The effect of brain state on rapid auditory processing in the rat

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Abstract of the Dissertation

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The correct interpretation of natural sounds, such as language, depends on an individual's ability to perform rapid auditory processing (RAP) - processing of auditory stimuli that occurs on a time scale of tens of milliseconds. Indeed, individuals who exhibit deficits in RAP also demonstrate impairments in the acquisition of normal language skills. Interestingly, auditory training that is designed to engage the attention of the subject can ameliorate these deficits. Even though the physiological basis of the improvement in language skills in these individuals is currently unknown, one possible correlate could be changes in brain state that occur through training. Indeed, changes in brain state have been shown to influence neuronal responsiveness to sensory stimuli. In addition, changes in the level in alertness, or attention, are associated with changes in the degree of cortical activation.

In this thesis, I explored the relationship between brain state and RAP by recording the simultaneous responses of large neuronal populations in the rat auditory cortex to temporally structured auditory stimuli. I systematically quantified the magnitude of evoked responses across different brain states, both under anesthesia and during wakefulness, and assessed the efficiency of RAP by estimating how well the type of

sensory stimulus could be predicted from the population activity.

First, RAP was assessed during the inactivated state. Surprisingly, even though response amplitude varies systematically with the phase of the slow oscillation, the efficiency of RAP does not. Second, RAP was examined across the different global activated and inactivated states observed under urethane anesthesia. This showed that RAP is overall more efficient in the activated state because of the ability of auditory populations to strongly respond to temporally structured stimuli. Finally, the effect of changes in the instantaneous level of activation during wakefulness on RAP was studied in chronically implanted rats, again showing that the efficiency in the processing of temporally structured stimuli increases with the level of cortical activation.

Together, these results establish a significant link between cortical activation and RAP performance. This suggests that improvement in language ability after training in humans might reflect an increased ability to produce cortical activation when required.

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1-3. Background and Significance

1.1. Rapid auditory processing

Natural sounds, such as language, have a complicated spectral and temporal structure, which varies across fast time scales. For example, the syllables ba and da differ in their spectral structure only within the first 40 ms of pronunciation (see, e.g., Fitch et al., 1997b). Thus, the correct interpretation of the acoustic environment in general, and language perception in particular, requires rapid auditory processing (RAP) - the processing of auditory stimuli on the time scale of tens of milliseconds. Even though various approaches and models are employed in the in the study of RAP, a common paradigm used in RAP studies is the presentation of a sound-pair separated by different inter-stimulus-intervals (ISIs). This paradigm enables the assessment of different variables, both physiological and psychophysical, that reflect the ability of individuals process temporally structured stimuli. For example, one psychophysical measure of RAP is the ability of the subject to perceive the correct identity of both elements of a sound pair as a function of the ISI which separates them (e.g., Tallal and Piercy, 1973). The correct perception of both sounds in a pair is modulated by variables such as the spectral-composition/duration/amplitude of the sounds, and the duration of the ISI (Moore, 1995). Generally, the correct perception of the second sound of sound-pairs presented with very short ISIs is impaired, a psychophysical phenomenon named ‘Forward Masking’ (Moore 1995). This phenomenon can also be studied psychophysically in animals. A clever paradigm used for this purpose is paired-pulse-inhibition (PPI). In this paradigm, the

physical reflexive expression of a startle response due to the unanticipated presentation of a very loud auditory stimulus (Pilz et al., 1987) is inhibited by a preceding cue (i.e., the 'inhibiting paired-pulse', Hoffman and Ison, 1980; Fendt et al., 2001; Swerdlow et al., 2001). The inhibiting cue can be either an auditory stimulus such as a tone, a silent gap in a continuous background sound, or a sensory stimulus of a different modality such as a light flash. This paradigm was successfully adapted to study RAP by the use of temporally complex stimulus as the cue, where the test is designed so that suppression of the startle response occurs only due to correct perception of the cue (for a review see Fitch et al., 2008).

Physiologically, studies of RAP focus on whether the physiological signal (such as the firing rate of single neurons, or EEG signal) is able to follow and respond to different components of a temporally structured stimulus. Specifically, for single neurons, the ability of the neuron to follow stimuli is measured as the relative response amplitude (firing rate) to different sound elements. The degree to which response amplitude is reduced when the sound is preceded by another sound compared to when it is presented alone, is defined as the degree of response suppression. This response suppression (also referred to as 'forward suppression') is thought to be the physiological basis of forward masking (e.g., Bartlett and Wang, 2005; Brosch and Schreiner, 1997; Wher and Zador, 2005). Studies employing two identical sound stimuli in fast succession generally report reduced response amplitude to the second sound in a pair; this response suppression is correlated with the ISI which separates the sound-pair (Tan et al., 2004; Wher and Zador, 2005). However, when the sounds in the pair are different, e.g., they had different

amplitudes or frequencies, more complex patterns are observed, such as second sound facilitation or suppression depending on the frequency tuning of the recorded cell (Shamma and Symmes, 1985; Calford and Semple, 1995; Brosch and Schreiner, 1997; Sutter et al., 1999; Bartlett and Wang, 2005).

The mechanism underlying this relative suppression of response was originally thought to be recurrent inhibition (e.g., Tan et al., 2004). However, it has been shown that while inhibitory currents do participate in response suppression on short time scales (~100 ms), response suppression on longer time scales is not due to inhibitory conductances and most probably stems from presynaptic mechanisms such as synaptic depression (Wehr and Zador 2005; Asari and Zador, 2009).

The brain areas that are involved in RAP have been characterized using various methodologies including PET and MRI. Generally, responses to rapidly presented stimuli are stronger across the left hemisphere, than the right (Belin et al., 1998; Zaehle et al., 2004). Using PET, Belin et al., (1998) reported that while sounds with slow transitions activate both hemispheres, rapidly changing stimuli preferably activate the left hemisphere. Further insight onto the brain regions that are involved in RAP in humans comes from studies comparing physiological and structural elements in the brains of normal individuals and individuals who have deficits in performing tasks that require RAP (and suffer from dyslexia / specific-language-impairment, to be discussed in detail later). For example, the findings of Benasich and colleagues (2006) support the involvement of left hemispheric activation in RAP, as children with deficits in RAP show reduced activation in this region compared with controls. Further more, structural MRI

studies reveal that individuals that are deficient in RAP have abnormal hemispheric asymmetries (reviewed by Paterson et al., 2006). In addition, morphological abnormalities are also evident in the medial geniculate nucleus of the thalamus of dyslectic individuals (Galaburda et al., 1994), an observation that is also valid in an animal model of dyslexia (Herman et al., 1997).

1.2. Speech perception is impaired in individuals who demonstrate deficits in RAP, but can be improved through training

Tallal and Piercy (1973) found that a certain population of aphasic children, who suffered from specific language impairment (SLI), had severe deficits in processing rapidly presented sound stimuli, compared with controls. While normal children could easily sequence tone pairs separated by as little as 8 ms, SLI children could not perform above chance unless the sounds were separated by at least 305 ms. Some physiological correlates of these deficits were characterized later on through the use of EEG recordings, demonstrating abnormal responsiveness to a rapidly presented auditory stimulus in infants with family history of language impairment (F+) compared with controls (Benasich et al., 2006). Specifically, in this study, a passive oddball paradigm was used, in which tones of either high (300 Hz), or low (100 Hz) fundamental frequency were presented in pairs of an either low-low sequence (standard stimulus, presented 85% of the time), or a low-high sequence (deviant stimulus). These stimuli were presented in blocks in which the tones within a pair were separated by either 70 or 300 ms. The results of this study demonstrated that evoked responses in F+ infants differed from responses in

controls during the 70 ms condition only. Differences were observed in both the amplitude of the mismatched response and the onset latency of the N_{250} component, which was significantly delayed in F+ infants. Interestingly, the differences in the N_{250} component only were significantly related to language outcome in these infants after 6 months. Deficits in RAP can be seen as early as infancy in individuals with family history of language impairment (F+) also on a behavioral level (Benasich and Tallal, 2002; Benashich et al., 2006; Choudhury et al., 2007). For instance, (F+) infants perform worse on an auditory temporal processing threshold task than controls (Choudhury et al., 2007). Even though this topic is still under investigation via longitudinal studies, it has been proposed that future language deficits in these infants might be prevented by training with tasks designed to improve RAP (for a review, Benasich et al., 2002).

Training can significantly improve language skills in SLI children (Tallal et al., 1996; Merzenich et al., 1996). In their study, Tallal, Merzenich and colleagues designed two tasks presented in the form of engaging ‘audio-visual games’ in which the correct identification and sequencing of rapidly presented sound stimuli was rewarded (Merzenich et al., 1996). In one task, frequency modulated tone pairs were presented at different ISIs and the child had to report the order of their presentation. In the other task, modified speech sounds (designed to enhance differences across stimuli by prolonging the more rapid transitions, such as the first 40ms of the ba/da sounds) were used in a similar manner. Specifically, the stimuli used were consonant-vowel (CV) stimuli (such as ba/da) presented in rapid sequence and the child had to report the position of the target CV in the pair. While the total duration of the CV stimulus was constant, several

variables were modified: (1) the duration of the consonant element of the CV, and respectively the duration of the vowel element to complement the constant CV duration (2) the amplitude of the consonant intensity over vowel intensity, and (3) the ISI between presented CV pairs. Intensive training on these tasks for a period of 4-6 weeks resulted in marked improvements in these children. Not only did they reach the level of controls at performing the task, they also significantly improved their language comprehension and speech discrimination abilities (Tallal et al., 1996).

Even though the results of these studies were robust, and are now commonly used for language training (see www.scilearn.com), the underlying physiological basis both of the cause of these developmental deficits and of their improvement through training remains largely unknown.

In order to address these questions, in addition to studies in humans discussed above, animal studies using animal models of dyslexia and SLI are also used. The basis of this animal model comes from several postmortem studies performed by Galaburda and colleagues, which reported that cortical abnormalities, such as molecular layer ectopias and microgyria, exist in dyslectic individuals (Galaburda and Kemper, 1979; Galaburda et al., 1985; Humphreys et al., 1990). These findings led to the development of an animal model which emulates these cortical abnormalities (specifically microgyria) by the use of freezing lesions applied to the cortical plate of newborn rats on the first day of life (Humphreys et al., 1991; Rosen et al., 1992). By using this model, Fitch and colleagues were able to demonstrate that microgyric rats are impaired in RAP (Fitch et al., 1994; Fitch et al., 1997a; Herman et al., 1997; Clark et al, 2000a) thus establishing a possible

link between microgyria and language deficits in humans. Specifically, they used a go/no-go auditory discrimination task in which tone-pairs separated by an ISI were presented and the performance of the microgyric rat group was evaluated relative to a sham control group as a function different ISIs. Even though microgyric rats were able to perform the task well with ISIs as long as 300 ms, they were significantly deficient relative to the control group at short ISIs (225 ms). This animal model is particularly strong since, similarly to SLI children, auditory training ameliorates RAP deficits in these animals. (Threlkeld et al., 2009).

Interestingly, deficits in RAP in microgyric rats have been shown to occur independently of the locus of the cortical lesion (Herman et al., 1997), suggesting that the locus of RAP deficits may not be exclusively the auditory cortex. On the other hand, abnormalities in the auditory nucleus of the thalamus (the medial geniculate nucleus, MGN) have also been reported in these animals, indicating that perhaps MGN damage can occur through cortical abnormalities, which then may finally result in RAP deficits. Indeed, postmortem analysis of dyslexic brains has also shown MGN anomalies (Galaburda et al., 1994).

In addition to the physical cause of SLI and Dyslexia, another important question is the physiological basis of the improvement in language skills that occur in SLI and dyslexic individuals as a result of auditory training. A clue to a possible physiological correlate of these improvements comes from the fact that the increased performance in RAP tasks, language skills and speech perception, is the result of training with tasks specifically designed to engage the subject's attention (Merzenich et al., 1996). Given that heightened levels of alertness and attention are associated with a specific constellation of diverse

physiological markers (including EEG desynchronization and shifts in patterns of neuromodulation) that are generically known as brain activation (which is a physiological correlate of arousal level), it is possible that the improvement in RAP after training is correlated with a shift in the level of cortical activation in these individuals. I will now elaborate on this possibility by describing in detail some physiological characteristics and behavioral correlates of the different brain states (including activation) and how they influence sensory processing.

2. Global brain states

Electroencephalographic (EEG) activity reflects changes in synaptic discharge of large neuronal populations. Berger (1929) was the first to show that EEG activity during sleep and wakefulness is different in respect to the spectral properties of the EEG signal. EEG activity during slow-wave-sleep (SWS) displays low frequency, high amplitude oscillations, and is characteristic of what is referred to as the synchronized, or inactivated state. In contrast, EEG activity during REM sleep and wakefulness generally displays faster, low amplitude EEG fluctuations, which can occasionally be synchronous in higher frequency ranges, and is characteristic of the desynchronized, or activated state (see, e.g., Steriade and McCarley, 2005). The differences in global activity seen across these states are the result of a shift in the relative activation of neuromodulatory subcortical systems (Moruzzi and Magoun, 1949). This shift (discussed in detail below) induces changes in both single cell physiology, and network dynamics.

2.1. The inactivated state is dominated by slow oscillations

The inactivated state is characterized by low frequency oscillations (< 4 Hz) and spindle oscillations (7-14 Hz). Prior to a series of studies conducted by Steriade and colleagues, the slow rhythm (< 1 Hz) was grouped along with the delta rhythm (1-4 Hz) as SWS oscillations (Steriade et al., 1993a-c). These two rhythms, however, were shown to originate at different structures: Delta oscillations are generated in the thalamus through the intrinsic properties of thalamocortical neurons (McCormick and Pape, 1990; Dossi et al., 1992), whereas the slow oscillation is thought to originate within the cortex: slow wave generation occurs in the cortex of cats in which the thalamocortical connections have been severed (Steriade et al., 1993b) and the thalamus of decorticated cats does not display slow oscillations (Timofeev and Steriade 1996). In addition, a slow oscillation homologous to the one observed during SWS can be generated in a cortical slice (Sanchez-Vives and McCormick, 2000).

The most striking feature of the slow oscillation is the alternation of the intracellularly recorded membrane potential of single neurons between periods of pronounced hyperpolarization (known as ‘downstates’) and periods of sustained depolarization where spiking activity is usually observed (known as ‘upstates’), a phenomenon which had been first observed in the striatum (Wilson and Groves, 1981). The fact that the slow oscillation is observed in the EEG signal implies that these alternations are coordinated across large neuronal populations. In fact, it was observed to be present in the majority of neurons of the thalamic-cortical circuit (Steriade et al., 1993a). The slow oscillation is coherent across different brain regions, as evidenced by the fact that the slow rhythm

recorded intracellularly in single cells is highly synchronous with the slow oscillation of a simultaneously distally recorded EEG signal (Steriade et al., 1993a-c; Isomura et al., 2006). However, in a later study utilizing simultaneous extracellular and intracellular recordings from remote cortical structures, it was shown that there is a temporal lag in the phase of the oscillation between cortical areas, which is correlated with distance (Amzica and Steriade, 1995). This is suggestive of the slow oscillation propagating across the cortical surface.

Even though up-downstate transitions were originally characterized in the deeply anesthetized preparation (Wilson and Groves, 1981; Steriade et al., 1993a) and were mainly thought to be a property of the deeply inactivated state which occurs naturally during slow-wave sleep (Steriade and McCarley, 2005), large membrane potential fluctuations were also recently observed in the awake preparation (Crochet and Petersen, 2006; Poulet and Petersen, 2008; DeWeese and Zador, 2006), although these are typically shorter than upstates during deep anesthesia. In the somatosensory cortex of the awake mouse during quiet immobility, these large fluctuations are synchronized across cell pairs (Poulet and Petersen, 2008). These 'inactivated' dynamics are abolished as the mouse begins to whisk, substituted by more high-frequency fluctuations that are correlated with whisker position (Crochet and Petersen, 2006), but which are more weakly correlated between neurons. These intracellular studies confirm previous extracellular measurements suggesting that increases in low-frequency power can also be observed during wakefulness (Buzsaki et al., 1988; Gervasoni et al., 2004).

2.2. Physiological characteristics of the up-downstate alternation

Within the inactivated state, the physiological properties of neurons vary with the phase of the slow oscillation. Membrane voltage (V_m) and membrane input resistance (R_{in}) are continuously modulated throughout the slow oscillation. During the upstate, V_m is relatively depolarized (~ - 60 mV; Steriade et al., 2001; Timofeev et al., 2001), and R_{in} is low compared with downstate (Contreras et al., 1996; Rudolph et al., 2005). The relative depolarization of neurons contributes to their excitability, producing tonic neuronal spiking activity throughout the duration of the upstate (Steriade et al., 2001). However, as the upstate progresses, firing rates decrease and excitability subsides, finally resulting in a transition into a downstate period. The underlying physiological basis of the upstate to downstate transition is not yet clear. However, it is thought to occur through a combination of factors effectively producing adaptation, such as synaptic depression (Contreras et al., 1996) or a Na^+ dependent K^+ current (Sanchez-Vives et al., 2000; Compte et al., 2003a). During the downstate phase, on the other hand, the V_m of neurons is more hyperpolarized, and their R_{in} is higher (Contreras et al., 1996; Rudolph et al., 2005). R_{in} increases throughout the downstate, so that, by the end of it, neurons are more compact and ready to be excited (Contreras et al. 1996). At this point the initiation of the upstate occurs. Upstates can originate within the cortex. This is supported by the observation that (1) the cortex can independently initiate and maintain regular up-downstate transitions (Steriade et al., 1993b; Amizca and Steriade, 1995; Sanchez-Vives and McCormick, 2000), and (2) cortical pace-maker neurons which fire continuously at

low rates during the slow oscillation have been characterized (Wang and McCormick, 1993; Le Bon-Jego and Yuste, 2007) and computational models suggest that they could suffice to initiate upstates (Compte et al., 2003a). Upstates can also be initiated by thalamocortical excitation. Studies in rat somatosensory cortex (Hasenstaub et al., 2007) and auditory cortex (Luczak et al., 2009; Curto et al., 2009) have shown that brief sensory stimuli delivered during the downstate can induce upstates, and thalamic stimulation itself has also been shown to be able to initiate upstates in thalamocortical slices (Beierlein et al., 2002; Shu et al., 2003a; MacLean et al., 2005).

2.3. The activated state

The major difference observed in the activity of the EEG across the inactivated and activated states, is the suppression of low frequency oscillations. Although in sensory cortical areas in cats, monkeys and humans activation is also sometimes associated with increased in the power of high frequency oscillations (~ 30-80 Hz, gamma range), in rodents the most striking changes in the spectral content of local field potentials (LFPs) occur at lower (< 20 Hz) frequencies (Gervasoni et al., 2004) across a wide variety of sleep and non-sleep conditions. Non-SWS EEG patterns are observed in a variety of conditions: they are characteristic of REM sleep, alert wakefulness and can also be observed under some anesthetics like urethane (Vanderwolf 1969; Steriade et al., 1993d; Destexhe et al., 1999; Steriade 1999; Steriade et al., 2001; Gervasoni et al., 2004; Détári and Vanderwolf, 1987; Murakami et al. 2005; Clement et al., 2008). As the low frequency power of the EEG signal observed during SWS decreases, so does the global synchrony

on that frequency range, with neuronal populations becoming sometimes synchronized at higher frequencies (beta range, 15-30 Hz, and gamma range, 30-80 Hz) with a more local spatial extent, e.g., within a cortical column (Steriade et al., 1993d; Steriade et al., 1996, Destexhe et al., 1999). Although wakefulness is generally associated with cortical activation, further changes in the structure of EEG fluctuations are observed with variations in the level of alertness, attention and engagement in specific motor behaviors (Vanderwolf 1969; Fries et al., 2001; Pesaran et al., 2002; Gervasoni et al., 2004; Poulet and Petersen, 2008). In one study, Fries and colleagues showed that multi-unit spiking activity and local field potential (LFP) fluctuations where less (more) synchronous in the low (high) frequency range when a monkey attended to the spatial location overlapping with the receptive field of neurons being recorded, than when attention was directed elsewhere (Fries et al., 2001). A recent study also showed that synchronous large amplitude fluctuations in the intracellularly recorded V_m of two simultaneously recorded neurons in mouse primary somatosensory cortex, largely disappeared when the animal went from quiet wakefulness to active whisking (Poulet and Petersen, 2008). Thus, increases in the level of attention or behavioral engagement lead to increases in the level of cortical activation, i.e., lower power in the low frequency range of the EEG or LFP and sometimes elevated power in the high frequencies.

2.4. Neuromodulators involved in brain activation

The differences in global activity seen across the activated and inactivated states are the result of a shift in the relative activation of neuromodulatory subcortical systems located

in the mesancephalic reticular formation (MRF). Moruzzi & Magoun, 1949, were the first to demonstrate that electrical stimulation of the MRF in deeply anesthetized cats causes the suppression of the slow oscillation and the emergence of faster, lower amplitude fluctuations. The activity of the neurons of the MRF neuromodulatory nuclei, follow the wake-sleep cycle: Both the noradrenergic (NA) neurons of the locus coeruleus (LC), the cholinergic (ACh) neurons of the Laterodorsal-Pendunculo Pontine Tegmental areas (LTD-PPT), and the serotonergic (5HT) neurons of the dorsal raphe nucleus (DR) exhibit high firing rates during wakefulness and low firing rates during SWS (Hobson et al., 1975; Aston-Jones and Bloom, 1981; Lydic et al., 1987). On the other hand, during REM sleep the LC and DR nuclei become silent and the PPT neurons alone continue to spike at a high rate (Hobson et al., 1975; Aston-Jones and Bloom, 1981; Lydic et al., 1987). Even though other MRF neuromodulatory nuclei are active during arousal (DR and LC), activation of the PPT alone (either spontaneous or through electrical stimulation) is sufficient to induce robust cortical activation as seen during REM (Szerb, 1967; Hobson et al., 1975; Aston-Jones and Bloom, 1981). In addition, the involvement of ACh in brain activation has also been shown through the application of antagonistic drugs such as scopolamine, whose delivery suppresses brain activation (Steriade et al., 1993e). Brain activation is also mediated by another cholinergic nucleus, the forebrain Nucleus Basalis (NB). Indeed, its relation to arousal has been shown not only in relation to the wake-sleep cycle, but also to specific behavioral correlates such as locomotion (Buzsaki et al., 1988). In awake-behaving rats, NB firing rates increase with locomotion, and decreased during immobility (as the low frequency EEG power increased; Buzsaki et al., 1988). In

addition, while thalamic lesions did not disrupt the distinct EEG patterns observed during the sleep-wake cycle, NB lesions significantly decreased cortical high frequency power (Buzsaki et al., 1988). Finally, transient brain activation can also be induced during inactivated periods, by electrical stimulation of the MRF, PPT, or NB (Moruzzi and Magoun, 1949; Steriade et al., 1993e; Buzsaki et al., 1988; Metherate et al., 1992), or by the application of noxious stimuli to the animal such as a tail pinch (Duque et al., 2000). In addition, spontaneous brain activation can occur under certain anesthetics, such as urethane (Détári and Vanderwolf, 1987; Murakami et al., 2005; Clement et al., 2008). The fact that brain activation can be induced through these manipulations under anesthesia has facilitated the investigation of physiological correlates of different brain states and of their effect on sensory processing (Rudolph et al., 2005; Castro-Alamancos and Oldford 2002; Curto et al., 2009).

The mechanisms by which ACh release lead to brain activation have been characterized in detail. Application of ACh to thalamocortical cells results in sustained membrane depolarization, which causes the firing mode of these cells to change from bursty (as seen during SWS), to tonic spiking (McCormick and Prince, 1986). In the thalamus (densely innervated by the PPT; Hallanger et al., 1987), the depolarization observed after ACh application occurs through two separate ACh channels: one is mediated by a nicotinic receptor and induces fast Na^+ influx. The other is a slower muscarinic receptor-type K^+ channel that is blocked by ACh (McCormick, 1991). In the cortex (which is innervated by the NB; Divac, 1975; Mesulam and Van Hoesen, 1976), ACh induces depolarization and spiking activity (reviewed by McCormick, 1992). This occurs through the ACh-mediated

reduction of several K^+ conductances such as I_M, a muscarinic receptor-type, voltage sensitive channel, that activates upon depolarization (McCormick and Wiliamson, 1989). Another K^+ conductance that is blocked by ACh is the Ca^{2+} after-hyperpolarization current, which is a Ca^{2+} sensitive channel that activates by small concentrations of Ca^{2+} and causes long hyperpolarization (McCormick 1992). Thus, in the thalamocortical system, ACh has an excitatory effect on single neurons, promoting membrane depolarization and tonic spiking activity in both thalamocortical and cortical neurons.

2.5. Acetylcholine and neural plasticity in auditory cortex

As discussed above, brain activation occurs through the increased activity of the brain stem neuromodulatory systems (section 2.4). In addition to the effect of these neuromodulators on the level of brain activation, they are also known as key modulators of neuronal plasticity (for reviews see: Gu, 2002; Weinberger, 2003; Irvine 2007). The involvement of the acetylcholine – nucleus-basalis system in plasticity has been thoroughly characterized. These studies were conducted under various conditions in both anesthetized and awake animals using diverse types of stimuli which range from pure-tones to noise bursts, presented either alone, or in more complex temporal structures. Together, these studies demonstrate that acetylcholine release via the activity of nucleus basalis has a significant effect on neural responses in cortex. For instance, multiple repetitions of tone-presentations along with nucleus-basalis stimulation (NB-stim) result in substantial re-mapping of the tonotopy of auditory cortex (Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998a, Bjordahl et al., 1998). Specifically, this pairing

protocol results in the shifting of the tuning of a significant number of neurons towards the paired tone frequency, thus producing re-organization of cortical tonotopy. Interestingly, even though tonotopic re-organization can occur after an intensive stimulation protocol (e.g. Kilgard and Merzenich, 1998a administered 300-500 pairings per day for a period of 20-25 days) substantial changes in tonotopy can also be observed after moderate pairing protocols, such as after a single session consisting of only 40 NB-stim-tone pairings (Bjordahl et al., 1998).

NB-stim can also facilitate long-term changes in neural responses to temporally complex stimuli. Even though most cortical neurons are unable to reliably follow and respond to auditory stimuli that have rapidly changing components (De Ribaupierre et al., 1972; Creutzfeldt et al., 1980; Calford and Semple, 1995; Brosch and Schreiner, 1997; Wehr and Zador, 2005), NB-stim pairing with the presentation of such stimuli, can facilitate neural responsiveness to temporally structured sounds as well. For instance, pairing NB-stim with trains of tone-pips presented at high frequencies significantly increases the number of neurons that are able to respond reliably to all tone-pips presented in a train (Kilgard and Merzenich, 1998b). In addition, in this study Kilgard and Merzenich (1998b) demonstrated that facilitation of responses to trains of tones can be induced for different repetition frequencies, both high and low. Thus, pairing NB-stim with 15 Hz tone-train facilitates response amplitude and reliability to stimuli presented at high repetition frequencies, and pairing NB-stim with 5 Hz tone-train facilitates responses to stimuli presented at low repetition frequencies. A point worth noting is that this response facilitation occurred only when the tone carrier frequency was varied across paring trials;

if the same tone frequency was used in all trials, no facilitation occurred (even though tonotopic changes did). Another study conducted by Kilgard and Merzenich (2002) demonstrated that NB-stim pairing can also induce plasticity to stimuli that have both rapidly changing elements and a complex spectral structure. In their study, the pairing of a sequence of high frequency tone – low frequency tone – white noise with NB-stim results in facilitation of neural responses to the later elements of the sound, (which are suppressed under control conditions). In addition, this response facilitation does not occur for the individual components of the sound, but for the full sequence (with the exception of responses to the white-noise element that are facilitated regardless of the order of the tones, as long as it is preceded by a double-tone presentation).

The link between naturally occurring plasticity and acetylcholine release was also demonstrated through behavioral and pharmacological approaches. First, the paring of a conditioned-stimulus (e.g. a pure tone) with an unconditioned stimulus (e.g. shock) leads to receptive field modifications similar to those observed through tone-NB-stim pairing, i.e., neurons of the primary auditory cortex shift their tuning towards the paired tone frequency (for reviews see: Weinberger, 1993; Weinberger, 2003), Second, the administration of acetylcholine muscarinic antagonists such as atropine and scolopamine (Hars et al., 1993; Bakin and Weinberger, 1996; Miasnikov et al., 2001) and NB lesions (Kilgard and Merzenich, 1998a) suppresses receptive field plasticity and tonotopic re-mapping. In humans, administration of scolopamine leads to impaired performance in tasks that require processing of rapidly presented visual stimuli (Wesnes and Warburton,

1984). Finally, learning and acetylcholine release in hippocampus and cortex are correlated (Orsetti et al., 1996).

On the other hand, even though brain activation and cortical plasticity appear to be complimentary phenomena, plasticity can also occur in the absence of brain activation (or acetylcholine release). For example, presenting two tones in brief succession (8-12 ms) can alter the tuning of a neuron in the auditory cortex of anesthetized ferrets (Dahmen et al., 2008), but this shift is not long-lasting (the change is sustained for a number of minutes). In addition, environmental enrichment also changes response properties of single neurons in the auditory cortex of rats (Engineer et al., 2004; Percaccio et al., 2005). In these studies, Kilgard and colleagues demonstrated that a few days in an enriched environment were sufficient to produce sharpening of the receptive fields of neurons, increase response amplitude, and increase suppression of 2nd stimulus responses on paired-tone presentation (i.e., worsen responses to temporally structures sounds). Interestingly, in a recent study (Percaccio et al., 2007) demonstrated that cortical modifications due to enrichment were not blocked by selectively destroying the cholinergic neurons of the basal forebrain, thus indicating that the plastic modifications that occurred in auditory cortex were not related to acetylcholine release via nucleus basalis.

2.6. Similarities between the upstate and the activated state

The upstate phase of the slow oscillation and the activated state share a number of physiological properties, which has lead to the suggestion that the upstate might serve as

a transient homologue of the awake-activated state (Destexhe et al. 2007; Castro-Alamancos 2009; McCormick et al., 2003). This is relevant because it might serve as a clue to the functional significance of upstates in particular and of the slow oscillation in general, which is largely unknown, and also because it might establish the upstate (which is easily observed under anesthesia and in vitro) as a valid experimental model for the investigation of information processing in the activated state.

The homology between the upstate and the activated state is expressed on several levels: First, neurons in both states are relatively depolarized (V_m fluctuates around – 60 mV), and have lower input resistance compared with the downstate phase (Contreras et al., 1996; Steriade et al., 2001; Timofeev et al., 2001, Rudolph et al., 2005). Second, tonic, irregular spiking is preserved in both states by a balance of inhibitory and excitatory currents, where inhibition is strong and serves to prevent epileptiform-type activity (Shu et al., 2003a; Rudolph et al., 2007; Compte et al., 2003a). Finally, at the population level, neurons can display locally coherent activity at high frequencies in both states (Destexhe et al., 1999; Compte et al., 2008).

3. The effect of global brain state on sensory processing

As the state of the brain of an animal, or person changes, be it from sleep to wake, from an upstate to a downstate, or from inattention to high vigilance, changes occur in the physiological and network properties of the neuronal population. These changes have been shown to significantly influence neuronal processing and representation of incoming sensory stimuli. Multiple methodologies have been employed to study these influences,

including single cell recordings, imaging, and behavioral measurements. The findings of studies conducted across different modalities and species are described below.

3.1. Sensory processing during different phases of the slow oscillation

Sensory processing is often studied in the anesthetized animal model, in which the most commonly observed cortical activity pattern is the SWS-like slow oscillation. As described above, the physiological properties of single cells and cortical circuits differ between the upstate and the downstate. These physiological differences would, presumably, lead to very different response properties depending on the phase of the slow oscillation in which a stimulus arrives and could, therefore contribute significantly to trial-to-trial variability in the responses to the same sensory stimulus (Arieli et al., 1996; Azouz and Gray 1999; Kisley and Gerstein, 1999; Massimini et al., 2003; Curto et al., 2009). It has long been known that sensory responses in the cortex are variable (see e.g., Dean 1981; Tolhurst et al., 1983; Shadlen and Newsome 1998; but see DeWeese et al. 2003 for an example of reliable responses), and this variability can have a strong impact on information processing, specially if it is correlated across neurons (Zohary et al., 1994; Averbeck et al., 2006) as one would expect if it arises from coordinated changes in the instantaneous state of the local circuit during the slow oscillation.

Interestingly, the way in which sensory responses change as a function of the phase in the slow oscillation is complex, and different studies using different techniques and in different sensory modalities have reported modulations of responses with opposite sign: Some studies in the cat visual (Arieli et al., 1996; Azouz and Gray 1999) and motor

(using thalamic electrical stimulation; Timofeev et al., 1996) cortices, and in ferret cortical slices using current injections (McCormick et al., 2003; Shu et al., 2003b) found responses during upstates to be generally larger than during downstates. On the other hand, studies in rat somatosensory (Haslinger et al., 2006; Sachdev e al., 2004; Hasenstaub et al., 2007) and auditory (Curto et al., 2009) cortices and in ferret cortical slices (Shu et al., 2003a) found upstate responses to be significantly smaller than downstate responses. Further research is needed to explain the factors underlying the different findings in these studies. In addition, the studies above focused on the modulation of responses to brief, single stimuli. Real life stimuli tend to have more complex temporal structures, especially in the auditory cortex during RAP. Therefore, in order to better understand stimulus processing in the inactivated state, processing of such stimuli must be studied as well.

3.2. Stimulus processing across the activated and inactivated states

Several studies have compared sensory processing in the activated and inactivated states, generally indicating that stimulus representation is improved in the activated state. Receptive fields of the majority of single neurons in both auditory thalamus and cortex are sharper during activation (Wörgötter et al., 1998; Edeline, 2003). In addition, the spatial spread of activity evoked by stimuli across the cortical surface is attenuated in the activated state (Castro-Alamancos 2004b, Ferezou et al., 2006). Both these observations suggest that in the activated state neuronal responses are more stimulus-specific, which may facilitate a more accurate cortical representation. On the other hand, single, brief

stimuli tend to evoke larger cortical responses in the inactivated state (Fanselow and Nicolelis, 1999; Castro-Alamancos 2004a; Crochet and Petersen 2006). However, this difference in size may not indicate better stimulus processing per se, as other response features such as response variability, also play an important role in stimulus representation, and as we have previously discussed, inactivated responses are known to vary with the phase of the slow oscillation (Section 3.1). In addition, it has been proposed that reductions in response amplitude may reflect a shift from a non-specific population response to a more stimulus-specific and reliable activation of smaller cells groups (Kohn and Whitsel, 2002).

Responses to high frequency stimuli also differ across global brain states, with stronger cortical and thalamic adaptation observed during inactivated states (Fanselow and Nicolelis 1999; Castro-Alamancos, 2004a; Poggio and Mountcastle, 1963). Stimuli delivered at frequencies above 10 Hz are strongly suppressed in the rat somatosensory cortex during quiescent, but not alert states (e.g. Fanselow and Nicolelis 1999). In an elegant study, Castro-Alamancos, (2004a) found differences in the degree of adaptation to passive whisker stimulation across different behavioral states. During quiet immobility, sensory responses adapted (to trains of stimuli of frequencies of 5 Hz and higher) more strongly than during active exploration. Interestingly, the same trend was observed during acquisition of an avoidance task. As the animal's performance increased (a period presumably corresponding to higher alertness and attention), the degree of adaptation was lower than during steady state correct performance, in which the task becomes 'routine'. These findings have important consequences for RAP, and suggest that processing of

stimuli with complex temporal structure might improve with the level of cortical activation.

3.3. Stimulus processing within the activated state, effects of vigilance and attention

The effect of activation level on sensory processing is often studied in the context of attention, working memory and performance measures such as reaction time. Most studies have generally focused on how these behavioral conditions modulate two distinct features: response amplitude and the spectral content of population responses.

Generally, increased attention is correlated with increased response amplitude. This phenomenon has been shown in various modalities and species (e.g. Haider, 1964; Eason et al., 1969; Picton et al., 1971; Hubel et al., 1959; Miller et al, 1972; Roelfsema, 1998; Fritz et al., 2007, for a review Raz and Buhle, 2006; Reynolds and Chelazzi, 2004). For example, in a study conducted in humans using EEG recordings, shock punishment was used to control attention level during a visual detection task (Eason et al., 1969). This study elegantly showed that evoked potentials to target stimuli during the 'shock-threat' condition were significantly larger than responses during a detection task with no shock, and than responses to passive stimulus presentations. Reaction times were also significantly shorter in the 'shock-threat' condition (Eason et al., 1969). Response increases during attention have also been reported at the level of single cells (for reviews see e.g., Reynolds and Chelazzi, 2004; Maunsell and Treue, 2006; Fritz et al., 2007). In a series of studies, Fritz and colleagues demonstrated that single neurons in the auditory cortex of the ferret, underwent long term receptive field changes that correlated with the

attentional demands of the task (Fritz et al., 2003; Fritz et al., 2005; Fritz et al., 2007). Specifically, focusing attention in order to detect a target tone within a complex sound envelope induced receptive field changes in single neurons that persisted for hours after the performance of the task (Fritz et al., 2005; Atiani et al., 2009). In addition, not only was response amplitude to the target tone elevated, in some cases responses to non-target tones were suppressed. These results complement findings from rat auditory cortex where the pairing of specific tones with Nucleus Basalis (NB) stimulation resulted in re-mapping of primary auditory cortex tonotopy in these animals (Kilgard and Merzenich, 1998). Thus, in both cases, increased activation (either through engagement in a demanding task, or through NB stimulation) had long term effects on neuronal tuning in auditory cortex.

Other series of studies have investigated how attention or short-term memory retention modulate the spectral content of different physiological signals. As mentioned above (Section 2.3), in monkeys and humans, where most studies on these types of higher cognitive functions are conducted, activation is associated an increase in the gamma frequency power of cortical EEG and LFP fluctuations. Characterizing these changes has, therefore, been the focus of most studies. In monkey (Fries et al., 2001; Womelsdorf and Fries, 2007) and human (Tallon-Baudry et al., 2001) visual cortex, gamma power has been shown to increase with attention. Fries et al., (2001) and Halgren et al., (1978) also reported decreases in low frequency power associated with increased attention (or task difficulty). During the delay period of a short term memory task, gamma power was

elevated in the local field potential (LFP) in monkey parietal cortex (Pesaran et al., 2002), but not in the spike trains of monkey prefrontal cortex (Compte et al., 2003b).

Although most studies of attention come from human or monkey experiments, a recent study addressed the effect of "non-selective" attention (i.e., engagement in a simple sound localization task) on response amplitude in rat primary auditory cortex (Otazu et al., 2009). This study found decreased response amplitude to sound stimuli (click-trains and pure tones) when the rats were performing a sound localization task as compared to a "passive" or "free" condition where the animal was awake but behavior was unrestricted. Otazu et al., 2009 point out that the lack of an attentional enhancement in their study is probably due to the fact that such enhancements were typically seen in "selective attention" (e.g., attending to a specific feature of a sensory stimulus, or to a specific spatial location) tasks, whereas their results describe a generalized non-specific effect that only appears to require involvement in any behavioral task, regardless of sensory modality. In this sense, the results of Otazu et al., 2009 would seem to confirm of a number of previous studies in somatosensory cortex which found decreased sensory responses during generally active (whisking, active exploration) compared to quiet or immobile behavioral conditions (Fanselow and Nicolelis, 1999; Castro-Alamancos 2004a; Crochet and Petersen 2006). However, it is not trivial that the free condition in Otazu et al., 2009 and the immobile/quiescent behavioral conditions in these studies can be readily identified (see also Discussion in Chapter 3), because the free condition in Otazu et al., 2009 does not specify, for instance, how behaviorally "active" the animal was. In most experiments, behavior was unrestricted during the free condition, and

possibly includes periods of active exploration of the behavioral arena, whereas other experiments in Otazu et al., 2009 were conducted in the head-fixed condition, in which by definition there is no locomotion (also see Discussion in Chapter 3).

4. Outline of Research Program

The evidence reviewed above can be summarized as follows: Language skills can be improved in children who suffer from specific learning impairment through training that is focused on rapid auditory processing. The tasks used for this training were designed to keep the children alert and to engage their attention. Increased alertness and attention is associated, on the one hand, with increased levels of cortical activation and, on the other hand, with increases in the amplitude of sensory evoked responses and with decreases in the degree of sensory adaptation to temporally structured stimuli. Although, together, these lines of evidence suggest that the efficiency with which the cortex processes rapidly changing sensory stimuli is strongly affected by brain state (with better processing during cortical activation) a number of issues remain open before a strong link between RAP and brain state can be established.

First, studies which directly assessed processing of temporally structured stimuli across different brain states were conducted in the somatosensory cortex, not on the auditory cortex where the first stages of RAP take place. Second, a systematic characterization of the representation of temporally-structured stimuli across different brain states under anesthesia and wakefulness, has not yet been performed. Third, an in depth characterization of the ability of cortical circuits to represent sensory information requires

knowledge of the simultaneous activity of large neuronal populations. Existing studies, however, have only reported the activity of single neurons or multi-unit clusters.

In this thesis I will investigate the hypothesis that the quality of representation of the types of auditory stimuli used to assess RAP in children with specific language impairment generally increases with the level of cortical activation. In order to do this, I will perform a systematic characterization of the responses of simultaneously recorded neuronal populations to these types of stimuli in the rat auditory cortex during the activated and inactivated states obtained under urethane anesthesia. In addition, I will also analyze responses of multiunit clusters to temporally structured stimuli from the auditory cortex of awake rats. This research program will be structured in three different chapters.

Chapter 1. Rapid auditory processing during the different phases of the slow oscillation

In this chapter, the ability of neuronal populations to discriminate a brief click sound from silence or a click from two clicks presented in quick succession will be investigated as a function of the phase of the slow oscillation in which the stimuli arrive under urethane anesthesia. This will elucidate, first, whether previous findings from other sensory areas on the differences between evoked responses to single stimuli arriving during upstates or downstates extend to the auditory cortex, and second, the extent to which discrimination between one and two rapidly presented auditory stimuli changes during the slow oscillation.

Chapter 2. Differences in rapid auditory processing between the activated and inactivated brain states

In this chapter, the type of analysis in Chapter 1 will be extended to responses during the activated state under urethane anesthesia, and the discrimination ability of the simultaneously recorded neural populations will be compared across the activated and inactivated states. This will allow us to directly test whether RAP is more efficient during cortical activation. In addition, it will also allow us to test the hypothesis that the upstate phase of the slow oscillation is a transient homologue of the activated state, both from the point of view of processing simple and temporally complex stimuli.

Chapter 3. Effect of modulations in the level of activation during wakefulness on rapid auditory processing

In this chapter, I will examine how the evoked multiunit responses to trains of clicks delivered to an awake rat in an unrestricted behavioral condition vary with the instantaneous level of activation prior to stimulus onset. This study is a first step towards characterizing the effect of brain state on RAP during wakefulness. In particular, it will elucidate, first, whether subtle changes in cortical state within a relatively homogeneous behavioral condition are associated with systematic differences in evoked sensory responses to temporally complex stimuli, and second, whether these differences are consistent with better stimulus-representation in trials preceded by larger activation levels.

5. General Methods

In this section I describe the experimental methods for Chapters 1 and 2, and the analysis methods used throughout the whole Thesis. Specific experimental or analysis methods used only on a given Chapter will be described there.

Surgery and recording

The data in Chapters 1-2 was collected under anesthesia. Sprague-Dawley rats (250-400 g) were anaesthetized with urethane (1.3-1.6 g/kg) and ketamine (25-40 mg/kg), supplemented by further doses of urethane (0.15 g/kg) as required. Animals were held with a custom naso-orbital restraint that left the ears free and clear. After preparing a 3 mm square window in the skull over left auditory cortex, the dura was removed and silicon microelectrodes (Neuronexus technologies, Ann Arbor MI) were inserted. Probes had either eight shanks each with eight clustered recording sites (Bartho et al., 2004), or four shanks each containing two tetrode configurations; shank spacing was 200 μm in both cases. Once a stable recording site was established, the cortex was covered with 1% agar/ACSF. The location of the recording sites was estimated to be primary auditory cortex by stereotaxic coordinates and vascular structure (Doron et al., 2002; Rutkowski et al., 2003; Sally and Kelly, 1988). Electrodes were estimated to be in deep layers by field potential reversal (Kandel and Buzsaki, 1997), most likely layer V based on the presence of units of high background rate (Sakata and Harris, 2009). Neuronal signals were high-

pass filtered (1 Hz) and amplified (1,000X) using a 64-channel amplifier (Sensorium Inc., Charlotte, VT), recorded at 20 kHz, 16-bit resolution using a PC-based data acquisition system (United Electronic Industries, Canton, MA) and stored on disk for later analysis. Spike detection and sorting was software-based, using previously described semi-automatic clustering methods (Harris et al., 2000; Hazan et al., 2006; see, www.klustakwik.sourceforge.net and www.klusters.sourceforge.net). LFP traces were obtained by digitally low-pass filtering (1.25 kHz) the broadband data.

Acoustic Stimuli

Acoustic stimuli consisted of single or double clicks (5 ms square pulses, 75 dB SPL, 50 ms inter-click interval, 2.5 s separation between stimuli). Experiments took place in single-walled sound isolation chamber (IAC, Bronx, NY) with sounds presented free field (RP2/ES1, Tucker-Davis, Alachua, FL). Sound level calibration was performed with an ACO-7012 microphone placed next to the right ear.

Unit selection criteria and response definition

To ensure only stably isolated units were used for analysis, the following criteria were applied: (1) mean firing rate of at least 0.5 Hz throughout the recording period, (2) stability throughout the recording period, assessed by comparing baseline firing rate during the first and second halves of the recording (100 ms window before each stimulus; cells for which differences were significant at $p<0.05$ were rejected). As cortical activation frequently occurred in restricted epochs of the recording, both criteria were

applied independently in either state to derive a separate stable population for the activated and inactivated states. Responses to each click were computed within windows of 50 ms, offset by 6 ms to compensate for the minimum possible delay for signals to reach auditory cortex (1st click responses: 6 to 56 ms, 2nd click or no-2nd click responses: 56 to 106 ms, baseline: –44 to 6 ms, all relative to 1st click onset). Rates were computed in Hz by dividing the number of spikes in the response window by 50 ms.

Statistical methods

To nonparametrically assess statistical significance of differences in a quantity between groups, we used an unpaired randomization method ('shuffle test'). A test statistic |s| was computed as the absolute value of the difference between the means of the two groups. Significance was by comparison to a null distribution where |s| was recomputed 5000 times after random reassignment of groups. The p-value for assessing significance was computed as the quantile of |s| relative to this null distribution.

Linear Discriminant Analysis

Stimulus reconstruction was performed using linear discriminant analysis (LDA) (see e.g. Hastie et al., 2001). For each stimulus presentation, the firing rates of all cells were collected into an array giving the spike count of each neuron in the corresponding time window. Performance was evaluated by cross-validation: trials were randomly divided into four equally-sized groups, with each taking a turn as the "test set" used to evaluate the performance of a classifier trained on the remaining three (the training set).

Discrimination boundaries were computed using standard linear discriminant analysis (Hastie et al., 2001). Problems associated with sparseness in the responses were avoided by regularizing the within-group covariance matrix $\Sigma$to $(1-\lambda)\Sigma + \lambda\overline{V}I$ where $\overline{V}$ is the average variance (i.e. the average of the diagonal elements of the covariance matrix), I is the identity matrix, and $\lambda = 0.1$.

Spike Count Correlations

Spike count correlations were calculated using a shift-corrector (for a closely related measure, see Bair et al., 2001) to exclude the effect of slow covariations in activity between cells. If $n_1(i)$ is the spike count of cell 1 in trial i, the corrected covariance between cells 1 and 2 was given by

$$c_{12} = \sum_{i=1}^{N_T} n_1(i)n_2(i)/N_T - \sum_{i=1}^{N_T-2} n_1(i)n_2(i+2)/(N_T-2)$$

where N_T is the total number of trials in the recording session (the inter-trial-interval was 2.5 s). The (normalized) correlation coefficient was then given by $r_{12} = c_{12}/\sqrt{c_{11}c_{22}}$.

Classification of cortical state

Periods of cortical activation were detected based on the dynamics of multiunit activity (MUA), generated by merging the detected spikes from all recorded channels. The recording was divided into successive 50 ms bins, and bins containing no MUA were detected (‘empty slots’). Periods of activation were indicated by the absence of any empty slots during a 10 second bin (i.e. no downstates). Results from this method were

confirmed visually by checking the corresponding local field potential (LFP) traces. Other methods, such as running averages of the variance of the MUA rate gave similar results .

Separation trials according to the phase of the slow oscillation

Trials occurring during the inactivated state were further subdivided according to the phase of the slow oscillation at which the stimulus was presented. Phases were defined using an automated procedure, which began with detection of downstates and upstates. Downstates were defined as continuous periods of at least 40 ms with zero MUA rate; upstates were defined as periods of at least 40 ms in which no two successive 10 ms bins had zero MUA rate (this slightly more relaxed criterion was used to avoid splitting upstates due to a single empty bin). For each experiment, the median length of spontaneous up- and downstates was computed from the periods between 0.5 s after the onset of each stimulus and the onset of the next stimulus. Across experiments, the average of the median downstate and upstate lengths were 160 ms and 210 ms respectively. Stimuli presented in downstates were classified in the “early downstate” phase if the time between the start of the downstate and the stimulus presentation was less than the median spontaneous downstate length, and “late downstate” phase otherwise. A similar criterion was used for stimuli presented in upstates.

A minimal requirement of 20 trials per stimulus was applied for all experimental conditions (including all inactivated phases, and full activated and inactivated states). Thus, results from late downstate trials were obtained from 320 neurons (5 experiments),

early downstate trials 320 neurons (5 experiments), late upstate trials 263 neurons (3 experiments), and early upstate trials 453 neurons (6 experiments).

6. Chapter 1 - Rapid auditory processing during the different phases of the slow oscillation

6.1. Rationale

The anesthetized cortex, similarly to the cortex during slow wave sleep, generally displays a slow oscillation (< 1 Hz) in which alternations between periods of increased network activity (upstates) and silence (downstates) occur regularly (Steriade et al., 1993a-c). Different phases of the slow oscillation are characterized by differences in neuronal physiological properties such as resting membrane potential and excitability (Contreras et al., 1996; Steriade et al., 2001; Timofeev et al., 2001; Rudolph et al., 2005). Processing dynamics of sensory stimuli is modulated by the phase of the slow oscillation, albeit in a complex manner. In the somatosensory cortex of the rat, brief stimuli presented in the upstate evoke reduced response amplitude compared with downstate responses (Sachdev et al., 2004; Haslinger et al., 2006; Hasenstaub et al., 2007; Haider et al., 2007). However, other studies found evidence for the opposite trend (Arieli et al., 1996; Azouz et al., 1999; Timofeev et al., 1996), specially in the visual cortex.

Not only does the slow oscillation affect sensory responses, sensory stimuli can also alter the course of the slow oscillation. In particular, brief sensory stimuli have been shown to induce ‘phase-flips’, terminating upstates into downstates and initiating upstates from downstates (Hasenstaub et al., 2007; also occurs via electrical stimulation of thalamocortical projections: Beierlein et al., 2002; Shu et al., 2003a; MacLean et al., 2005). Through its effect on the phase of the slow oscillation, brief stimuli can have a

relatively long lasting effect (~ 100 ms), which would influence the processing of subsequent stimuli within that time scale. Processing of pairs of clicks with an inter-stimulus interval (ISI) of 50 ms, the type of stimuli used to test RAP (Tallal and Percey, 1973; Benasich et al., 2006), might thus be affected in a complicated way by the ongoing slow oscillation.

In this chapter we investigated how populations of neurons in the auditory cortex responded to single clicks and click pairs (50 ms ISI) and the extent to which the discriminability between these two stimuli changed throughout the slow oscillation.

6.2. Results

In order to study cortical representations of rapid auditory stimuli, we recorded neuronal populations in auditory cortex using high-density silicon probes. Stimuli consisted of brief, loud click stimuli (5 ms duration, >70 dB) presented either alone, or in pairs separated by 50 ms. Our data set consisted of a total of 453 well-isolated single neurons, obtained from 4 animals from which 6 recording sessions were made. Data was only considered from periods in which the cortex was inactivated (see Methods).

Correlation between population baseline activity and single cell responses

Cortical activity alternated between periods of high excitability and spiking (upstates) and silence (downstates) comprising the slow oscillation. As a first step to investigate the effect of this ongoing oscillation on the evoked responses in our dataset, we measured the correlation across trials of single cell responses and baseline MUA (blMUA, computed as

the summed activity of all well isolated single cells (excluding the cell under investigation) during a baseline window of 50 ms). In Fig. 1.1a, responses of a single cell are sorted according to blMUA for single (left) and double (right) click trials. This example is representative of the trend observed for most single cells, where high blMUA trials tend to evoke smaller overall responses than low blMUA trials. This trend is quantified below (Fig. 1.1b) for the entire single cell population. For each neuron we computed the correlation

coefficient (CC) across trials between its response and the blMUA. Responses were measured in four different time windows: a baseline window (44 ms preceding, to 6 ms after stimulus onset), a 1st click window (6 to 56 ms after stimulus onset), a 2nd click window (56 to 106 ms after stimulus onset in double click trials), and a no-2nd click window (56 to 106 ms after stimulus onset in single click trials). Each panel in Fig. 1.1b displays the distribution of CCs across the whole population for each time window. During the baseline period, single cells had a mean correlation with blMUA of 0.27, confirming that the activity of single cells is positively correlated across the population during the slow oscillation. Although there is large variability across neurons, the mean CC for 1st click responses was negative (-0.036), indicating that on low blMUA trials neuronal responses were larger than on high blMUA trials. This suggests that a similar modulation of sensory responses to brief stimuli takes place in the auditory and somatosensory cortices across the slow oscillation. A negative mean CC was observed for all response epochs: for 2nd click responses it was –0.054, and for no-2nd click responses it was –0.09. Interestingly, this trend became stronger as a function of time from stimuli

presentation. The mean 1st click CC was significantly less negative than both the mean 2nd click and the mean no-2nd click CCs (mean shuffle test p-value<0.05 p<0.001 respectively). These results indicate (1) that similarly to the case in somatosensory cortex, brief sensory stimuli evoke weaker responses when arriving on periods of high activity (presumably upstates), and vice-versa (Fig. 1.1b) and (2) that the level of baseline activity at stimulus onset has a long lasting effect, influencing both early and late sensory responses.

Classification of trials according to the phase of the slow oscillation

In order to investigate more systematically how sensory responses varied across the slow oscillation, we divided trials according to the phase of the slow oscillation (PSO) assessed in the baseline period just prior to stimulus onset. Trials occurred at any point during the ongoing slow oscillation (Fig. 1.2a). We considered four trial types (Fig. 1.2b): the late downstate (blue), the early downstate (green), the late upstate (pink) and the early upstate (orange). Early or late were defined with respect to the median up- or downstate durations (see Methods). We checked that our separation into different PSOs was meaningful by comparing the average firing rates and their trial-to-trial variability across the different phases. Baseline rates were significantly different (p<0.001 all cases) from the overall inactivated rate (2.06±0.10 Hz, measured in the baseline window): late upstate, 3.41±0.20 Hz, early upstate, 4.07±0.22 Hz (both late and early downstate baseline rates were 0 Hz by definition), Fig. 1.2c. Baseline variability was quantified for all PSOs using the Fano-factor (FF = spike count variance / spike count mean).

Separation into PSOs successfully reduced the baseline variability of each type of trial, compared with the variability of all inactivated trials as a whole (Fig. 1.2d. Inactivated 9.82±1.35, late upstate 3.40±0.97, early upstate 3.30±0.78, both cases $p<0.01$). Note that downstate baseline rate is zero by definition and therefore is not variable. Next, we examined how sensory responses varied across PSOs.

Response amplitude as a function of the phase of the slow oscillation

We quantified sensory responses to the 1st and 2nd clicks by counting spikes in 1st and 2nd click windows respectively (Fig. 1.3a-b). Overall, sensory responses to brief stimuli arriving on a downstate were larger than those arriving on an upstate (Fig. 1.3c).

The mean response to the 1st click in late-downstate trials was significantly larger than in upstate trials (both cases, $p<0.005$). A similar effect was observed in early-downstate trials, but the difference only reached significance when compared with late-upstate trials ($p<0.05$ vs. $p>0.08$ for early-upstate). The mean response to the 1st click was not significantly different ($p>0.3$, both cases) between the early and late parts of either type of trial. The same trend was observed for the mean response to the 2nd click (Fig. 1.3d). Mean 2nd click responses in both types of downstate trials were significantly larger than in both types of upstate trials cases, $p<0.002$), and no significant differences between early and late trial types was observed.

Quantifying the effectiveness of the 2nd click in evoking a sensory response

Although responses to the 2nd click in downstate trials are larger than in upstate trials (Fig. 1.3d) this does not necessarily mean that the 2nd click is more effective in driving a response in either trial type. It is also possible that the different firing rates of the neurons after 2nd click presentation originate from late components of the response to the 1st click. This is supported by previous findings in the somatosensory cortex showing that brief whisker deflections could induce 'phase-flips', such that upstates can be terminated by the stimulus and turned into downstates and vice-versa (Hasenstaub et al., 2007). Thus, by 'resetting' the slow oscillation, a brief stimulus can have a long lasting effect. Consistent with our previously found negative correlation between no-2nd click responses and blMUA (Fig. 1.1b), we observed 'phase-flips' in our dataset (Fig. 1.4a-b). When comparing activity during the no-2nd click window, relative to baseline activity (Fig. 1.4c), we found that no-2nd click activity evoked from downstate trials was high and significantly different from 0 (t-test $p<0.05$), and that no-2nd click evoked activity on upstate trials was significantly suppressed ($p<0.05$).

Thus, as predicted, since stimuli delivered during downstate trials evoke high activity even in the absence of a 2nd click (due to upstate initiation), at least some of the activity measured during the 2nd click response window is induced by the 1st click, not the 2nd click. In these conditions, it is reasonable to measure the efficiency of the 2nd click in evoking a response by comparing activity between the 2nd and no-2nd windows. The effectiveness of the 2nd click measured in this way was low, evoking responses of ~ 1.5 Hz (Fig. 1.4d). Surprisingly, it was mostly uniform across PSOs (Fig. 1.4d; 5 out of 6 pair-wise comparisons had, $p>0.1$), with the exception that the late downstate response

was slightly smaller than the early upstate response ($p<0.05$, but larger than 0.03). Therefore, even though activity in the no-2nd click window was on average larger during downstate trials, this activity did not reflect a higher average efficiency of the 2nd click in evoking spiking activity, as compared to upstate trials.

Stimulus Prediction Analysis

The results from the previous sections quantify the average ability of the 1st and 2nd clicks to drive populations of neurons in the auditory cortex throughout the slow oscillation. However, the extent to which downstream targets will be able to detect (and further process) these stimuli depends, not only on the mean difference in activity caused by the stimuli, but also on the trial-to-trial variability in the responses across the population. Such trial-to-trial variability, especially if it is correlated across cells, can have a large impact on the ability of downstream targets to decode the stimulus (Zohary et al., 1994; Dayan and Abbott 1999; Aberbeck and Lee 2006). Although trial-to-trial variability, and the extent to which it is correlated across neurons, can be estimated when one records simultaneously the activity of a neural population, we took the approach of directly estimating their effect on the discriminability of the sound clicks. In order to do this, we used a "stimulus-reconstruction" approach (Oram et al., 1998), in which the particular stimulus presented to the animal is predicted from the pattern of neuronal population response it evokes. Specifically, we asked how well population activity could discriminate, (1) presentation of a single click from no sound presentation, and (2) double click presentation from single click presentation. The population response to each

stimulus presentation (or lack thereof) was summarized by a 'response vector' containing the spike counts of all neurons in the appropriate window. Classification was performed using linear discriminant analysis (LDA; see e.g., Hastie et al., 2001

see also Methods). We first investigated the performance with which population activity could distinguish the response to a single click from baseline activity. To do this, we trained the classifier to discriminate response vectors computed from the 1st click and baseline windows. To illustrate how population activity can be used to decode stimuli, we divided the population into two randomly assigned groups, and plotted the mean spike count within each group against each other across trials (Fig. 1.5a-b, left). Spike counts during the 1st click period form a cloud that can be easily separated from the cloud computed from spike counts during the baseline period (Fig. 1.5a, left). Consistent with this picture, classification of responses to the click from baseline responses using LDA had a high performance (Fig. 1.5a, right).

Next, we used LDA with the response of each neuron in the population taken as an independent variable (see Methods). 1st click vs. baseline discriminations was very accurate for all PSO, with performances of ~ 90 % (Fig. 1.5c, left). Although slightly better in downstate trials, performance was not statistically different across all PSOs and compared to performance in the inactivated state as a whole (Fig. 1.5c, left; downstate trials vs. other conditions, $0.04<p<0.1$ for all cases). These results suggest that even though the mean response of the neurons to the 1st click during downstate trials was larger than in upstate trials, the evoked trial-to-trial variability in downstate trials is also

larger, so that discrimination of single click presentations from no click presentations is similarly effective in both types of trials during the slow oscillation.

To investigate the ability of auditory cortical population activity to distinguish double-click from single-click stimuli, we next trained the classifier to discriminate response vectors computed from the 2nd click and no-2nd click windows. Overall, 2nd vs. no-2nd click discrimination was poorer than discrimination of 1st click vs. baseline activity, with performances of ~ 75% (Fig. 1.5c, right), consistent with the relatively low average efficiency of the 2nd click in driving the population (Fig. 1.4d). In addition, single- versus double-click discrimination was also equivalent across all PSOs (Fig. 1.5c, all cases, $p>0.7$).

We conclude that, despite large changes in the properties of neurons between upstates and downstates, auditory cortical populations are similarly capable of discriminating temporally structured stimuli across the different phases of the slow oscillation.

6.3. Discussion

The three main results we found in this chapter are, first, that the overall magnitude of sensory responses arriving in the upstate phase where smaller than in the downstate phase, second, that the ability of the neuronal population to detect single clicks from click pairs is not influenced by PSO, and, third, that detection of the presence or absence of a brief sound (1st click vs. baseline discrimination), was consistently better than detection of whether the stimulus had temporal structure (2nd vs. no-2nd click discrimination), suggesting that RAP is uniformly impaired across the slow oscillation.

The effect of the phase of the slow oscillation on responses to brief stimuli

We found that the timing of stimuli presentation relative to the PSO had a significant effect on the response amplitude of single neurons, whereby upstate responses were significantly smaller than downstate responses (Fig. 1.3). Our findings in rat auditory cortex complement some findings in the rat somatosensory cortex, where a similar trend was observed in responses to whisker deflections (Hasenstaub et al., 2007; Sachdev et al., 2004; Haslinger et al., 2006). Increased responsiveness during upstates, however, has also been reported, especially in the visual cortex (Arieli et al., 1996; Azouz and Gray, 1999; Timofeev et al., 1996; Shu et al., 2003b). Several factors could account for these seemingly inconsistent results, including the effective strength of the stimulus used and inter-areal differences in stimulus processing. Hasenstaub et al. (2007) confirmed that the upstate PSO is more excitable, i.e., neurons respond more strongly to injection of small synthetic EPSCs during upstates. Thus, sensory inputs which are effectively 'small' might elicit stronger responses in upstates than in downstates (Shu et al., 2003b). Hasenstaub et al., (2007) suggested that sensory responses are a 'network' effect which has an 'explosive' or non-linear character, which is evidenced by the fact that brief stimuli can evoke upstates from downstates and vice-versa ('phase-flipping'). Our results suggest that the auditory and somatosensory cortices might be similar in this respect.

In addition, even though neurons respond to stimuli in the upstate, it seems like, probably due to strong adaptation mechanisms, the cortical circuit is not capable of sustaining a response once it's been active for several tens of milliseconds. Indeed, responses in these

cortical areas have been characterized as very transient or 'punctate' (Petersen et al., 2003; DeWeese et al., 2003), even when the stimulus is sustained. Neuronal responses to visual stimuli, in contrast, can be sustained. Drifting gratings at the preferred velocity and spatial frequency of a neuron elicit sustained spiking during several hundreds of ms (see, e.g., Azouz and Gray, 1999). Given that neuronal responsiveness is increased during periods of depolarization, if the 'phase-flip' phenomenon was not characteristic of visual cortex, this could explain why, when the visual stimulus arrives on a more depolarized phase, it elicits quicker and larger responses from neurons in the visual cortex (Arieli et al., 1996; Azouz and Gray, 1999). In agreement with this interpretation, the effect of spontaneous depolarization on visual responses seems to be 'additive' or 'linear' (Azouz and Gray, 1999), in contrast to the 'phase-flip' phenomenon, which is intrinsically non-linear.

Single versus double click discrimination is poor across all phases of the slow oscillation

Surprisingly, we found that despite significant differences in the amplitude of responses to both single and double click stimuli during upstates and downstates (Fig. 1.3), discrimination of whether a single or a double click stimulus was presented was (1) worse than detection of a single click from silence, and (2) similarly effective in both PSOs (Fig. 1.5). This is associated to the fact that the first click in the pair could evoke a 'phase-flip', inducing an upstate from a downstate and vice-versa (Fig. 1.4). Because of this, 2^{nd} click responses, relative to no-2^{nd} click activity are always weak: if the 1^{st} stimulus arrives on a downstate, 2^{nd} click responses appear large (Fig. 1.3d) but no-2^{nd}

click activity is also elevated because of the induced upstate (Fig. 1.4c). Similarly, if the 1st stimulus arrives on an upstate, no-2nd click activity is low because of the induced downstate, but 2nd click responses are only moderate. In both cases, it seems like the 2nd click is not able to significantly change the level of activity induced by the 1st click (Fig. 1.4d). What could be responsible for this difference in the effectiveness of the 1st and 2nd clicks?

The median upstate and downstate durations were 210 and 160 ms respectively, which are significantly longer than the 50 ms between clicks. This suggests that the mechanisms that regulate spontaneous transitions in phase, have a longer time constant than 50 ms, and are probably not responsible for the different effectiveness of the 1st and 2nd clicks. Instead, this difference could be due to adaptation of inputs from the thalamus, impeding the relay of the 2nd click to the cortex. It is known that, specially during the inactivated state, thalamic neurons (Diamond et al., 1992; Fanselow and Nicolelis, 1999; Chung et al., 2002), and thalamocortical synapses (Chung et al., 2002; Castro-Alamancos and Oldford, 2002; Boudreau and Ferster, 2005) strongly adapt to trains of sensory stimuli. An adapted thalamic response to the 2nd click could make the cortical responses to the 2nd click less effective, making it equally difficult to infer whether a 2nd click was, or was not, presented in either phase of the slow oscillation.

7. Chapter 2 - Differences in rapid auditory processing between the activated and inactivated brain states

7.1. Rationale

During the activated state (typical of REM sleep and attentive wakefulness) the high-amplitude, low-frequency EEG or LFP oscillations typical of the inactivated state are suppressed and replaced by a more desynchronized pattern of EEG and LFP fluctuations (Berger, 1929; Vanderwolf 1969; Steriade et al., 1993d; Destexhe et al., 1999; Steriade 1999; Steriade et al., 2001; Gervasoni et al., 2004).

Sensory responses have been shown to be strongly modulated by brain state (Worgotter et al., 1998; Castro-Alamancos, 2004a; Edeline, 2003; Crochet and Petersen, 2006; Fanselow and Nicolelis, 1999). While higher amplitude responses to simple stimuli have been reported in the inactivated state (Fanselow and Nicolelis, 1999; Castro-Alamancos, 2004a), other response features such as receptive field sharpness (Worgotter et al., 1998; Edeline, 2003) and the degree of response adaptation (Castro-Alamancos, 2004a; Fanselow and Nicolelis, 1999), suggest that the processing of temporally structured stimuli might be more efficient in the activated state. In particular, impaired processing in the inactivated state might arise from interactions between the temporal structure of the sensory stimuli and the cortex's own dominant intrinsic dynamics (i.e. the slow oscillation).

Previous studies on the interaction between brain state and temporally structured stimuli were conducted in the somatosensory cortex. As a first step towards establishing whether

differences in the degree of cortical activation might be associated with better processing of complex auditory stimuli and speech, we investigated auditory responses from simultaneously recorded neuronal populations in the auditory cortex in both the activated and inactivated states. As in the previous chapter, we assessed the ability of neural populations to discriminate the same type of single and double click stimuli which are used to assess RAP in humans (Tallal and Piercy, 1973; Benasich et al., 2006; Choudhury et al., 2007).

Sensory processing across brain states can be studied under urethane anesthesia, as this type of anesthetic has been shown to induce spontaneous alternations between periods of cortical activation and inactivation (Détári and Vanderwolf, 1987; Murakami et al. 2005; Clement et al., 2008). In addition to enabling the comparison of the activated and inactivated states as a whole, this methodology makes it possible to directly compare sensory processing in the activated state and during the upstate phase of the slow oscillation. Based on the similarity between some of their physiological properties, these two states have been suggested to be homologous (Destexhe et al., 2007; Castro-Alamancos, 2009; McCormick et al., 2003), but so far sensory processing in the two states has not been directly compared.

7.2. Results

Under urethane anesthesia, in addition to inactivated pattern described in detail in the last chapter, population activity displays transient epochs of cortical activation in which downstates are generally absent (Fig. 2.1), as shown previously (Detari and Vanderwolf,

1987; Murakami et al., 2005; Clement et al., 2008). In this chapter, we compared population responses to temporally structured stimuli between the activated and inactivated states. We used identical stimuli as in the last chapter, namely, brief, loud click stimuli (5ms duration, >70 dB) were presented either alone, or in pairs separated by 50 ms. Our data set consisted of a total of 453 well isolated, single neurons recorded in the inactivated state, and 361 neurons recorded in the activated state, obtained from 4 animals from which 6 recordings were made.

Stimulus prediction analysis

To quantitatively investigate the effect of global cortical state on population coding of rapid auditory stimuli, we used the same stimulus-reconstruction approach as in the last chapter (Fig. 1.5, see Methods). Specifically, we asked how well population activity could discriminate (1) presentation of a single click from no sound presentation, and (2) double click presentation from single click presentation.

We first investigated the performance with which population activity could distinguish the response to a single click from baseline activity. To do this, we trained the classifier to discriminate response vectors computed from 1st click and baseline time windows. To illustrate how population activity can be used to decode stimuli across states, Fig. 2.2a shows the mean spike counts of two randomly chosen cell groups of one experiment, plotted against each other. As evident in the figure, 1st click and baseline responses are easily discriminable in both the activated and inactivated states, which is reflected in the high performance of the classifier (Fig. 2.2c). Across experiments, no

significant difference between activated and inactivated performance was observed (Fig. 2.2e, left, 90.33±2.69% vs. 89.17±2.68% correct respectively, p=0.66).

To investigate the ability of cortical population activity to distinguish double-click from single-click stimuli, we next trained the classifier to discriminate response vectors computed from the 2nd click and no-2nd click time windows. Unlike discrimination of single clicks from baseline, discrimination of 2nd click and no-2nd click responses was worse in the inactivated state (Figs. 2.2d-e, right hand side, 91.17±0.95% vs. 79.17±4.00% correct, $p<0.005$), and this effect was consistent across experiments (Fig. 2.2e. right). Thus, although the ability of auditory cortex to detect the presence of a sound is similar in both states, its ability to represent the fine temporal structure of the sound is state dependent.

Response features potentially contributing to discrimination performance

We next investigated which features of neuronal population responses could be responsible for this difference in discrimination performance. We reasoned that one or both of two factors could contribute. The first factor is average response magnitude. In somatosensory cortex, the size of cortical responses to whisker stimuli and the degree of adaptation to stimulus trains have been reported to vary with cortical state (Castro-Alamancos, 2004a; Fanselow and Nicolelis, 1999). The second factor is increased trial-to-trial variability due to the up/down oscillation itself. The discriminative capacity of neural codes is diminished by noise, an effect that is more severe if this noise is correlated (Zohary et al., 1994; Averbeck et al., 2006). As described in Chapter 1, the

inactivated state shows globally coordinated spontaneous fluctuations. It has been shown that these fluctuations are structurally homologous to sensory responses (Luczak et al., 2009), and can affect sensory responses in a nonlinear manner (Hasenstaub et al., 2007; Curto et al., 2009). Therefore, it may both degrade sensory responses and display population activity that appears stimulus-evoked in the absence of sensory stimuli.

Response magnitudes

Responses of a single example neuron to single and double clicks as a function of state are shown in Fig. 2.3a. We systematically compared mean responses to 1st clicks (relative to baseline) and 2nd clicks (relative to no-2nd click presentation) for all cells in our database across states. Average 1st click responses were significantly larger across the population in the inactivated state (Fig. 2.3b left; 2.55±0.28 Hz, n=453 neurons vs. 1.15±0.41 Hz, n=361 neurons, $p<0.005$). This result is perhaps surprising, given the equivalent performance of 1st click discrimination in both states, but is consistent with reports from rat somatosensory cortex that described larger responses to transient whisker stimuli in the inactivated state (Castro-Alamancos, 2004a). In contrast, average 2nd click responses were larger in the activated than inactivated state (Fig. 2.3b right; 2.49±0.24 Hz vs. 1.41±0.14 Hz, $p<0.001$). Thus, equivalent discrimination of 1st click responses from baseline occurs despite smaller response magnitude in the activated state. In the case of 2nd click discrimination, however, smaller responses in the inactivated state are consistent with poorer LDA performance.

Response variability

The apparent lack of a consistent relationship between classification performance and mean response differences could be due to differences in trial-to-trial variability across the different conditions. To investigate this possibility, we next examined differences in response variability, quantified using the Fano-factor (FF), across states. Fig. 2.4a shows FFs computed for all cells individually in the four response windows. Because the number of spikes occurring in a 50 ms window is typically small, single-cell FFs were all close to 1. Nevertheless, variability was slightly but significantly higher in the inactivated condition for the baseline (FF=1.12±0.01 vs. 1.01±0.01, $p<0.0001$) and 2nd click windows (0.96±0.01 vs. 0.93±0.01, $p<0.05$). Given that the deleterious effects of noise on discrimination performance can be enhanced by correlations (Zohary et al., 1994), we next measured pair-wise correlations for each response window, using a 5 s shift corrector to correct for covariations in response due to slow changes in network excitability (see Methods). Fig. 2.4b shows the distributions of pair-wise correlations for both states in one experiment, in the four response windows. On average, correlations in the baseline and 2nd click windows were significantly larger in the inactivated state than in the activated state ($p<0.005$, all experiments). During the 1st click and no-2nd click windows, a weaker effect was seen ($p<0.05$ for 3/6 experiments in both response windows). To test the degree to which correlation can affect population variability, we next computed the population FF (the FFof the summed responses of all simultaneously recorded cells) in each response condition. For all conditions, except 1st click responses, population FFs were higher in the inactivated state ($p<0.005$, baseline;

p=0.09, 1st click; p<0.01, 2nd click; p<0.05, no-2nd click). These results clarify the relationship between discrimination performance and mean response differences: although the mean difference between 1st and baseline responses was larger in the inactivated than in the activated state (Fig. 2.3b), this was counterbalanced by increased population variability during the baseline period (Fig. 2.4c), which could explain the similar performance. In contrast, for 2nd versus no-2nd discrimination, activated responses were both more different on average (Fig. 2.3b), and less variable in the activated state (Fig. 2.4c), consistent with better discrimination.

Variability and discrimination performance in the absence of population correlation

To investigate the extent to which correlated variability contributes to impaired 2nd click discrimination in the inactivated state, we created a shuffled data set, in which correlations between cells were destroyed by randomly reassigning trials independently for each neuron. Fig. 2.5a shows population FFs for the shuffled data, compared with the original data. Shuffling reduced the population FFs in all conditions ($p<0.05$ for all four response windows, in activated and inactivated states), confirming a significant contribution of correlations to population-level variability in this data. Furthermore, after shuffling, population FFs no longer differed between activated and inactivated states ($p>0.1$ for all response windows).

We next assessed the effect of this shuffling manipulation on stimulus reconstruction using discriminant analysis. We reasoned that if shuffling also eliminated the state-dependence of 2nd click discrimination, this would indicate a major contribution of

variability to this phenomenon. No effects of shuffling were found on 1st click discriminability (Fig. 2.5b). Furthermore, while shuffling produced a small improvement in 2nd click discriminability in the activated state ($p<0.05$), it did not eliminate the state-dependence of 2nd click discriminability ($p<0.05$), suggesting that variability did not play a major role in poorer 2nd click discrimination in the inactivated state (Fig. 2.5c).

Responses magnitudes as a function of the phase of the slow oscillation

Although discriminating the fine structure of a complex sound is better in the activated than in the inactivated state (Fig. 2.2e), population activity in the inactivated state is temporally heterogeneous, alternating between upstate periods of excitability and spiking and downstate periods when the network is silent. It is thus conceivable that restricting the analysis of inactivated responses to trials in which the stimuli arrived on upstates would reveal similar performance between these trials and the activated state as a whole. In fact, it has been suggested that the upstate and the activated state are homologous, and that the upstate could be used as a model for the study of the activated cortex (Destexhe et al., 2007; Castro-alamancos, 2009; McCormick et al., 2003). We therefore asked whether upstate responses resembled activated responses in our data, and whether stimuli presented during upstates could be discriminated with accuracy comparable to that found in the activated state. We began by analyzing responses to single click stimuli as a function of the phase of the slow oscillation (PSO), just as we did in the previous chapter (Fig. 1.2), but this time in comparison with activated responses (Fig. 2.6a). 1st click responses in downstate trials (compared to baseline) were significantly larger than

in upstate trials. The same responses in the activated state were more similar to those in upstate than in downstate trials, although they were slightly larger (Fig. 2.6b). The late period of the response (activity in the no-2nd click window compared to baseline) in the activated state was also similar to the late response in upstate trials, showing significant suppression with respect to baseline (Fig. 2.6c). Thus, for single-click responses, upstate responses do indeed resemble activated responses, consisting of a relatively small initial response followed by a period of suppression.

Unlike responses to single clicks, 2nd click responses (relative to no-2nd click activity) were smaller in all phases of the inactivated state, compared with activated state responses (Fig. 2.7; all cases, $p<0.005$). Thus, activated and upstate responses to double clicks were different, with significantly more attenuation in upstate than in activated trials. Presentation of the 1st click in any PSO therefore induces a 'refractory period' during which responses to subsequent clicks are attenuated: whether the 1st click was presented in a downstate and induced a long-lasting response, or was presented in an upstate and induced a period of suppression, presentation of a 2nd click caused only a small increase over this 1st click-induced ongoing activity. In the activated state, however, unlike the upstate, 2nd click responses are visible, or accentuated, on top of the suppression caused by the 1st click.

Response variability as a function of the phase of the slow oscillation

We previously found that trial-to-trial response variability was higher in the inactivated state than in the activated state for 2nd but not 1st click responses (Fig. 2.4). To what

extent does this higher variability reflect the fact that stimuli arrive at different PSOs? To address this question, we computed response variability within each PSO separately (Fig. 2.8). In Chapter 1, we showed that the trial-to-trial baseline variability within each PSO is smaller than in the inactivated state as a whole (Fig. 1.2). Here we further compare variability in upstate trials with variability in the activated state across the different response windows. During baseline, upstate and activated variability were similar (Fig. 2.8a). In contrast, evoked responses during upstates were consistently more variable than activated responses (Fig. 2.8b-d). This points to another difference between activated and upstate dynamics: not only is the cell population more refractory in the upstate, responses are also less reliable, which might further impair the ability of the inactivated cortex to discriminate auditory stimuli with a complex temporal structure.

Stimulus discriminability as a function of the phase of the slow oscillation

Finally, we asked how phase separation would affect discrimination performance. Despite slightly improved performance during downstate trials, discriminability of a sound from silence (1st click from baseline activity) was not statistically different between any PSO and the activated state (Fig. 2.9a). However, discrimination of 2nd click responses from no-2nd click activity was significantly impaired in all PSOs, compared with the activated state (Fig. 2.9b; all cases, $p<0.02$). These findings confirm that despite similarities in single click responses between the activated state and the upstate (Fig. 2.6), differences in 2nd click evoked activity (Fig 2.7) and in trial-to-trial variability (Fig. 2.8) impair RAP in

upstate trials to the same degree as in downstate trials ($p>0.7$, both cases), and the inactivated state as a whole ($p>0.7$, all cases).

7.3. Discussion

In this chapter we characterized the responses of auditory populations to single and double click stimuli in the activated and inactivated states under urethane anesthesia. Our main finding was that neurons in the auditory cortex can 'follow' changes in the acoustic environment more effectively in the activated state. This finding held even if the activated state was only compared with the upstate phase of the slow oscillation. Thus, processing of temporally structured stimuli, such as required in RAP, is significantly state-dependent, and more efficient in the activated state.

Responses to brief stimuli in the activated and inactivated states

In agreement with other studies, (Faneslow and Nicolelis, 1999; Castro-Alamancos 2004a; Crochet and Petersen 2006), we found that cortical responses to brief stimuli during activation were smaller than during inactivation (Fig. 2.3). These results could be explained by differences in the level of synaptic depression across the activated and inactivated states. Since in the activated state thalamocortical synapses are tonically active, they are thought to be constantly suppressed (Castro-Alamancos and Oldford, 2002; Castro-Alamancos 2004a; Otazu et al., 2009, more). This constant suppression reduces the amplitude of response to stimuli in the activated state, relative to the inactivated state in which these synapses are not constantly suppressed due the transient

(bursty) firing mode of the thalamocortical neurons (McCarley et al., 1983; Steriade et al., 1993c; Castro-Alamancos and Oldford, 2002). Even though this difference in synaptic depression might result in overall smaller responses in the activated state, these responses could still be beneficial for stimulus representation if they were associated with an increase in response 'selectivity', i.e., by promoting the responsiveness only of neurons which are best tuned to the stimulus (Wörgötter et al., 1998; Edeline, 2003; Castro-alamancos 2004b; Kohn and Whitsel, 2002). Indeed, other response variables also become 'sharper' in the activated state, such as the size of receptive fields (Wörgötter et al., 1998; Edeline, 2003) and the degree of spread of response across the cortical surface (Castro-Alamancos, 2004b; Petersen et al., 2003). Our results suggest that improved representation might also be found in the activated state despite overall smaller responses, through a reduction in baseline and trial to trial variability (Figs. 2.4 and 2.8).

Upstate versus activated responses to temporally complex stimuli

Recording under urethane anesthesia enabled us to examine the hypothesis that the upstate phase of the slow oscillation is a homologue of the activated state. This hypothesis was supported in the case of evoked responses on single click trials. Suppression of population activity following the 1st click and good 1st click discriminability from baseline, were observed under both conditions (Figs. 2.6 and 2.9). However, even though both the upstate and the activated state are considered to be 'high conductance states', in which neurons are thought to be able respond quickly to transient stimuli (Destexhe et al., 2003), we found that the processing of double click stimuli was

significantly better in the activated state (Figs. 2.7 and 2.9). Thus, despite the physiological similarities observed in these two conditions (depolarized V_m and general excitability), differences in physiological variables such as R_{in} and neuromodulatory influences might affect the ability of the neuronal population to process temporally complex stimuli. Differences in R_{in} could explain the difference in double click responses we observed here: higher R_{in} in the activated state would make cortical neurons relatively more compact, which will increase the efficacy of stimuli, while the relative V_m depolarization will maintain them in an excitable state, facilitating responses to incoming synaptic inputs.

Another significant difference between the upstate and the activated state that may have contributed to better double click discrimination on activated trials is the firing mode of thalamocortical cells. As discussed previously, during the inactivated state, thalamocortical cells exhibit a bursty firing mode, which is thought to limit the temporal frequency of inputs that thalamocortical neurons can relay to the cortex. During the activated state, however, more depolarized thalamocortical neurons firing tonically are able to relay inputs more effectively, thus facilitating cortical responsiveness to high frequency stimuli (Castro-Alamancos and Oldford, 2002; Castro-Alamancos, 2004a).

Thus, even though the upstate and the activated state share certain physiological features and display similar response properties to simple stimuli, these similarities are not sufficient to facilitate the processing of high frequency information by the cortical population during upstates, resulting in overall better RAP performance in the activated state.

8. Chapter 3 - Effect of modulations in the level of activation during wakefulness on rapid auditory processing

8.1. Rationale

Although the main differences in brain state are observed across the wake-sleep cycle (Berger, 1929; Steriade and McCarley, 2005), or under certain anesthetic regimes (Detari and Vanderwolf, 1987; Murakami et al., 2005; Clement et al., 2008), modulations in brain state occur also within the activated state, as a function of behavioral or cognitive variables (Gervasoni et al., 2004; Jung et al., 1997; Crochet and Petersen, 2006; Fontanini and Katz, 2008). These modulations can be assessed by both behavioral (e.g. reaction time) and physiological measurements (e.g. degree of EEG synchronization in different frequency bands). In this chapter I will explore how modulations in the degree of cortical activation during wakefulness affect processing of temporally structured stimuli in the auditory cortex of chronically implanted rats.

Changes in the level alertness, attention or cognitive load, are associated with different patterns of EEG activity, with more attentive states usually corresponding to higher overall desynchronization, and sometimes with oscillatory activity in the high frequencies (Destexhe et al., 1999; Gervasoni et al., 2004; Jung et al., 1997; Fries et al., 2001; Tallon-Baudry et al., 2001; Pesaran et al., 2002). These changes also influence cortical responsiveness to sensory stimuli, albeit in a complex manner. Whereas available evidence suggests that the overall amplitude of sensory responses is higher in quiescent, immobile awake states than in states of active engagement in behavior (Castro-

Alamancos 2004a; Crochet and Petersen 2006), within actively behaving human and non-human subjects, increased attention or alertness generally increases the amplitude of sensory responses (Hubel et al., 1959; Haider, 1964; Eason et al., 1969; Picton et al., 1971; Roelfsema et al., 1998; Fritz et al., 2003; Reynolds and Chelazzi, 2004; Raz and Buhle, 2006). At the same time it has been shown that increased alertness is associated with a lower degree of sensory adaptation (Castro-Alamancos 2004a; Fanselow and Nicolelis, 1999), which suggests that processing of temporally structured stimuli might be better in more attentive and alert states.

Here, we investigated RAP in auditory cortex of the awake, freely moving rat. Even though the rat was consistently awake throughout the recording sessions, it displayed various behaviors such as exploration, grooming, and quiet sitting. Since the most salient feature of inactivated brain states is the existence of large amplitude, low frequency oscillations (Destexhe et al., 1999; Gervasoni et al., 2004), we assessed the degree of cortical activation/inactivation on a trial by trial basis by measuring the power in the low frequencies of multi-unit or LFP recordings in the auditory cortex during a period of a few seconds before the presentation of the sensory stimulus. Although the differences in the degree of activation are smaller than during anesthesia, there was a significant amount of trial-to-trial variability in the degree of cortical activation, which we found to be associated with differences in the effectiveness of the sensory stimuli. Click trains either of 5 Hz, or 20 Hz frequency, evoked consistently higher amplitude responses during more activated periods.

8.2. Specific methods.

Experimental Methods (adapted, with permission, from Otazu et al., 2009)

Animals. All experiments were conducted in a single-walled sound booth (Industrial Acoustics Company). Rats were water deprived under a protocol approved by the Cold Spring Harbor Laboratory Animal Committee (Supplementary Table 1 online). Subjects in all experiments were adult male Long Evans rats (Taconic Farms).

Task versus free conditions. In the task condition, the subject was trained to poke its nose into the center port, thereby triggering the onset of the nontarget stimulus, which consisted of a train of diotic clicks (white-noise bursts, 5-ms duration), followed by the target stimulus. The onset of the train was preceded by a random delay of 400–600 ms. The nontarget stimulus lasted for 1.8 s, after which the target stimulus was presented. The target stimulus consisted of a monoaural, 0.3-s broadband sound, formed by 16 tones between 1 and 16 kHz, that were uniformly distributed in the logarithmic space. The subject remained in the center port until the end of target delivery. The target stimulus indicated the location of the reward port on that trial. Subjects performed a trial every 9.03 ± 0.16 s (mean ± s.e.m.) for ~200 trials per recording session. In the free condition, the three ports were blocked and the same sequence of stimuli was delivered (every 9.37 ± 0.28 s, mean ± s.e.m., ~100 stimulus repetitions before and ~100 stimulus repetitions after the rat performed the task).

Surgery. All procedures were approved by the Cold Spring Harbor Laboratory Animal Committee. Rats were anesthetized with an intraperitoneal injection of a mixture of

ketamine (60 mg per kg of body weight) and medetomidine (0.51 mg per kg). Wounds were infiltrated with lidocaine. For tetrode implants in left auditory cortex, the temporal muscle was recessed and a craniotomy and a durotomy were performed. Electrodes were implanted between 4.5 and 5.0 mm posterior to bregma and 6.4 mm left of the midline. We also attached a plastic ring next to, but not touching, each pinna, which we could use to screw the earphones into place. After surgery, rats were left to recover for several days before resuming water deprivation.

Stimulus delivery. Stimulus was delivered through earphone; on each recording day, an earphone (ER-6i Isolator, Etymotic Research) was screwed into the earphone holder without anesthetizing the rat. The earphone had a soft silicone cover, which allowed us to adjust it in place without causing discomfort to the rat. Sound intensity was determined with a Brüel & Kjær type 4939 free-field microphone, Type 2670 1/4-inch Microphone Preamplifier and Type 2690A0S2 2-Channel Microphone Conditioning Amplifier (Brüel & Kjær Sound & Vibration Measurement A/S) positioned 5 mm in front of the earphone. At this position, the intensity of the chord was 69dB RMS SPL (74 dB SPL peak value) and the click was 76dB RMS SPL (82 dB SPL peak value).

Electrophysiology. We implanted polyimide-coated nichrome wires (H.P. Reid, wire diameter of 12.5 mm) that were twisted in groups of four as tetrodes (each wire was gold plated to o0.5-MΩ impedance at 1 kHz). We implanted six independently movable tetrodes using a custombuilt drive. We recorded spiking activity and LFPs with a Cheetah32 32 Channel System (Neuralynx). For the head-fixed rats, we used a single tungsten electrode (Model TM33C10, World Precision Instruments) with an impedance

of 1 MΩ, amplified using a CyberAmp 380 (Molecular Devices) and recorded using Matlab custom software.

For the multi-unit activity (MUA) analysis, events were included if they exceeded a threshold of 50 mV on any of the four channels of the tetrode.

To obtain LFPs, we filtered the signal from one of the leads of each tetrode or the tungsten electrode used for the head-fixed behavior between 1 Hz and 475 Hz. After acquisition at 3,225 Hz, we applied a high-pass four-pole Butterworth filter (10 Hz). Each day, each tetrode or tungsten electrode was independently advanced until we could observe stable spiking activity.We did not specifically sample for sites that were responsive to our stimulus ensemble. We advanced the tetrodes at least 40 mm every day to avoid having multiple recording sessions with the same subset of cells. We used a skull screw as a ground. We used a nearby nichrome wire as a reference for the tetrode recordings.

Analysis methods

MUA from a given tetrode was selected for further analysis was selected only if the stability criterion described in the general Methods was satisfied.

Activation level measures

The level of activation on each trial was determined by using the power spectrum of the MUA firing rate (calculated with time bins of 1 ms) in a period of 3 s prior to click train onset. Each MUA firing rate trace was first z-transformed by subtracting its mean and dividing by its standard deviation. Activation level was assigned on the basis of the mean

power of the z-transformed MUA firing rate in the frequency range of 0-5 Hz. A similar analysis was conducted for LFP traces, in a 2 s period prior to the onset of click trains. Activation values were positively and significantly correlated across MUA and LFP activation levels. Although most of the analysis in this chapter was done using the activation measure based on MUA activity, similar results were obtained using the measure based on the LFP.

We assessed whether the level of activation on each trial had an effect on sensory responses by comparing sensory responses in two types of trials: more-activated (MA) and less-activated (LA) trials. Since cortical activation is negatively correlated with the level of low frequency power, MA (LA) trials were defined as those in the lower (higher) 30^{th} percentile of the distribution of power in the 0-5 Hz frequency range (Fig 3.1). This classification was applied to each recording session separately.

Local Field Potential (LFP) responses

For each session, the least noisy LFP channel was selected for analysis (the channel with the smallest degree of variance). LFP responses to click trains were averaged across trials, thus producing one 5 Hz click train and one 20 Hz click train LFP response trace per session per trial type (MA or LA). LFP response amplitudes to individual clicks were assessed as the minimum of the LFP trace within a 20 ms window after click presentation.

LFP response adaptation was assessed as the amplitude of each click within the train divided by the amplitude of the first click in that train.

LFP response magnitude was computed relative to the responses on MA trials. Thus, for each session, the amplitude of each click within the train was calculated by dividing by the amplitude of the first click of the MA train in that session.

MUA responses

MUA responses were assessed as the average MUA firing rate across specified response windows. Unless specifically stated, MUA responses were computed from separate tetrodes. When the 'full MUA' was used, the MUA traces from all tetrodes in a single session were merged into a single MUA trace.

8.3. Results

In order to study the effect of modulations in the level of cortical activation within wakefulness on rapid auditory processing (RAP) we used data recorded from awake, chronically implanted rats (courtesy of Gonzalo Otazu, from the laboratory of Anthony M Zador, Cold Spring Harbor; see Otazu et al., 2009). This dataset consisted of MUA and LFP traces collected from 2 animals across a total of 15 recording sessions (6 in one, 9 in the other). On each session, neuronal activity was recorded during two different conditions, the task-condition and the free-condition. During the task-condition, the rat would initiate a trial by inserting its nose into a nose poke port. A variable time after the nose poke (400-600 ms) a click train with a frequency of either 5 Hz, or 20 Hz would play for 1.8 s. The click train had no behavioral significance to the rat. At the offset of the click train a chord was presented to either the left, or the right ear of the rat, indicating the position of the port in which the rat would receive a liquid reward. In the free-condition,

the nose poke and reward ports were obstructed and no specific behavior was expected of the rat while the same stimuli (i.e. a 5/20 Hz click train followed by a chord) were presented with a variable inter-trial interval of 9-9.5 s (for full details, see specific Methods in this chapter).

Assessing the degree of cortical activation during wakefulness

In our analysis of anesthetized data in Chapters 1-2 we used the local density of downstates in 10 s windows as a measure of the degree of cortical inactivation. In this data set, however, this measure was not applicable since the behavioral condition of the rat was awake and mobile, a condition in which downstates are generally absent (see e.g., Destexhe et al., 1999). Therefore, we used the amount of low frequency power in the multi-unit activity (MUA) in order to measure variations in the level of cortical activation across trials. Specifically, we used the mean power in the 0-5 Hz frequency range of the MUA traces measured in a window of 3 s prior to trial onset (similar results were obtained with LFP, see specific Methods on this chapter). Fig. 3.1c shows an example of the distribution of low frequency power across trials in the activated and inactivated states taken from an anesthetized experiment and from an awake recording session of the present dataset (no distinction was made between the free- and task-conditions in this figure). Across sessions, the mean low frequency power for the awake data was significantly smaller than in the activated state, suggesting that, in the awake condition, the local circuit seems to be even more activated than our previous activated data set under urethane anesthesia (Fig. 3.1d). In Fig. 3.1a, four example MUA traces from

anesthetized inactivated and activated periods and from the awake dataset (whose locations in the distributions in Fig 3.1c are indicated by arrows) are shown. The inactivated MUA shows clear up-down transitions, while in all other conditions the MUA looks more desynchronized (power spectra corresponding to these four traces are shown in Fig. 3.1b). Note also that, although there is trial-to-trial variability in the degree of low-frequency synchronization in the awake condition, the magnitude of such variability is much smaller than in the anesthetized condition as a whole, and than the variability in the anesthetized activated and inactivated states taken separately, and this is regardless the fact that the awake data includes both the task- and the free-conditions. Thus, changes in the degree of activation during wakefulness are substantially smaller than under urethane anesthesia.

Distributions of low frequency power in the task- and free- behavioral conditions

In order to determine what was the best condition (task-condition vs. free-condition) to analyze the effect of modulations in the level of cortical activation on sensory processing, we assessed the range of activation values across trials measured in each condition separately (Fig. 3.2). During the task-condition, the rat was consistently engaged in the same behavior, initiating trials and receiving water reward approximately every 3 s. During the free-condition, on the other hand, the rat exhibited a wider range of behaviors (including active exploration, grooming, and quiet sitting). As expected, the condition with a larger range of behaviors also had a wider range of activation levels (Fig. 3.2a-b). Although the two distributions overlapped considerably, the range of activation values

during the task-condition over all sessions was significantly smaller than during the free-condition (Fig. 3.2b, $p<0.05$). Therefore, in order to analyze the effect of brain state on RAP we restricted our analysis to trials from the free-condition only, where the range of activation values was larger. Given that the task- and free-conditions have been shown to be associated with different sensory responses (Otazu et al., 2009), and that this difference in response might be due to factors beyond the overall level of cortical activation in each condition (for instance, overall firing rates and trial-to-trial variability differs between conditions, data not shown), in order to isolate the effect of the level of activation on auditory responses we discarded grouping the two behavioral conditions together.

In all further analyses, we assessed the effect of the level of cortical activation by classifying trials into two types: the more activated (MA) and the less activated (LA) trials. MA (LA) trials were defined as those in the lower (higher) 30^{th} percentile of the distribution of low frequency power (0-5 Hz) across trials. This classification was applied to each session in the free-condition. As an illustration, the average power spectra for LA trials and MA trials in both the free and the task-conditions are shown in Fig. 3.2c for one recording session.

Response adaptation as a function of activation level

Previous studies in rat somatosensory cortex have demonstrated that adaptation to high frequency stimuli is modulated by the behavioral condition of the rat. Specifically, adaptation during quiescent periods was stronger than during alert periods (Castro-

Alamancos, 2004a). In addition, response amplitude to stimuli was negatively correlated with alertness level (Castro-Alamancos, 2004a; Crochet and Petersen, 2006). Since the behavioral state of the rat in the free-condition was unrestricted, in order to establish a link between our MA and LA trials and the alert/quiescent states characterized in these studies, we examined response amplitude and adaptation in our data set (Fig. 3.3), using the peak LFP response (Fig. 3.3a) in a window of 20 ms after click presentation.

We found that both 1st click responses and the responses to most subsequent clicks for both 5 and 20 Hz click trains were significantly larger in MA trials (Fig. 3.3b; stars mark cases with $p<0.05$). Next, adaptation was assessed as the ratio between the response to each click in the train and the response to the 1st. We found no significant difference between the degree of adaptation in MA and LA trials (Fig. 3.3c). Thus, although response magnitude seems to correlate positively with the degree of activation prior to stimulus onset, the dynamics of the peak responses to temporally structured stimuli is similar for MA and LA trials. If our MA and LA trials were representative of the alert and quiescent data described in Castro-Alamancos (2004a) one would expect that, at frequencies of 5 and 20 Hz, adaptation should be significantly larger in LA trials, and so should 1st stimulus responses. The fact that we do not see this suggests that our MA/LA distinction does not correspond to the alert/quiescent distinction made by Castro-Alamancos (2004a) and that our results might be better understood within the context of attention studies conducted in the sensory cortices of other species (Hubel et al., 1959; Haider, 1964; Eason et al., 1969; Picton et al., 1971; Fritz et al., 2003), which generally report larger response amplitude as the animals' attentional focus increases (see

Discussion). Nevertheless, the previous analysis shows that there are clear differences in sensory responses between MA and LA trials (Fig. 3.3b) and that, therefore, this distinction is appropriate for studying how the instantaneous level of activation of the cortex affects RAP during wakefulness. To this effect, similarly to the approach we took in the previous chapters, we first analyzed spiking responses to single stimuli, and then to later components of the click train.

Effect activation level on 1st click responses

We found that responses to clicks during wakefulness are sometimes very transient, lasting only a few ms (Fig. 3.4a), which is significantly shorter than the 50 ms response windows we used in Chapters 1-2. Therefore, in order to make sure that click-evoked responses were not "washed away" by counting spikes after the neurons firing rate had gone back to baseline, responses to the 1st click were quantified as the mean MUA rate measured from individual tetrodes at three different response window sizes: 6, 20 and 50 ms, all beginning 6 ms after 1st click onset (i.e. 6 to 12 ms, 6 to 26 ms and 6 to 56 ms). Baseline activity was also measured at complementary windows, in the period immediately preceding the 1st click response period (i.e. 0 to 6 ms, -14 to 6 ms, and –44 to 6 ms). Consistent with our previous LFP results (Fig 3.3b), 1st click response magnitude was larger for MA trials (Fig. 3.4b). Since responses were very transient, this difference reached significance only in the 6 ms window size ($p<0.05$, other cases, $p>0.3$).

To investigate whether this difference in average response led to better discriminability of the 1st click from silence on a trial-by-trial basis, we again tried to predict the stimulus from the simultaneously recorded MUAs from several tetrodes (2-5 tetrodes per session) using linear discriminant analysis (LDA, see Methods). Discrimination between 1st click responses and baseline activity was significantly better in the MA than in LA trials for all response windows (Fig. 3.4c, $p<0.01$, all cases). These results suggest that the ability of downstream targets to detect the presentation of a single click is better when the instantaneous state of the cortex is more activated.

Since the shortest response window (6 ms) reflects the difference between the MA and LA trials best, from now on results will only be presented for the 6 ms response window (results obtained considering longer response windows were not different).

Effect of activation level on responses to successive clicks

We assessed the quality of RAP by quantifying the responses to successive clicks of the 20 Hz click train. Studying responses to multiple clicks enabled us to examine two types of response dynamics: one that occurs on a short time scale right after the onset of the click train, and one that occurs on a longer time scale, once stimulus responses have reached a steady state.

In order to study RAP on a short time scale, we examined responses to the 2nd click (Fig. 3.5a; spike count in a window 56 to 62 ms after stimulus onset). We found that 2nd click responses between MA and LA trials were not significantly different. All average response measures: 2nd click response, no-2nd click response (obtained from responses to

the 5 Hz click trains) and their relative magnitude (2nd click – no-2nd click), were not significantly different (Fig. 3.5b, all cases, $p>0.8$). Given that the presence or absence of a 2nd click evoked similar average responses in MA and LA trials, it is to be expected that the presence or absence of a 2nd click would be equally discriminable in the two trial types as well. Indeed, we found that discrimination between 2nd click and no-2nd click responses was not significantly different LA and MA trials (Fig. 3.5c, LA 73±11%, MA 74±3%, $p>0.8$).

Even though 2nd click responses were not significantly different between MA and LA trials (Fig. 3.5), our analysis of LFP responses (Fig. 3.3) suggests that responses to subsequent click stimuli might be stronger in MA trials. In Fig. 3.6a-b, an example of averaged MUA responses to a 20 Hz click train from one tetrode in one recording session is shown. Notice that even though peak MUA responses do not appear different, inter-click activity is more suppressed in MA trials. This effect was quantified by measuring click evoked rates, and pre-click baseline activity in 6 ms windows for every click stimulus in the train, beginning with the third click. We present results obtained using the responses of the full MUA activity (merged activity of all tetrodes in one session, see specific Methods in this chapter. The same results were obtained using responses from single tetrodes, data not shown). We found that in 13/15 sessions, pre-click baseline was significantly higher in LA trials (Fig. 3.6c; full dots represent sessions with $p<0.05$). Click responses, however, were not different between the two trial types (Fig. 3.6d; 6/15 sessions were significantly larger in LA trials, 4/15 were significantly larger in MA trials and 5/15 did not vary significantly across the two classes). Importantly, click responses

compared to pre-click baseline were significantly higher on average in MA trials (Fig. 3.6e; 11/15 sessions). Thus, although the firing rate of the neurons in both types of trials was similar after the stimulus, the change in firing rate due to the stimulus, i.e., the effectiveness of the stimulus in eliciting activity from the neuronal population, was larger in MA trials. Interestingly, this suggests that the LFP response (Fig. 3.3) is a better predictor of the effectiveness of the stimulus in driving the cells (i.e., of the difference between evoked and baseline activity) than of the actual evoked response it elicits from the population (Fig. 3.6d).

We confirmed that processing of the steady-state responses was more effective in MA trials by training a classifier (using LDA) to discriminate between pre-click activity and click-evoked responses. As expected, in 11/15 sessions, discrimination was better in the MA than in LA trials (Fig. 6f). Thus, it is easier to detect the presence or absence of both the 1st click (Fig. 3.4) and of clicks during the steady state response (Fig. 3.6) in trials during the free-condition in which the level of cortical activation prior to stimulus onset was higher.

8.4. Discussion

The results we obtained in Chapter 2 strongly suggested that RAP might be more efficient during states with a higher degree of cortical activation. The data in Chapter 2, however, was obtained from an anesthetized preparation, and the general motivation in this Thesis was to investigate whether improvements in RAP after training might be associated with attention-related changes in the level of cortical activation. A more rigorous test of this

hypothesis requires examination of data from the awake preparation, which we undertook in this chapter by analyzing multi-unit activity from the auditory cortex of chronically implanted rats (Otazu et al., 2009).

Rats in the awake condition display an overall activated brain state

First, we established how the level of activation in the awake condition compares with the different brain states we observed under anesthesia. Profound changes in the dynamics of cortical networks have been reported during wakefulness. In a series of recent studies, for instance, Petersen and colleagues (Crochet and Petersen, 2006; Poulet and Petersen, 2008) have established that in the somatosensory cortex of awake mice, large, low-frequency fluctuations (typical of the inactivated anesthetized preparation) in membrane potential are observed during quiet immobility, and are replaced by less coherent and faster fluctuations as the mice begin to whisk. It is thus possible to observe both urethane-like activated and inactivated states during wakefulness. Nevertheless, we found that the auditory cortex of the rats from Otazu et al. (2009) was more activated than the anesthetized activated state we analyzed in Chapter 2 (Fig. 3.1). Indeed, at least extracellularly, we did not observe the type of large, low frequency fluctuations during wakefulness that have been reported in the somatosensory cortex (Crochet and Petersen, 2006; Poulet and Petersen, 2008). Nor did we observe periods of generalized silence (downstates) in the free-condition, unlike what was found (in intracellular recordings) during non-whisking periods in the studies of Petersen and colleagues (Crochet and Petersen, 2006; Poulet and Petersen, 2008) and in the auditory cortex (DeWeese and

Zador, 2006). In addition, we found responses in MA trials were generally larger than in LA trials, but adapted to the same extent, (Fig. 3.3), whereas in the somatosensory cortex responses in the quiescent state tended to be larger and to adapt more strongly than in the alert state, especially if the alert state involved whisking (Fanselow and Nicolelis, 1999; Castro-Alamancos, 2004a). This difference in activation levels across studies might be due to the fact that, even though the behavior of the rats in the free-condition in Otazu et al. (2009) was relatively uncontrolled, the rat was mostly active: it moved around the cage, groomed, etc, and was not allowed remain motionless for a period of more than several seconds. Our results on the differences between responses in MA and LA trials should not, therefore, be interpreted in the context of differences between responses during quiescence versus alertness.

Activation levels differed across the task and free-conditions

We restricted our analysis to trials in the free-condition, in which the rats were not performing the auditory localization task, because we observed a larger range of activation values in this condition (Fig. 3.2). Interestingly, the distribution of activation values between the two conditions differed primarily in the existence of a subset of trials in the free-condition displaying larger activation levels than any trial in the task-condition (Fig. 3.2a). This subset of trials biases the distribution of activation values making the overall level of activation in the free- larger than in the engaged condition. It is difficult to interpret this result without detailed access to the behavior of the animal. One possible interpretation is that the behavioral state of the animal in the free-condition is more

"heterogeneous", and includes periods of rest/immobility (although these would not be very long, as stimulus presentations following more than 5 s of immobility were excluded from further analysis) together with periods of active exploration of the experimental apparatus (the data I analyzed does not include head-fixed experiments in Otazu et al., 2009). Although a detailed description of the behavior of the animal during the free condition is not provided in (Otazu et al., 2009), this interpretation is supported by their Fig. S1a, where it is shown that the distribution of overall movement of the animal (pixel difference between images sampled at 3 Hz) has heavier tails in the free- compared to the engaged condition. It is therefore possible that MA trials in the free condition correspond to trials where the "pixel difference" in Fig. S1a in Otazu et al., 2009 was higher. It would be interesting to know whether the distribution of activation values in the head fixed experiments in Otazu et al., 2009 follows the same pattern as the one I have described here.

It might have been expected that trials during the engaged condition would display the highest level of activation, given that the animal should presumably be more "attentive" during performance of the task, and that attention has been associated with desynchronization of the EEG. However, the sound localization task is relatively easy and probably involves a relatively mild attentional load. In one of their control experiments, however, Otazu et al., 2009 used a more difficult, and presumably attention-demanding task. Although they reported that sensory responses were still suppressed during performance of this task compared to the passive condition, it would be interesting to explicitly compare whether the overall level of activation in this more difficult task

was higher than during the easier sound localization task, and how it compared to more activated trials during the free condition.

Higher levels of activation were associated with larger response amplitude, and better stimulus representation

A higher degree of activation was associated with larger initial and steady state LFP responses (Fig. 3.3) and with larger initial and steady state evoked-versus-baseline spiking responses (Fig. 3.6e-f). These larger steady state spiking responses were due to a difference in baseline pre-click activity between MA and LA trials (Fig. 3.6c). Although these findings suggest that processing of temporally structured stimuli was more efficient in MA trials, we did not observe any difference in the responses to the 2nd click of the train (Fig. 3.5) between MA and LA trials, which was unlike our findings for steady state responses (Fig. 3.6) and for the anesthetized data (Chapter 2). However, responses to the 2nd click in the awake dataset seem, in this respect, exceptional, as they are different from the trend observed both for the 1st (Fig. 3.4) and for steady state responses (Fig 3.6). Looking closely at Figs. 3.5a and 3.6a, it is apparent that the response to the 1st click is 'extended', and does not decay to the same baseline level in 50 ms as the responses to subsequent clicks. The response to the 2nd click, which rides on top of this late phase of the 1st click response appears to be independent of the level of activation prior to click-train onset.

These results appear at odds with previous studies suggesting that "active" behavioral conditions (characterized by higher levels of activation) are associated to suppressed

sensory responses when compared with “quiescent” behavioral conditions, characterized by lower levels of activation. However, at least in the data-sets that I analyzed, the free condition is not associated to inactivated MUA patterns. In fact, the trials with the least degree of activation in the free condition were more activated than the trials with the least degree of activation in the engaged condition (Fig. 3.2a). During the slow oscillation under urethane anesthesia, the same measure (MUA power under 5 Hz) reveals much lower degrees of activation than during the free condition, further suggesting that the free condition is not associated with the types of inactivated patterns typical of quiescence/ immobility (Buzsaki et al., 1988; Gervasoni et al., 2004).

It is tempting to speculate on why the MA trials during the free condition are associated to enhanced sensory responses compared to LA trials. One possibility would be to assume that MA trials correspond to situations where the rat was paying more attention to its acoustic environment, in which case enhanced responses during MA trials might reflect previously reported attentional enhancements (for reviews see e.g., Raz and Buhle, 2006; Reynolds and Chelazzi, 2004). However, Otazu et al., 2009 still saw suppression in control experiments where the auditory task was more difficult, presumably requiring higher levels of attention, and also saw the same amount of suppression regardless of what sensory modality (auditory vs. olfactory) this attention was directed to, demonstrating that the types of manipulations of attention involved in these control experiments still don’t lead to an overall enhancement of auditory responses.

The difficulty in interpreting these results probably comes from an over-simplified picture of our characterization of the animal’s level of engagement and attentional state,

of their relationship to the level of desynchronization of the EEG and of how the two previous variables affect sensory processing. In order to specify better these relationships it will be helpful to conduct future studies where, in addition to physiological measures of brain activity, one will have the maximum possible control over the behavioral and cognitive state of the animal.

As a whole, the results presented in this chapter suggest that small differences in the instantaneous level of cortical activation during wakefulness are sufficient to evoke small but reliable differences in auditory responses, and that these differences are such that temporally structured auditory stimuli are slightly but consistently easier to detect when the cortex is more activated prior to stimulus onset, from the activity of the neuronal populations in the auditory cortex. These results are consistent with selective attention studies, which generally found, first, larger sensory responses as the overall level of attention of the subject increased (e.g. Eason et al., 1969; Picton et al., 1971; Hubel et al., 1959; Haider, 1964; Miller et al, 1972; for reviews see Raz and Buhle, 2006; Hromádka and Zador, 2007) or as attention was directed towards the spatial location or stimulus features in the receptive field of the recorded neurons (for reviews see e.g., Reynolds and Chelazzi, 2004; Maunsell and Treue, 2006; Fritz et al., 2007) and second, that attention is positively correlated with cortical activation (Fries et al., 2001; Tallon-Baudry et al., 2001; Pesaran et al., 2002; Jung et al., 1997; Halgren et al., 1978), but seem at odds with studies relating sensory responses to alertness/arousal (Fanselow and Nicolelis, 1999; Castro-Alamancos 2004a; Crochet and Petersen 2006; Otazu et al., 2009; see previous paragraph).

9. General Discussion and Future work

The overall theme of this Thesis has been the investigation of how brain state affects auditory processing of temporally structured stimuli. This research program was motivated by two observations. First, it is possible to improve rapid auditory processing (RAP) in children with specific language impairment through training with tasks designed to engage the children's attention (Merzenich et al., 1996; Tallal et al., 1996), and second, increased attention has generally been be associated with a higher degree of cortical activation (Fries et al., 2001; Tallon-Baudry et al., 2001; Pesaran et al., 2002; Jung et al., 1997; Halgren et al., 1978). These observations led us to hypothesize that processing of temporally structured stimuli by neuronal populations in the auditory cortex might be more efficient with increased levels of cortical activation. This hypothesis was investigated by making recordings of simultaneous neuronal populations in the auditory cortex in the rat in different brain states, initially under urethane anesthesia, and finally by analyzing previously collected data from awake rats. We assessed the efficiency of RAP by estimating how well single clicks could be discriminated from contiguously presented click pairs (the same type of stimuli used to assess RAP in human subjects) based on the activity of the recorded neuronal populations.

Our results generally validated our original hypothesis: RAP was significantly more efficient in the activated than the inactivated state under urethane anesthesia. Moreover, we found that RAP in the activated state was better than in the inactivated state even after separating trials in the inactivated state according to the phase of the slow oscillation on

which the stimulus arrived, and focusing on upstate responses (Chapter 2). Interestingly, within the inactivated state, while the phase of the slow oscillation had a clear effect on cortical responses to brief stimuli, cortical processing of temporally structured stimuli was similarly impaired across all phases (Chapter 1). During wakefulness, the pattern of results was more complicated, but pointed to a higher efficacy of rapidly changing auditory stimuli to evoke discriminable responses during trials in which the cortex was more activated (Chapter 3).

9.1. Addressing experimentally the "activation" hypothesis for training-induced changes in RAP

Because we found that the discriminability of temporally structured stimuli (RAP performance) was positively correlated with the level of activation, and given that improvements in RAP occur in SLI children after auditory training (Tallal et al., 1996; Merzenich et al., 1996), our results suggest that a possible mechanism for this training-induced changes could be that training is associated with changes in the level of brain activation of the subjects.

An experimental paradigm designed to address this "activation" hypothesis for training-induced changes in RAP should include: (1) A training paradigm through which subjects (controls/impaired) improve their performance on an auditory task requiring RAP, and (2) recordings of physiological variables allowing the characterization of the "state" of brain regions of interest (ideally also of the simultaneous activity of populations of single neurons) during performance of this task. Furthermore, the results of these experiments

should show that (a) the level of cortical activation after training (either overall during task performance or during stimulus presentation) increased relative to the that measured in the same period before training, and (b) physiological measures of the efficiency of processing of temporally modulated stimuli (such as the discriminability measure I used in my thesis) should increase in par with performance in the task.

In chapters 1 and 2, we assessed the effect of brain state on sensory responses under anesthesia, providing evidence in favor of point (b). On chapter 3, we did the same thing during wakefulness, but there was no training, and the recordings were obtained in conditions in which RAP was not behaviorally relevant (Otazu et al., 2009). Thus, whereas our results demonstrate that discriminability of temporally modulated auditory stimuli correlates positively with the level of cortical activation, our design cannot address whether training-induced changes in the level of cortical activation are responsible for improvement of RAP in SLI children.

In order to assess whether levels of brain activation increase in children who suffer from SLI as a result of training, one could record EEG activity from these children throughout the course of training. The level of activation in brain regions of interest, e.g., the auditory cortex, would be quantified by the absence of low-frequency power in the EEG signal, as I did in Chapter 3, and/or by increases in power in the gamma-range. Activation levels could be analyzed in various ways: overall activation across training sessions, activation during passive listening to temporally structured auditory stimuli before and after training, activation as a function of trial-by-trial performance, and activation during different periods within the task, for example during the waiting period, the stimulus-

presentation period or the decision and execution period. Also, the amplitude of evoked responses as a function of the level of activation and performance could be assessed under all of the above conditions.

Animal experiments using appropriate behavioral tasks could be used to explore the relationship between neural representations (at the single cell or population level), brain state, and RAP. The paired-pulse inhibition (PPI) paradigm (Hoffman and Ison, 1980; Fendt et al., 2001; Swerdlow et al., 2001; see also section 1.1) could be used to investigate this problem. In the PPI paradigm, a startle response induced by a startling stimulus (e.g., a loud sound) is inhibited if the startling stimulus is preceded by a perceived neutral stimulus. The presence or absence of the startle response when properties of the neutral stimulus are varied is used as a measure of whether these properties are perceived by the animal. When the PPI paradigm is used to study RAP, the neutral stimulus (which could be a tone pair (e.g., high-low) with a short ISI) is a deviant and the standard is another tone pair (e.g., low-high). If the animal can perceive the difference between the two tone pairs, the deviant inhibits the startle response (for a review see Fitch et al., 2008). In order to study the effect of brain state on RAP using this procedure, the neutral stimulus could be presented in different behavioral contexts. For instance, if many consecutive trials of the PPI task used, the behavioral context will be "task engaged" which will (depending on the difficulty of the discrimination) presumably engage attention. On the other hand, the auditory stimuli could also be presented outside the context of a session (e.g., during free exploration in the cage, during drowsiness, etc…). Measuring behavioral (presence/absence of a startle response) and physiological

responses (EEG, LFP, unit/population activity) in these different conditions would allow assessment of the extent to which different behavioral states and their corresponding brain states, affect RAP performance and the activity of neuronal populations in the auditory cortex.

In the previous experiment, differences in behavioral/brain state occur spontaneously. One could also directly manipulate brain state pharmacologically.

For example, application of acetylcholine antagonists such as scopolamine suppresses cortical activation in cats (Buzsaki et al., 1988; Metherate and Ashe, 1992; Steriade et al., 1993e). Thus, application of scopolamine specifically to auditory cortex during performance of the RAP-PPI task could be used in order to observe whether reduction in cortical activation levels alters the ability of the animal to discriminate the deviant from the standard (this should be done with simultaneous physiological recordings to insure that the antagonist is effective at inactivating the cortex). Another possible intervention could be to stimulate the nucleus basalis (NB) during performance of the task. NB stimulation is an established method for brain activation (Buzsaki et al., 1988; Metherate et al., 1992; Steriade et al., 1993e). One could use NB stimulation at specific times during the trial to test the effect of increased brain activation on task performance.

A more direct link with the human studies could be established by using animals with induced microgyria, an animal model for SLI and dyslexia (Humphreys et al., 1991; Rosen et al., 1992), in behavioral tasks requiring RAP, such as the one described above. Fitch and colleagues demonstrated that rats with induced microgyria are impaired in a go/no-go auditory discrimination task (Fitch et al.,1994; Fitch et al., 1997a; Herman et al.,

1997; Clark et al., 2000, also see section 1.2). This animal model is particularly strong since, similarly to SLI children, auditory training ameliorates RAP deficits in these animals. (Threlkeld et al., 2009).

9.2. The validity of urethane-activation as a model of activation during alert-wakefulness

Global patterns of brain activity vary during wakefulness, with behavioral alert/attentive/ information-processing conditions displaying "activated" dynamics, characterized by tonic activity and absence, or significant reduction, of large-amplitude, low frequency EEG, LFP or MUA fluctuations when compared to passive/quiescent/quiet-immobility behavioral conditions (Buzsaki et al., 1988; Gervasoni et al., 2004; Castro-Alamancos 2004a; Crochet and Petersen, 2006; Poulet and Petersen, 2008).

Although more complex, these changes are analogous to those observed during the sleep cycle. Thus, REM sleep is similar, at least at the level of EEG and related measures of global activity, to alert awake conditions, and correspondingly SWS is similar to quiescent /immobile awake conditions.

The global changes that occur across the sleep cycle, also occur under urethane anesthesia. Under urethane, as opposed to most anesthetics, spontaneous transitions between periods of activation and inactivation occur regularly (Vanderwolf 1969; Steriade et al., 1993d; Destexhe et al., 1999; Steriade 1999; Steriade et al., 2001; Gervasoni et al., 2004; Détári and Vanderwolf, 1987; Murakami et al. 2005; Clement et al., 2008).

This concomitant desynchronization of the EEG (and related measures of activity) is the basis for the urethane model of awake activation.

In addition to the similarity of spontaneous global activity fluctuations between the activated/inactivated states under urethane and the alert/quiescent conditions, processing of sensory information across these different states under urethane mirrors differences observed across quiescence and alertness in the un-anesthetized rat somatosensory cortex (Castro-Alamancos, 2004a). Specifically, in this study, results obtained across the inactivated/activated states under urethane anesthesia were later found to hold in the quiescent/alert behavioral conditions. Importantly for the purposes of my study, the equivalence between urethane and awake activated states extended to the processing of temporally structured stimuli (trains of whisker deflections).

Activation during wakefulness and under urethane differ, however, in their pattern of neuromodulation. Multiple loci and neuromodulators are involved in the alert-awake condition (Dringenberg and Vanderwolf, 1998), such as the nucleus basalis (acetylcholine), the raphe nuclei (serotonin), the locus coeruleus (noradrenaline). The activity of these different nuclei is modulated by specific behavioral contexts that can vary on a fast time scale, thus the substrates of awake cortical activation and the neuromodulators that cortical neurons are exposed to can change rapidly. The activity of neurons of the locus coeruleus, for instance, increases specifically in situations where the animal is highly alert and attentive, such as during the performance of a demanding task (e.g., Aston-Jones et al., 1991). These fast changes in neuromodulatory levels and composition change response properties of single neurons through changes in their

physiology (McCormick 1992). These changes are not additive (i.e., more alertness does not result in higher firing rate), but are more complex. For example, noradrenaline reduces baseline firing rate and increases response amplitude, while acetylcholine increases both spontaneous and evoked activity levels (Foote et al., 1975; Waterhouse and Woodward, 1980; Kasamatsu and Heggelund, 1982; Manunta and Edeline, 1997; Edeline, 2003; Hirata et al., 2006).

Brain activation under urethane, however, occurs exclusively through acetylcholine modulation, in a similar way to activation during REM (Clement et al., 2008). This was thoroughly demonstrated by Clement and colleagues (2008) by the use of both pharmacological and physiological approaches. First, application of acetylcholinesterase inhibitor promoted long lasting activation, as did application of muscarinic agonists. Correspondingly, acetylcholine antagonists produced long lasting inactivation. Next, in order to verify that the monoaminergic systems that are involved in awake activation, but not in REM activation are not involved in urethane-induced activation, the monoaminergic vesicular stores were depleted by the use of reserpine. Even though this manipulation was effective at inducing the symptoms related to monoaminergic deficits during wakefulness, under urethane anesthesia the cycle of brain inactivation and REM-like activation was not disrupted. Finally, infusion of lidocaine to the forebrain area suppressed the spontaneous occurrence of periods of brain activation, thus identifying the nucleus basalis as the locus responsible for cortical activation.

Since activation under urethane and during REM sleep both occur through acetylcholine, differences in sensory processing between wakefulness and REM may be applied to

activation during urethane. In humans, the major difference in cortical responses during REM sleep and wakefulness occurs in late response components: early responses are processed similarly under both states (human < 20 ms), but later response components are relatively suppressed (Pare and Llinas, 1995; Kahn et al., 1997). Also, during wakefulness sensory stimuli can induce gamma coherence in different regions (Ribary et al., 1991; Llinas and Ribary, 1993). During REM, even though stimuli can reset gamma, they do not induce coherence across different regions (Llinas and Ribary, 1993). On the other hand, certain similarities also exist in features of sensory processing across REM sleep and wakefulness. For instance, features such as receptive field size and response latency are preserved across these conditions in guinea pig auditory cortex (Edeline et al., 2001).

To summarize, activation under urethane and wakefulness show similarities, mainly at the level of global patterns of EEG activity, and differences, mainly in their neuromodulatory content. Nevertheless, although these differences suggest caution when extrapolating results obtained under urethane activation to the alert-awake condition, one should keep in mind that urethane activation is a significant step forward in the search for an anesthetized model of information-processing states, as compared to the inactivated state obtained under urethane and other anesthetics.

9.3. Caveats associated to data analysis

My conclusions regarding the “quality” of representation of temporally structured stimuli in the auditory cortex were based on a classification-prediction method. In particular, I

trained a classifier to distinguish between the population responses to two types of stimuli (in my case these were almost always the activity evoked by a 2nd click and the activity in the same time window when no 2nd click was presented) using some fraction of the data, and then uses this classifier to predict which type of stimuli was present on each trial in the remaining fraction of the data. The logic of this analysis is that, if the two types of stimuli are easily differentiable by a simple classifier, then it is presumed that they will also be easy to distinguish by networks downstream from the area one records.

Given that we don't know the algorithms that these downstream networks use to "read out" the activity we are recording, one should be careful in relying too much on any specific read-out, or classification method. The safest way to deal with this problem is to use a diversity of classification methods, and to make sure that they all agree qualitatively. If different methods lead to different results, then the conclusion of each analysis reveals properties of the method. If this is the case, the conclusions are necessarily weakened, given our ignorance about the actual methods used by the brain.

The use of a single method, however, would not be too problematic if this method is very simple/general, the assumption being that if a very simple method is able to reveal meaningful differences, more sophisticated methods will as well, including whichever methods are used by downstream networks.

I used Linear Discriminant Analysis (LDA, see e.g., Hastie et al. 2001) with cross-validation as my classification-prediction method. Using LDA, I found that population activity was more "different" (more easily classifiable) in the 2nd click and no-2nd click periods (Chapter 2) and in the evoked and baseline periods within a click train (Chapter

3) for higher levels of cortical activation. LDA is a standard linear classification method. It finds hyper-planes (planes in more than two-dimensions), and assigns data points to a given category depending on where it falls between these hyper-planes. The hyper-planes are chosen so that the variance of the data within each category is minimal, and the variance of the data across categories is maximal. This method is optimal if the underlying distributions of data in each category are Gaussian and have the same covariance matrix, but it can still be used if these conditions are not met. I used LDA because it is the simplest method that uses the covariance of the data, i.e., it takes advantage of the fact that the activity of multiple neurons is recorded simultaneously. This is important because, in principle, classification performance can strongly depend on the covariance of the data.

There are two caveats to consider when using LDA. First, because LDA requires estimating the covariance of the data (in my case, how correlated the spike counts of different neurons are in each condition), it requires a significant amount of trials or high firing rates. If not enough data is available to estimate the covariance matrix reliably, large numerical errors can occur. To avoid this problem, I used a regularized version of LDA, in which one replaces the covariance matrix Σ of the data by $(1-\lambda)\Sigma + \lambda\overline{V}I$ where $\overline{V}$ is the average variance (i.e. the average of the diagonal elements of the covariance matrix), I is the identity matrix, and λ is a regularization parameter. This is equivalent to setting a threshold on the magnitude of the different principal components of the covariance matrix. If the principal component is much larger than λ, it is effectively unchanged by this procedure. If the principal component is much smaller than

λ, which would make it very difficult to estimate it from the data, it is effectively replaced by λ. I found that except for $\lambda = 0$, the value of λ did not significantly alter my results, so I did not vary it, and set it equal to $\lambda = 0.1$ everywhere.

Second, because my data is very high-dimensional (each data point has a number of dimensions equal to the number of single neurons), there is the danger of over-fitting. In short, when the data is very high-dimensional, for any given data set it is possible to find a hyper-plane which successfully separates arbitrarily defined categories even if these categories are not really there. I avoided this problem by using cross-validation. In this procedure, the classifier is trained (hyper-planes are defined) based only on a subset of the data (the training set), and performance is assessed based on the remaining data. If cross-validation is used, classification performance does not get better simply by using high-dimensional data.

The LDA method is particularly simple because it is linear and because it only requires fitting of a single covariance matrix (all categories are assumed to have the same covariance). Many more complicated methods can be used. For instance, non-linear methods find non-linear boundaries (hyper-surfaces) between categories. Other methods try to fit higher-order statistical properties of the data. These methods, however, being more complex, require more data to be specified appropriately.

Although we do not know which "read-out" method is used by neural circuits in the brain, LDA is effectively finding linear combinations of the spike counts from each neuron which are useful for distinguishing responses to different stimuli, a computation that could, for instance, be carried away by a single neuron post-synaptic to the

population I recorded with an appropriate set of synaptic weights. These weights would depend on the covariance of the neuronal responses. Although estimating the covariance, or correlation, of the spike counts of different cells is not a trivial computation, the results of my analysis in Chapter 2 (Fig. 2.5c) show that 2nd click and no-2nd click activity are still easily separable even if neuronal correlations are artificially removed, suggesting that estimation of the covariance of the data is not necessary for finding a linear combination of the population responses which successfully separates 2nd click and no-2nd click activity.

A final caveat is that, although LDA was not able to easily distinguish 2nd click from no-2nd click activity under cortical inactivation, this does not preclude that more sophisticated methods would. Whereas it is conceivable that one could find a method able to distinguish with little error 2nd-click from no-2nd click activity in the inactivated state, the fact that LDA cannot would make that result method-dependent and thus weaker. Thus, our analysis, while not univocally showing that downstream networks are able to better decode the identity of the stimulus under cortical activation than under inactivation, convincingly shows that such decoding under cortical activation is easier.

9.4. Functional role of different brain states

The major changes in brain state occur throughout the sleep cycle (Berger, 1929; Steriade and McCarley, 2005), during which the brain is not using sensory stimuli to guide behavior. During wakefulness, changes are not so pronounced, but brain state also varies. In behavioral states where the animal is alert, attentive, or generally engaged in a

behavioral task, the large-amplitude low frequency oscillations seen during slow wave sleep (SWS) are generally abolished. In the neocortex they are replaced with broad band desynchronization, or with more spatially confined synchrony in the gamma frequency range (Steriade et al., 1993d; Steriade et al., 1996; Destexhe et al., 1999; Buzsaki et al., 1988; Gervasoni et al., 2004). In the hippocampus, oscillations are prominent during different behaviors, and, in particular, active exploration is associated with strong rhythmicity in the theta (and gamma) ranges (see e.g., Buzsaki, 2006). SWS-like patterns, however, have been seen in sensory cortices during quiet wakefulness or immobility (Gervasoni et al., 2004; DeWeese and Zador, 2006; Crochet and Petersen, 2006; Luczak et al, 2007; Poulet and Petersen, 2008; Luczak et al., 2009). The functional role of these large, coordinated fluctuations in activity during wakefulness is unknown. During SWS, they have been suggested to be involved in memory consolidation through the replay of sequences of neural activity experienced during wakefulness (Wilson and McNaughton, 1994; Nadasdy et al, 1999; Ji and Wilson, 2006; Euston et al., 2007; Peyrache et al., 2009). Consistent with this role, sequences of spiking across neuronal populations have been observed in the auditory cortex during upstate onsets under anesthesia and during the quiet awake condition (Luczak et al., 2007; Luczak et al., 2009), suggesting that perhaps functions related to memory consolidation already take place during the awake state when the cortex is not engaged in the active processing of sensory information. Alternatively, the cortex could switch into this inactivated pattern for metabolic reasons. Synaptic activity is costly from an energetic point of view (see e.g., Lauritzen, 2001) and it might be efficient to transiently ‘turn off’ a network if the animal is not processing

information with it, even during wakefulness. Related to this idea, the exact spatial extent of the inactivated pattern during wakefulness is not known. Although during anesthesia the slow oscillation seems to be a fairly global phenomenon (Steriade et al., 1993a-c; Isomura et al., 2006), less evidence is available on this issue during the awake state. Large waves of activity were seen to propagate across the somatosensoy cortex in awake mice (Ferezou et al., 2006), which suggests that they could be a global cortical phenomenon, but the fact that this pattern of activity is readily abolished by whisking (Crochet and Petersen, 2006; Poulet and Petersen, 2008) suggests that it might be local to the somatosensory system. Since the degree of activation of a local circuit can be assessed extracellularly, it would be interesting to record simultaneously from several sensory areas during a behavioral task requiring multi-modal sensory processing, to investigate the extent to which the degree of activation is coherent across different sensory areas.

Similarly, it is interesting to ask why attentive processing takes place in a more desynchronized pattern characterized by tonic spiking. Again, the fact that tonic synaptic activity is metabolically expensive suggests that there probably is an advantage to this mode of operation of the cortical circuit for information processing. One possibility is that in a more desynchronized state the local cortical circuit operates more ‘linearly’ and is able to track changes in synaptic input more faithfully (Curto et al., 2009). Network states characterized by global tonic synaptic activity, i.e., ‘high conductance states’, have also been suggested to be able to track synaptic inputs more efficiently due to an overall reduction in the effective time constant of the neurons (Destexhe et al., 2003), and to the

fact that there is always a small fraction of neurons close to threshold and ready to fire (Tsodyks and Sejnowski, 1995; VanVreeswijk and Sompolinsky, 1996; Silberberg et al., 2004). According to this general view, when the network operates in a more activated regime, it 'listens' more closely to long-range inputs (thalamocortical or from other cortical areas), whereas more inactivated regimes would be present for internal functions of the local circuits which do not require external information.

9.5. Modulation of sensory processing by brain state

Our work confirms a large number of previous studies showing a strong modulation of sensory responses by brain state. Work in the rat somatosensory cortex under anesthesia and wakefulness suggests a picture where the instantaneous degree of cortical activation is negatively correlated with the magnitude of sensory responses to brief, temporally unstructured input. Indeed, during the inactivated state, responses during upstates to whisker deflections are smaller than during downstates, and responses during alertness seem to also be smaller than responses during immobility (Fanselow and Nicolelis, 1999; Sachdev et al., 2004; Castro-Alamancos, 2004a; Crochet and Petersen 2006; Haslinger et al., 2006; Hasenstaub et al., 2007; Haider et al., 2007), as if stimuli arriving on a network where neurons were firing tonically were less capable of producing a strong sensory response than the same stimuli arriving in a transiently inactive, or more strongly fluctuating network. However, the effect of the instantaneous level of cortical activation on sensory responses is more complicated than this picture suggests, since a substantial amount of evidence shows that, within the awake state, higher alertness or attention is

positively correlated with low frequency desynchronization (Destexhe et al., 1999; Gervasoni et al., 2004; Jung et al., 1997; Fries et al., 2001; Tallon-Baudry et al., 2001; Pesaran et al., 2002), and with enhanced sensory responses (Hubel et al., 1959; Haider, 1964; Eason et al., 1969; Picton et al., 1971; Fritz et al., 2003; Roelfsema et al., 1998; Reynolds and Chelazzi, 2004; Raz and Buhle, 2006). Thus, relationship between the magnitude of sensory responses and the level of cortical activation seems to be non-monotonic, at least as far as responses to simple, brief stimuli are concerned. Our results are consistent with this picture. We observed weaker responses to single clicks in the activated than in the inactivated state under urethane anesthesia (Chapter 2), but stronger responses to the 1st click of a train for more activated trials during wakefulness in an overall activated background (Chapter 3). Most likely, this is a hint that the effect of brain state on sensory processing is complex, and cannot be reduced to a one dimensional continuum of activation levels.

9.6. The relationship between brain state and the perception of temporally modulated stimuli

The inability of an individual to correctly perceive the identity of sounds preceded by the presentation of other sounds is called ‘forward masking’ (Moore, 1995). As mentioned previously (section 1.1), most studies concerning forward masking focus on the effect of varying the physical properties of different components of the sound (such as amplitude/ spectral content/duration etc), on their perceptibility by the subject. However, I was unable to find many studies concerning the effect of either the behavioral condition, or

the brain state of the animal on forward masking. Even though in our studies we did not measure the effect of state on perception directly, our results indicate that state may influence forward masking nevertheless. Our study complements other physiological studies in animals that approached the mechanisms of forward masking (also referred to as 'forward suppression') with the working hypothesis that response suppression is the physiological basis of masking (e.g., Bartlett and Wang, 2005; Brosch and Schreiner, 1997; Wher and Zador, 2005). Our results showed that suppression is stronger with increased levels of inactivation (Figure 2.2; Figure 3.3). This observation corresponds to studies that measured response amplitude to high frequency stimuli in somatosensory cortex across different behavioral conditions and reported stronger adaptation under more inactivated conditions (Fanselow and Nicolelis, 1999; Castro-Alamancos, 2004a). Thus, our analysis of population responses in auditory cortex suggests that the degree of forward masking would be reduced with increased cortical activation.

10. References

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