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Development of complex sound representations in the primary auditory cortex

by

Michele Nerissa Insanally

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy in Neuroscience

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Shaowen Bao, PhD., Chair

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Professor Keith Johnson

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Prologue

How does the brain give rise to consciousness? Somehow this enormous sack of cells comprised of proteins and fats is responsible for our thoughts, emotions, decisions and desires. We have used our brain to create nations, wars, nail files, credit default swaps, the Internet, schools, insurance companies, paint remover, buildings and every known human invention. As one of my mentors once said, "We live in the collective imagination of others." The field of neuroscience attempts to understand what this imagination is and how it is created.

Abstract

Development of complex sound representations in the primary auditory cortex

by

Michele Nerissa Insanally

Doctor of Philosophy in Neuroscience

University of California, Berkeley

Professor Shaowen Bao, PhD., Chair

The brain has a tremendous ability to change as a result of experience; this property is known as plasticity. Our mastery of soccer, rhetoric, agriculture and instrumentation are all learned skills that require experience. While the brain is plastic throughout life, during early development, the brain demonstrates a heightened sensitivity to experience. This unique epoch during development in which the brain is particularly susceptible to change is called a *critical period*. During the critical period, sensory experience results in significant modifications in structure and function. The set of studies described in this dissertation aim to investigate how complex sound representation develops during the critical period in the rat primary auditory cortex.

Previous examinations of the critical period in the auditory cortex have typically used simple tonal stimuli. Repeated exposure of rat pups to a tone, for instance, has been shown to selectively enlarge cortical representation of the tone and alter perceptual behaviors. However, probing cortical plasticity with a single-frequency tone might not reveal the full complexity and dynamics of critical period plasticity. After all, natural, biologically important sounds are generally complex with respect to their spectrotemporal properties. Natural sounds often have frequencies that vary in time and amplitude modulation. Psychophysical studies indicate that early experience of complex sounds has a profound impact on auditory perception and perceptual behaviors. Experience with speech, for instance, shapes language-specific phonemic perception, enhancing perceptual contrasts of native speech sounds and reducing perceptual contrasts of some foreign speech sounds. At the electrophysiological level, auditory cortical neurons preferentially respond to certain complex sounds, such as species-specific animal vocalizations. It is unclear how such selectivity for a complex sound emerges, and whether it is innate or shaped by early experience.

In order to address this question, we exposed rat pups to a frequency-modulated (FM) sweep in different time windows during early development, and examined the effects of such sensory experience on sound representations in the primary auditory cortex (AI). We found that early exposure to an FM sound resulted in altered characteristic frequency representations and broadened spectral tuning in AI neurons. In contrast, later exposure to the same sound only led to greater selectivity for the sweep rate and direction of the experienced FM sound. These results

indicate that cortical representations of different acoustic features are shaped by complex sounds in a series of distinct critical periods.

Next, we confirmed this model of brain development in a set of experiments that examine how exposure to noise affects these various critical periods. We examined the influence of pulsed noise experience on the development of sound representations in AI. In naïve animals, FM sweep direction selectivity depends on the characteristic frequency (CF) of the neuron—low CF neurons tend to select for upward sweeps and high CF neurons for downward sweeps. Such a CF dependence was not observed in animals that had received weeklong exposure to pulsed noise in periods from postnatal day 8 (P8) to P15 or from P24 to P39. In addition, AI tonotopicity, tuning bandwidth, intensity threshold, tone-responsiveness, and sweep response magnitude were differentially affected by the noise experience depending on the exposure time windows. These results are consistent with previous findings of feature-dependent multiple sensitive periods. The different effects induced here by pulsed noise and previously by FM sweeps further indicate that plasticity in cortical complex sound representations is specific to the sensory input.

Identifying how the developing brain processes sensory information provides a foundation for understanding more complex behaviors. These results advance our understanding of the neuronal mechanisms underlying sensory development and language learning. Specifically, they elucidate the age-dependent effects of complex sound exposure on spectral tuning and complex sound representation in the rat primary auditory cortex. In addition, they provide a foundation for subsequent studies investigating the neural basis of language development.

Chapter 1. Introduction: Developmental plasticity in the rat primary auditory cortex

1.1 Introduction to developmental cortical plasticity

Imagine a ship at sea, guided by the illumination of a distant lighthouse while unremittingly jostled by a tempestuous tide. Similarly, neurons use chemical guides managed by the genetic code to find their ultimate synaptic destination very early during development. Shortly after this period of travel, neural circuits are refined by experience, allowing the organism to sensibly interact and adapt to its environment. Early life experiences can result in significant modifications to brain structure, function, and ability. Infants, for example, learn a unique language depending on the environment they are raised in - and will eventually acquire native fluency. Similarly, juvenile songbirds must hear tutor song early in life, typically from their father, to accurately reproduce the song later in life (Marler and Peters 1982). A biological handle on the effects of early life experience on changes in neural circuitry is essential to understanding brain function and ultimately behavior.

In the early twentieth century, Konrad Lorenz conducted a series of important studies on the behavioral consequences of early life experience (Figure 1). He extensively examined imprinting behavior in Greylag geese and observed that goslings (when incubator-hatched) will imprint on a moving object within thirty-six hours of hatching, often on Konrad himself (Lorenz 1937). Lorenz was the first to refer to this unique developmental epoch in which animals are especially sensitive to external sensory stimuli as a *critical period*.



Figure 1. Konrad Lorenz with Greylag geese (http://www.dacherpwd.com/science-puppy_development.html).

This exquisite ability of the brain to adapt to its environment and to learn is present throughout life. However, extensive studies conducted in a variety of animals indicate that there are unique differences between the learning that occurs during development versus adulthood. For example, early in development exposure to passive stimuli can result in significant changes to the brain

(Zhang et al., 2001; Chang and Merzenich, 2003; de Villers-Sidani et al., 2007; Han et al., 2007), whereas in adult animals active attention to stimuli and engagement of neuromodulatory systems are typically required for changes in cortical responses (Kilgard 2003; Froemke et al., 2007)

Electrophysiological studies of experience-dependent cortical plasticity

Studies on neuroplasticity have become a leading focus of intense scientific research. The sub-field of neuroplasticity intersects many other disciplines including but not limited to: psychology, linguistics, physics, biology, robotics, mathematics, sociology, anthropology, and chemistry. Currently, neuroplasticity is being studied on many axes ranging from molecular to theoretical. There are also many approaches to the different levels: anatomical, electrophysiological, biochemical, and mathematical. Specifically, studies range from investigating changes in neural activation (such as small changes in synaptic current) to structural changes (as in the growth of tiny sprouts on the tips of dendrites or large scale reorganization of neural circuitry). The field of neuroplasticity can be furthered categorized by a few common study types such as 1) learning and memory, 2) functional recovery following pathology, 3) experience-dependent neuronal reorganization, and 4) neuronal development. The focus of my dissertation falls into the third categorization; specifically, how complex sound representations develop in rat primary auditory cortex (AI) using electrophysiological recordings of multiunit brain activity.

Some of the very first electrophysiological studies on developmental plasticity were conducted in the visual system. In the early 1960s, David Hubel and Torsten Wiesel (Figures 2 & 3) discovered that monocular deprivation (the suturing of one eye) in kittens resulted in a shift of ocular dominance towards the non-deprived eye (Wiesel and Hubel, 1963). This effect is only seen when monocular deprivation is performed from the third postnatal week up to three months; hence, there is a *critical period* for this manipulation.



Figure 2. David Hubel (left) and Torsten Wiesel (right). Recipients of the Nobel Prize in *Physiology and Medicine* in 1981 (<http://www.ftpress.com/articles/article.aspx?p=1431818>)

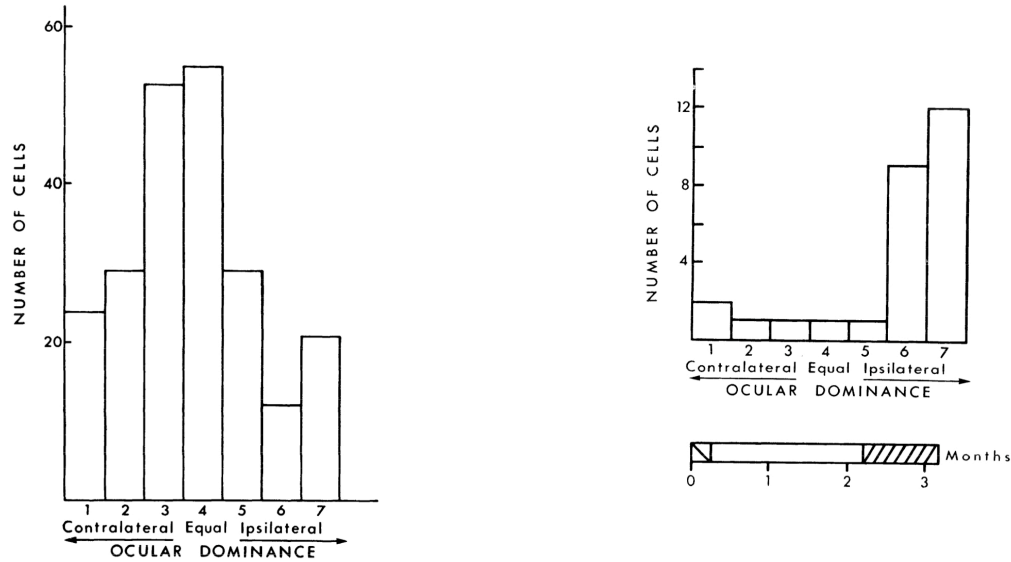


Figure 3. Summary of main results from Wiesel and Hubel, 1963. The figure on the left is data from a normal adult cat while the figure on the right is data from a cat that underwent monocular deprivation. Note the shift in ocular dominance towards the non-deprived eye (figure on the right). This seminal study demonstrated that the visual system is highly plastic during development.

Since then, we have learned much about the characteristics of developmental plasticity, not only in the primary visual cortex (V1), but in other cortical areas as well. For example, injury to the whiskers of rat pups during a critical period results in a loss of barrel representation in somatosensory cortex (Van der Loos & Woolsey, 1973). In addition, early life experience can have profound consequences for brain development and function in the primary auditory cortex. For example, exposing animals to a specific acoustic environment during the critical period results in permanent large-scale reorganization of A1. Specifically, rearing rat pups in a single pulsed pure tone environment, for instance, results in an enlarged cortical representation for the experienced tone (Zhang et al., 2001; Chang and Merzenich, 2003; de Villers-Sidani, 2007; Han et al., 2007). In fact, the critical period for frequency representation in A1 has been defined to be from postnatal days 11 to 13 (de Villers-Sidani et al, 2007). This important study was the first to delineate the exact days during postnatal development in which frequency representation can be manipulated in A1. Interestingly, a study by Yoon Han, a former undergraduate student in our lab, examined the functional consequences of such changes in cortical representation. This study demonstrated that early exposure to a pure tone enlarges cortical representation of the experienced tone, but impairs perceptual discrimination near that frequency (Han et al., 2007). Moreover, discrimination of nearby underrepresented frequencies improved. Collectively, these studies provide strong evidence for the manipulation of cortical sound representation during development.

In addition to pure tone stimuli, broadband white noise is a stimulus that has also been widely used to study critical period plasticity. For example, rearing rats during the first month of life in pulsed white noise disrupts tonotopicity, degrades frequency tuning, and reduces temporal correlation between populations of neurons in A1 (Grecova et al., 2009; Zhang et al., 2002). In contrast, exposing animals to continuous environmental noise, rather than periodic bursts of noise, for the first month of life prolongs the duration of the critical period (Chang & Merzenich,

2003). In other words, the critical period remains open, such that subsequent exposure to a single pure tone after the first month of life results in greater representation for that tone. Therefore, the temporal structure of noise inputs results in different plasticity effects in A1. It's important to also note that early exposure of animals to a continuous tone prevents the closure of the critical period (Zhou et al., 2008). These studies indicate that the closure of the critical period window in AI requires temporally structured input.

It has also been shown that critical period closure in AI is modulated locally by sound input dynamics; exposing animals to spectral band-notched noise results in critical period closure for the portion of AI representing those absent frequencies while exposing animals to band-pass noise kept the critical period open for those frequencies (de Villers-Sidani et al., 2008).

Taken together, these studies indicate that the spectral structure of sound stimuli influence the formation of frequency tuning characteristics, whereas the temporal structure modulates the closure of the critical period in rat A1.

Auditory cortical responses to complex stimuli

Many studies of the critical period in the primary auditory cortex have typically used simple tonal stimuli. Thus, the very first studies of response properties in subcortical structures primarily used simple tonal or click stimuli. The use of simple sounds to probe cortical processing in the auditory system is, in part, a result of the traditional view that its primary function is spectral decomposition. Indeed, as far as we know, the cochlea performs a simple Fourier analysis on sounds. However, natural, biologically relevant sounds are generally complex in their spectral and temporal structures (Singh and Theunissen, 2003). Psychophysical studies indicate that early experience of complex sounds has a profound impact on auditory perception and perceptual behaviors. Experience with speech, for instance, shapes language-specific phonemic perception, enhancing perceptual contrasts of native speech sounds and reducing perceptual contrasts of some foreign speech sounds (Werker and Tees, 1984; Kuhl et al., 1992; Kuhl et al., 2006).

Although the primary auditory cortex responds robustly to pure tones, establishing a fine tonotopic map - there is increasing electrophysiological evidence that the primary auditory cortex responds to complex sound features (Theunissen et al., 2000; Klein et al., 2000; deCharms et al., 1998). Complex sounds are processed in the auditory cortex by neurons that are selective for sound features on multiple acoustic dimensions, such as spectral composition, amplitude modulation, and frequency modulation (Nelken and Versnel, 2000; Loftus and Sutter, 2001; Lu et al., 2001; Linden et al., 2003; Atencio et al., 2007). For example, auditory cortical neurons are selective for the direction and rate of frequency-modulated sweeps (Ye et al., 2010; Zhang et al., 2003; Orduna et al., 2001). In addition, animals in which the auditory cortex has been bilaterally ablated can still perform frequency discrimination but cannot discriminate sweep direction (Ohl et al., 1999; Wetsel et al., 1998). Moreover, auditory cortical cells are very sensitive to the properties of the stimulus. For example, marmoset cortical neurons are selective for within-species vocalizations and cortical responses are significantly diminished when the stimulus is distorted (Wang, 2000; Wang et al., 1995).

While previous studies have shown that spectral and temporal selectivity are shaped by sensory inputs during the critical period (Zhang et al., 2001; Chang and Merzenich, 2003; Chang et al., 2005; de Villers-Sidani et al., 2007; Han et al., 2007), it remains to be determined whether selectivity for complex sound features, such as frequency modulated sweeps, can be altered by early sound exposure. The studies set forth in my dissertation are designed to address this question and elucidate how complex sounds develop in the primary auditory cortex.

1.2 Characterization of the auditory system

Motivation for using the rat as a model system

Traditionally, a variety of species have been used to study the auditory system, including but not limited to: cats, owls, bats, ferrets, and gerbils. More recently however, it has become more common to use rats for the study of the developing auditory system (Figure 4). Firstly, the rat auditory system is altricial and continues to develop well after birth. Presumably, this protracted process allows the developing auditory system to adapt to its environment. Secondly, there are very practical reasons for using rats; husbandry and handling are relatively simple in contrast to higher order animals. Rats also have the ability to learn novel tasks relatively quickly, making them useful for behavioral experiments. Perhaps most importantly, rats use their auditory system to interact with the environment and communicate with conspecifics.



Figure 4. Sprague-Dawley rat used in the experiments described in subsequent chapters (<http://www.flickr.com/photos/jepoirrier/422469518/in/set-72157594329856603>).

Tonotopic organization of the auditory system

Historically, the auditory system has been extensively studied at the level of subcortical structures. During the early 20th century, Georg von Békésy first described the tonotopic organization in the cochlea. Using human cadavers, he dissected out the inner ear and observed that the basilar membrane of the cochlea moved as a wave when stimulated by sound. Due to the mechanical structure of the basilar membrane, each sound corresponds to a distinct pattern of vibrations along the basilar membrane. Additionally, von Békésy theorized that the hair cells of

the cochlea are arranged tonotopically, such that hair cells respond to a particular frequency depending on their placement along the cochlea (Bekesy 1974; Goldstein 2001). For example, hair cells located near the base of the cochlea respond selectively to high frequencies while cells near the apex respond preferentially to low frequency sounds (see Figure 5). This tonotopic organization seen in the cochlea is maintained throughout the entire auditory pathway.

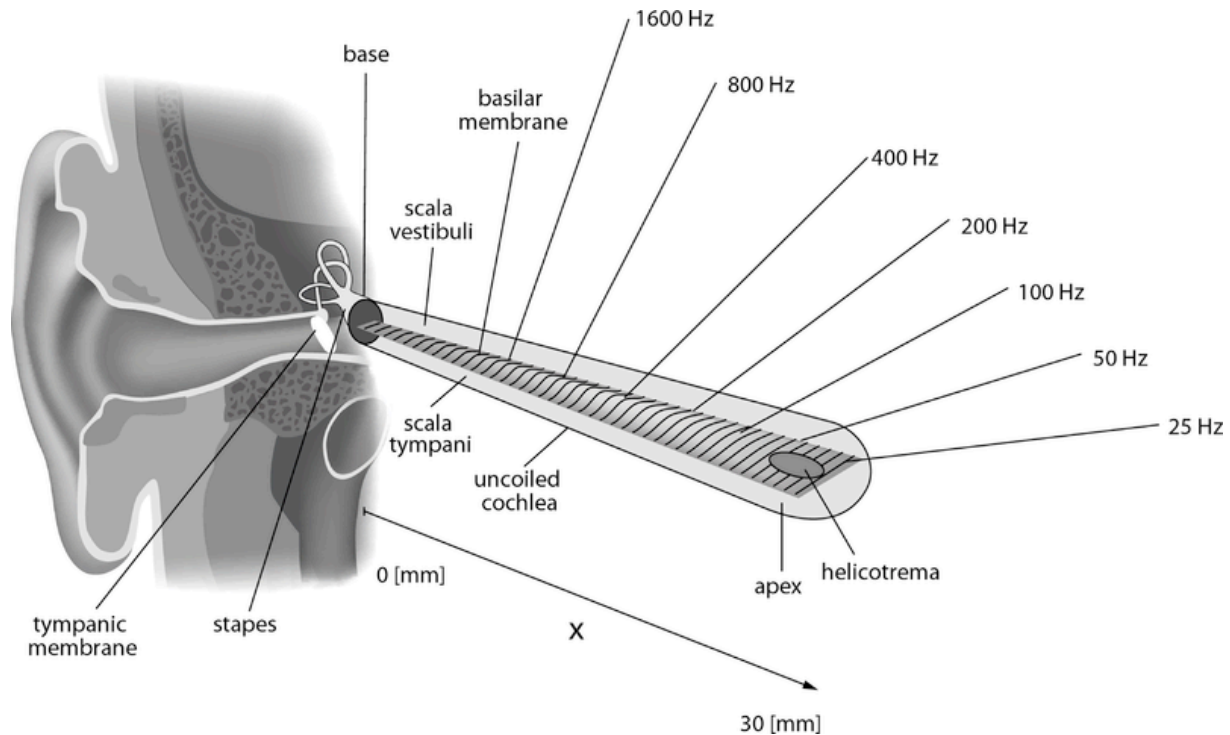


Figure 5. Uncoiled cochlea with basilar membrane (Kern et al., 2008)

Therefore, the primary auditory cortex has a fine tonotopic gradient such that neurons located caudally preferentially respond to low frequency sounds; whereas neurons located rostrally preferentially respond to high frequency sounds (Figure 6). Traditionally, the primary auditory cortex has been defined to represent frequency ranges from 0.5 - 32 kHz largely due to hardware challenges; however, using more sophisticated speakers, there is recent evidence that it extends up to 74 kHz (H. Kim, personal correspondence).

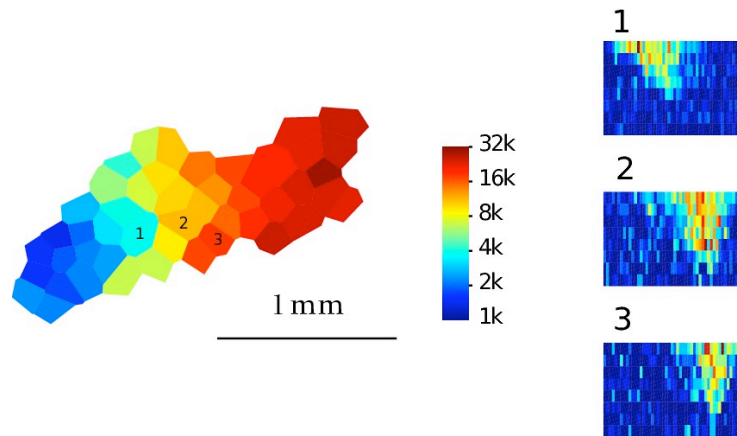


Figure 6. Representative cortical frequency map and tuning curves from a naive adult rat. The figure on the left is a normal cortical CF map, where each polygon represents a multiunit recording site. Cool colors represent low frequencies and warm colors represent high frequencies. Note the fine tonotopic gradient. The horizontal axis on the figure on the right depicts logarithmically scaled frequency from 1 to 32 kHz. The vertical axis depicts tone intensity from 0 to 70 dB SPL.

Motivation for studying A1

The primary auditory cortex is functionally defined by sharp, V-shaped tuning curves and a fine tonotopic gradient (Figure 6). These properties facilitate the study of A1 as this organized distribution of frequencies along the rostral-caudal axis provides a stereotyped substrate in which to detect changes in frequency representation. Hence, the majority of studies investigating auditory cortical plasticity are limited to A1 despite knowledge of other auditory cortical areas (Figure 7, Polley et al., 2007)

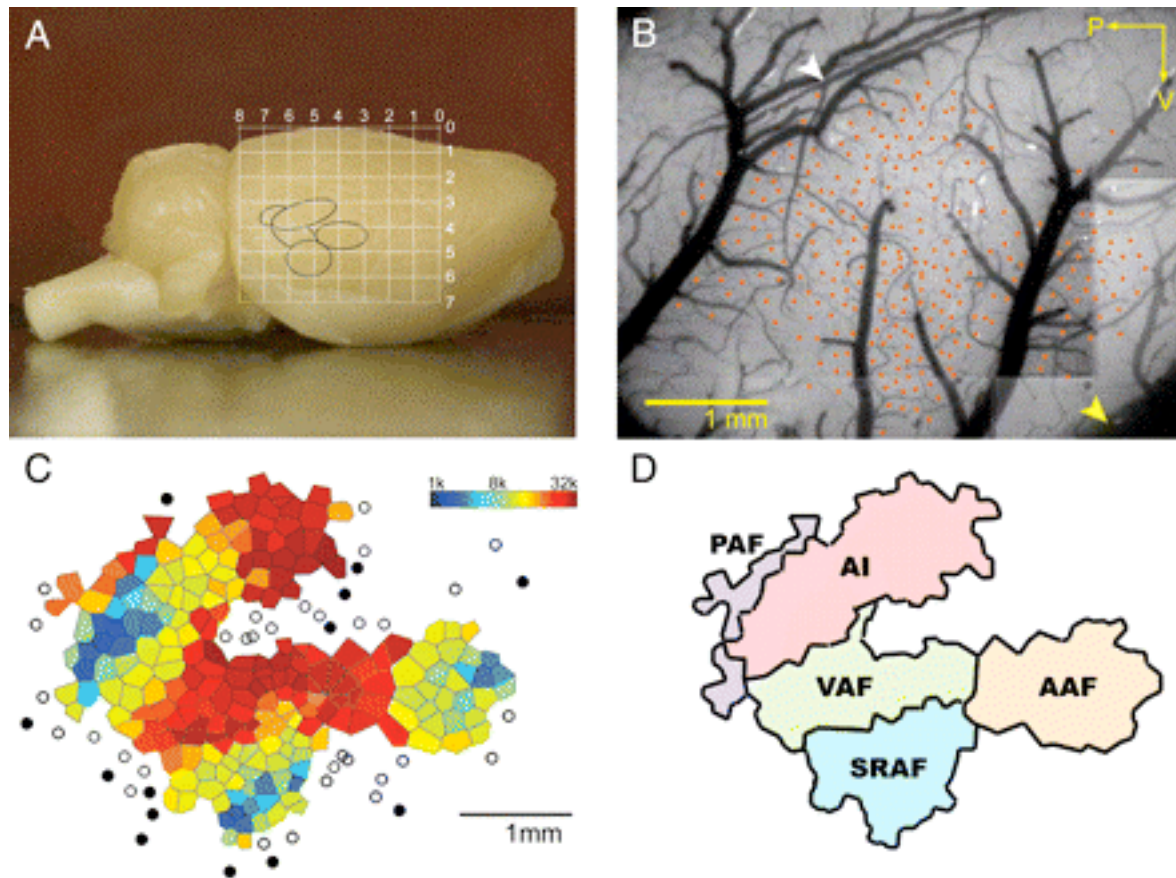


Figure 7. Tonotopic organization of several auditory areas in the albino rat (Polley et al., 2007).

Development of AI

Sound-evoked unit responses can be recorded from rodent primary auditory cortex during the second postnatal week (Zhang et al., 2001; Gael-Dor et al., 1993). Subsequently, a fine tonotopic gradient matures rapidly. From P11 onwards, mid-range frequencies around 6 to 8 kHz develop first, shortly followed by an elaboration of mid to high frequencies from around 9 to 32kHz (de Villers-Sidani et al., 2007; Zhang et al., 2001). Finally, the lower frequency bound of 1 to 5 kHz develops rounding out the full frequency spectrum by approximately P16 (de Villers-Sidani et al., 2007; Zhang et al., 2001). During this short period of tonotopic map development a few changes in unit response properties occur: 1) receptive field tuning broadens 2) the sound intensity response threshold decreases and 3) the response latencies become shorter (see Figure 8 & de Villers-Sidani et al., 2007). It should be noted that these response properties were determined under an anesthetized preparation and may be different in the awake cortex. To summarize, the mature rat primary auditory cortex is traditionally characterized as representing frequencies from 1 to 32 kHz, with tone-evoked response latencies of 7 to 12 milliseconds and reaches maturity by P16.

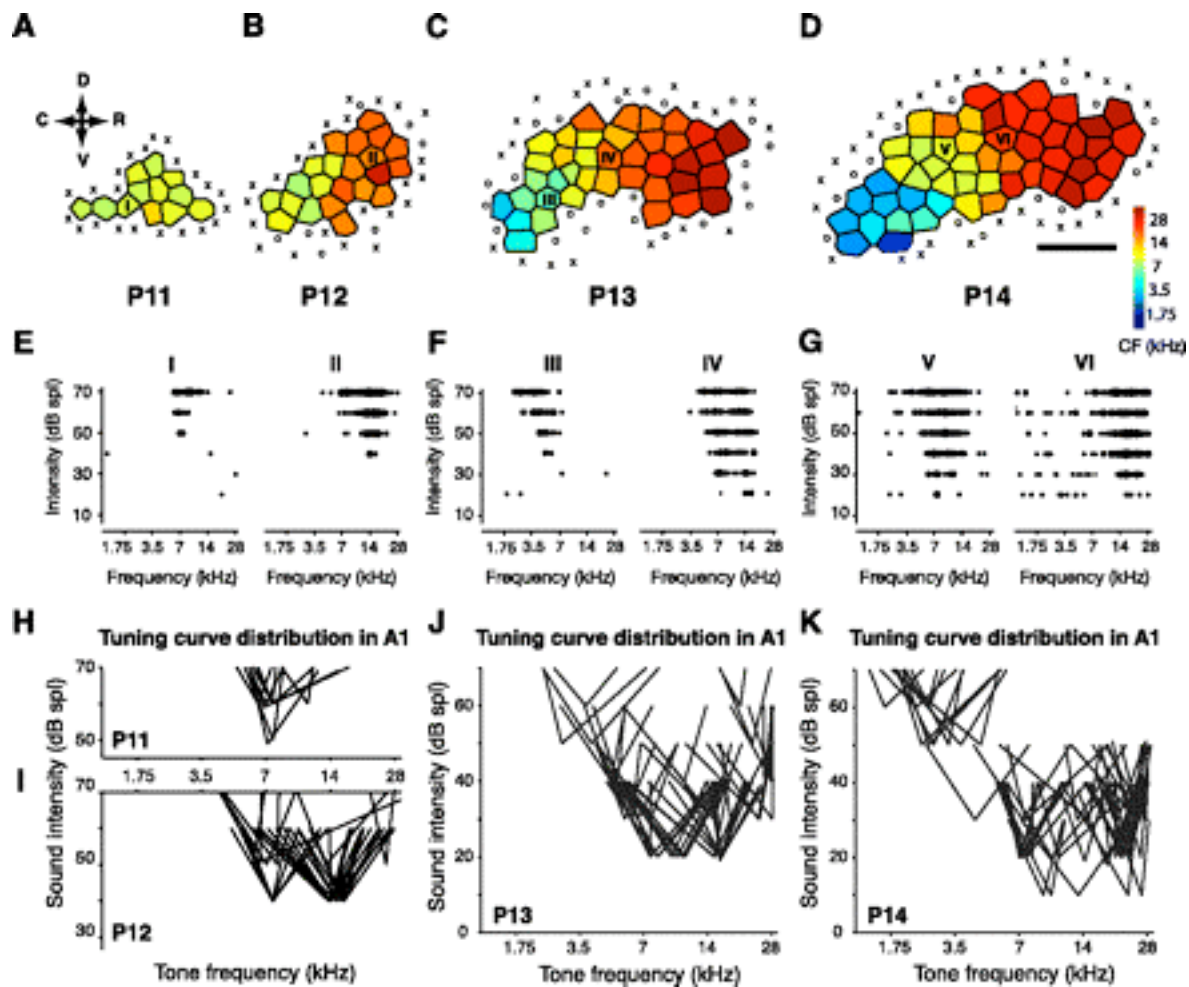


Figure 8. Tonotopic development of rat primary auditory cortex (de Villers-Sidani et al., 2007).

1.3 Summary and Motivation

Studies of the primary auditory cortex have just begun to delineate the properties of developmental plasticity. The vast majority of neuroplasticity studies in A1 have used simple tonal stimuli. However, probing cortical plasticity with a single-frequency tone might not reveal the full complexity and dynamics of critical period plasticity. After all, natural sounds comprise multiple frequency components with time-varying amplitude and frequency modulations. Considering the importance of complex sound processing to the auditory system, a complete understanding of how complex sounds are represented in the brain is of immense relevance. Thus, in order to gain a greater understanding of complex sound development, we investigated the effects of FM sweep rearing in the rodent primary auditory cortex. We then tested our model of critical period plasticity using pulsed white noise.

Chapter 2. Feature-dependent sensitive periods in the development of complex sound representation

2.1 Preface

It has been demonstrated quite extensively that passive exposure to simple tonal stimuli early in development can reorganize cortical sound representations. However, the effects of complex sound experience on cortical development remain to be determined. In order to address this, I exposed rat pups to a frequency-modulated (FM) sweep at different times during early development, and examined the effects of such sensory experience on sound representations in the primary auditory cortex (AI). Here, I show that early exposure to a FM sound resulted in altered characteristic frequency (CF) representations and broadened spectral tuning in AI neurons, whereas later exposure to the same sound only led to greater selectivity for the sweep rate and direction of the experienced FM sound. This work establishes for the first time that cortical representations of different acoustic features are shaped by complex sounds in a series of distinct sensitive periods that increase in complexity during development.

Portions of this chapter have been published in the *Journal of Neuroscience* (Insanally et al., 2009).

2.2 Methods

Acoustic Rearing

The UC Berkeley Animal Care and Use Committee have approved all procedures used in this study. Litters of rat pups (female, Sprague-Dawley) and their mothers were placed in an anechoic sound-attenuation chamber for a brief period, comprising one or a combination of four time windows (P8-P15, P16-P23, P24-P31, P32-P39). Trains of logarithmic frequency modulated (FM) sweeps (frequency varied exponentially with time from 50 to 0.5 kHz at a rate of -80 octaves/second, 60 dB SPL, five sweeps in a train at 5 Hz, with 500-ms quiet periods between trains; all speakers had less than 3% spectral distortion, and their output levels were calibrated for the entire output frequency range) were played to the animals 24 hours/day. In addition, the acoustic environment could also contain vocalization sounds produced by the animals, mainly in a frequency range above 30 kHz. After the exposure period, the animals were returned to standard housing conditions.

Electrophysiological recording

The primary auditory cortex of sweep-exposed and naïve animals were mapped under pentobarbital anesthesia (50 mg/kg for induction, 10-20 mg/kg supplemental, as needed; intraperitoneal injections) at ages P40 to P60. Animals in different groups were roughly age-matched. The primary auditory cortex was defined by its tonotopic organization and reliable neuronal responses to tone pips of selective frequencies. Neurons were evenly sampled from the primary auditory cortex using parylene-coated tungsten microelectrodes (2 M Ω) advanced 500-

600 micrometers below the pial surface (layer4/5), and responses to 25-ms tone pips of 50 frequencies (1 to 30 kHz, 0.1 octave spacing) and 8 sound pressure levels (0–70 dB SPL, 10 dB steps) were recorded three times to reconstruct the response-frequency tuning curve. Responses to a series of FM sweeps (frequency range, 0.5-50 kHz; sweep rates, 30, 45, 60, 75 and 90 octaves/second, in both up and down directions) were also recorded three times to determine the neurons' selectivity for FM sweep rates. Tone pips and FM sweeps were intermixed and presented in a pseudorandom order.

Analysis

The CF and tuning bandwidth of each neuron were determined automatically, which involves smoothing and thresholding of the receptive field. The CF was calculated as the center of mass of the receptive field. To generate CF maps, Voronoi tessellation was performed to create tessellated polygons, with each polygon corresponding to an electrode penetration site, and assigned the CF of the site. The magnitudes of responses to FM sweeps were determined in 30-ms response windows that were automatically set to best fit the response latencies at all the different sweep rates. Under the assumption that the neuronal response was triggered by a specific frequency (denoted as f in kHz) in the FM stimuli, for upsweeps, $FM_Rate \times (T - T_{min}) = \log_2(f \times 0.5^{-1})$, in which T is the measured latency of the response to the sweep at the FM_Rate , and T_{min} is the minimal response delay. A regression analysis was performed on FM_Rates and Ts to derive T_{min} and f . The expected latency was then calculated as $T_{exp} = \log_2(f \times 0.5^{-1}) \times FM_Rate^{-1} + T_{min}$. A 30-ms window centered at T_{exp} was used as the response window. The number of spikes in the response window was counted as the response magnitude. Spontaneous firing was determined from the 30-ms pre-stimulus period, and was subtracted from the response to the FM sweeps. For downsweeps, $FM_Rate \times (T - T_{min}) = \log_2(f^{-1} \times 50)$, and $T_{exp} = \log_2(f^{-1} \times 50) \times FM_Rate^{-1} + T_{min}$. Our visual inspection indicated that the response windows consistently captured the phasic cortical responses to the sweeps (See Figure 13).

A sweep direction selectivity index (SDSI) was calculated as $(R_{up} - R_{down}) / (R_{up} + R_{down})$, in which R_{up} is response magnitude to the up-sweep, and R_{down} is response magnitude to the down-sweep. To avoid bias by small responses, we calculated a chi-squared (χ^2)-based statistic of up-sweep selectivity: $\chi^2\text{-SDSI} = (R_{up} - R_{down}) |R_{up} - R_{down}| / (R_{up} + R_{down})$.

Determining the locations of recorded sites on the tonotopic axis

A line connecting the site with the highest CF and that with the lowest CF was defined as the tonotopic axis. The maps were rotated to orient the tonotopic axis horizontally. For all recorded sites in each map, a linear regression was performed in the form of $\log(CF) = k(X - c)$, in which CF is the characteristic frequency in kHz, and X is the x-coordinate, of the recorded site. Then, X - c is used as the location of the recorded site on the tonotopic axis.

Unless stated otherwise, statistical significance was quantified with ANOVA and post-hoc Bonferroni's test using StatView (SAS Institute). Data are presented as mean \pm SEM.

2.3 Exposure to FM sweeps alters the characteristic frequency and bandwidth of cortical neurons

An analysis of spectral tuning revealed that exposure to the FM sound from P8-15 significantly reduced the number of neurons tuned to frequencies below 4 kHz (χ^2 -test, $p < 0.05$; see Figure 9). More neurons in P8-15 animals were tuned to 4-8 kHz (χ^2 -test, $p < 0.05$), suggesting that there was a shift from low to high characteristic frequencies. This effect was not seen in animals exposed to the sound in later time points.

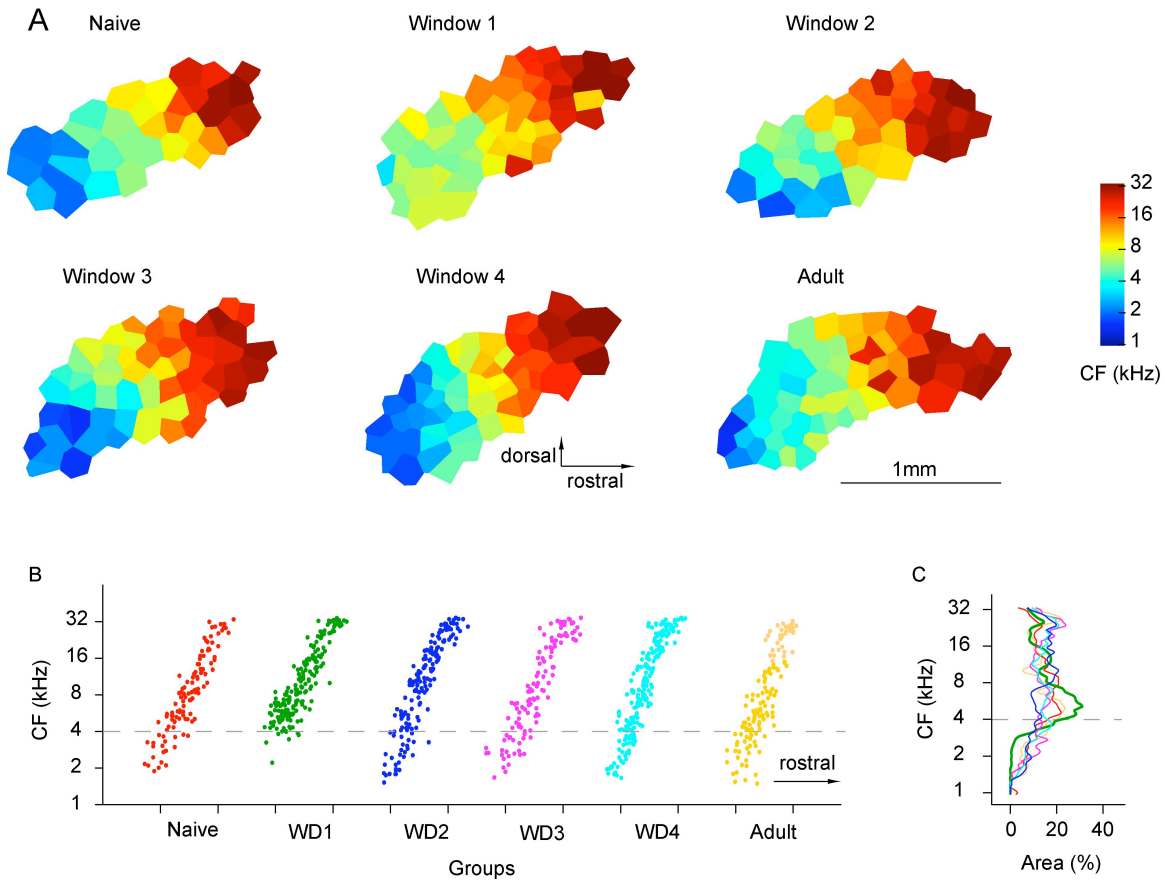


Figure 9. Exposure to downward FM sounds in the first time window alters the cortical CF map. A, Representative cortical CF maps of naive, window 1, window 2, window 3, window 4, and adult animals. B, CF distribution along the tonotopic axis. Recordings from four animals were included for each group. C, Percent primary auditory cortical area representing frequencies in a 0.4-octaves frequency band. Color codes for experimental groups are the same as in B. Note that only W1 animals had reduced representations of frequencies below 4 kHz.

The bandwidths of the animals that were exposed to the FM sweeps from P16-23 were significantly broader than those of the other groups at high sound pressure levels from 50 to 70 dB (Figure 10, ANOVA, $p < 0.05$). The effect was seen in neurons of all CF ranges. The bandwidth of auditory cortical neurons undergoes rapid developmental changes in a brief period from P16 to P18 (see results below and Figure 6). Our results indicate that exposure to broadband stimuli during this period shapes broadly selective receptive fields. Exposure to the

same downward FM sweep for four weeks did not change cortical CF maps (Figure 9) or bandwidths (Figure 10) in adult animals (> 3 months).

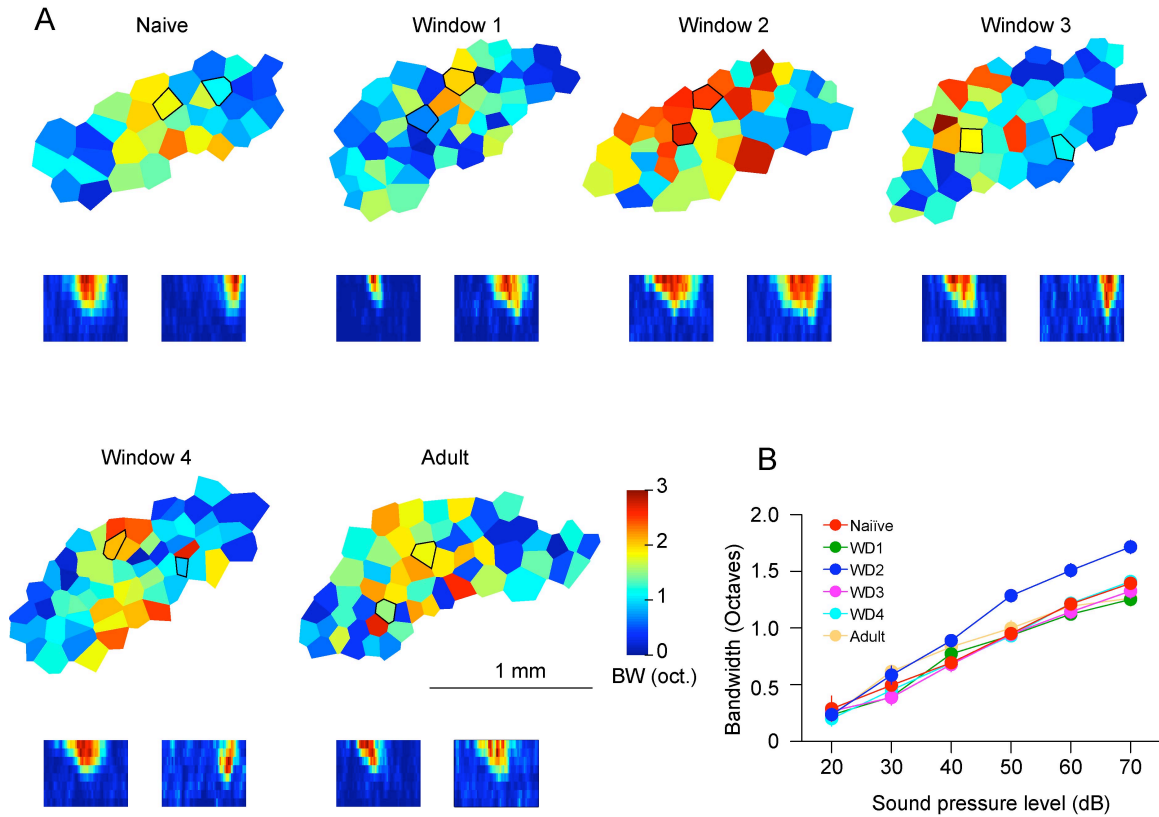


Figure 10. Exposure to downward FM sounds in Window 2 broadens frequency tuning. A. Representative cortical tuning bandwidth maps based on BWs measured at 70 dB SPL. Insets are representative receptive fields that had been recorded in the locations outlined in the bandwidth maps. The vertical axis of the receptive field plots depicts sound intensity from 0 to 70 dB SPL. The horizontal axis depicts frequencies from 1 to 32 kHz. B. Tuning bandwidth as a function of sound pressure level. Error bars depict SEM, the majority of which are occluded by the data symbols. Only Window 2 animals showed broadened frequency tuning.

2.4 A sensitive period for cortical FM direction selectivity

Auditory cortical neurons are selective for FM directions at 80 octaves per second, which corresponds to the highest sweep rate at which rats can discriminate sweep directions (Zhang et al., 2003; Gaese et al., 2006). To probe plasticity, we exposed rat pups to downward logarithmic FM sweeps (50-0.5 kHz, 60 dB SPL, - 80 octaves per second, 5 sweeps in a train at 5 Hz, with 500 ms of silence between trains) in one of four time points (P8-15, P16-23 P24-31 and P32-39, see Methods). Cortical spectral and FM representations were examined in both sweep-exposed and control animals at ages P40 to P60. Auditory cortical neurons typically responded to the testing FM sweeps with a brief burst of spikes (Figure 11, A-B). This FM response was quantified using the number of spikes in a 30-ms response window (automatically determined with an analysis program; for details, see Methods). To investigate whether direction selectivity was altered by sweep exposure, a sweep direction selectivity index (SDSI) was calculated as (R_{up}

$-R_{\text{down}}/(R_{\text{up}} + R_{\text{down}})$. Because SDSI can be biased by weak responses, we calculated a χ^2 -based statistic that measures the significance of sweep direction selectivity: $\chi^2\text{-SDSI} = (R_{\text{up}} - R_{\text{down}}) |R_{\text{up}} - R_{\text{down}}|/(R_{\text{up}} + R_{\text{down}})$. Neurons selective for upward FM sweeps will have a positive SDSI and a positive χ^2 -SDSI.

Animals that were exposed to the downward FM sweeps from P24-31 and P32-39 showed significantly lower SDSIs and χ^2 -SDSIs near the exposure FM rate of 80 octaves/s (at 75- and 90-octaves/s, Figure 12A and 12B; $p < 0.05$, ANOVA, compared to all other groups), indicating increased selectivity for the experienced downward FM sweep. Examination of the SDSI of individual neurons revealed that in naïve animals, most direction selective neurons preferred upsweeps (Figure 11C). In the animals that were exposed to the downward FM sweep from P32-39, fewer neurons selected for upsweeps and more neurons preferred downsweeps (ANOVA group comparison, $p < 0.05$).

Exposure to downsweeps can alter the number of low-CF neurons (see results below and Figure 9). To avoid potential bias caused by this difference in CFs, we calculated SDSI only for neurons with CFs higher than 4 kHz. The results of this subset analysis did not differ from those of analysis of all neurons (ANOVA, $p > 0.1$). Adult animals (more than 3 months old) that had been exposed to the same downward FM sweep for four weeks showed no changes in sweep direction selectivity (Figure 12) ($p > 0.5$).

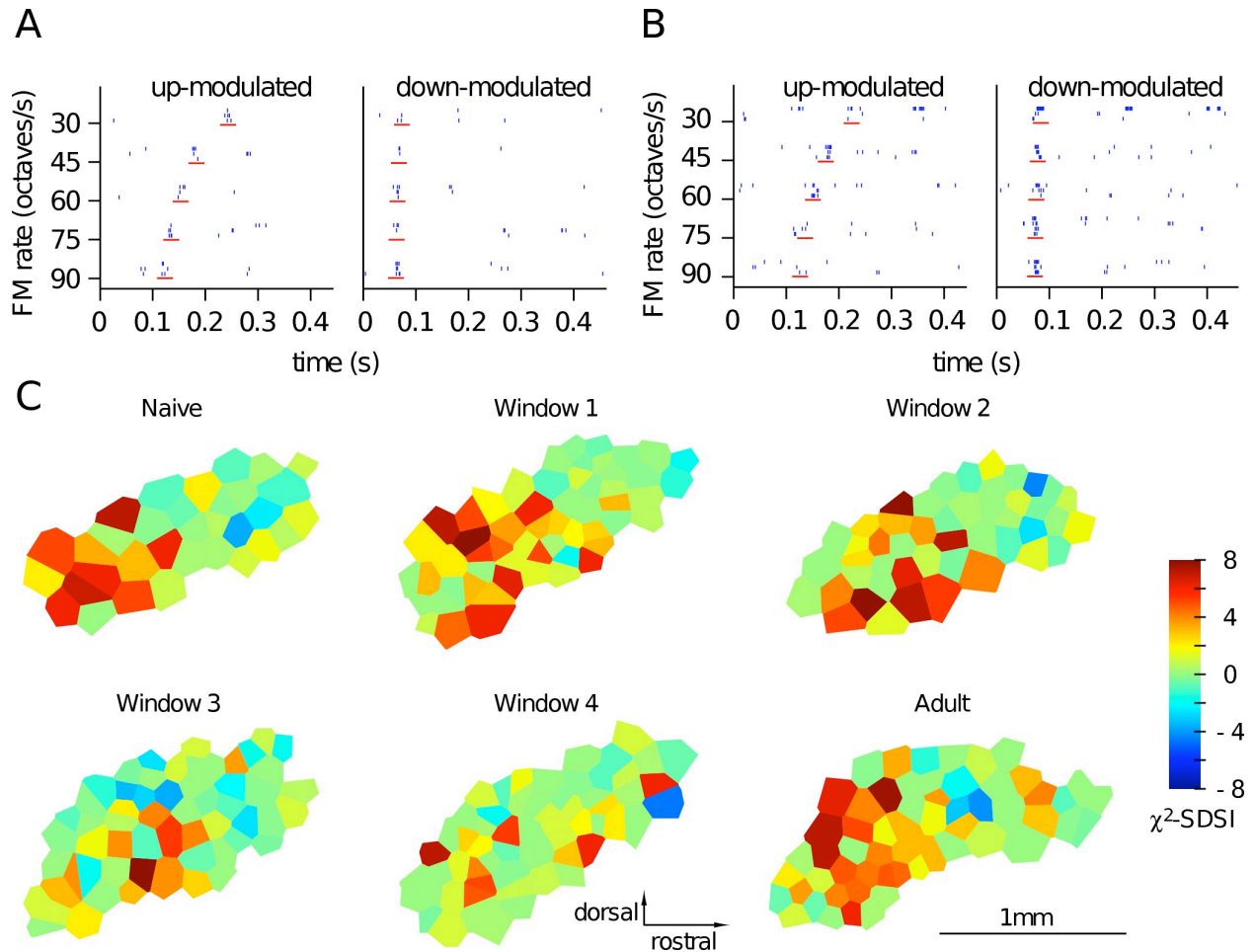


Figure 11. Exposure to frequency-modulated sounds in the third and fourth time windows alters sweep direction selectivity. **A, B**, Raster plots to FM sounds recorded from high frequency selective neurons in naive and FM-exposed animals, respectively. The red horizontal bars denote the 30 ms response windows. Note the selective response to downward-modulated sounds in **B**. **C**, Representative cortical maps of the χ^2 -based sweep direction selectivity. Direction selectivity index was calculated from responses to upward and downward sweeps at a rate of 90 octaves/s. Experimental animals were exposed to downward sweeps in four different time windows. Animals that had heard the downward sweeps in window 4 showed a negative shift of the selectivity index, indicating that the neurons were more selective for downward sweeps than those of the other groups.

We also identified the sweep rate that activated the strongest response in each neuron, which we defined as its best FM rate. Initial examination of the best FM rate showed no significant difference among the Naïve, P8-15 and P16-23 groups, or between the P24-31 and P32-39 groups; we therefore collapsed those conditions for all further analyses. Neurons that had a best FM rate at - 90 octaves/s were more frequently observed in P24-31/P32-39 animals than in Naïve/P8-15/P16-23 animals (Figure 12C; χ^2 -test, $p < 0.05$). In P24-31 and P32-39 animals, more neurons had best FM rates at - 90 octaves/s than at - 30 octaves/s (Figure 12C; χ^2 -test, $p < 0.05$). The downward/upward response ratios were also significantly increased in the P24-31/P32-39 animals for 75 and 90 octaves/s rates when compared to the slower rates, and when compared to the ratios in the Naïve/P8-15/P16-23 animals for the same 75 and 90 octaves/s rates (Figure 12D; χ^2 -test, $p < 0.05$). These results indicate that some cortical neurons shifted their FM rate selectivity to that of the experienced sweep. The sensitive period for FM direction

selectivity observed in this study coincides with the maturation of inhibitory areas in the frequency-intensity receptive field (Chang et al., 2005), which may contribute to FM direction selectivity (Zhang et al., 2003; Razak and Fuzessery, 2007).

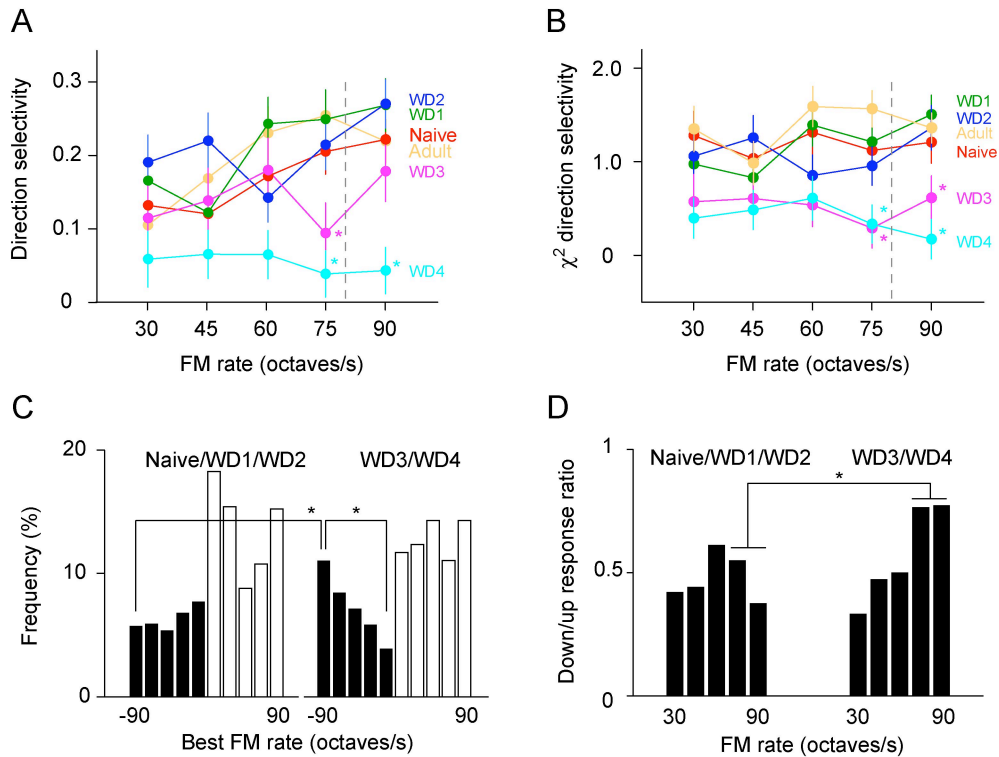


Figure 12. Exposure to frequency-modulated sounds alters sweep direction and rate selectivity. A. Sweep direction selectivity index (SDSI) at different sweep rates. Exposure to 80-octaves/s downward FM sounds (marked by the dashed line) in W3 and W4 resulted in downward shifts of SDSI near the exposed sweep rate. B. χ^2 -based sweep selectivity. C. Histogram of best FM rates that activate the strongest responses in cortical neurons. More neurons in W3 and W4 animals became tuned to -90 octaves/s, which is near the exposure FM rate. D. The ratio of neuronal responses (number of spikes in a 30-ms window) to downward vs. upward FM sweeps. The ratio is greater for W3 and W4 animals than for the other groups. * indicates $p < 0.05$.

We examined the auditory cortex of animals that had been exposed to 80 octaves/s downward FM sweeps from P8-23, and found reduced representations of frequencies below 4 kHz (χ^2 -test, $p < 0.05$), broadened tuning bandwidth (ANOVA, $p < 0.05$), but no downward shift of FM direction selectivity (Figure 13). Animals that had been exposed to the downward FM sweeps in all four time points showed reduced representations of frequencies below 4 kHz (χ^2 -test, $p < 0.05$) and an increase in downward FM direction selectivity (Figure 123 ANOVA, $p < 0.05$) in primary auditory cortical neurons. The neuronal tuning bandwidth, however, was not broadened (Figure 13D, ANOVA, $p > 0.5$). Thus, sound experience from P8-15 did not prevent subsequent broadening of frequency tuning in P16-23 animals or shift FM direction selectivity in P24-31 or P32-39 animals. Furthermore, although exposure to downward FM sweeps from P24-31 and P32-39 does not change the bandwidth (Figure 10, $P > 0.5$), it can re-normalize the broadened tuning caused by earlier experience from P16-23, suggesting that the plasticity effects induced in different sensitive periods may be interactive (i.e., reduction of bandwidth during P24-31 and

P32-39 depends on the increase of bandwidth during P16-23). Results for all six conditions are summarized in Table 1.

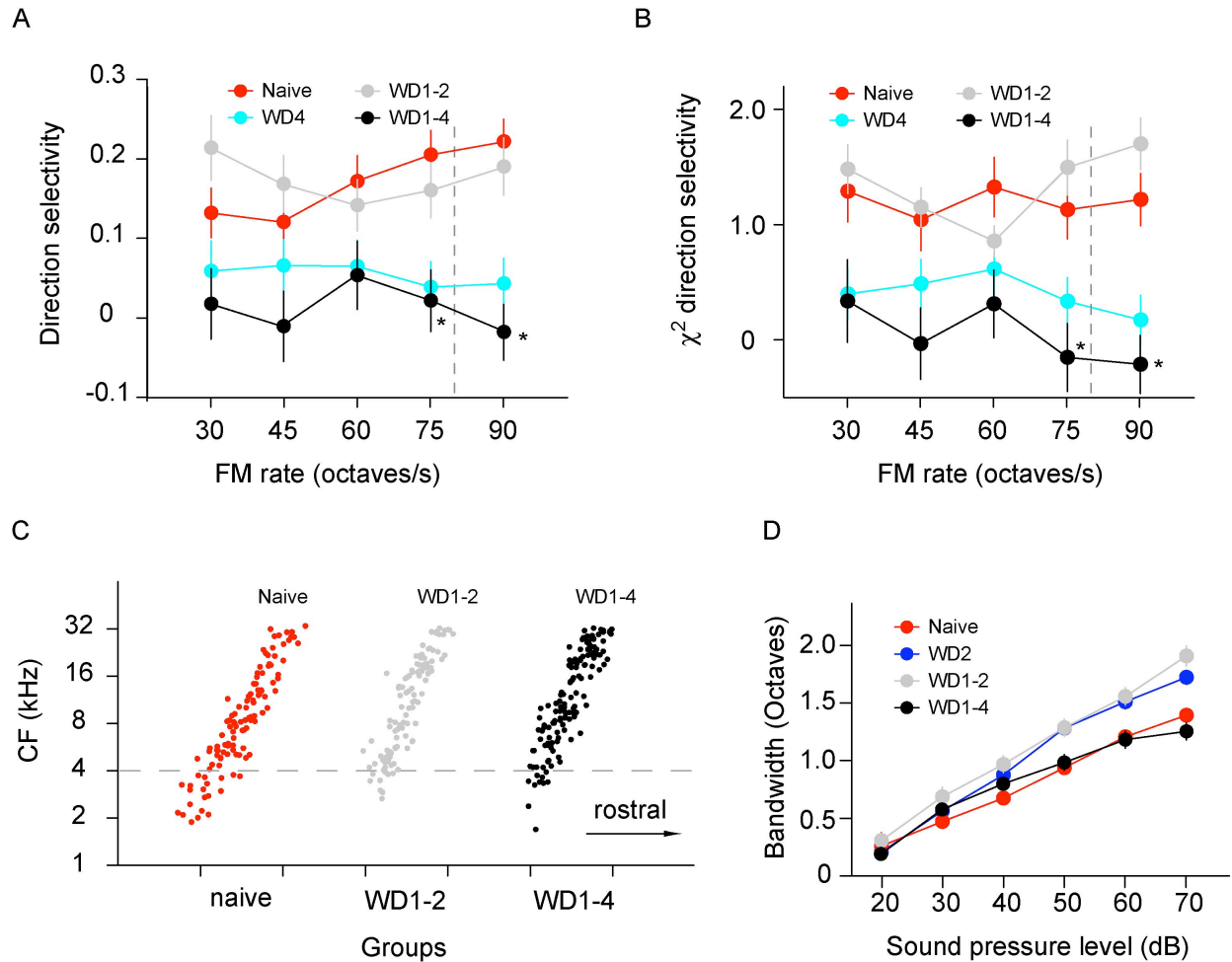


Figure 13. Exposure to downward sweeps in all four windows alters CF distribution and sweep direction selectivity. A. The SDSI was down-shifted in animals that had been exposed to downward sweeps in Windows 1 through 4, but not in animals exposed to the sweep in Windows 1 and 2. B. χ^2 statistic of sweep direction selectivity. C. Characteristic frequency distribution along the tonotopic axis. Both Window 1-2 and Window 1-4 groups had fewer neurons tuned to frequencies below 4 kHz. D. Tuning bandwidth as a function of sound pressure levels. The Window 1-2 group, but not the Window 1-4 group, showed broadened frequency tuning at 50-70 dB SPL.

Table 1. Effects of sweep exposure on neuronal response properties

	Window 1	Window 2	Window 3	Window 4	Window 1-2	Window 1-4
CF	Shifted				Shifted	Shifted
BW		Broadened			Broadened	
SDSI			Shifted	Shifted		Shifted

2.5 Development of cortical responses

To determine whether the three sensitive periods are correlated with specific developmental events, we examined the development of cortical acoustic representations in *naïve* animals. By ~P15, the CF map of the primary auditory cortex of the naïve group became finely tonotopic, and AI neurons showed narrow spectral tuning as reported previously (de Villers-Sidani et al., 2007). Most AI neurons represented middle frequencies, with less representation of frequencies below 4 kHz or above 25 kHz (for example, see Figure 15). Spectral tuning rapidly became broader between P16 and P18 (Figure 14B), coinciding with the sensitive period for tuning bandwidth. Tuning remained broad throughout P24-31 and P32-39. Cortical responses to FM sweeps were direction-selective in all four time points, with no significant difference between the strengths of sweep direction selectivity in the four time points (Figure 14C) ($p > 0.5$). The magnitude of cortical responses to FM sweeps was quantified with the maximum and mean responses to the 10 sweeps used in the experiment (i.e., upward- and downward-modulated sweeps at 30, 45, 60, 75, 90 octaves/sec). Both the maximum and the mean response magnitudes to FM sweeps increased rapidly between P24 to P26, coinciding with the *onset* of the sensitive period for FM sweep direction selectivity (for example see, Figure 14C). The coincidences between the emergence/maturation of sound feature representations and the sensitive period of the corresponding features suggest that sensory development and plasticity involve the same cortical substrates.

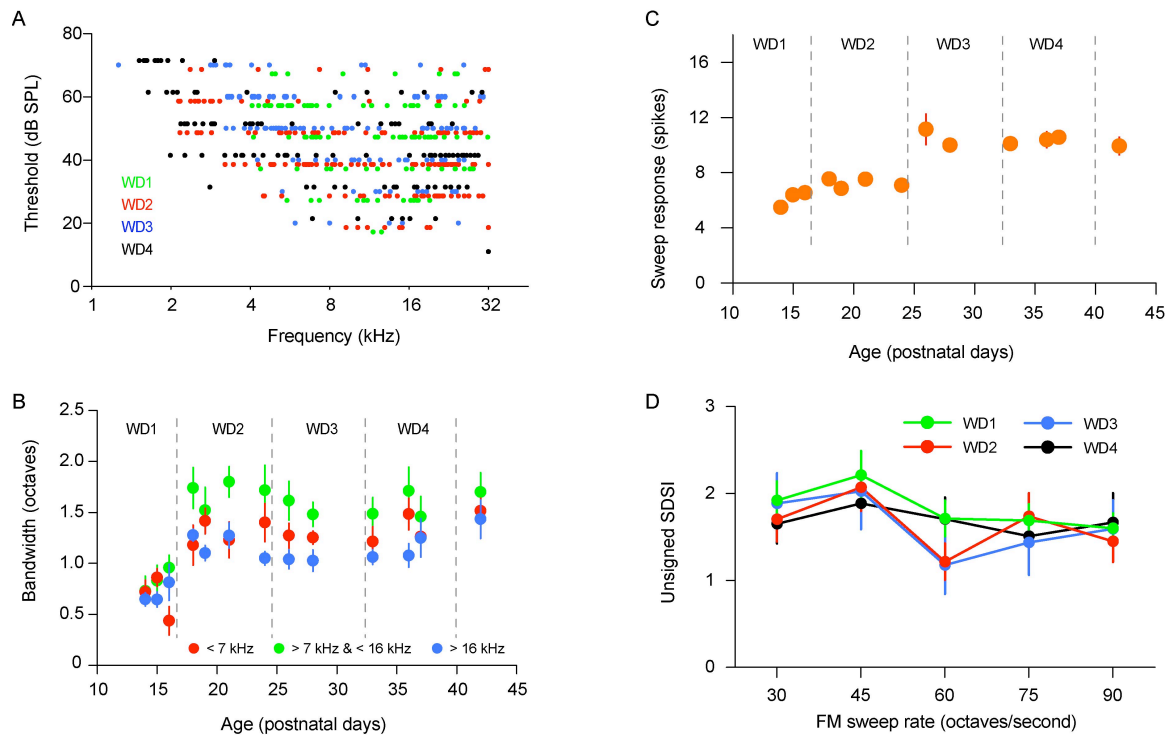


Figure 14. Development of response threshold, tuning bandwidth and sweep response in different time windows. A. Frequency-dependent development of response threshold. Low frequency responses emerge after the age of P16, whereas the response thresholds for middle- and high-frequency neurons were not different among the four time windows. A 1-dB plotting offset was introduced between groups for better group distinction. B. Tuning bandwidth increases in window 2. Error bars depict SEM. C. Sweep response magnitude increases in Window 3. The spike response to FM sweeps was counted in 30-ms windows. Some of the error bars are occluded by the data symbols. D. The strength of sweep direction selectivity did not change during development. The mean of the absolute values of the χ^2 -based SDSI is used so that selectivity for up and downsweeps are equally considered.

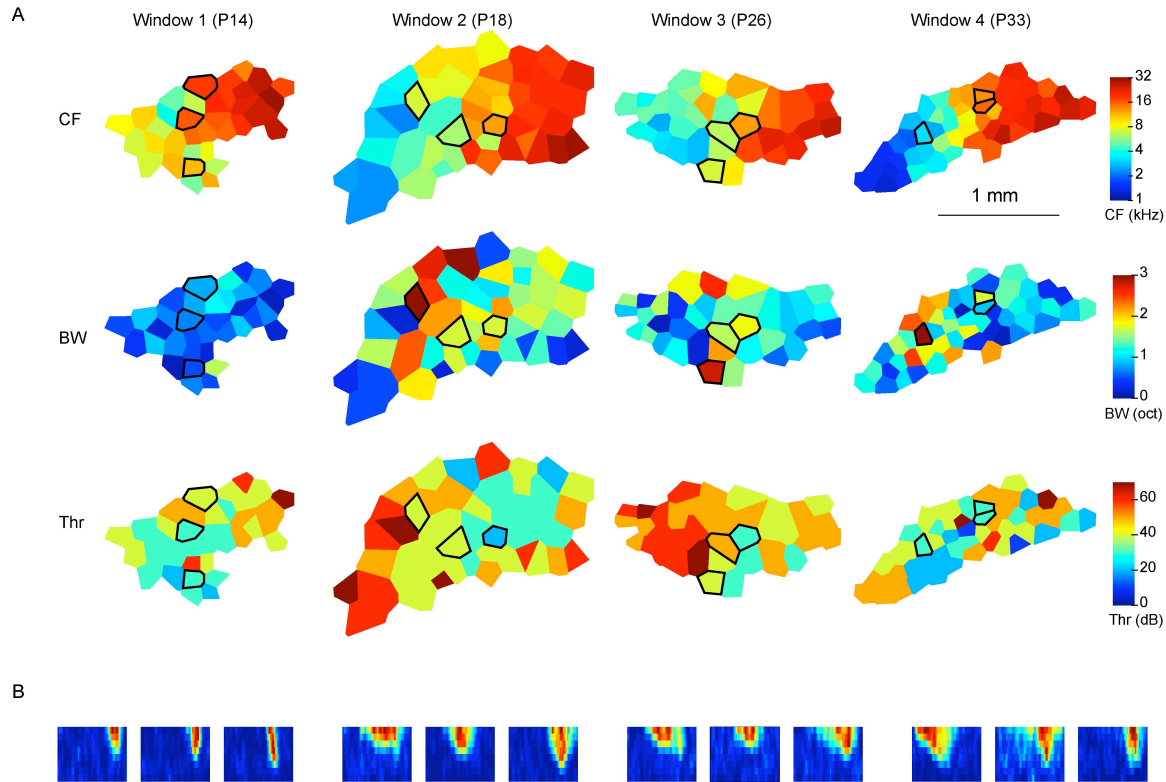


Figure 15. Example cortical maps and receptive fields. A. Representative cortical CF, BW and response threshold maps obtained in four different developmental windows. B. Representative receptive fields of the neurons whose recording sites are outlined in the maps. The vertical axis of the receptive field plots depicts sound intensity from 0 to 70 dB SPL. The horizontal axis depicts frequencies from 1 to 32 kHz. Neurons in the primary auditory cortex of the P14 animal had narrower frequency tuning than the older animals, while their response thresholds were comparable. The size and shape of the functionally determined primary auditory cortex were more variable in juvenile animals (< P40) than in adults (> P40). The difference in AI sizes seen here was not significant

2.6 Summary

The results of the present study indicate that primary auditory cortical representations are shaped by different features of the same sound in distinct time windows. We referred to these time windows as sensitive periods in the present study to indicate the different time windows of feature sensitivity *within the critical period* of heightened cortical plasticity. Thus, the critical period of sensory development in the primary auditory cortex is not a unitary event, but comprises a series of feature-dependent sensitive periods. Sensitive periods for more complex sound features tend to occur later, with each sensitive period approximately coincidental with the emergence or maturation of the cortical representation of the corresponding feature—the sensitive period for the cortical tonotopic CF map reside in Window 1, when the cortical map first emerges (de Villers-Sidani et al., 2007); the sensitive period for bandwidth plasticity is located in Window 2, when bandwidths of cortical neurons increase normally; plasticity for FM direction selectivity occurs in Windows 3 and 4, when strong cortical responses to FM sounds develops (Zhang et al., 2001; Chang and Merzenich, 2003; Chang et al., 2005; de Villers-Sidani

et al., 2007). These findings suggest that the sensitive periods described in the present study are determined by normal experience-dependent development of sensory representations.

Chapter 3. Pulsed noise experience disrupts complex sound representations

3.1 Preface

In Chapter 2, I described a series of experiments, the results of which demonstrate that the development of auditory feature representations in the primary auditory cortex is organized hierarchically as a series of sensitive periods sequenced from rudimentary to more complex representations. I tested this staggered sensitive period model of auditory feature representations by exposing litters of rats to pulsed broadband noise during finite time points during development. The results from this study indicate that the disruptive effects of pulsed broadband noise are restricted to the elaboration of a particular auditory feature during development. Furthermore, A1 tonotopicity, tuning bandwidth, intensity threshold, tone-responsiveness, and sweep response magnitude were differentially altered by noise experience depending on the exposure time windows. These results are consistent with my previous finding of multiple sensitive periods in A1.

3.2 Methods

Acoustic rearing

The UC Berkeley Animal Care and Use Committee have approved all procedures used in this study. Litters of rat pups (female, Sprague-Dawley) and their mothers were placed in a sound-attenuation chamber for a brief period, comprising one of four time windows (P8-P15, P16-P23, P24-P31 and P32-P39). Pulsed trains of uniform white noise were generated with a LabView program and I/O card and played through a Vifa speaker (XT25TG30-04) placed in the sound attenuation chamber 24 hours/day. The peak sound pressure level was measured with a BK microphone and conditioning amplifier over a 5-ms window and in the 1-32 kHz range, and was set at 65 dB SPL. Figure 16 shows the spectral density of the noise. Each noise pulse was 50-ms long with 5-ms on- and off-ramps. Each noise pulse train consisted of 6 noise pulses played at 6 Hz. Trains were separated by 500 ms quiet periods. In addition, the acoustic environment may have also contained vocalization sounds produced by the animals, mainly in a frequency range above 32 kHz. After the exposure period, the animals were returned to normal animal rooms.

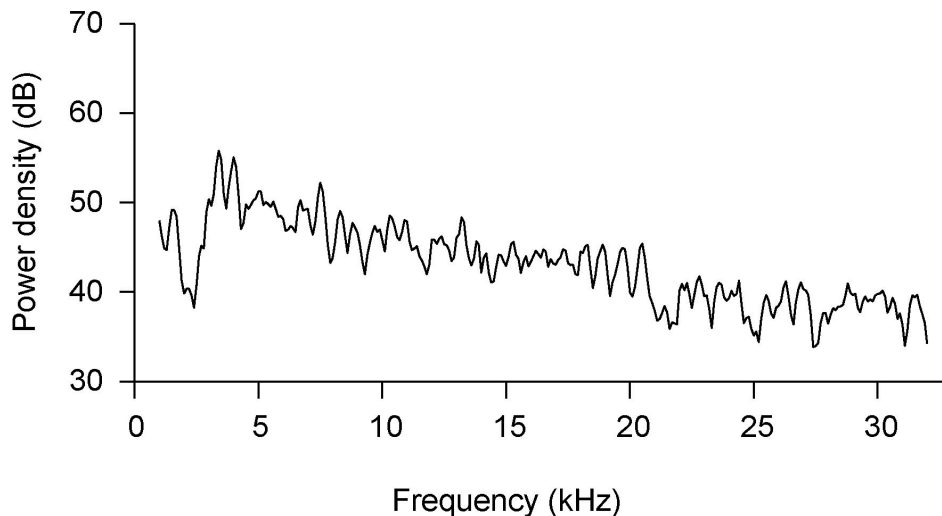


Figure 16. Power spectrum density of the pulsed noise.

Electrophysiological recording

The A1 of pulsed noise-exposed and naïve animals (four animals for each group) were mapped under pentobarbital anesthesia (50 mg/kg for induction, 10-20 mg/kg supplemental, as needed; intraperitoneal injections) at ages P40 to P60. Animals in different groups were roughly age-matched. The primary auditory cortex was defined by its tonotopic organization and reliable unit responses to tone pips of selective frequencies. Units were evenly sampled from the primary auditory cortex using parylene-coated tungsten microelectrodes (2 M Ω) advanced 500-600 micrometers below the pial surface (layer4/5), and responses to 25-ms tone pips of 50 frequencies (1 to 32 kHz, 0.1 octave spacing) and 8 sound pressure levels (0-70 dB SPL, 10 dB steps) were recorded three times to reconstruct the response-frequency tuning curve. Responses to a series of exponential FM sweeps (frequency range, 0.5-50 kHz; sweep rates, 30, 45, 60, 75 and 90 octaves/second, at 60 dB SPL, in both up and down directions) were also recorded three times to determine the units' selectivity for FM sweep rates. Tone pips and FM sweeps were intermixed and presented in a pseudorandom order through a custom made STAX speaker. A tucker-Davis Technologies system (TDT System 3) was used for speaker calibration, sound delivery, and electrophysiological recording.

Analysis

The receptive field was analyzed automatically with a custom-made program. In brief,, all responses in the receptive field were thresholded at 28% of the maximum response magnitude, and smoothed with a 3x3 median filter. A frequency-intensity response area was defined as three continuous pixels of responses of at least one spike per tone over the threshold. The CF and tuning bandwidth of each unit was derived from all response areas of the receptive field. The CF was calculated as the center of mass, and the tuning bandwidth was defined as the bandwidth of all response areas. Responsiveness of a unit to tones was quantified with the sum of all spikes in the response areas over the threshold. A unit was considered non-responsive to tones if there was not a response area in the receptive field. To generate CF maps, Voronoi tessellation was

performed to create tessellated polygons, with each polygon corresponding to an electrode penetration site, and assigned the CF of the site.

The magnitudes of responses to the exponential FM sweeps were determined in 30-ms response windows that were automatically set to best fit the response latencies at all the different sweep rates. Under the assumption that the unit response was triggered by a specific frequency (denoted as f in kHz) in the FM stimuli, for upsweeps, $FM_Rate \times (T - T_{min}) = \log_2(f/f_{min})$, in which T is the measured latency of the response to the sweep at the FM_Rate , and T_{min} is the minimal response delay. A regression analysis was performed on FM_Rates and Ts (peak response latencies for each FM rate derived from 20-ms smoothed PSTH) to derive T_{min} and f . The expected latency was then calculated as $T_{exp} = \log_2(f/f_{min}) \times FM_Rate^{-1} + T_{min}$. A 30-ms window centered at T_{exp} was used as the response window. The number of spikes in the response window was counted as the response magnitude. Spontaneous firing was determined from the 30-ms pre-stimulus period, and was subtracted from the response to the FM sweeps. T_{min} and f are derived separately for up and downsweeps. For downsweeps, $FM_Rate(T - T_{min}) = \log_2(f_{max}/f)$, and $T_{exp} = \log_2(f_{max}/f) \times FM_Rate^{-1} + T_{min}$. Our visual inspection indicated that the response windows consistently captured the phasic cortical responses to the sweeps.

A sweep direction selectivity index (SDSI) was calculated as $(R_{up} - R_{down})/(R_{up} + R_{down})$, in which R_{up} is response magnitude to the upswing, and R_{down} is response magnitude to the downswing. To avoid bias by small responses, we calculated a χ^2 -based statistic of upswing selectivity: χ^2 -SDSI = $(R_{up} - R_{down})|R_{up} - R_{down}|/(R_{up} + R_{down})$.

Determining the locations of recorded sites on the tonotopic axis

A line connecting the site with the highest CF and that with the lowest CF was defined as the tonotopic axis. The maps were rotated to orient the tonotopic axis horizontally. For all recorded sites in each map, a linear regression was performed in the form of $\log(CF) = k(X - c)$, in which CF is the characteristic frequency in kHz, and X is the x-coordinate, of the recorded site. Then, X - c is used as the location of the recorded site on the tonotopic axis.

Unless stated otherwise, statistical significance was quantified with ANOVA and post-hoc Bonferroni's test using StatView (SAS Institute). Data are presented as mean \pm SEM.

3.3 Exposure to pulsed noise disrupts cortical frequency tuning and tonotopicity

We exposed rat pups to pulsed white noise (50-ms noise pulses, 5-ms ramps, 65 dB SPL, 6 pps with 500 ms of silence between trains) in one of four time windows (P8-15, P16-23, P24-31 and P32-39) and examined cortical sound representations from P40 to P60. Comparison of frequency-intensity receptive fields indicates that exposure to pulsed noise during P8-15 led to a greater number of presumptive AI sites that did not respond to tones (as marked in gray in Figure 17A; also see Figure 17B-5). Such poorly responsive sites were less frequent in P16-23, P24-31 and P32-39 groups ($p < 0.0001$; P8-15, 19 ± 5 ; P16-23, 3 ± 1 ; P24-31, 4 ± 1 ; P32-39, 3 ± 1), and were not seen in naïve animals. Because AI sites are generally defined by their response to tones,

defining non-responsive AI sites was somewhat arbitrary, especially for the bordering sites. However, the large number of non-responsive sites in W1 animals was not likely due to over sampling of non-AI sites because the sizes of AI were not significantly different among the five groups (W1, $1.18 \pm 0.05 \text{ mm}^2$; W2, $1.15 \pm 0.06 \text{ mm}^2$; W3, $1.09 \pm 0.11 \text{ mm}^2$; W4, $1.18 \pm 0.03 \text{ mm}^2$; naïve, $1.21 \pm 0.04 \text{ mm}^2$; $p > 0.5$). In addition, the non-responsive sites were located mostly in the AI area defined by tone selective sites and the appropriate tonotopic gradient. These non-responsive sites were approximately evenly distributed along the tonotopic axis—a KS test revealed the distribution of the non-responsive sites was not significantly different from a uniform distribution ($p > 0.5$; see Figure 18A).

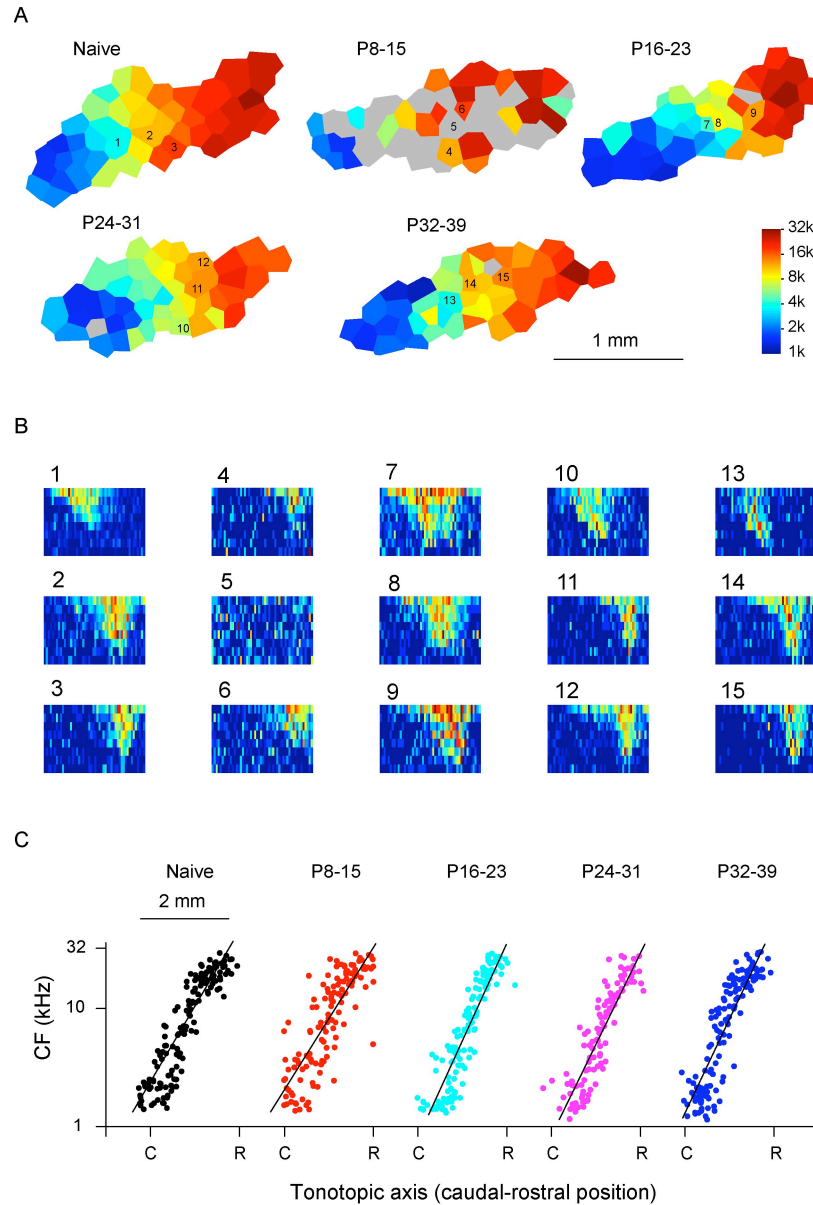


Figure 17. Exposure to pulsed noise alters AI tone responsiveness and the tonotopic CF map. A. Representative cortical CF maps of control, P8-15, P16-23, P24-31 and P32-39 animals. Sites that did not respond to tones (i.e., did not have a response area) are plotted in gray. B. Receptive fields of the numbered sites in A. The

horizontal axis depicts logarithmically scaled frequency from 1 to 32 kHz. The vertical axis depicts tone intensity from 0 to 70 dB SPL. C. CF distribution along the tonotopic axis. The linear regression lines are plotted. The points are more scattered for the P8-15 group than for all the other groups, indicated by significantly great residual of the linear regression analysis ($P < 0.0001$).

To determine whether the occurrence of the non-responsive sites was due to a general reduction of cortical responses in all units, or a reduction of responses in a specific subpopulation of units, we quantified the size of the frequency-intensity response area of the tone-responsive units and found that the P8-15 group had significantly smaller response areas compared with the P16-23, P32-39, and naïve groups ($p < 0.05$; Figure 18, B and C). In addition, the response magnitude to tones in the response area was reduced in the P8-15, P24-31, and P32-29 groups compared with the naïve group ($p < 0.01$; Figure 18, D and E). Among the noise-exposed groups, the response magnitude was lower for the P24-31 group than the P8-15 and P16-23 groups ($p < 0.01$) and was lower for the P32-39 group than the P16-23 group ($p < 0.05$).

Analysis of the receptive field indicates that the noise exposure also altered tone response threshold (Figure 18F). Units from the P8-15 group had significantly higher thresholds than did the P16-23, P24-31 and the P32-39 group ($p < 0.05$; Figure 18F). The threshold of the P32-39 group was significantly lower than that of the naïve control group.

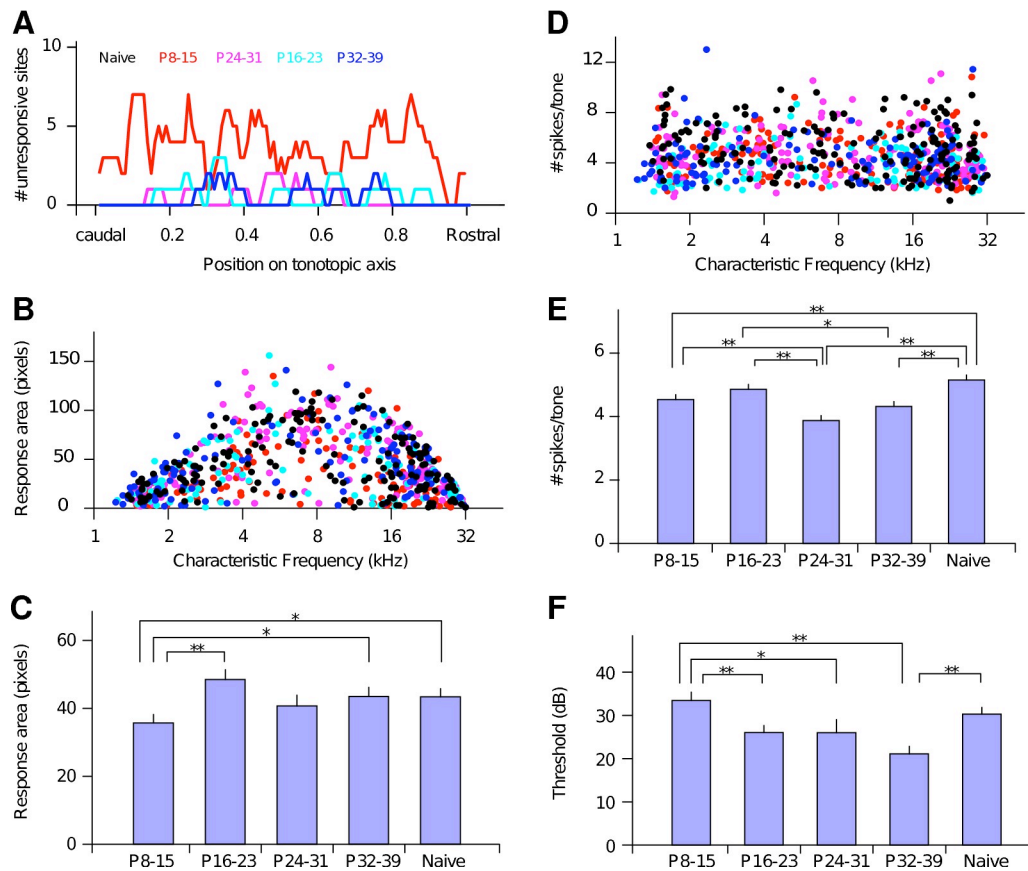


Figure 18. Exposure to pulsed noise alters AI receptive field properties. A. Number of non-responsive sites along the tonotopic axis. The bin size is 5% of the length of AI along the tonotopic axis. B and C, the size of the response areas as a function of the CFs. D and E, number of spikes per tone in the response areas. F, response threshold. Error bars show SE. * $P < 0.05$, ** $P < 0.01$.

The basic tonotopic gradient from caudal low frequencies to rostral high frequencies was present in all groups (Figure 17C). However, fine tonotopic organization was disrupted in P8-15 animals, as indicated by the wider scattering of points in its CF-tonotopic axis plot (Figure 17C). The residual of a linear regression analysis was greater for the P8-15 group than for other groups (P8-15, 0.29 ± 0.02 mm; P16-23, 0.17 ± 0.01 mm; P24-31, 0.19 ± 0.01 mm; P32-39, 0.21 ± 0.01 mm; naive, 0.20 ± 0.01 mm; $p < 0.0001$).

Unlike exposure to downward FM sweeps during P8-15, which results in reduced representations of frequencies lower than 4 kHz (Insanally et al. 2009), exposure to pulsed noise did not cause a distortion in the proportional allocation to any given range of CFs (Figure 17A and 2C and Figure 20C).

3.4 Exposure to pulsed noise broadens frequency tuning bandwidth

We calculated the frequency tuning bandwidth from units that had frequency-intensity receptive fields. The bandwidths of units in the P16-23 animals were significantly broader than those of the other groups at sound pressure levels 50 to 70dB (Figure 17B and Figure 19A; $p < 0.05$). To determine whether the bandwidth differences were an epiphenomenon of the noise exposure-induced threshold shifts (Figure 18D), we measured the bandwidth relative to the response threshold. The bandwidth was significantly broader for the P16-23 group than for all the other groups at 40 dB above threshold (Figure 19B, $p < 0.05$). These findings indicate that exposure to a broadband stimulus in P16-23 results in broader receptive field tuning, as shown in a previous report (Insanally et al. 2009). In addition, the bandwidth was significantly narrower for the P32-39 group than for all the other groups at 30 and 40 dB above threshold (Figure 19B; $p < 0.05$).

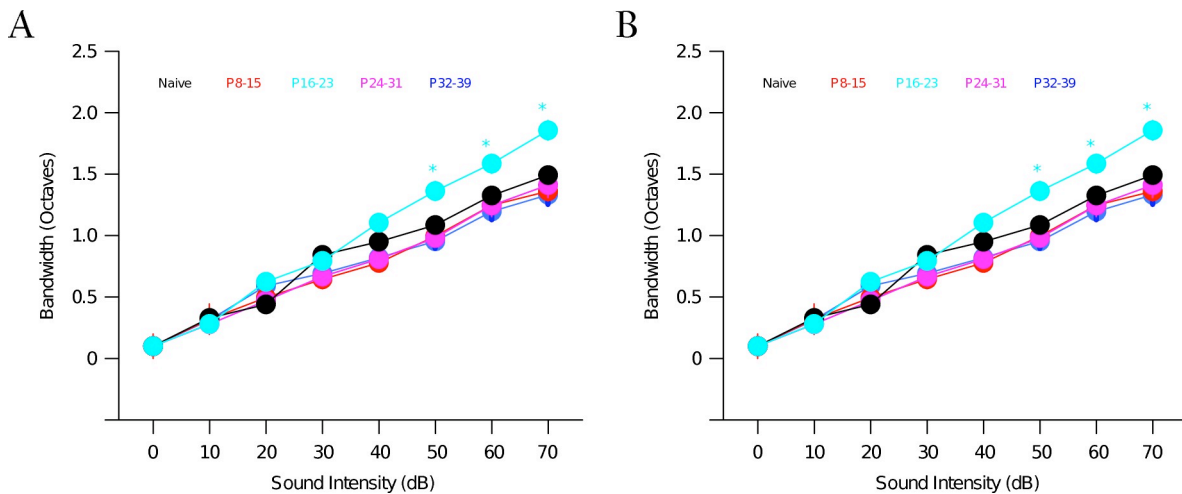


Figure 19. Exposure to pulsed noise alters bandwidth tuning of AI units. **A.** Bandwidth measured at different sound pressure levels. **B.** Bandwidth measured at various sound levels above the threshold. Error bars depict SEM, some of which are smaller than, and occluded by, markers of the plot. *, $p < 0.05$; **, $p < 0.01$. ANOVA compared with all the other groups.

3.5 Exposure to pulsed noise alters FM sweep direction selectivity

All experimental groups were affected by pulsed noise exposure in different ways. We observed a loss of CF dependence on direction selectivity in animals exposed to pulsed noise from P8-15, P24-31 and P32-39. In addition, the sweep response magnitude was significantly lower for all noise-exposed groups.

We recorded cortical responses to up and downward sweeps at five rates (30, 45, 60, 75 and 90 octaves/s) from tone responsive units (see 3.2 Methods for definition). The non-responsive AI units were either weakly or not responsive to the exponential FM sweeps and were not analyzed further (mean response magnitude to all ten FM sweep sounds was 0.86 ± 0.07 spikes/sound for non-responsive AI sites and 3.26 ± 0.21 spikes/sound for responsive AI sites, $p < 0.0001$). We calculated a χ^2 -based sweep direction selectivity index (χ^2 -SDSI) that measures the level of sweep direction selectivity (see 3.2 Methods). Units that are selective for upsweeps will have a positive χ^2 -SDSI, those selective for downsweeps will have a negative χ^2 -SDSI.

Previous studies reported that high-CF units tend to select for downward sweeps and low-CF units for upward sweeps (Heil et al. 1992; Zhang et al. 2003). We observed this CF dependence of sweep direction selectivity in AI units of naïve animals for high sweep rates at 75 and 90 octaves/s (Figure 20), but not for low sweep rates at 30, 45 and 60 octaves/s. Exposure to pulsed noise during P8-15, P24-31 and P32-39 resulted in a loss of CF dependence of sweep direction selectivity. Figure 20A depicts the χ^2 -SDSI at the sweep rate of 90 octaves/s as a function of CF. A linear regression analysis revealed that the slopes of the regression line are significantly negative for the P16-23 and naïve control groups ($p < 0.05$), and are not different from 0 for P8-15, P24-31 and P32-39 groups ($p > 0.05$). When grouped by CFs into 1-octave bins, χ^2 -SDSI is a decreasing function of CF only for the P16-23 and naïve control groups ($p < 0.05$, comparing CF < 8 kHz vs. CF > 16 kHz), but not for the other groups ($p > 0.05$). The same results were seen for the sweep rate of 75-octaves/s ($p < 0.05$ for the P16-23 and naïve control groups but not the other groups).

When compared between groups, the χ^2 -SDSI is significantly lower for P16-23 and naïve groups than for the other groups at 16-32 kHz, and is significantly lower for P8-15 group than for the other groups at 8-16 kHz (Figure 20B). This loss of CF dependence is not due to a general loss of direction selectivity because the strength of direction selectivity, as measured by the mean absolute value of the χ^2 -SDSI is not different among the five groups for any of the sweep rates ($p > 0.1$). Furthermore, the general distributions of the χ^2 -SDSI are not different among the five groups (Figure 20D, pair-wise KS tests, $p > 0.05$).

We also calculated the magnitude of the cortical responses to the best FM sweep, the one that activated the unit strongest. All noise exposed animals showed weaker sweep responses compared to naïve animals (Figure 20E, $p < 0.05$). In addition, the sweep response was weaker for the P8-15 group than for the P16-23 group ($p < 0.05$).

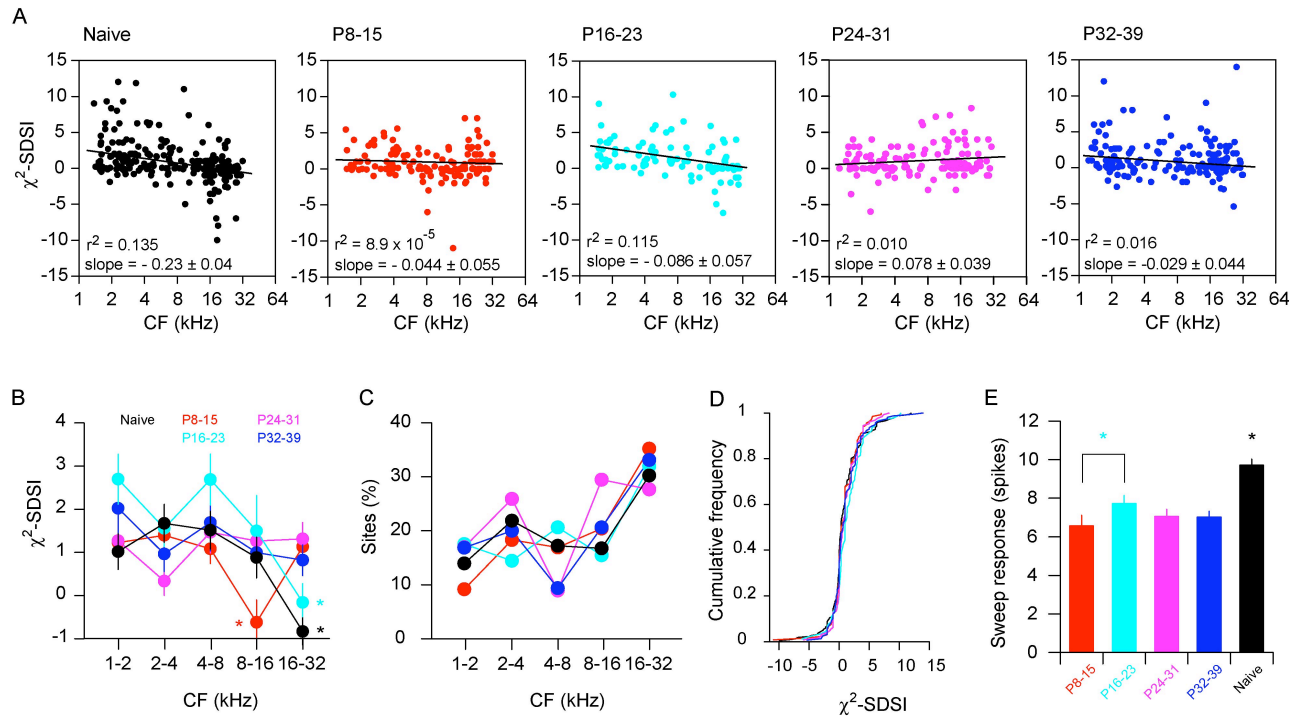


Figure 20. Exposure to pulsed noise alters FM sweep representation. A. χ^2 -SDSI as a function of CF for P8-15, P16-23, P24-31, P32-39 and control animals. Each point represents a unit for which sweep responses have been recorded. The linear regression line is plotted, and the 95% confidence interval of the slope is indicated. Only the P16-23 and the control groups had significantly negative slopes. B. Average χ^2 -SDSI as a function of CF for all groups. Note the lack of CF dependence in P24-31 and P32-39 groups. P8-15 group also lacked the typical downward FM direction selectivity at high frequencies. The χ^2 -SDSI index value is different ($p < 0.05$) between the low (1-8 kHz) and high (16-32 kHz) frequency bins for P16-23 and control groups, but not for the other groups. C. Number of recorded sites in each frequency bin. D. Cumulative distributions of the χ^2 -SDSI. The distributions are not significantly different among the five groups. E. The magnitude of responses to the FM sweeps. * indicates $p < 0.05$. A sweep speed of 90 octaves/second was used in the analysis for these figures.

3.6 Summary

Here, we examined the influence of pulsed noise experience on the development of sound representations in the rat primary auditory cortex. In naive animals, FM sweep direction selectivity depends on the CF of the unit - low CF units tend to select for upward sweeps and high CF units for downward sweeps. Such a CF dependence was not observed in animals that had received weeklong exposure to pulsed noise in periods from postnatal day 8 (P8) to P15 or from P24 to P39. In addition, A1 tonotopicity, tuning bandwidth, intensity threshold, tone-responsiveness, and sweep response magnitude were differentially affected by the noise experience depending on the exposure time windows.

Chapter 4. Conclusions and Implications

4.1 Summary of Results

The two sets of comprehensive studies described in Chapter 2 and 3 demonstrate that experience of complex auditory stimuli can have profound effects on cortical sound representations in rat A1. In Chapter 2, I described a set of studies, the results of which indicate that early exposure to exponential FM sweeps effects the development of complex sound representations. Namely, by exposing rats to a complex sound we showed that there are a series of multiple sensitive periods in A1, where representations of complex sound features build upon earlier established, simpler sound features. Specifically, early exposure of rat pups to FM stimuli results in changes to the cortical CF map and BW tuning; whereas, later exposure to the same sound results in changes to FM sweep direction and rate selectivity. We then confirmed our model of multiple critical period plasticity by exposing animals to pulsed broadband noise in the same time windows as in the first study. In this case, we again found the plasticity effects to be restricted to different time windows. These results are consistent with the previous findings of feature-dependent multiple sensitive periods described in Chapter 2. The different exposure time-dependent effects induced here by pulsed noise and previously by FM sweeps further indicate that plasticity in cortical complex sound representations is specific to the sensory input.

4.2 Discussion of Results

Multiple critical period plasticity using FM sweeps as stimuli

The results of the present study indicate that primary auditory representations are shaped by different features of the same sound in distinct time windows. We referred to these time windows as sensitive periods in the present study to indicate the different time windows of feature sensitivity *within the critical period* of heightened cortical plasticity. Thus, the critical period of sensory development in the primary auditory cortex is not a unitary event, but comprises a series of feature-dependent sensitive periods. Sensitive periods for more complex sound features tend to occur later, with each sensitive period approximately coincidental with the emergence or maturation of the cortical representation of the corresponding feature—the sensitive period for the cortical tonotopic CF map reside in Window 1, when the cortical map first emerges (de Villers-Sidani et al., 2007); the sensitive period for bandwidth plasticity is located in Window 2, when BWs of cortical units increase normally; plasticity for FM direction selectivity occurs in Windows 3 and 4, when strong cortical responses to FM sounds develops (Zhang et al., 2001; Chang and Merzenich, 2003; Chang et al., 2005; de Villers-Sidani et al., 2007). These findings suggest that the sensitive periods described in the present study are determined by normal experience-dependent development of sensory representations.

Earlier studies have suggested that the sensitive period for visual direction selectivity may occur later than that for ocular dominance plasticity (Daw and Wyatt, 1976; Li et al., 2006). The two types of plasticity were induced with different procedures in those studies, and involved different sensory inputs, which might have contributed to the observed differences in the timing of the sensitive periods. Experience-dependent plasticity occurs in auditory brainstem in specific time

windows (Clopton and Silverman, 1977; Knudsen, 1985; Yu et al., 2005), and brainstem responses to different types of sounds may develop sequentially (Johnson et al., 2008). Acoustic input/feedback also drives sensory and motor phases of bird song learning in different time windows (Doupe and Kuhl, 1999). In this study, we have demonstrated that the auditory cortex extracts different information from the same stimuli to shape its cortical circuits in different developmental stages, revealing the progressive nature of the development of complex sound representations. Our findings suggest that the sensory inputs must match the processing capacity of the sensory system to improve processing efficiency.

The distinct sensitive periods suggest that CF, BW, and sweep direction selectivity are determined by different cellular circuitries, and cortical plasticity in those response properties occurs in corresponding circuitries (Hensch, 2004). In our experiments, the downward FM sweep activates high-CF neurons before it activates low-CF neurons, possibly enhancing intracortical excitatory connections from high-CF to low-CF neurons through spike-timing dependent plasticity (Yao and Dan, 2001; Fu et al., 2002), and causing an upward shift in CFs. Several recent studies have shown balanced excitatory and inhibitory frequency input to auditory cortical neurons, suggesting that frequency tuning is mediated primarily by excitatory input (Fu et al., 2002; Wehr and Zador, 2003; Zhang et al., 2003; Tan et al., 2004). The slightly lagged inhibitory input may serve to shape sweep selectivity and the precise timing of cortical responses (DeWeese et al., 2003; Zhang et al., 2003; Kandler, 2004; Razak and Fuzessery, 2007). The inhibitory circuit may also be involved in sculpting the excitatory circuit in cortical plasticity (Zheng and Knudsen, 1999; Fagiolini and Hensch, 2000; Fagiolini et al., 2004; Froemke et al., 2007). Recent studies have also suggested that the CF and BW may be determined differently by thalamocortical and intracortical connections (Kaur et al., 2004; Metherate et al., 2005; Liu et al., 2007). Thus, the three sensitive periods in the primary auditory cortex might be mediated separately by thalamocortical, intracortical excitatory and intracortical inhibitory circuits.

Multiple critical period plasticity using broadband pulsed noise as stimulus

Our results are in general agreement with a previous report of multiple sensitive periods for the developing auditory cortex. In addition, we show that the same response features may be altered in different sensitive periods. For example, bandwidth tuning was broadened by pulsed noise exposure during P16-23, and narrowed during P32-39. Similarly, the frequency dependence of sweep direction selectivity was disrupted by pulsed noise exposure during P8-15 and P24-39. Because these response features are determined by multiple (e.g., excitatory and inhibitory) cortical circuits (Galindo-Leon et al; Razak and Fuzessery 2009; Zhang et al., 2003), our results are consistent with multiple sensitive periods for the development of these different circuits.

The differences between the noise-induced effects reported here and FM sweep-induced effects reported in Chapter 2 indicate that plasticity effects of simple and complex sound representations depend on the spectrotemporal structures of the sensory input. For example, rearing rat pups in downward FM sweeps P8-15 resulted in reduced representations of frequencies below 4 kHz (Insanally et al. 2009). By contrast, animals reared in pulsed noise during the same period showed apparently normal low frequency representations, but disrupted tonotopicity. In addition, exposure to FM sweeps and pulsed noise during P24-31 and P32-39 also produced different outcomes to sweep selectivity of primary auditory cortical units. Exposure to downward FM

sweeps resulted in a significant increase in the number of units selective for downsweeps (Insanally et al. 2009), whereas exposure to pulsed noise eliminated CF dependence of χ^2 -SDSI. Animals that had been exposed to pulsed noise during P8-15 also lacked CF dependence of χ^2 -SDSI. Because sweep selectivity is presumably mediated by temporally offset excitatory and inhibitory receptive fields, altered excitatory receptive fields in P8-15 animals (Figure. 2 and 3) may have contributed to the disruption of sweep direction selectivity.

Exposing rat pups to pulsed noise from P9-P28 results in broadened (e.g., multi-peaked or flattened) tuning curves and degraded tonotopic maps (Zhang et al. 2002). In our study, the two effects occurred in different time windows--degraded tonotopicity in window 1 and broadened tuning curves in window 2. Thus, they might be mediated by different mechanisms that are active in different developmental stages. In addition, we have also observed a large number of non-responsive sites in the window 1 group that did not respond well to tones or sweeps. The mechanisms that lead to such diminished tone responses are unknown.

The findings that exposure to pulsed noise from P32 to P39 resulted in reduced threshold and narrower tuning bandwidth are unexpected. Broadband noise activates both the excitatory and inhibitory areas of the receptive field, and the interactions between the excitation and inhibition are likely important in determining the specific form of cellular plasticity that pulsed noise induces. It is possible that the period from P32 to P39 represents a unique window, in which broadband noise-activated excitation and inhibition interact to shape stronger connections from a narrower range of thalamic input. It is also possible that broadband noise enlarges sideband inhibition to shape narrower frequency tuning, and subsequent homeostatic excitatory plasticity leads to lower response thresholds.

To standardize data analysis, we used a program to automatically determine unit response properties such as CF, bandwidth, and sweep response magnitude. The CF was calculated as the center of mass of the frequency-intensity receptive field (See Chapter 3: Methods). These CFs are biased by responses tuned at high intensity levels, and tend to be lower than the CFs determined at the threshold sound level. Nevertheless, the center-of-mass CFs are highly correlated with the threshold CFs. In addition, the center-of-mass CFs may be more relevant in analyzing CF dependence of the FM sweep selectivity, because the FM sweeps were played at a high intensity level of 60 dB SPL.

Sweep direction selectivity is presumably mediated by sideband inhibition (Galindo-Leon et al. 2009; Razak and Fuzessery 2009; Zhang et al. 2003). Sideband inhibition for high-CF neurons is located mostly on the low frequency side, giving rise to its selectivity for downward sweeps. Likewise, low-CF neurons, having sideband inhibition mostly on the high frequency side, prefers upward sweeps. A recent study indicates that the development of GABA-mediated sideband inhibition is experience-dependent, and both the development and maintenance of FM direction selectivity requires appropriate experience (Razak and Fuzessery 2009; Razak et al. 2008). It is likely that the pulsed noise used in the present study dominated the acoustic environment, and masked/diluted the FM sound features in the natural acoustic environment, thereby disrupting normal development of inhibitory circuits and the CF dependence of sweep direction selectivity.

4.3 Implications

Studies of cognitive development have revealed multiple, hierarchical sensitive periods (Ruben, 1997; Werker and Tees, 2005). The sensitive period for phonological development, for instance, precedes those for syntactic and lexical development. Even within phonological development, speech experience alters vowel perception before shaping the perception of acoustically distinct consonants (Kuhl et al., 1992; Polka and Werker, 1994; Werker and Tees, 2005). We may consider static vowel sounds to be analogous to pure tones, and dynamic consonants to be more similar to FM sweeps. Thus, the multiple sensitive periods shown in the present study may be relevant to sequential phonological development.

Conclusions

In conclusion, acoustic experience has a significant impact on cortical complex sound representations. When complex sounds are used, a more nuanced understanding of critical period plasticity emerges; complex sound feature representation builds upon simpler features established earlier during development. This hierarchical system is successive and progressive in nature, such that frequency and bandwidth changes occur before that of sweep direction selectivity. This property of auditory cortical development allows for altricial species to adapt appropriate sensory representations.

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