP prefers 70 dB) (Fig. 5.10B). Because the large spikes of the unit were not completely filtered out by our lowpass filter, the LFP contains spike artifacts (Fig. 5.10C). The influence of the spike power is seen in the high frequency bands of the LFP spectrum (Fig. 5.10D, green lines). The effect is restricted to the high frequency bands as power in frequencies below 100Hz is unchanged with sound level. It turns out to be critical to design a simple algorithm to remove high SNR spikes from the LFP before performing analyses on the gamma bands. This is relatively straightforward since spikes have stereotypical shapes and are easily

detectable. Unfortunately, we filtered our signal in hardware and did not retain the whole signal which is necessary to perform spike removal (see Discussion).

### 5.3.9 Covariation of firing rate and LFP power

We computed covariation of trial firing rates and LFP power at each site where both a single-unit and LFP were simultaneously recorded. To avoid spiking artifacts in the LFP, we restricted our analysis to <20dB SNR units. We divided the lower bands of the LFP spectrum into more traditional ranges used for the EEG ( $\delta$ =.7-4.2,  $\theta$ =4.2-7.5,  $\alpha$ =7.5-12,  $\beta$ =12-20,  $\gamma$ =20-50,  $h\gamma$ =40-120, and  $uh\gamma$ =100-300Hz). The rate-power correlations are plotted for each band in Figure 5.11A. A few surprising features emerged. First, since  $\delta$  is prominent in SWS when arousal is low, we had expected negative correlations of spike rate with  $\delta$  energy, but  $\delta$  band-spike rate correlations were non-existent ( $p_{\text{awake}}=.73$ ,  $p_{\text{SWS}}=.44$ ,  $p_{\text{REM}}=.47$ , t-test,  $n_{\text{awake}}=116$ ,  $n_{\text{SWS}}=133$ ,  $n_{\text{REM}}=118$ ). Second, we had expected that  $\beta$  energy might show positive correlation with firing rate since  $\beta$  is an indicator of arousal. Instead, correlations between firing rate and any of the low frequency bands ( $\theta$ ,  $\alpha$ , and  $\beta$ ) were negative. None reached significance at the  $p<.01$  level (t-test,  $n_{\text{awake}}=116$ ,  $n_{\text{SWS}}=133$ ,  $n_{\text{REM}}=118$ ). Third, co-variance of spike rate and LFP power in the  $\gamma$ ,  $h\gamma$ , and  $uh\gamma$  bands exhibited positive correlation (significant at the  $p<10^{-10}$  levels for the  $h\gamma$  and  $uh\gamma$  bands, t-test,  $n_{\text{awake}}=116$ ,  $n_{\text{SWS}}=133$ ,  $n_{\text{REM}}=118$ ). Taken alone this is not a surprising result. The suggestion that  $\gamma$  power correlates with improved neural firing has been made before (Fries et al., 1997; Fries et al., 2001). What is unexpected about our data is that gamma band correlations also exist in sleep. In fact, rate-gamma correlations in SWS were significantly better than those in awake ( $p_{h\gamma}=.002$ ,



$p_{\text{uh}\gamma}=.001$ , Wilcoxon rank sum,  $n_{\text{awake}}=116$ ,  $n_{\text{SWS}}=133$ ). This runs contrary to the idea that gamma has a special role in enhancing neural firing during aroused states such as attention. The mechanism by which gamma energy can lead to (or be the result of) improved neural firing is operative in sleep. For comparison, we measured correlations between driven and spontaneous rates on the same trials (Fig. 5.11A, far right bars). Correlations with spontaneous rate were as high as any of those with the LFP. So the excitability of a neuron immediately before a stimulus is played can predict driven firing. Like gamma energy, spontaneous rates may be an indirect measure of local activity.

#### *5.3.10 Controlling for spike SNR in spike rate-LFP power covariation*

To better estimate the effects of leakage from the spike signal on the LFP, we re-measured rate-power correlations as a function of spike SNR, LFP power, and spike rate. We reasoned that leakage would depend to some degree on all three of these factors. If a neuron has high SNR or a high firing rate, more spike power would be found in the LFP leading to spurious correlation between the LFP and spiking. Likewise, if the LFP is of sufficiently high power, it may be more robust to the influence of spikes. A trend of increasing rate-power correlations was found in units with high spike SNRs (Fig. 5.11B). This effect was restricted to the gamma bands and did not influence lower frequency bands. Spikes above 30dB SNR had the most influence, but below 30 dB, the relation was mostly flat (Fig. 5.11B). This suggests that the 20 dB cut-off used in the rate-power correlation analysis of Figure 5.11A was more than sufficient. Unlike spike SNR, the mean number of spikes elicited had a weaker effect with curves at all frequencies being mostly flat (Fig. 5.11C). And mean LFP power, high or low, had little influence on how

well the LFP correlated with single-unit firing rates (Fig. 5.11D). If anything, a negative trend was present so that a stronger LFP limited the correlation that could be induced by spike leakage. The highest three frequency bands ( $\gamma$ ,  $h\gamma$ , and  $uh\gamma$ ) were clearly separate (higher correlation values) from the lower frequency bands under all conditions (Fig. 5.11, B-D). This was the case regardless of how large spikes were, how many spikes were elicited, or how much power was contained in the LFP.

#### *5.3.11 Spike and LFP coherence*

Finally, we measured the coherence between individual spikes and the LFP waveform. The spike-triggered average (STA) of the LFP is a measure of the activity immediately preceding and following a spike (Fries et al., 2001) (see Methods). The raw STA showed a clear negative deflection preceding spikes in all states and a longer, larger positive deflection following spikes (Fig. 5.12A). The negative deflection lasted for only ~50ms, but the longer post-spike deflection lasted for up to 200ms. SWS shows a clear gain in STA power over awake and REM (mean gain=45%,  $p=0$ , t-test,  $n=337$ ) (Fig. 5.12B). However, if the raw STA is normalized by the amplitude of the LFP used in spike-triggering, then SWS had a similar STA waveform to those in awake and REM (Fig. 5.12C), and power of the normalized STA is similar between awake and SWS (mean gain=-7%,  $p=.07$ , t-test,  $n=337$ ) (Fig. 5.12D). This suggests that the strength of the SWS STA is because of stronger LFP amplitudes in SWS.

If the Fourier transform of the STA is taken, the spike-field coherence (SFC) is obtained. The SFC is a measure of how coherent spikes are with different frequency bands of the LFP (Fries et al., 2001). For example, studies in the visual system have

shown an increase in the gamma range of the SFC when attention is directed inside a cell's receptive field. It is possible that spikes lock to different brain rhythms during sleep and wakefulness. Of special interest were the low frequencies corresponding to slow waves since these could induce the correlations between neurons that we observed earlier (Figure 5.4A). In Figure 5.12E, the SFC did show improved locking of spikes in SWS to frequencies below 10Hz. At higher frequencies, awake, SWS, and REM all had similar SFC's. A more accurate measure is to normalize the SFC by the mean LFP spectrum. In doing so, the normalized SFC captures any improvements in spike synchronization independent of enhancement in the regularity of the LFP oscillations. In the normalized SFC, the observed trend in the low frequencies remained; spikes during SWS were more coherent with low frequencies than those in awake and REM (Fig. 5.12F). The normalized SFC above 100Hz became elevated, but this may be because the normalization values were very small (power is weak above 100Hz). Also, above 100Hz, spike contamination becomes an issue, so high coherence may reflect the fact that the high frequency range of the LFP overlaps the lower end of spike power.

## **5.4 Discussion**

### *5.4.1 Disorganization of single neurons during sleep*

The responses of single-neurons can be up- or down-regulated in SWS and REM. In the present study, we did not find any evidence of spatial organization of these activations and deactivations. Driven rates of nearby neurons could be modulated in

opposite directions. The result is that the map of firing rates across the cortical surface or in depth is randomly modified. This is somewhat surprising given that neurons in the thalamus are modulated much more homogeneously (Livingstone and Hubel, 1981; Edeline et al., 2001). If thalamic projections to cortex are organized, then neighboring cortical neurons would be expected to be more homogeneous. This result is also surprising from the perspective of the dynamics of sleep. We supposed that during certain sleep cycles activity is regulated in a consistent fashion. But even in neurons recorded simultaneously within the same sleep cycle, modulation was random. This spatially random behavior has been suggested before by a few groups (Livingstone and Hubel, 1981; Pena et al., 1999; Edeline et al., 2001). Our study is the first to directly confirm it by systematically recording from pairs of neurons simultaneously. Combined with the finding in Chapter 1 that REM and SWS independently modulate firing rates, the picture we put forth is one of a network of neurons that is randomly spatially modulated in two different patterns, one pattern that recurs in every cycle of SWS and one pattern that recurs in each cycle of REM.

Although mean firing rates can differ between units, we observed a tendency for increased trial-by-trial firing rate correlations in SWS. This came at the cost of disrupting correlation patterns normally present in awake and to some degree in REM. This finding is consistent with work showing improved neuronal correlations in spontaneous firing during SWS (Noda and Adey, 1970) and with work showing that slow-waves induce coherence across space (Destexhe et al., 1999). In awake and REM, LFPs are less coherent across space (Destexhe et al., 1999) which may relate to decreased neural correlations. Another possible explanation for the increase in correlated firing in

SWS comes from data in Chapter 4 showing that inhibition decreases in SWS. This may lead to unmasking of more inputs including common inputs. Regardless, our result implies that neurons in SWS fire less independently which may limit their coding capacity (Abbott and Dayan, 1999). Neurons in awake and REM have baseline levels of correlation which may simply reflect common thalamic inputs to neighboring neurons. Alternatively, the awake-like correlations observed in REM may be related to dreaming. In such a scenario, correlations present in the preceding awake period would carry over into dreams during REM. One way to test this hypothesis would be to re-measure awake and REM correlation patterns in later sleep cycles and see if these patterns change and are re-expressed in REM.

#### *5.4.2 LFP responses during sleep*

This study is the first to report the effects of sleep on LFP sensory responses. Previous work correlated LFP sensory responses with single-unit responses during the awake state (Henrie and Shapley, 2005; Liu and Newsome, 2006; Kreiman et al., 2006; Kayser et al., 2007). The main finding of those studies is that the gamma frequency bands ( $>20\text{Hz}$ ) correlate strongly with single-unit tuning. We found weak evidence that sleep modulated LFP gamma and no evidence of correlation between sleep modulation of LFP gamma and single-unit modulation. Our original reason for collecting the LFP was that we thought it would tell us what the thalamic input is during sleep. We thought that the LFP would clearly decrease during sleep since the thalamus is depressed in sleep. Instead, changes in LFP gamma averaged near 0%. Either the thalamus is actually active in sleep or the LFP is a poor proxy for thalamic input. A more direct way to assess

thalamic input to cortex during sleep may be to collect the current source density (CSD) and measure the strength of the layer 4 sink (Mitzdorf, 1985; Heynen and Bear, 2001).

Although the LFP was not a good predictor of sleep modulation of single unit responses, it was a good predictor of trial-by-trial variations in firing rate. Especially in the gamma range, there were significant positive correlations between gamma power and firing rates. Low frequency bands manifested a weaker but significant negative correlation with spiking rate. This trial-by-trial correlation was present in all three states (awake, SWS, and REM). Apparently, similar mechanisms are operative in sleep and awake.

Given that previous work had suggested a special role for gamma power in attention (Fries et al., 2001), we were surprised to find that SWS had the strongest gamma power-firing rate correlations (Fig. 5.11A). This observation resembled our finding that pairs of units showed higher correlations in SWS (Fig. 5.4A). We believe the two are related. Improved spike rate-LFP gamma correlations probably imply improved correlations between units. It is not clear what the causal order is. Neurons could be coordinated through the gamma oscillation, or coherent firing of neurons could induce gamma oscillations. A third possibility is that increased firing rates lead to both higher neuron coherence and higher gamma oscillations (de la Rocha et al., 2007), but we have no reason to believe that SWS increased firing rates. Either way, correlations between units (Fig. 5.4A) and between units and LFP gamma (Fig. 5.11A) were closely related in our data because they were both strongest in SWS, significantly stronger than in awake or REM. This adds evidence to the idea that SWS involves coordinated activity (Destexhe et al., 1999). The novel finding is that this coherence may lead to improved gamma band

correlations. Such gamma band correlations had normally been considered the domain of enhanced processing states like attention (Fries et al., 2001). This view may have to be revised to include SWS. The mechanism for improved population correlations seemed to at least partly involve the low frequencies of the EEG as spikes were more coherent with frequencies below 10 Hz in SWS than in any other state (Fig. 5.12F)

When we examined the correlation of spiking activity during the stimulus to spontaneous activity immediately preceding, there was also a strong correlation. Background firing rates, like high gamma activity, can predict the changes in evoked response strength. As shown in Chapter 3, though, changes in spontaneous activity could not predict changes in overall driven activity during sleep. This parallels the finding here that the sleep modulation of the LFP does not correlate with single-unit modulation. The LFP and spontaneous activity seem to be a good measure of the excitability of neurons locally in time but not modulations of firing rate on the episode timescale during sleep.

#### *5.4.3 Technical issues with recording the LFP*

Interpretation of the LFP is limited. For one thing, the LFP resides in the frequency range between the EEG and spikes. From one side, the EEG can confound the LFP, and from the other, spikes and multi-unit activity can mix with the LFP. We did find strong amplitude changes in the SWS EEG which led to consistent changes in the LFP (Fig. 5.6). But these changes were uncorrelated with single-units. Low frequency portions of the EEG are likely too gross in their spatial scale to apply at the neural level. We had difficulty examining the higher gamma bands of the LFP because, above 100Hz, spiking can lead to energy in the LFP. This was despite strong online hardware filtering. Lowpass filtering can only completely remove periodic signals, but, being impulses,

spikes contain energy in all frequencies. Post-hoc we developed a spike removal algorithm that managed to clean-up LFPs, but for the analyses described above, we could not apply this algorithm and instead restricted our analyses to LFPs collected when spike SNRs were small. Spike SNRs  $< 20\text{dB}$  seemed to be sufficient for examining the LFP independent of spike energy (Fig. 5.11B). In the future, it is best to record the LFP on a separate electrode when using high impedance electrodes that routinely yield large spike waveforms. This precaution may not be necessary when using low impedance electrodes or recording multi-unit activity (Fries et al., 2001). An alternative is to remove spikes from the unfiltered waveform before lowpass filtering below 300Hz, but this would have to be done offline.

We adopted conventional frequency domain measures of the LFP in our analyses, but in order to correlate LFP data with the wealth of evoked potential studies during sleep (for reviews see Cote, 2002; Bastuji et al., 2002), future LFP work will need to examine properties of the LFP in the time domain. Features of the LFP waveform may correspond to different components of thalamic and cortical processing. Most studies to date have focused on the first 50ms of the LFP since this is most reliable. Changes in wave shape with cortical depth make any further analysis difficult without first co-registering the LFP with laminar location (Mitzdorf, 1985; Heynen and Bear, 2001). In evoked potential studies, however, the measured signal contains stable late components that can be interpreted to have a cortical basis (Bastuji et al. 2002; Hennevin et al., 2007). Since the evoked potential is many steps removed from spiking responses, it is difficult to extend interpretations much further. Ultimately, a multi-scale approach will be necessary to characterize the cortical network under different behavioral states.



## 5.5 Methods

See Chapter 2: General Methods for details of electrophysiological recordings and sleep scoring.

### 5.5.1 LFP recordings

Local field potentials (LFP) were recorded in two hemispheres of two animals. In one animal, LFPs were recorded in both A1 and LB (43q). In the second animal, LFPs were recorded exclusively in LB (16s). The findings of this study were unaffected when restricted to LB (Fig. 5.7), so the results from both A1 and LB were pooled. LFPs were measured on the same electrode as single-units. 2-4M $\Omega$  tungsten microelectrodes were used. After the first stage of filtering (1-10000Hz, 100x), the electrode signal was split and filtered separately to obtain the spike (300-3750Hz, 100x) and LFP (1-300Hz, 100x) signals. The spike signal was digitized at 8kHz for later use. A 1kHz sampling rate was used for digitizing LFP signals. LFPs were DC subtracted and notched filtered offline at 60Hz and higher harmonics (120Hz and 180Hz) using a fourth order elliptical filter (stopband attenuation=40dB, passband ripple=.1).

### 5.5.2 Rate correlation

Correlations between two simultaneously recorded neurons responding to the same stimulus were computed using a simple normalized measure adapted from Bair et al., 2001:

$$\rho = \sum_{i=1}^{trials} \frac{(r_{i,1} - \mu_1)}{\sigma_1} * \frac{(r_{i,2} - \mu_2)}{\sigma_2} = \sum_{i=1}^{trials} z_{i,1} * z_{i,2}, \text{ where } r_i \text{ are the firing rates on the } i\text{th trial}$$

which are mean subtracted and normalized by the standard deviation of firing across all trials to obtain a z-score  $z_i$ . Rate correlations were bootstrapped by randomly permuting the trials of one neuron in the pair and recalculating correlation (n=25).

### 5.5.3 Spike synchrony

To measure spike timing synchrony between neurons, a cross-correlation between spike trains was first computed using (Bair et al., 2001):

$$CCH_{1,2}(\tau) = \frac{1}{T - |\tau|} \frac{1}{N} \sum_{i=1}^N \sum_{t=1}^T \frac{x_{i,1}(t)x_{i,2}(t + \tau)}{\sqrt{\sum_t x_{i,1} \sum_t x_{i,2}}}, \text{ where } \tau \text{ is the time delay } (\tau = -100 \text{ to}$$

100ms),  $T$  is the length of the spike train, and  $N$  is the total number of trials. This measure normalizes spike trains by their average firing rate before cross-correlating and corrects for edge effects using a triangular window. Shift-predictor CCH's were generated by re-computing and averaging the CCH for all trials  $i, j$  where  $i \neq j$ . We computed the standard deviation of individual shift predictor trials from the mean and used a cut-off of 2\*sd to determine if synchrony was significant. Few CCH's passed this criterion in our sample which is a reflection either of low connectivity in auditory cortex, the low number of trials collected, not optimizing the stimulus for both neurons, or not eliciting enough spikes to achieve statistical significance.

#### *5.5.4 Frequency analysis of LFP*

Similar to previous work (Kreiman et al., 2006), we computed the power spectral density using the Welch method with either a 100 or 128 point Hamming window and 50% overlap of segments. To ensure that absolute power was preserved in the spectrum estimate, we normalized the spectrum by the sum of all the frequencies and multiplied by the power of the raw signal. This yielded units of power ( $V^2$ ) per Hz. The power was computed from the spectrum using two different schemes:  $\delta=7-4.2$ ,  $\theta=4.2-7.5$ ,  $\alpha=7.5-12$ ,  $\beta=12-20$ ,  $\gamma=20-50$ , high- $\gamma=40-120$ , ultrahigh-  $\gamma=100-300$  Hz, or 0-10, 10-40, 40-120, and 100-300 Hz. Spectra were computed separately during the pre-stimulus period (baseline) and during the first 100ms of stimulation. For trial-by-trial correlations with single-unit firing rates, LFP spectra were computed for the whole signal (pre-stimulus, stimulus, and post-stimulus periods). In addition to computing the spectrum on each trial, we computed the peak-to-peak value of the stimulus-triggered average of the LFP. Spectral power or peak-to-peak amplitudes of LFPs were considered significant if they were  $3 \times \text{sem}$  above levels during the baseline period and were considered significantly different in two states if separated by more than  $2 \times \text{sem}$ .

#### *5.5.5 Spike signal-to-noise ratio*

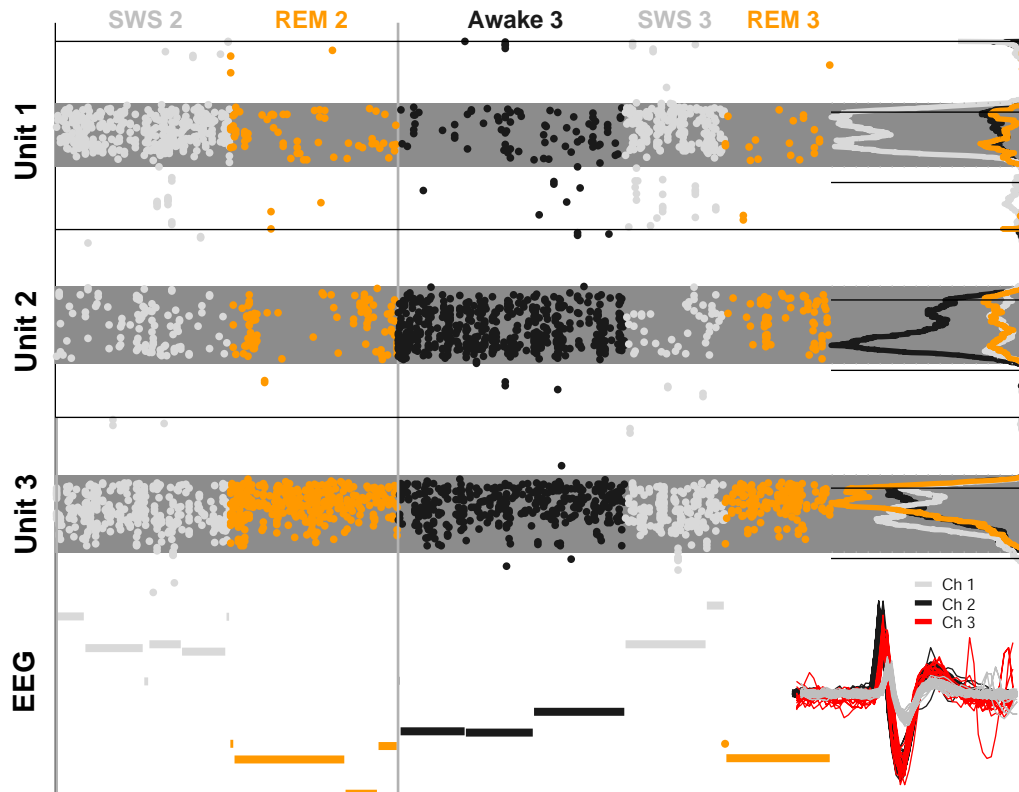
100 spike waveforms were averaged and the peak-to-peak value was compared to the noise in the 10ms preceding the spike according to  $\text{SNR} = 20 \times \log_{10}(V_{pp}/\sigma)$ .

#### *5.5.6 Spike-triggered average of LFP*

The spike-triggered average (STA) of the LFP was taken by averaging the LFP signal  $\pm 200$ ms about each spike. The STA was averaged across all spikes elicited in a neuron whether spontaneous or evoked and regardless of stimulus used. The shift predictor was computed by permuting LFP trials and re-computing the STA 10 times. This randomization is important because it accounts for any stimulus induced correlations in spikes and LFP activity. Often, in the first 100-200ms following stimulation the increase in spike rate and LFP power can artificially induce correlations. A second bootstrap procedure we used was to randomize spike times within a trial before computing the STA. This procedure yielded weaker shift predictors than randomizing trials. We found it better to use the first, more conservative trial-shift predictor. STAs that reached absolute values  $> 2.5 \times \text{sd}$  after shift predictor subtraction were considered significant.

#### *5.5.7 Spike field coherence*

The spike-field coherence (SFC) is a measure of the degree of synchrony between spikes and field potentials in different frequency bands. The raw SFC is simply the power spectrum of the STA. In order to distinguish a change in coherence from a change in the strength of oscillatory patterning of the LFP, the raw SFC is normalized by the mean spectrum of all LFPs used in calculating the STA (Fries et al., 2001).



**Figure 5.1.** Example of 3 simultaneously recorded units.

Three single-units were recorded simultaneously on one electrode. Units were isolated on the basis of their different spike shapes (inset). The EEG (bottom) reaches a peak in SWS (gray), falls in REM (orange), and returns to intermediate levels in awake (black). Data was not collected continuously. Each horizontal bar represents the mean rms EEG power of a continuous measurement. Breaks between bars indicate separate measurements. Data were recorded from all three units in two different episodes, episodes 2 and 3, of sleep (EEG returns to a high value in a second episode of SWS after awake period).

The spiking behavior of each unit was different. The top unit responded most strongly in SWS (gray) (rasters oriented vertically, each column represents a single trial). The second unit responded most strongly in awake (black), and the third unit responded equally well in awake, SWS, and REM. The PSTH's (far right, oriented sideways) show the averages of activity across all trials in each state. SNRs for the units (top to bottom) were 27, 39, and 28 dB. Data collection from all three units spanned a 100 minute period.

**Figure 5.2.** Sleep modulation of nearby units.

(a) SWS gains for unit pairs recorded within 200 $\mu$  of each other. Usually, units were recorded sequentially (dark gray), but, in some cases, unit pairs were recorded simultaneously in the same sleep cycle (light gray). Gain for the first unit recorded is plotted against the gain of the second unit recorded. No correlation was observed in sleep modulation of units in a pair ( $r^2=.02$ ,  $p=.08$ ,  $n=199$ ) even if recorded simultaneously ( $r^2=.07$ ,  $p=.08$ ,  $n=46$ ).

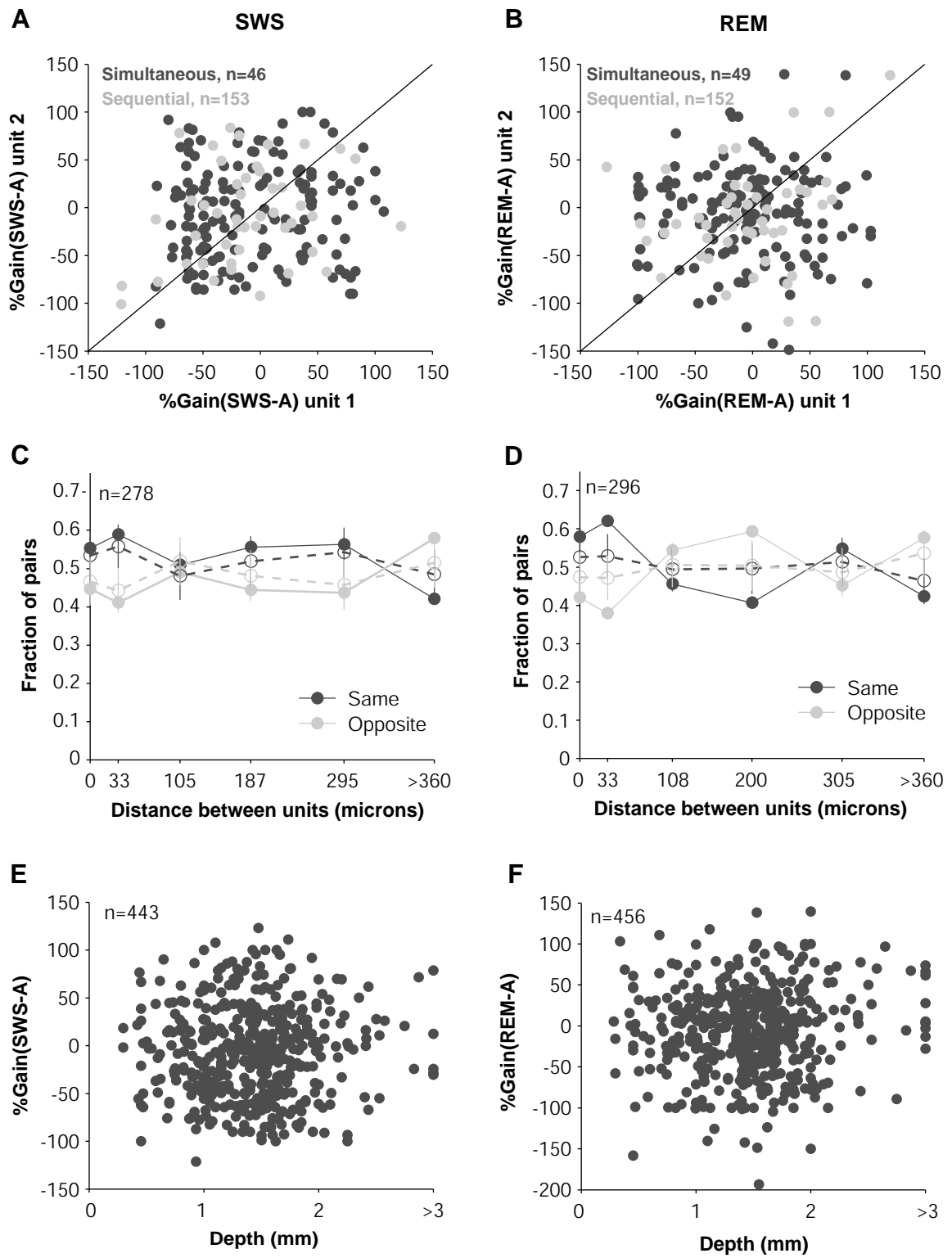
(b) Same as (a) except for REM. Gains of nearby units were uncorrelated ( $r^2=.001$ ,  $p=.67$ ,  $n=201$ ) even if recorded simultaneously ( $r^2=.01$ ,  $p=.44$ ,  $n=49$ ).

(c) The direction of modulation in SWS was equally likely to be in the same (dark gray) or opposite direction (light gray) for neurons recorded on the same track at various distances. Distances labeled are centers of distance quintiles (0 corresponds to simultaneously recorded units). Given the distribution of gains, we obtained a bootstrap estimate of how often two gains randomly drawn from this distribution would be in the same (dark gray dashed line) or opposite (light gray dashed line) directions (error bars represent  $\pm$ sd,  $n=100$ ).

(d) Same as (c) except for REM. In general, REM modulation of a neuron was just as likely to be in the same or opposite direction as other neurons recorded in the same track.

(e) SWS gain as a function of depth from the cortical surface. No tendency exists in gain with depth ( $r^2=.001$ ,  $p=.61$ ,  $n=443$ ).

(f) REM gain as a function of depth from the cortical surface. No tendency exists in gain with depth ( $r^2=.004$ ,  $p=.16$ ,  $n=456$ ).



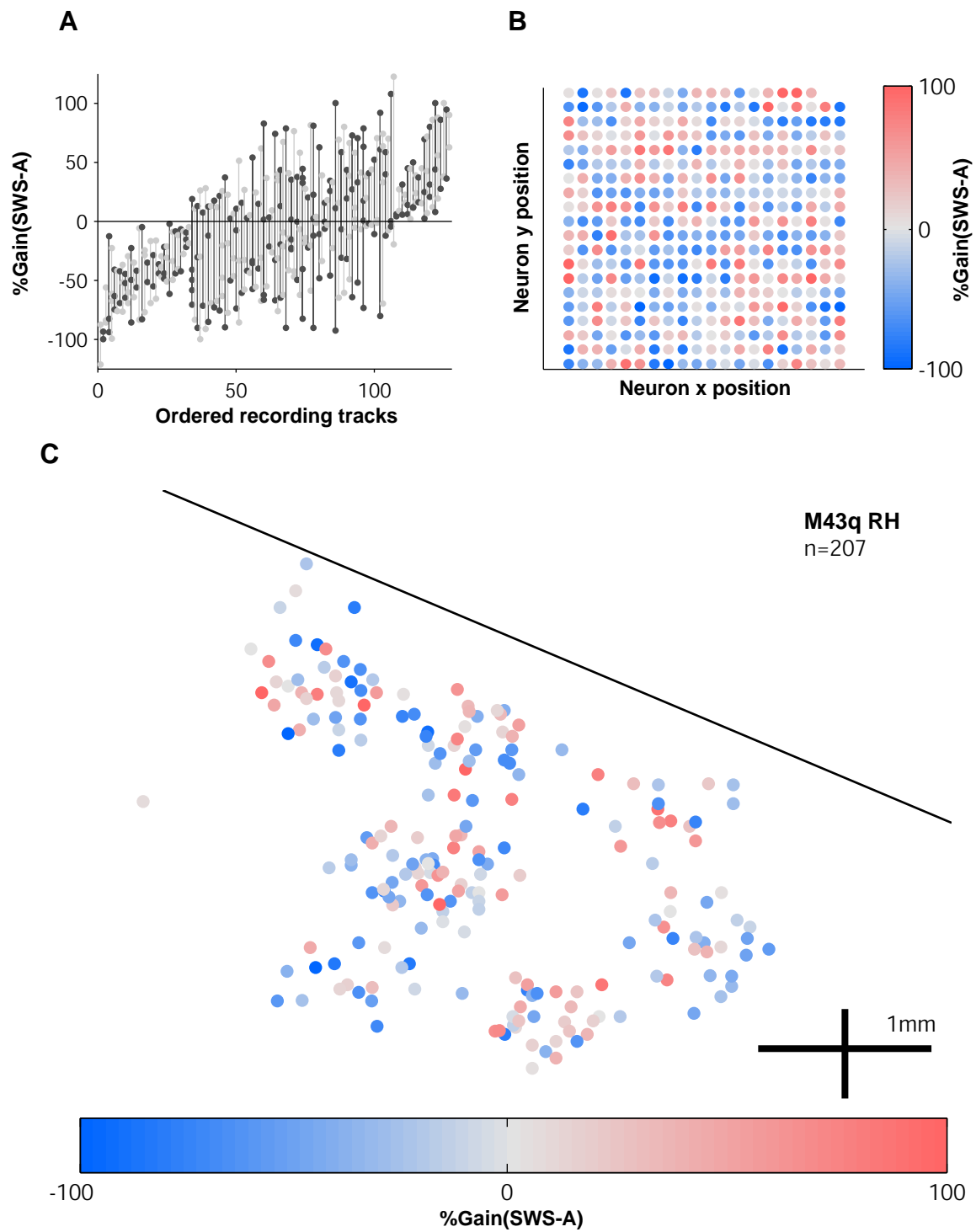
**Figure 5.3.** Illustration of the lack of spatial organization in SWS.

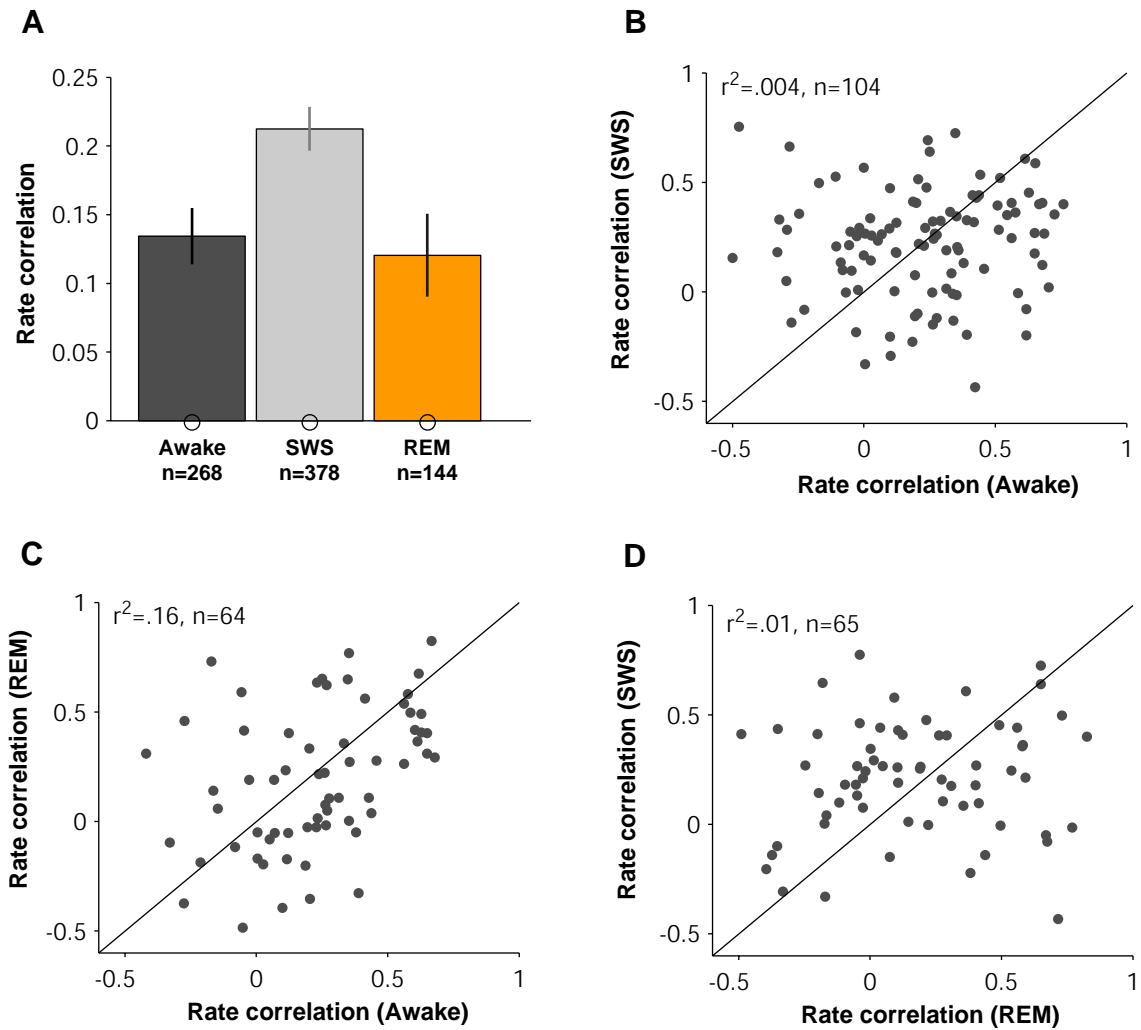
(a) All tracks where more than one unit was recorded ( $n=127$ ). Each dot represents a unit, and all units from the same track are connected by a line. Tracks are first ordered by how consistent the sign of the gain was between units (all positive, all negative, or mixed sign) and then ordered by the mean gain. On some tracks, units displayed consistent modulation (far right and left tracks), but on the majority (middle tracks), gains widely varied. So sleep modulation was not consistent on any given night.

(b) To simulate how disorganized cortical firing rates would be during SWS, we used the pairwise gain values obtained in Figure 5.2a. We used these values as measures of pairwise correlation and arrayed the gain values in a grid (every two neurons represents a pair from Fig. 5.2a).

(c) Actual data across the cortical surface from the most extensively mapped hemisphere (M43q, right hemisphere). SWS modulation varies quite randomly. The map of firing rates across auditory cortex is highly modified.







**Figure 5.4.** Covariation of firing rates in awake, SWS, and REM.

(a) Trial-by-trial fluctuations in firing rate were strongest in SWS (light gray) but also significantly present in awake (dark gray) and REM (orange) (error bars represent  $\pm$ sem; open circles are bootstrapped estimates found by randomly assigning trial numbers).

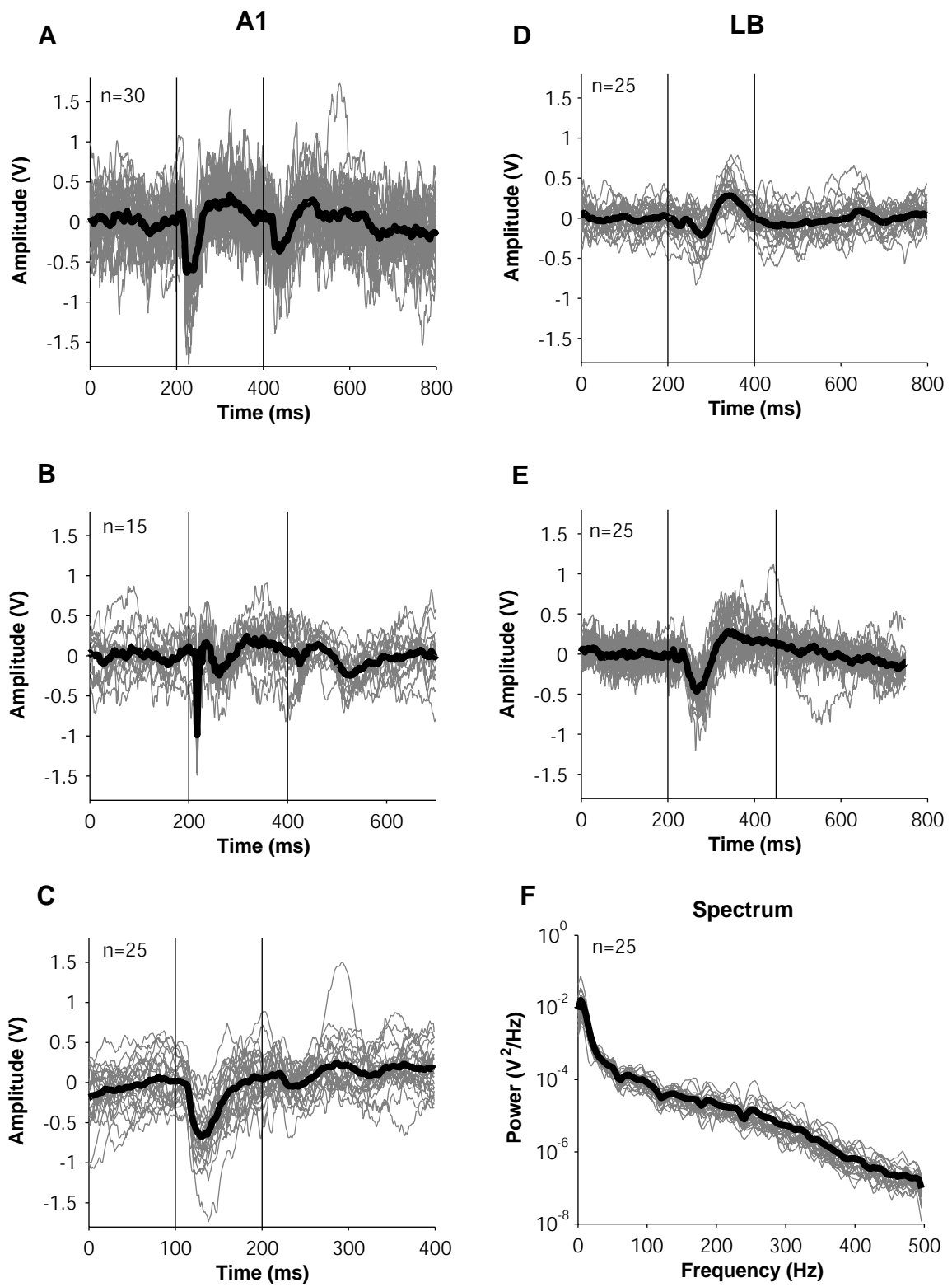
(b) Strengths of rate correlations present in SWS were uncorrelated with those in awake ( $r^2=.004$ ,  $p=.54$ ,  $n=104$  stimuli).

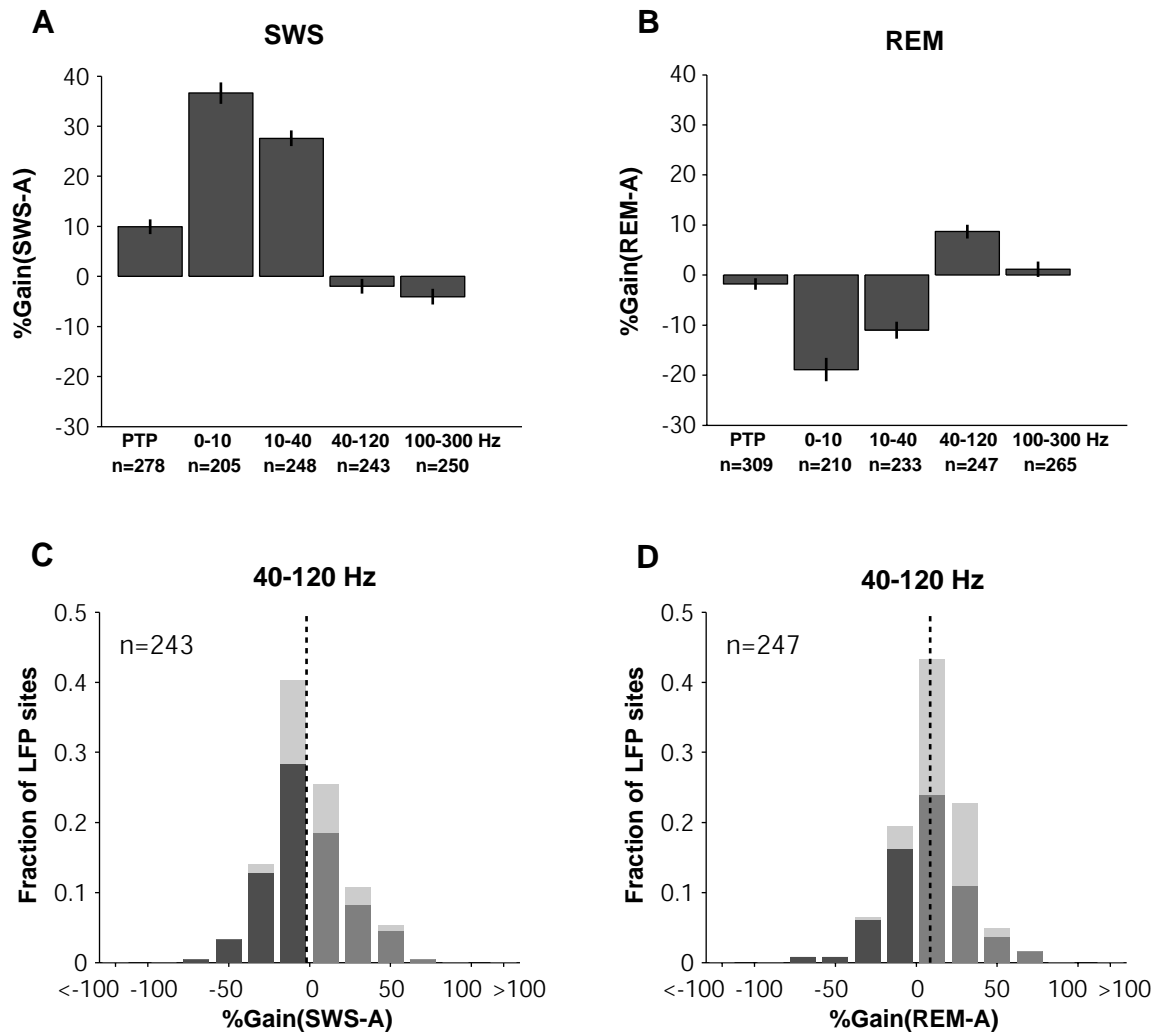
(c) Pattern of rate correlations in awake and REM was similar ( $r^2=.16$ ,  $p=.001$ ,  $n=64$  stimuli).

(d) SWS rate correlations were dissimilar from REM rate correlations ( $r^2=.01$ ,  $p=.41$ ,  $n=65$ ).

**Figure 5.5.** Example LFPs recorded from A1 and LB.

- (a) LFP waveforms from individual trials are plotted in gray. The mean LFP waveform is overlaid (black). Negative LFP deflections are clear at the onset and offset of the stimulus.
- (b) Another example LFP from A1 showing a fast negative component at stimulus onset.
- (c) Example LFP from A1 that shows a broader waveform than in the examples of (a) or (b).
- (d) In LB, waveforms were generally broad and did not reach as high amplitudes as in A1. In some cases, a small negative potential can be discerned at the short latencies seen in A1. This was followed by a larger, slower negative waveform.
- (e) Small negative potential before main LFP deflection can be seen in this example taken from LB.
- (f) The power spectrum of the individual LFP waveforms on each trial in (e) (gray). Mean spectrum is overlaid (black). The LFP has a lowpass nature with most energy concentrated in the EEG range (<20Hz).





**Figure 5.6.** Sleep modulation of LFP.

(a) SWS increased PTP amplitude and the power in the low frequencies of the LFP. Energy in the high and ultrahigh gamma (40-120 and 100-300 Hz) bands did not change strongly when the animal entered into SWS.

(b) In REM, power in the low frequencies decreased (opposite of SWS effect, see (a)). PTP and gamma energy did not appear to change strongly.

(c) SWS Modulation of high-gamma (40-120 Hz) energy was centered near 0 (mean -2%,  $p=.18$ , t-test,  $n=243$ ). Distribution was narrow. Average magnitude of modulation was only 18%, but 77% of LFPs were significantly modulated in SWS ( $>2 \times \text{sem}$  difference from awake) (darkly shaded bottom portions of bars represent significant gains; lightly shaded top portions represent insignificant gains).

(d) Same as (c) except for REM. Gain in high-gamma energy tended to be positive in REM (mean 9%,  $p=8 \times 10^{-10}$ ,  $n=247$ ). 64% of LFPs were significantly modulated. Magnitude of modulation only averaged 17%.

**Figure 5.7.** Comparison of LFP in A1 and LB.

(a) Peak-to-peak LFP amplitudes were generally larger in A1 than in LB whether in awake (dark gray) or SWS (light gray). Note that LFP amplitude tends to be higher in SWS than awake (error bars represent  $\pm$ sem). PTP values were taken for all stimuli tested in a minimum of 5 reps in awake and SWS and having a response  $>3 \times$ sem above baseline in either awake or SWS.

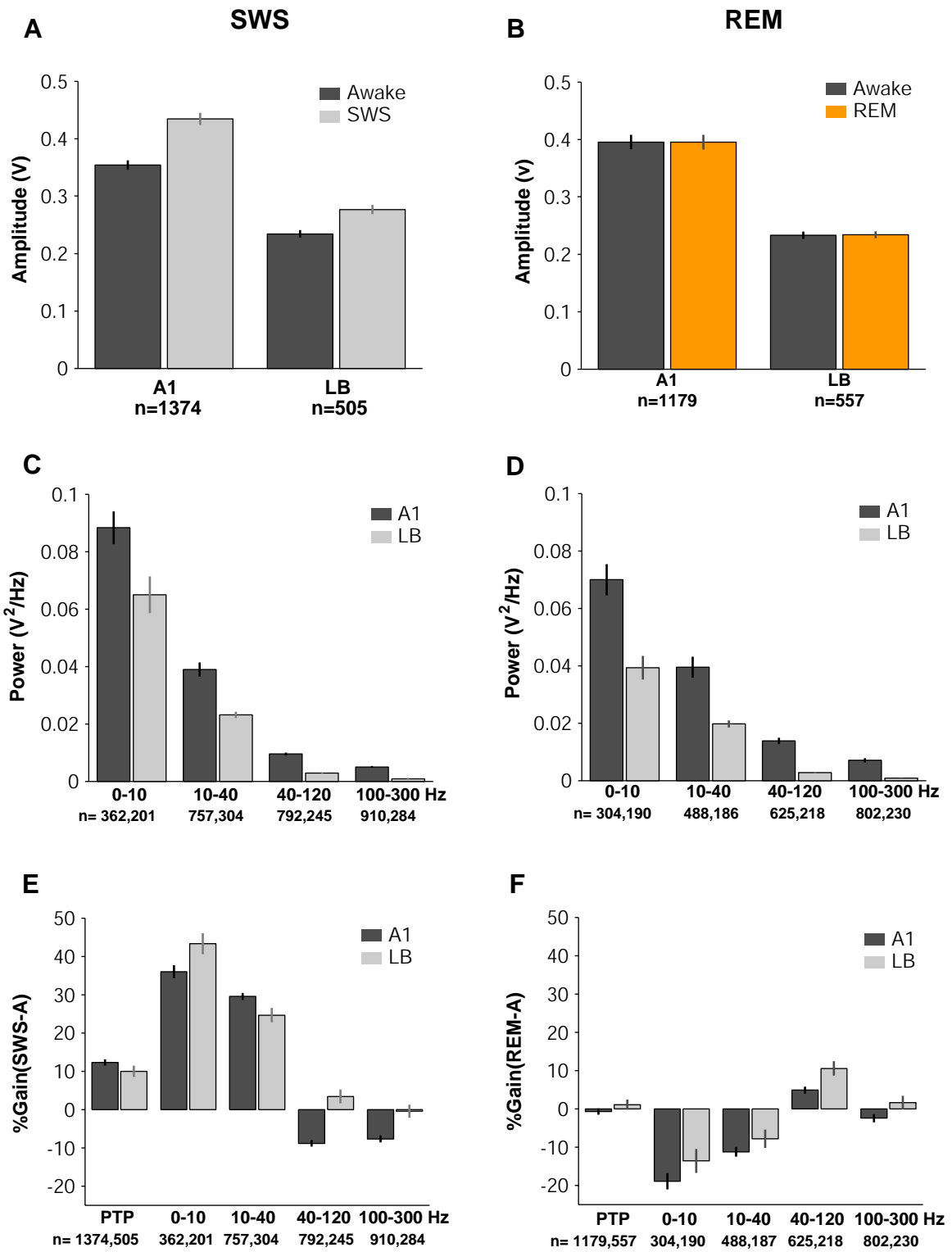
(b) Same as (a) except REM substituted for SWS. Response amplitudes in A1 were stronger than those in LB for awake and REM states.

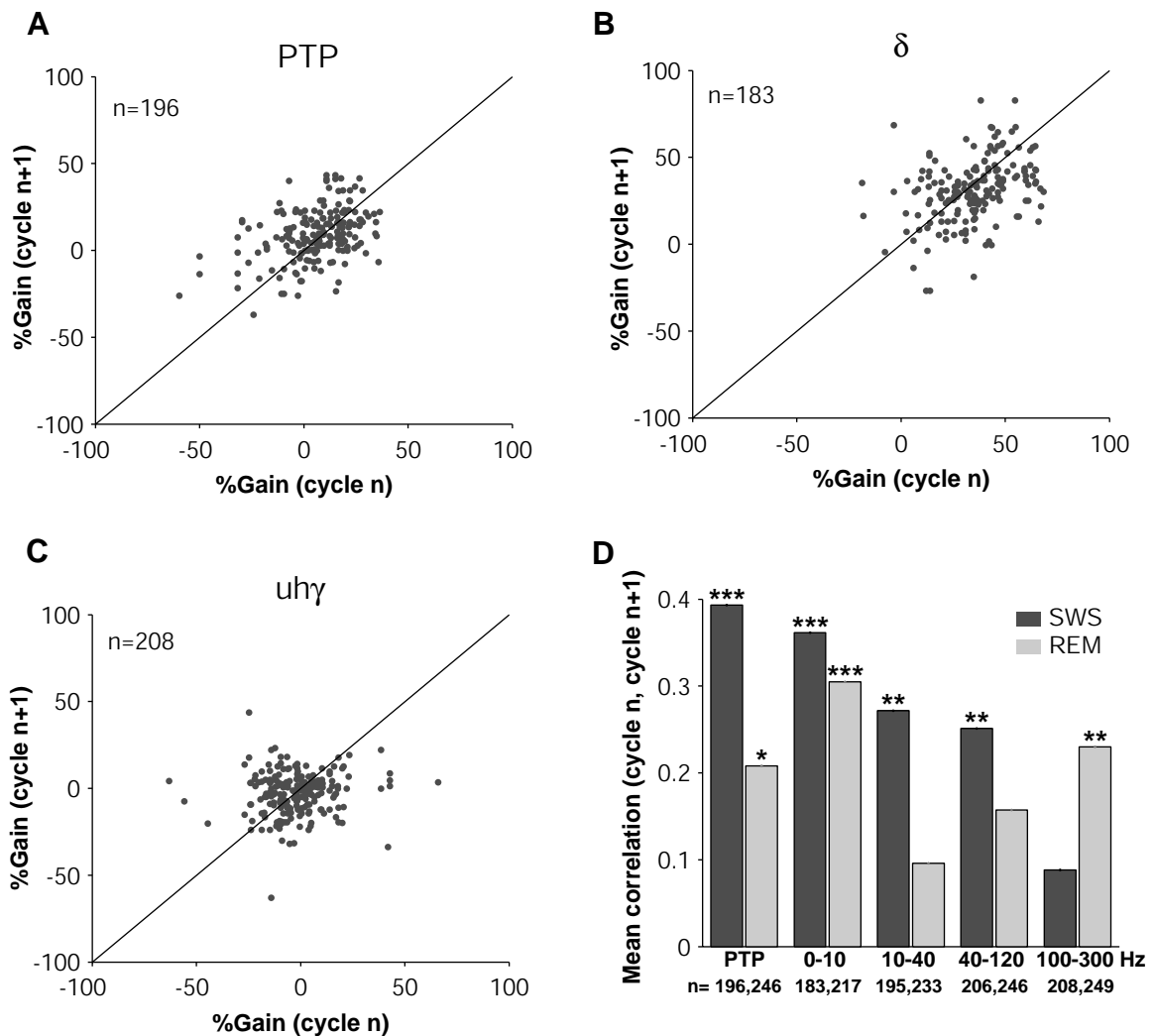
(c) Comparison of LFP power in A1 and LB across frequency bands. Power is generally higher in A1 (dark gray) than in LB (light gray) regardless of frequency range. Power was computed as the mean of awake and SWS values for all stimuli eliciting a significant modulation of power ( $>3 \times$ sem from baseline) in at least 1 state (error bars represent  $\pm$ sem).

(d) Same as (c) except for REM. A1 power during REM was greater than LB power for all frequency bands.

(e) Although A1 may have had higher LFP amplitude (a) and power (c) than LB, SWS modulation of amplitude (PTP, far left) and power were similar in A1 and LB (error bars represent  $\pm$ sem).

(f) Same as (e) except for REM. Pattern of negative gains at low frequency bands seen earlier in pooled data (Fig. 5.6b) was present in both A1 (dark gray) and LB (light gray) even though amplitude (b) and power (d) tended to be higher on average in A1.





**Figure 5.8.** Consistency of LFP modulation across sleep cycles.

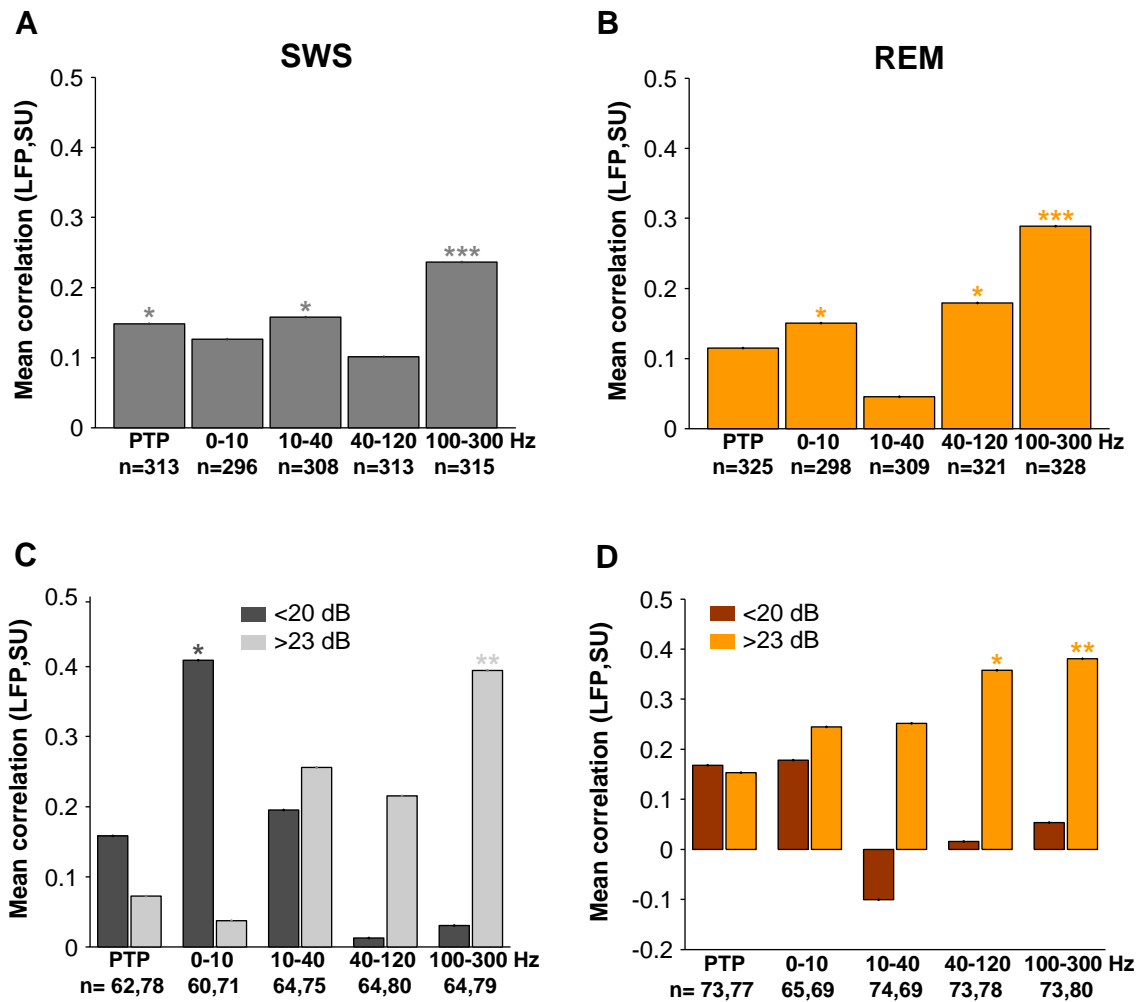
(a) In many cases, LFPs were re-measured in a later cycle of sleep at a different site (i.e. in conjunction with a different isolated single-unit). SWS modulation of PTP amplitude was correlated between cycles ( $r^2=.15$ ,  $p=1*10^{-8}$ ,  $n=196$ ).

(b) Modulation of delta power was somewhat similar from one cycle to the next ( $r^2=.13$ ,  $p=5*10^{-7}$ ,  $n=183$ ).

(c) Modulation of high-gamma (40-120 Hz) power was weak but present from cycle to cycle ( $r^2=.06$ ,  $p=.0003$ ,  $n=206$ ).

(d) In general, correlation of sleep modulation between cycles was only present for SWS (dark gray). REM modulations (light gray) were not as consistent. The correlation between nearby episodes of SWS may reflect the waxing and waning trend of slow-wave amplitude throughout the night. This general effect on waveform amplitude likely trickles into higher frequency bands (10-40 and 40-120 Hz) even though slow-waves are restricted below 5 Hz suggesting a multiplicative (modulatory) rather than additive effect of slow-waves.





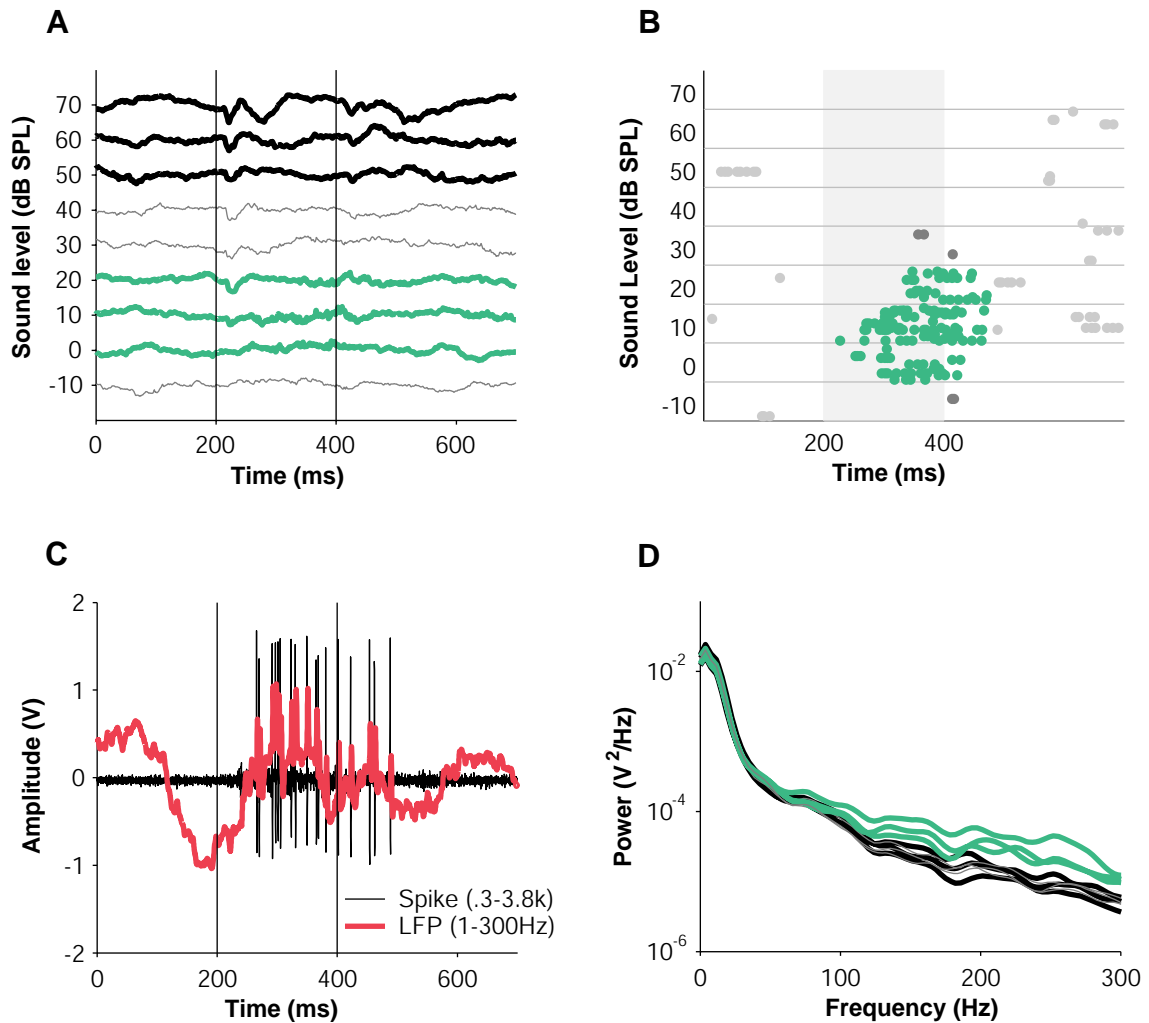
**Figure 5.9.** Correlation between single-unit and LFP modulations during sleep.

(a) LFP recordings were made in conjunction with single-units. The modulation of LFP amplitude (PTP) and power during SWS was compared to modulation of single-unit firing rates during SWS. Only the ultra-high gamma (100-300Hz) band showed a strongly significant correlation (\* $p < .01$ , \*\* $p < .001$ , \*\*\* $p < .0001$ ). Sites were selected based on the presence of LFPs at least 1.5\*sem above baseline and single-unit firing rates at least 2\*sem above spontaneous.

(b) In REM, power in the ultra-high gamma band was also modulated in a manner consistent with single unit modulations.

(c) Redoing the analyses in (a) separately for low SNR (<20 dB, dark gray) and high SNR (>23 dB, light gray) single-units reveals that ultra-high gamma correlations with firing rate modulation are only present in high SNR units. Correlations return almost to 0 in the high and ultra-high gamma bands for low SNR units.

(d) Similar to SWS, REM modulation showed strong correlation in the high and ultra-high gamma bands only for high SNR units (orange), but for low SNR units the high and ultra-high gamma bins were near 0.



**Figure 5.10.** Example LFP contaminated by simultaneously recorded spikes.

(a) LFP amplitude generally increases monotonically with sound level of stimulus. At quiet levels (green and gray), LFP waveform is weak.

(b) Single-units in auditory cortex often exhibit non-monotonic tuning with sound level. This particular unit only responds to 0-20 dB SPL (green).

(c) Example stimulus trial at 10dB SPL. The LFP (red) has no clear stimulus locked component at this quiet sound level. However, the LFP has artifacts from the spiking signal (black). Energy from the 35 dB SNR spikes leak into the signal despite lowpass filtering.

(d) Normally the LFP spectrum has the most power for loud sound levels (thick black lines), but in this case power is greatest for quiet sound levels (thick green lines) where driven spiking of unit artificially boosted power. Note that differential effect is restricted to frequencies above 100 Hz. Lower frequencies are generally not affected by spike power. Only the high part of the LFP spectrum is susceptible.

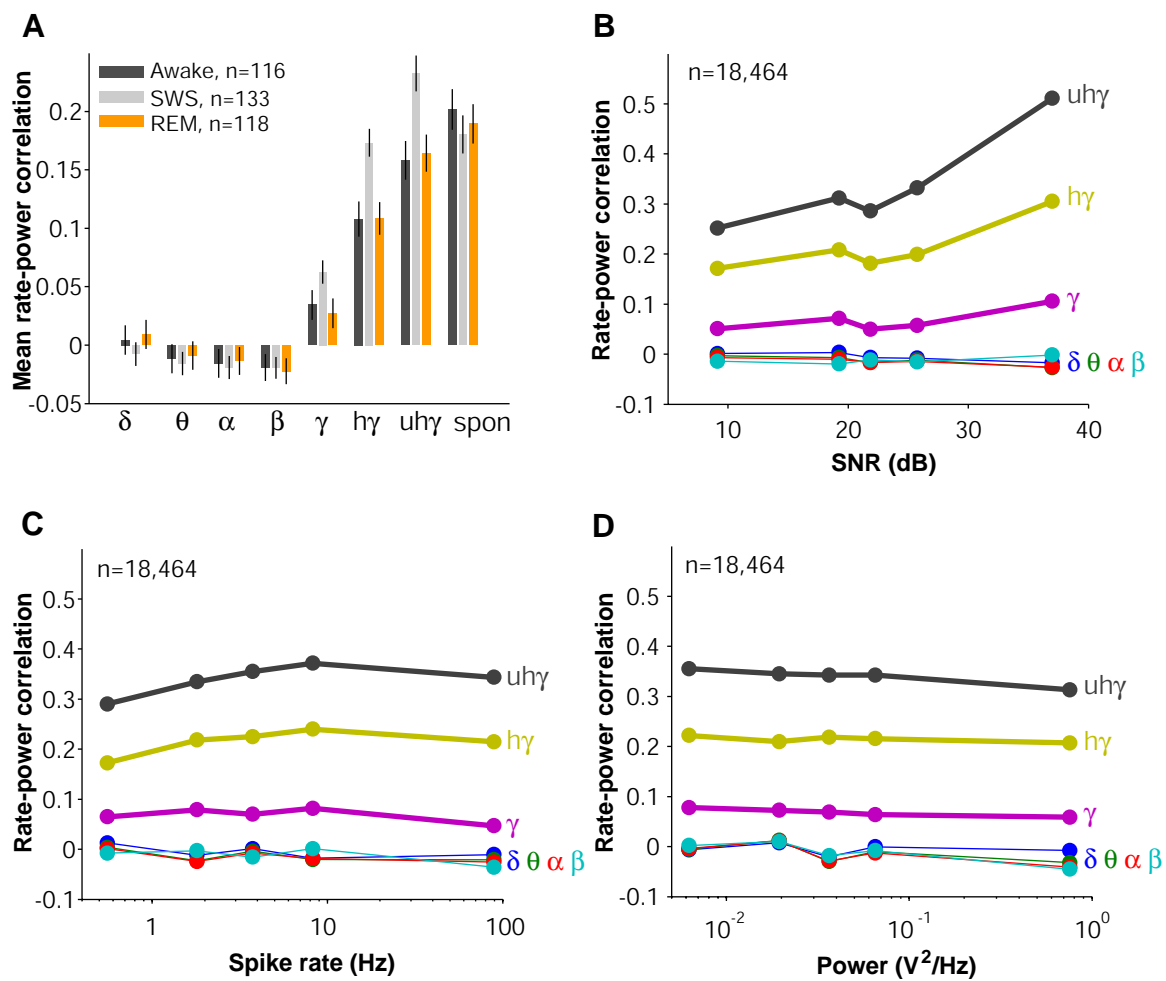
**Figure 5.11.** Spike rate-LFP power correlation.

(a) In general, low frequency LFP power (<20Hz,  $\beta$  corresponds to 12-20Hz) was negatively correlated with spike rate. Power in the gamma bands (20-50, 40-120, and 100-300 Hz) positively correlated with spike rate fluctuations. Surprisingly, gamma-rate correlations were strongest in SWS (light gray). For comparison, correlation between spontaneous (spon) rate immediately preceding driven activity is shown. Sites were selected based on the presence of an LFP recorded simultaneously with a low SNR (<20 dB) single-unit. No criteria for driven rate or LFP significance were used. Rates included spontaneous and driven spikes, and LFP power was taken across the complete trial duration. (error bars represent  $\pm$ sem)

(b) Spike rate-LFP power correlation during SWS plotted as a function of spike SNR for all stimuli ( $n=18,464$ ) where LFP and single-unit data were obtained. SNRs were divided into quintiles and dots represent centers of each quartile. The low frequency bands show no correlation with spike rate regardless of SNR. Although the gamma bands show improved correlation with spike rate at high spike SNRs, strong correlation is still present even at the lowest SNRs (10 dB) suggesting that gamma-rate correlations are not completely attributable to spike artifact.

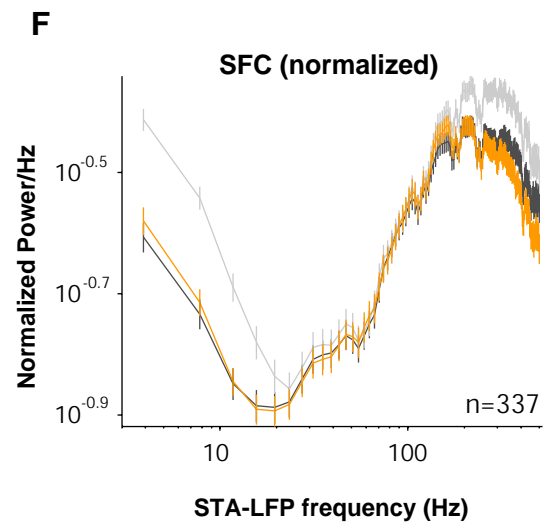
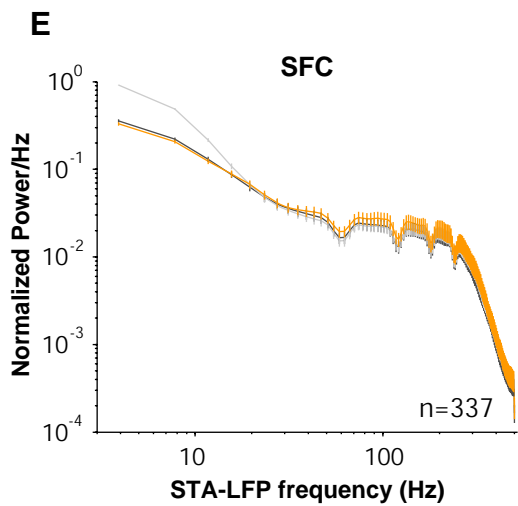
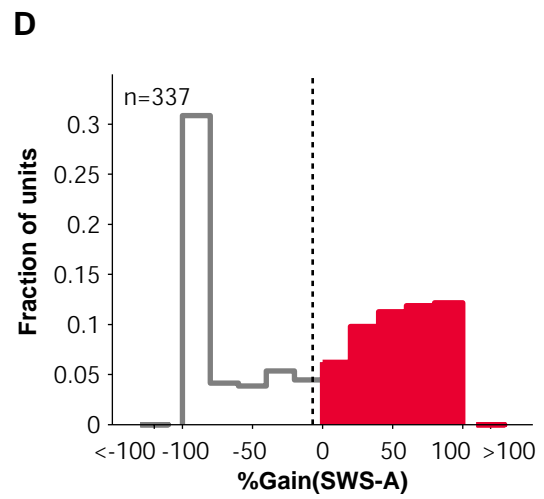
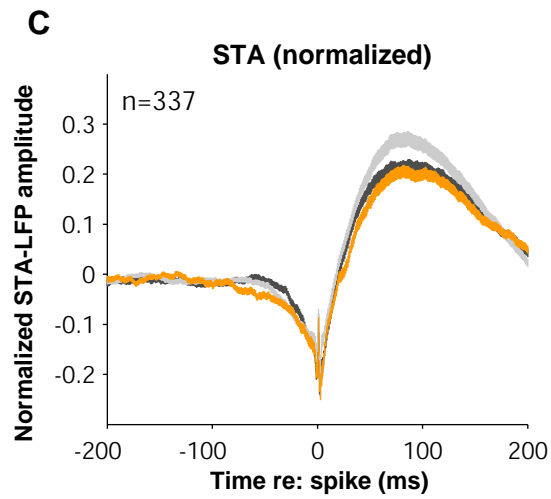
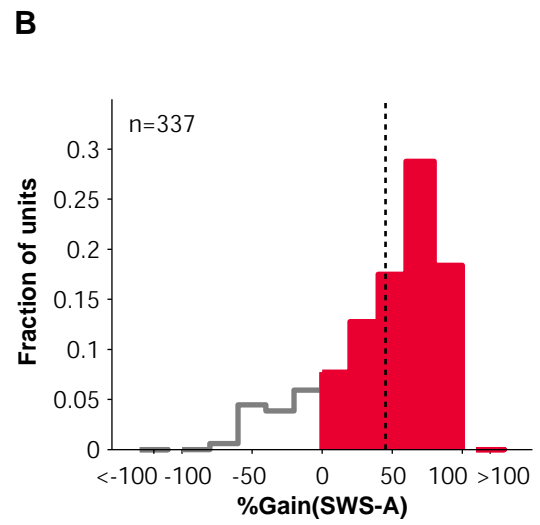
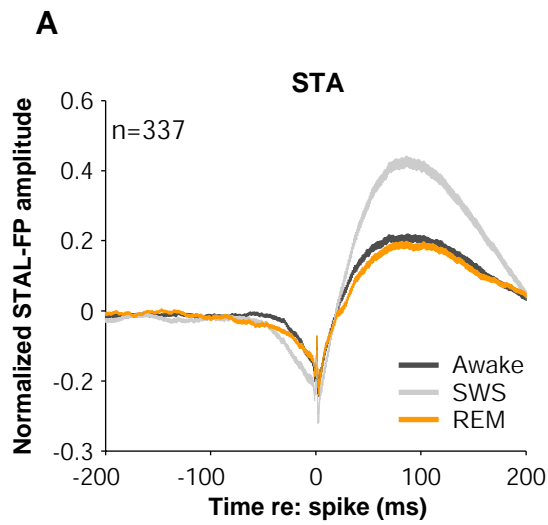
(c) Spike rate-LFP power correlation during SWS as a function of mean spike rate. Although the gamma bands show a slight increase in rate-power correlation for spike rates above 1Hz, the effect is weak. Spike size (b) seems to have a stronger influence than spike rate on rate-power correlations.

(d) Spike rate-LFP power correlation during SWS as a function of LFP power (summed energy from 1-50 Hz). A negative trend is barely discernable. Strong rate-LFP correlations are present in the gamma bands regardless of LFP power. Even when LFP power is high and spike artifacts may have less influence, correlation above 0.2 is seen in the high and ultra-high gamma bands.



**Figure 5.12.** Spike-triggered average of LFP and spike-field coherence.

- (a) For each unit recorded simultaneously with the LFP in all three states, the average LFP waveform 200ms preceding and following the spike was computed. Individual unit STAs that reached a value at least  $2.5 \times \text{sd}$  above the shift predictor were averaged to generate the population STA. The population SWS STA (light gray) is stronger than the awake (dark gray) and REM (orange) STA's (error bars represent  $\pm \text{sem}$ ).
- (b) The gain of individual unit STA's was high in SWS (mean 45%,  $p=0$ , t-test,  $n=337$ ) reflecting the increase in SWS STA amplitude seen in (a) (light gray curve). Gain was computed using the power (mean of squared amplitude) of the STA in SWS and awake where  $\% \text{Gain} = (\text{SWS} - \text{A}) / \max(\text{SWS}, \text{A})$ .
- (c) When individual unit STA's are normalized by average LFP amplitude before population averaging, the SWS curve (light gray) becomes more similar to the awake (dark gray) and REM (orange) curves. This suggests that the strong SWS STA seen in (a) is partially the result of a larger amplitude LFP in SWS. (error bars represent  $\pm \text{sem}$ )
- (d) The gain of LFP amplitude normalized STA's for individual units was not different from 0 (mean 7%,  $p=.07$ , t-test,  $n=337$ ).
- (e) The SFC was found by averaging the power spectra of all individual unit STAs that reached a value at least  $2.5 \times \text{sd}$  above the shift predictor. For frequencies below 10 Hz, spikes and LFP appear to be especially coherent in SWS (light gray) (error bars represent  $\pm \text{sem}$ ).
- (f) Normalizing the individual unit SFC's by the mean of all LFP power spectra did not affect the trend in (e). SWS still showed enhanced coherence between spikes and low frequencies of the LFP (error bars represent  $\pm \text{sem}$ ).



## Chapter 6: Concluding Remarks

In three main chapters, this thesis outlined properties of primate auditory cortex during sleep. Here, I will summarize the major findings, their implications, and future directions.

In a distilled sense, Chapter 3 showed that cortical neurons respond to sounds during sleep; Chapter 4 showed that neurons can only respond in a limited range because of reduced excitation and inhibition; and Chapter 5 showed that single neuron activations in sleep are not spatially organized but can co-modulate with local activity especially in SWS.

A better way to consider these observations is from the point of view of a homunculus trying to make sense of activity in auditory cortex during sleep (or the thought experiment of an asleep auditory cortex placed in an alert rest of the brain). What the homunculus would see is that a neuron in SWS could increase or decrease its response to a sound by 40% and that nearby neurons would also change their responses unexpectedly. Neurons that were once quiet in awake may suddenly respond very strongly to the sound in SWS. As if reading this new map of activity is not confusing enough, the situation becomes worse when noise is added to the system. Now, neurons start responding together so that some times they all respond slightly more as if more stimulus is present, but really it is common noise. This correlated firing makes it hard to determine how much signal is present in the noise. Finally, if temporal variations are introduced into the signal, the population of neurons will be slow to respond because

little dynamic range is available. At any given moment, the homunculus would have a hard time determining if the signal changed since activity changes little. These challenges the homunculus faces illustrate the multi-faceted effects of sleep. They affect rate coding, signal-to-noise calculations, and population dynamics. All of these would affect a population read-out of activity in auditory cortex (which is what the omniscient homunculus represents).

## **6.1 Chapter 3**

### *6.1.1 Summary*

The main finding of Chapter 3 is that neurons in auditory cortex are quite responsive to sounds even when the animal goes through various stages of sleep (SWS and REM). In a way, this was a pilot study intended to find out whether neurons could even be studied when the animal fell asleep. Not only did they respond, but they displayed a diversity of responses that became the subject of later chapters. Externally driven responses during sleep were on average ~10% reduced from those in awake. Responses were repeatable from one cycle to the next as if the network kept returning to the same state of sensory processing on each cycle. SWS and REM represent at least two different states of sensory processing as their responses were uncorrelated.

In the next stage of auditory processing, lateral belt, average responses were also mostly preserved during sleep. Originally, we had expected that in higher brain areas responses would be more attenuated in sleep. Our result, however, is consistent with



imaging work that shows activation in the extent of the human temporal lobe to sounds under a variety of altered states (sleep, vegetative, anesthetized) (Portas et al., 2000; Laureys et al., 2000; Davis et al., 2007). As a side note, we did place a lesion in the region of our belt recordings in one animal. Based on histologic examination, the lesion was well outside of A1 and may have been even as far as parabelt. So our finding may extend to the lateral limit of auditory cortex and certainly well beyond primary auditory cortex.

### *6.1.2 Significance*

The study in Chapter 3 yielded data similar to labs already doing sleep work (Pena et al., 1999; Edeline et al., 2001). In that sense, the suggestion that the brain is active during sleep is not entirely novel. If there was doubt, our study solidified earlier findings by performing arousal controls, recording in a higher area, and testing neurons across the whole night. In comparing to previous neurophysiology work, hindsight proves its worth. Many hints that were dropped in anecdotes or unlikely tendencies in published data became magnified in our study. The main theme from these studies is that neural responses are diverse (Livingstone and Hubel, 1981; Pena et al., 1999; Edeline et al., 2001). Neurons are not all down-regulated as might be expected if sleep is a low activity state. Diversity in cortical neurons has been noted before (Nelken, 2004). Why cortical responses are so heterogeneous could be traced to complicated connectivity, the variety of neuron types, non-stationarity of responses, or the higher level nature of processing.

Some imaging studies have found activity in human auditory areas during non-awake states (Portas et al., 2000; Davis et al., 2007). Since the imaging signal usually correlates better with synaptic activity, it does not determine whether the output of a given area is present (Viswanathan and Freeman, 2007). A strong imaging signal only suggests that an output could exist. Short of recording neurons directly in humans, our data strongly imply that the spiking output of auditory cortex is still at a high level during sleep.

The findings of Chapter 3 raised two major issues which became the subject of later chapters. (1) Despite the presence of activity, does sensory processing change? (Chapter 4) (2) Is this activity the result of or at least part of larger scale reorganization or ongoing sleep processes? (Chapter 5)

This chapter did provide some of the answers to two questions. (1) Are responses in the sleep state like the anesthetized state? Although we did not directly perform the comparison between the two states, we found during sleep a prevalence of driven responses in neurons even ones in the upper layers (anesthetized studies usually record from layer 4), awake-like sustained response patterns, and independent responses in REM, so it is safe to say that sleep is a unique state. Any analogies made with anesthetics are either superficial, uninformed, or tenuous. (2) Where are sounds gated from perception during sleep? By showing that a putative ‘gate’ would have to occur at a level higher than secondary auditory cortex, these results overturn the long held notion that the thalamus prevents the relay of signals into cortex during sleep (Steriade, 2003).

## **6.2 Chapter 4**

### *6.2.1 Summary*

The study in Chapter 4 found that auditory processing in SWS has limited dynamic range. Depending on the sound level of the acoustic event, neural responses cannot be driven or suppressed as strongly in SWS. These effects are less pronounced in REM. REM seems to have more awake-like processing. We were able to model the effects of sleep on firing rates using a simple scaling of the excitatory and inhibitory inputs into a cell. The main prediction of this model was that less inhibition in SWS could actually lead to stronger firing rates. This trend was seen in the population intensity and frequency tuning curves. In general, though, when we tested more complex stimuli, no striking deficits were seen in sleep processing – aside from slight shifts in various tuning properties (phase-locking, sound level threshold, non-monotonicity, and modulation tuning).

### *6.2.2 Significance*

The results of Chapter 4 significantly expand on our understanding of sensory processing during sleep. When we noticed the change in the underlying dynamic range of responses, it was a ‘makes sense’ type of result. It made sense from the point of view of lowered metabolic consumption in the brain during sleep (Hobson, 2005). Metabolism goes down in sleep and synaptic activity is known to be metabolically costly. It is expected that excitatory and inhibitory synaptic activity would suffer. Also, it made

sense from a mechanistic point of view of which the proposed conceptual model is a realization. For a long time, we had been puzzled by the fact that the thalamus is consistently found to be depressed in sleep (Edeline et al., 1999) and that cortex consistently shows up as more active than its thalamic input (Livingstone and Hubel, 1981; Edeline et al., 2001). We had suspected that one way this was possible was that inhibition is released during sleep. Seeing that less inhibition is present during SWS helped clarify a possible difference between thalamus and cortex.

Our finding was a nice fit to the idea that REM is a more awake-like state. External sounds are often incorporated into dreams (Ramsey, 1953; Berger, 1963; Burton et al., 1988), and generally people are more easily awakened from REM sleep (Bonnet, 1982). Classically, SWS is considered deep sleep because of its depth of behavioral unresponsiveness. We found that dynamic range was not as compromised in REM, and that responses in REM could be just as rapid as those in awake. This is an idea that emerges from the thesis; REM is not only an active state but may be close to awake as far as auditory processing. Originally, we had started by comparing SWS and REM as two sleep states whereas now we tend to think of REM in terms of awake.

The bigger conceptual challenge of this chapter is what exactly is meant by limited dynamic range in SWS. The first thing to mention is that we suspect the inhibition that we observed was likely of the broad (sums across all fibers, not tuned), normalizing (grows linearly with stimulus strength), slow type. This may have been a limitation of our methods or may have been the overriding strength on extracellular responses of this type of inhibition. Such inhibition has been considered important in response normalization across the network or gain control in different responses regimes

(Salinas and Their, 2000; Schwartz and Simoncelli, 2001). Most recently, this sort of inhibition was observed in context of leech behavior (Baca et al., 2008) but is prevalent in many other settings (Chance et al., 2002). Increasingly, inhibition has become a more prominent subject in neural studies. An attention study bolstered the idea that inhibition is an important sculptor of the existing excitatory network by showing that attention may exert its influence through inhibitory interneurons (Mitchell et al., 2007). These results including ours bring inhibition into view as a prominent player in cortical processing especially during behavioral state changes. As mentioned, we tried to examine direct consequences of this inhibition on single neuron processing and found little effect. Our current, untested speculation is that changes will be more strongly observed at higher levels of processing. In the read-out of the modified auditory cortical responses will the true consequence to processing be observed. This view receives indirect evidence from imaging studies showing that as soon as complicated discriminations of sounds are tested in sleeping or sedated subjects, higher frontal areas lose their response even though activity was present in the temporal lobe to simple stimuli (Portas et al., 2000; Davis et al., 2007). Ultimately, these deficits should manifest behaviorally. Unfortunately, sleeping subjects cannot be queried to report what they perceive while they sleep. Is it much attenuated in amplitude or is the sound transmitted only in a garbled form? Such processes go on subconsciously, and future studies will have to use more sophisticated approaches and record in higher areas to determine the nature of the cortical transformation of sounds during sleep.

## 6.3 Chapter 5

### 6.3.1 Summary

In this study, we set out to determine if any organization existed in the modulation of auditory cortex by sleep. We felt that organization could occur because of common thalamic input or because of patterned internal processes. When we examined correlations between neurons or between neurons and the LFP, we found little evidence of local organization. An interesting finding, though, was that the pattern of firing correlation between neurons in REM was similar to that in awake, so joint firing statistics may be similar between the two states. On the other hand, SWS joint firing statistics were modified compared to those in awake and REM, and SWS had the strongest incidence of joint firing. We found that the LFP was weakly correlated from one sleep cycle to the next, but neurons behaved independently with respect to these patterns on a long timescale. On a short timescale, gamma band correlations with single neuron firing rates persisted in both states of sleep suggesting that gamma oscillations are universal in brain activity.

### 6.3.2 Significance

The results of Chapter 5 couch our work in terms of more classical measures of sleep. For example, hippocampal studies of learning measure correlations between pairs of neurons during sleep (Wilson and McNaughton, 1994), and many studies of general activity (not sensory processing) use signals like the EEG and LFP and look for

coherence across space and time (Destexhe et al., 1999; Steriade et al., 2001). A major idea behind such work is that ensembles of neurons can display patterns of network-level organization during sleep (Harris et al., 2002; Harris, 2005). Our LFP results agree with this notion as there were consistent (repeatable over cycles) biases in different frequency bands of the LFP, and LFP activity on single trials correlated with single unit responses. Consistent with the idea of improved coherence by the common oscillations in SWS (Destexhe et al., 1999), neuron pairs became more correlated during SWS. This suggests to us some organization of activity. Supporting this further, neurons in SWS tended to firing more strongly with increases in gamma energy. We were somewhat surprised to find that gamma activity was an equally important determinant of single neuron firing in REM as in awake and was actually most salient in SWS. Low frequency LFP energy led to lower firing rates in awake and REM, not just in SWS. In other words, the LFP did not give a clean separation of what brain rhythms are more important in what states. The LFP during SWS had strong power in all frequency bands even those above the EEG range. We had expected some decrease at least in high frequency bands (because of decreased thalamic input). In the end, we found the analyses of this chapter quite important. Even if the results were hard to interpret, we achieved our goal to use network properties to differentiate awake, SWS, and REM. The evidence that SWS is a coherent state could not have been inferred from single-unit data. Future studies recording from a larger population of cells such as is done in the hippocampus or imposing daytime behaviors may be able to observe more organized activations in cortex. Or it could be that external stimulation is an artificial activation of the sleep network. Maybe measuring spontaneous activity patterns is the only means to finding sleep patterns.

## 6.4 Final Discussion

In the beginning of this thesis, I mentioned four possible hypotheses of sensory processing in sleep. They were termed the **Attention, Thalamic Gate, Mentation, and Vigilance** hypotheses. Some spoke at a mechanistic level (attention, thalamic gate) and some more at the functional level (mentation, vigilance), but all were broad. In my opinion, the thalamic gate hypothesis needs to be re-visited. I found it hard to believe that I was getting strong cortical responses, but it seemed like every time I doubted the response I was getting, a sleep active neuron would come along to re-convince me. I was especially convinced when I recorded three neurons simultaneously (Figure 5.1) and found that they behaved completely differently. Recording them simultaneously controlled for sleep cycle effects. The question could always come up that I was only seeing ‘sleep active’ neurons in aberrant sleep cycles where the animal was not fully asleep. In such a case, all neurons should be active. When I moved far away from A1 to record in LB, I hoped to find less responses, but even then neurons would respond during sleep and occasionally respond stronger than in awake. If the thalamus gates the signal from cortex, it appears to get re-amplified. A study of the effects of sleep in the auditory thalamus of the marmoset would be a valuable complement to the results in this thesis. I do not discount the idea that the thalamus is depressed, but this thesis suggests that is only part of the story; cortical responses exhibit unique behavior.

The attention hypothesis is also only part of the story. I did observe that the arousal of the animal especially the eyes being open helped improve responses by up to



10% (on the order of attentional improvements reported in V1 (McAdams and Maunsell, 1999)), but neurons did not behave in a graded fashion once the animal fell asleep. Some neurons would respond better in SWS and some better in REM even though both states constitute a loss of arousal. Attention effects in the visual literature suggest a saturation model (Reynolds et al., 2000; Reynolds and Chelazzi, 2004). Instead, we observed a loss of dynamic range involving inhibition (super-saturation). This model is more in line with the idea that metabolic and hence synaptic activity is lowered in SWS and may not be directly related to a top-down gating of arousal/attention.

The mentation and vigilance hypotheses were conceived to oppose each other. The mentation hypothesis states that sleep is focused on internal processes, while the vigilance hypothesis states that some resources are dedicated to monitoring the external environment for the safety and security of the sleeper. Our results suggest both hypotheses. Auditory cortex may be involved in vigilance since it can still respond to sounds. Even if there is less dynamic range which may limit the discriminative capacity of processing, the amount of stimulus-driven activity we observed suggests that sounds can still be detected. We do not doubt that internal processes activate cortical sensory areas (Ji and Wilson, 2007) whether in dreaming or in consolidation of new perceptual skills like those used in speech and music (Stickgold, 2005). Our study was not geared to directly expose these phenomena, but we would tend to believe that they exist. Neurons seemed to undergo complex and sometimes large modulations during our experiments.

## 6.5 Future Experiments

After I started doing the sleep experiments, I realized how much potential there is for studying sleep in the auditory system. It becomes immediately obvious how powerful it is to be able to play sounds and measure responses even while the animal sleeps. For one thing, it lets you assay how active neurons are during sleep. For another, it allows you to manipulate activity in the brain during sleep. The ability to measure and manipulate activity opens the door to a number of scientific questions that have yet to be asked. This luxury is not as easily afforded in other sensory modalities. Here are some future experiments suggested by the results of this thesis or by random musings on many a long night:

**Chronic recording.** Sleep is an ideal candidate for the use of implanted electrodes. From a practical point of view, implanting electrodes allows experiments to be done in a more natural setting for the animal. They can sleep in their home cage. Also, if sleep is at all involved in plasticity, then it becomes important to track how neurons change over long periods of time. Response behavior within a night was fairly consistent, but I always wondered how neurons would behave the next night or even the next day during daytime hours.

**Frontal cortex and sensory association areas.** Recording from these areas which may be closer to perception could reveal stronger effects of sleep on sensory responses. The only problem is that responses in these areas are not well-understood (Romanski, 2007). It would be interesting to see how active these areas are or if their

responses drastically change as the imaging data suggests (Portas et al., 2000; Davis et al., 2007).

**Plasticity and behavior.** Combining a behavioral task with sleep could be a powerful paradigm. Studies in V1 have looked at how neural activity during sleep affects ocular dominance plasticity (Frank et al., 2001; Jha et al., 2005; Krahe et al., 2005). They showed that cortical activity during sleep is important for plasticity to take place. In the auditory system, this idea can be taken one step further by inducing different patterns of activity during sleep. For example, if the animal is trained on a task to detect rising frequency modulated sweeps, rising sweeps can be used to stimulate the proper pattern of activity at night in order to improve behavior the next day. Alternatively, down sweeps can be used to disrupt ongoing sleep patterns or maybe even potentiate synapses in the opposite direction. The idea behind experiments like these is whether acoustically stimulating different patterns of activity in A1 on a regular basis during sleep can lead to systematic changes in neural maps or in behavior. This assumes sleep is an especially plastic period. A control would be to see if the same stimulation when the animal is awake could induce plastic changes.

**Replay of rhythmic sounds.** In the long-term, recording from many neurons simultaneously may become more routine, and patterns of activation across multiple neurons can be observed (Lee and Wilson, 2002). In the short term, sleep replay effects could be examined in single neuron firing patterns. Periodic stimuli, which are commonly used in auditory studies, seem like a good candidate. By training the animal on a modulation discrimination task or by simply overstimulating with sounds of a given periodicity, periodic patterns of neural replay could be observed in sleep. The prediction

would be that neural interspike intervals become biased toward the periodicity of the stimulus used.

**Attention, awake, asleep.** The influence of behavioral states has been repeatedly studied but rarely all in the same preparation. Despite all of the work looking at effects of altered states, it is not clear how they relate at the single neuron level. In this experiment, I propose comparing the effects of sleep to the effects of attention (behavioral task or controlled arousal) to see if they are the same in the same neural population or are achieved in different ways in each neuron. A gain control model seems useful for attention and suggests simple mechanisms, but as I have mentioned, the effects of sleep may be different.

**Intracellular recordings.** I am only aware of one study that recorded intracellularly from cortical neurons during sleep (Timofeev et al., 2001). Doing this is a heroic experiment and could be important given the results of this thesis. For one thing, what is the input like to a neuron and how does it relate to the output during sleep? Such studies have been performed in thalamus (Coenen and Vendrik, 1972; Hirsch, 1983) during sensory stimulation but not in cortex. Our work suggests that large changes are occurring in the excitatory and inhibitory inputs to a cell. Additionally, the stability achievable in a sleeping animal (as opposed to an animal performing a task) affords a unique opportunity to study behavioral state effects at the mechanistic level.

**Oddball paradigm.** In retrospect, we would have tested neurons using an oddball stimulus paradigm during sleep. At the time of our studies, we were not aware of the extensive evoked potential literature showing that the MMN is no longer present in SWS (Sabri et al., 2003; Sabri and Campbell, 2005) and only weakly appears in REM

(Loewy et al., 1996; Nashida et al., 2000; Atienza et al., 2000). The MMN is thought to be important since it might reflect higher level processing. Short-term memory is required to keep track of stimulus probability given preceding stimuli. In sleep, this higher order memory process appears to be compromised, although some have suggested a deficit in low-level transmission of the acoustic signal (Sabri and Campbell, 2005). It would be interesting to see which hypothesis single neuron data support.

**Background sounds.** When I first started the sleep project, we were really interested in the idea that calling someone's name could wake them up. We thought that testing neurons with vocalizations might reveal some special processing. This may yet be true, but we did not find evidence of a special allowance for vocalizations at least in primary auditory cortex. Maybe in higher areas it would be more apparent. What I began to realize, though, is that specializations may exist in early areas for filtering out background sounds. This perspective may prove valuable in future experiments. Functionally, it is important to stay asleep most of the time which means ignoring sounds off in the distance. In SWS and REM, responses to quiet sounds decreased suggesting that sounds <30 dB, which are likely part of background, are selected out. Also, quiet sound responses were weaker in neurons recorded during later sessions suggesting adaptation on the part of the animal. The other interesting piece of data was a large negative gain in sleep for environmental and colony sounds. No other class of sounds was nearly as suppressed. In addition to higher order spectral and temporal structures, these sounds were long in duration. Quite possibly, long, ongoing sounds are adapted to very strongly since they are probably not foreground events which would be more discrete in time. Future experiments could manipulate other defining properties of

background sounds to see if and how sleep selectively acts on these types of sounds.

This might require a better understanding of the statistics of background natural sounds.

Perhaps it will turn out that the most important function of hearing during sleep is to filter out the 99% of irrelevant sounds rather than select the 1% of relevant sounds.

**Biophysical model.** The studies embodied in this thesis were mainly phenomenological in nature. Our intent was to gather details of auditory processing during sleep since almost no data exist on this matter. Some of the observations we made could benefit from a detailed mechanistic modeling. Although we came up with a conceptual model using excitation and inhibition, this model did not include a thresholding nonlinearity or account for fast and slow processes of inhibition. The model also did not incorporate adaptation which would be important if we are to go beyond mean firing rates and model response profiles. A quantitative model would facilitate going between extracellular firing rates and underlying excitatory and inhibitory currents and may be able to explain the observations that large sleep modulations tended to occur at low firing rates and that extracellularly observed suppression was nonlinearly dependent on spontaneous rate. A successful model would be helpful in making predictions for future experiments.

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