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Behavioral Relevance of Fine Timing in Different Cortical Areas

A Dissertation Presented

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Yang Yang

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Yang Yang

We, the dissertation committee for the above candidate for the Doctor of Philosophy degree, hereby recommend acceptance of this dissertation.

Anthony M. Zador – Dissertation Advisor

Professor, Neuroscience, Cold Spring Harbor Laboratory

Glenn C. Turner – Chairperson of Defense

Assistant Professor, Neuroscience, Cold Spring Harbor Laboratory

Josh Dubnau – Committee Member

Assistant Professor, Neuroscience, Cold Spring Harbor Laboratory

Michael R. Deweese – Outside Committee Member

Assistant Professor, Helen Wills Neuroscience Institute, UC Berkeley

This dissertation is accepted by the Graduate School

LawrenceMartin

Dean of the Graduate School

Abstract of the Dissertation

Behavioral Relevance of Fine Timing in Different Cortical Areas

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Neurons in sensory cortices can lock with millisecond precision to the fine timing of some stimuli, but it is not known whether or how this precision is used behaviorally. I addressed three issues related to these questions. First I asked whether fine timing differences in neural activity of auditory cortex are sufficient to drive behavior. Second I asked whether different cortical areas are differentially able to exploit timing information behaviorally. Finally I asked whether sensory deprivation affect the ability of resolving cortical timing.

I used electrical microstimulation to establish a causal link between cortical timing information and behavior. I implanted two electrodes into the cortex of rats, and trained them to perform a two-alternative-forced-choice task in which the two stimuli to distinguish were simultaneous vs. sequential stimulation of the two electrodes. In this way I could bypass the sensory input and deliver stimuli directly to the cortex. I found that in auditory cortex, rats could exploit

time differences as short as 3 msec to drive decisions, which suggests that precise neural timing in auditory cortex is sufficient to drive behavior. I also found that different cortical areas were different in deriving behaviorally relevant information from the fine timing of neural activity. In the visual cortex, animals could be trained to resolve differences of 15 msec but not 5 msec, substantially longer than the 3 msec limit observed in the auditory cortex. The barrel cortex was even "faster" than auditory cortex, with a lower limit below 1 msec. To determine whether this ability was use-dependent, I deprived rats of sensory input into the barrel cortex by trimming their whiskers from birth until adulthood, and trained them to distinguish fine timing differences in barrel cortex. All sensory deprived rats showed significant defects in discrimination, indicating that the ability to exploit cortical fine timing information is use-dependent.

In conclusion, I found that: (1) precise cortical timing can drive behavior; (2) different sensory cortices have different thresholds; and (3) the ability to exploit timing information behaviorally is use-dependent. My findings have implications for the neural codes used by different sensory areas to encode information.

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Chapter 1

Introduction

How does brain work?

This is a question that has fascinated generations of neuroscientists. Though there is no answer to this question (yet), there is a consensus that neurons are the fundamental units of the brain, and that neurons represent and transmit information through trains of action potentials, or spikes (Crick 1994). To understand the brain, the first step is to understand the spikes.

There have been different views on how information is coded into spikes, and how spikes are read out (Softky and Koch 1993; Shadlen and Newsome 1998; deCharms and Zador 2000; Averbeck, Latham et al. 2006). In the cortex, people sometimes observed that spikes were highly irregular and noisy (Shadlen and Newsome 1998), and sometimes found that spikes were temporally precise and stimulus-locked (Buracas, Zador et al. 1998; Arabzadeh, Bathaie et al. 2002; Barbour and Wang 2003; DeWeese, Wehr et al. 2003). What we are interested in is whether this often-seen millisecond precision of neuronal responses in different sensory cortices can be utilized by animals in a behavioral context, and whether the ability of the cortex to decode this information depends on sensory experience. In this chapter, we are going to give a brief introduction to backgrounds on the precision of neural responses in different cortical areas, how electrical and optogenetic stimulation have been used to study neurobiology, and lastly how sensory deprivation during development affects the properties of barrel cortex.

1.1 Precision of Neuronal Responses in the Auditory Cortex

The early pioneering work of single-unit recordings in cat visual cortex showed that in response to sensory stimuli, cortical neurons responded with variable delay and spike counts (Hubel and Wiesel 1959). Similar results have later been reported in studies of visual and other sensory cortices (Adrian 1926; Werner and Mountcastle 1965; Tolhurst, Movshon et al. 1983; Tolhurst 1989; Britten, Shadlen et al. 1992; Tovee, Rolls et al. 1993; Petersen and Diamond 2000).

More recently, it was found that in the auditory cortex, some neurons respond to pure tones with high precision, in terms of both spike count and spike timing (DeWeese, Wehr et al. 2003). Deweese and colleagues found that the responses to repeated pure tone presentations were usually transient, and could lock with millisecond precision to the onset of the acoustic stimulus.

The transient responses could be explained by the balanced excitation and inhibition of neurons in the auditory cortex (Wehr and Zador 2003). Using whole-cell patch clamp recordings in anesthetized rats, Wehr & Zador found that inhibition and excitation occurred in a precise temporal sequence. The excitatory input was followed precisely by inhibition within 1-4 milliseconds. Excitation was rapidly quenched by inhibition, and therefore truncating the spiking response. The inhibition that immediately follows the excitation could possibly increase the temporal precision of neuronal spiking.

Similar results have also been observed in the auditory cortex of macaque

monkeys in a different experimental setup (Barbour and Wang 2003). Single unit recordings have shown that neurons in monkey auditory cortex can also fire precisely timed spikes in response to certain stimuli.

1.2 Precision of Neuronal responses in the Visual and Barrel Cortex

Traditional view is that in the visual cortex, neurons respond to visual stimuli over a long period of time and the information is included in the mean firing rate over several hundred milliseconds or even seconds (Schiller, Finlay et al. 1976; Dean 1981; Vogels, Spileers et al. 1989; Snowden, Treue et al. 1992; Britten, Shadlen et al. 1993).

However, with time-varying visual stimuli, it has also been found that neurons in certain parts of the visual cortex can respond with high temporal precision (Buracas, Zador et al. 1998). Buracas and colleagues presented alert monkeys with quickly drifting visual gratings while recording neuronal activities in MT (middle temporal visual area) with conventional single unit recording technique. The middle temporal visual area contains neurons that are selective to direction of moving objects (Maunsell and Van Essen 1983; Newsome and Pare 1988). It was found that when using the time-varying stimuli, the precision of the responses was in millisecond scale (time jitter ~3 ms). Temporally precise responses to time-varying stimuli have also been shown in other neural systems. (Bialek, Rieke et al. 1991; de Ruyter van Steveninck, Lewen et al. 1997; Borst and Theunissen 1999; Ahissar, Sosnik et al. 2000)

If the high temporal precision of neurons in visual cortex (monkey MT)

came as a surprise, there is no surprise that responses to whisker stimulation of neurons in barrel cortex were stimulus-locked and had a time jitter of only a few milliseconds (Shimegi, Ichikawa et al. 1999; Arabzadeh, Petersen et al. 2003), similar to the responses observed in auditory cortex and MT.

Barrel cortex is part of rodent somatosensory cortex, which processes tactile information from the facial whiskers (Simons 1978). It was called "barrel cortex" because layer IV of this cortical area show arrays of "barrels" with 2-Deoxyglucose staining (Land and Simons 1985). The special anatomical feature of the barrel cortex was first demonstrated by Hall & Lindholm (Hall and Lindholm 1974) and by Welker (Welker 1971; Welker 1976) using histologcal sections cut tangentially through layer IV from a flattened cortical hemisphere. Each whisker corresponds to one barrel, and barrel cortex is organized in the same spatial pattern as the facial whiskers.

Barrel cortex is well known for its precision in coding temporal and spatial aspects of the tactile stimuli (Ahissar and Arieli 2001). Each barrel corresponds to one principle whisker. The principal whisker evokes the strongest response and other whiskers evoke weaker responses (Welker 1971; Simons 1978; Armstrong-James and Fox 1987; Diamond, Armstrong-James et al. 1993). Stimulation of the principal whisker evokes spikes a few milliseconds earlier than the surrounding whiskers (Armstrong-James and Fox 1987; Armstrong-James, Fox et al. 1992), which suggests that spike timing may carry information about the location of the tactile stimulus.

1.3 Fine Timing and Behavior

Fine stimulus timing can be used to guide behavior. Inter-aural time difference (ITD) of a few microseconds is computed in subcortical nuclei to determine the spatial localization of sound.

In birds, ITDs are encoded by coincidence-detector neurons which receive excitatory inputs from two ears via axons of slightly different length (Jeffress 1948). The difference in axon lengths results in a difference in traveling time of spikes, and the time difference compensates for the ITD between the ears. Each coincidence-detector neuron fires maximally only when the time difference was compensated, and each neuron corresponds to a location in space.

In mammals, a different strategy was used. The precise timing of neuronal inhibition was involved in ITD processing in medial superior olive (MSO). (Brand, Behrend et al. 2002)

It is worth noting, however, that both in birds and mammals, the time difference was computed subcortically.

1.4 Microstimulation and Behavior

Electrical microstimulation is a technique that has been used in experimental and clinical neuroscience for over a century (Fritsch and Hitzig 1870). Although it has always been criticized to be imprecise, it has contributed greatly to both system neuroscience and clinical treatment in neural systems.

It was found that microstimulation could restore hearing to deaf patients by stimulating different regions of the cochlea (Bierer and Middlebrooks 2002;

Middlebrooks and Bierer 2002; Bierer and Middlebrooks 2004; Snyder, Bierer et al. 2004). It may also some day restore vision in blind patients (Schmidt, Bak et al. 1996; Zrenner 2002; Bartlett, DeYoe et al. 2005; Bradley, Troyk et al. 2005; DeYoe, Lewine et al. 2005; Merabet, Rizzo et al. 2005; Tehovnik, Slocum et al. 2005; Pezaris and Reid 2007; Pezaris and Reid 2009). Electrical stimulation of the basal ganglia is a very effective treatment for Parkinsonian patients and has been used extensively (Limousin, Pollak et al. 1995; Dostrovsky, Levy et al. 2000; Dostrovsky, Hutchison et al. 2002; Dostrovsky and Lozano 2002; MacKinnon, Webb et al. 2005). Deep brain stimulation of subgenual cingulate has been used to treat major depression (Mayberg, Lozano et al. 2005).

Aside from the clinical use, electrical microstimulation has played an important role in establishing a causal link between neural activity in various brain regions and behavior. The first experiment to directly link neural activity and perception using microstimulation technique was done by Newsome and colleagues (Salzman, Britten et al. 1990; Salzman, Murasugi et al. 1992; Murasugi, Salzman et al. 1993; Salzman and Newsome 1994). Monkeys were trained to perform a visual task. Monkeys sat in front of a screen with moving dots on display. Some percentage of the dots moved coherently in one direction while the others moved randomly. The monkeys were trained to report the direction of the coherent dot motion (Salzman, Britten et al. 1990; Salzman, Murasugi et al. 1992; Murasugi, Salzman et al. 1993; Salzman and Newsome 1994). Stimulations were done in the middle temporal visual area (MT). MT neurons are direction-selective, responding optimally to a certain direction of

motion and poorly or not at all to motion in the opposite direction. Neurons with similar preferred directions are clustered in the same column. It was found that stimulating certain MT columns could strongly bias the monkey's decisions toward the preferred direction of the stimulated column.

Electrical microstimulation can not just bias animal's behavior, but can also drive animal's behavior without natural stimuli. Romo and colleagues (Romo, Hernandez et al. 1998; Romo, Hernandez et al. 2000) trained monkeys to first discriminate the frequency of mechanical stimuli. The ability to make the discrimination was thought to depend on the quickly adapting (QA) neurons in primary somatosensory cortex (S1) (Mountcastle, Talbot et al. 1969; Mountcastle, Steinmetz et al. 1990; Recanzone, Merzenich et al. 1992). They then substituted electrical microstimulation of QA neurons for one of the mechanical stimuli, so that the monkey had to discriminate between the frequency of an artificial stimulus delivered directly to the cortex and a natural mechanical stimulation. The monkeys were able to perform the frequency discrimination between mechanical and electrical stimulations, and also when both stimuli were electrical. In this experiment, microstimulation alone was sufficient to produce reliable behavioral effects.

Microstimulation has also been used in studying attention (Moore and Fallah 2001; Moore and Armstrong 2003; Moore and Fallah 2004; Moore and Chang 2009) and "read-out" mechanisms (Groh, Born et al. 1997; Lisberger and Ferrera 1997; Nichols and Newsome 2002).

1.5 Sensory Deprivation and Plasticity in the Barrel Cortex

Experience-dependent plasticity in developing cerebral cortex has been studied extensively. In early days, Hubel and Wiesel found that in the visual cortex, receptive field properties are experience-dependent (Wiesel and Hubel 1965), and the plasticity has a critical period (Hubel and Wiesel 1970; Olson and Freeman 1980). Later, Simons and Land (Simons and Land 1987) found that visual cortex was not the only cortical area that exhibits experience-dependent plasticity: by depriving rats of sensory input from the facial whiskers, they could induce abnormality in the response properties of neurons in somatosensory cortex. They trimmed whiskers in row C, or all but row C, from P0 to P45 or P60. Single unit recordings were done after whiskers grew to full length. They found enlarged receptive fields, reduced angular tuning, increased responsiveness and altered temporal patterns of stimulus-evoked spikes in deprived barrels.

After the pioneering work by Simons and Land, there have been more studies on the anatomy (Micheva and Beaulieu 1995), physiology (Fox 1992), temporal coding (Ahissar and Arieli 2001), and behavioral effects (Carvell and Simons 1996) of plasticity in the barrel cortex, at different stages of development. In early post-natal stage (P0-7), plasticity mainly occurs in thalamocortical pathways (Crair and Malenka 1995; Isaac, Crair et al. 1997). Lesion before P4 could disrupt barrel formation (Van der Loos and Woolsey 1973; Woolsey and Wann 1976). Plasticity in Layer 2/3 peaks around P9-13 and lasts to adolescence (Fox 1992; Glazewski and Fox 1996; Fox 2002). Plasticity in Layer 4 drops rapidly between P0 and P4 and was no longer observed after P7 (Fox 1996; Fox

2002).

With new techniques available, plasticity in barrel cortex could be visualized *in vivo*. Using 2-photon microscopy, Svoboda and colleagues found that sensory deprivation during P11-13 markedly reduced the motility, but not density, length, or shape, of spines and filopodia in layer 2/3 of barrel cortex (Lendvai, Stern et al. 2000). They also found that in sensory deprived barrels, more spine turnovers were observed, and the plasticity was cell-type dependent. Regular spiking, simple, small apical tufted cells lost intracortical input, and the spine density decreased; intrinsically bursting, complex, large apical tufted cells gained intracortical input, and the spine density increased (Holtmaat, Wilbrecht et al. 2006; Holtmaat and Svoboda 2009).

1.6 Optogenetics, Optical Stimulation and Behavior

Channelrhodopsin-2 (ChR2) is a light-sensitive cation channel that is activated by 470mm blue light (Boyden, Zhang et al. 2005; Zhang, Aravanis et al. 2007).

ChR2 can be expressed stably in mammalian neurons at high levels without causing damage to the cells. ChR2 can reliably drive neuronal depolarization when activated by blue light (Boyden, Zhang et al. 2005). Direct activation using a train of light pulses can drive neurons to fire action potentials with millisecond precision (Boyden, Zhang et al. 2005; Zhang, Wang et al. 2006).

ChR2 can be expressed by injecting virus into neural tissues, by in-utero electroporating ChR2 plasmids, and by other genetic approaches. Using certain

neuronal subtype specific promoters, ChR2 can be expressed in different types of neurons (Lagali, Balya et al. 2008; Cardin, Carlen et al. 2010). And combining with other genetic approaches, ChR2 can also be expressed in a pathway-specific fashion (Lima, Hromadka et al. 2009; Cruikshank, Urabe et al. 2010). Therefore, it is possible to control neuronal activities optically in cell-type specific and pathway-specific ways.

ChR2 has been used broadly in controlling physiological properties of different brain structures (Hira, Honkura et al. 2009; Lima, Hromadka et al. 2009; Rizzi, Powell et al. 2009; Grossman, Poher et al. 2010). ChR2 has also been used to drive behavior (Huber, Petreanu et al. 2008; Rizzi, Powell et al. 2009). Svoboda and colleagues expressed ChR2 in the barrel cortex of mouse using in-untero electroporation. Then they trained the mice to perform a 2-alternative forced choice task based on optical stimulation. If the mouse could detect the stimulation, it had to go to one side to collect reward, and if there was no stimulation, the mouse had to go to the other side to collect reward. Mice could be trained to perform this task well above chance level. In terms of controlling behavior, ChR2 combined with optical stimulation is similar to but more specific than the traditional electrical stimulation method.

1.7 Thesis Outline

This thesis is divided into 8 chapters.

Chapter 1 contains backgrounds and introduction.

In chapter 2 (methods section), we will describe in detail how we did all

the experiments and how we analyzed the data, including: behavioral training, implantation surgery, multiunit recording, electrical stimulation, virus injection, optical stimulation, and whisker trimming.

In chapter 3, we will describe the first electrical stimulation experiment: electrical stimulation of auditory cortex. We first trained animals to detect electrical stimulation, and then to discriminate fine timing differences in the stimulation trains. We found that in rat auditory cortex, animals could be trained to use timing differences as short as 3 ms to drive decisions (Yang, DeWeese et al. 2008).

How about other cortices? Is extracting fine timing information a unique feature of auditory cortex, or is it common for all sensory cortices? In chapter 4, we will describe the experiment in a different cortical area, the visual cortex. Although it has been shown that neurons in monkey MT could respond precisely to visual stimuli, we found that in rat visual cortex, animals could not discriminate timing differences below 15 ms. And in most inter-stimulus intervals tested, visual cortex animals were worse than the auditory cortex animals.

Is auditory cortex special, or is visual cortex special? To find out, we decided to do the experiment in a third sensory cortex, the barrel cortex. (Chapter 5) We found that barrel cortex was even better than auditory cortex in extracting fine timing information, and performances in all inter-stimulus intervals were better than the performances from visual and auditory cortex experiments. The limit for barrel cortex is 1 ms.

Does the ability to extract fine timing information depend on sensory

experience? Auditory and tactile information often contains fast time-varying component, while visual scenes don't change rapidly in daily life. Given that sensory deprivation in barrel cortex is well-established, we decided to use the whisker system as our model. We trimmed one side of the facial whiskers of some rats from P0 till P60 and trained them on the timing task by stimulating the contralateral barrel cortex (Chapter 6). We found that with sensory deprivation, although the anatomical map of the barrel cortex remains intact, the ability to discriminate fine timing is severely impaired. The sensory deprived barrel cortex was almost as bad as visual cortex in fine timing discrimination. However, with training, some rats were able to improve and eventually perform as well as the control.

In chapter 7, we will describe the preliminary results from the optical sitmulation experiments. We were able to train 4 animals to detect optical stimulation in the auditory cortex, and 1 animal to perform 5 ms timing task.

Finally, in chapter 8, we will discuss the results and provide some perspectives.

Chapter 2

Experimental procedures

2.1 Experimental Animal

We used male Long-Evans rats as experimental animals.

For virus injection, animals we used were below 100g. For training, we used adult animals of 250-300g. For sensory deprivation experiments, we used new-borns. All rats used in all experiments were males.

2.2 Virus Injections

The virus we used in the optical stimulation experiment was AAV-CAGS-ChR2-Venus (Huber, Petreanu et al. 2008). The virus expresses at a high level in all cell types. The serotype of the virus was AAV 2/1 (Bartlett, Samulski et al. 1998)

We directly injected the virus into the left auditory cortex of 4-week-old young rats using glass micropipettes (Cetin, Komai et al. 2006; Lima, Hromadka et al. 2009). The tip of the glass pipettes was approximately 10-20 um in diameter. For each animal, we made 2 small craniotomies, each 0.2 mm in diameter.

We injected young animals because the expression of the virus was higher in younger animals.

We chose to make two small craniotomies instead of one big craniotomy the size of the implantation surgery, because we needed to open up the cortex

again for implanting the optical fibers. Big craniotomies in the first surgery often lead to massive bleedings in the second surgery. Re-growth of the dura at the site of the craniotomy after the first surgery was likely the cause of the bleeding, and small craniotomies could keep the dura from overgrowing.

We made 2 penetrations, one in each craniotomy, and injected virus into different depths of the cortex. We first advanced the pipettes about 1 mm into the cortex, then retract the pipette and injected at 1.0 mm, 0.8 mm, 0.6 mm, and 0.4 mm. Injecting into 2 sites with 4 different depths can cover most part of the primary auditory cortex.

We waited at least 3 weeks for the virus to fully express (Kuhlman and Huang 2008).

2.3 Sensory Deprivation of Whiskers

We obtained pregnant rats of E14-E16. We observed the pregnant rats closely. Immediately after they gave birth, we started trimming whiskers of the pups (normally within 4 hours of birth, no more than 12 hours). All whiskers were trimmed on one side, including the identified A-E rows, 1-5/8, and the alpha row (see figure 2.1). Whiskers were trimmed every 24 hours till adulthood (P0 ~ P60).

Before P12, rat pups were held in place by hand, and whiskers where trimmed without anesthesia. After P12, animals were anesthetized with 2% isofluorane before whisker trimming, and were insensible during the procedure. After P30, animals were anesthetized with 2.5% isofluorane. Half of the animals

were trimmed on the left side, and half were trimmed on the right side. Animals all behave normal after whisker trimming.

2 control animals were raised alongside their littermates without whisker trimming, and went through the same procedures every day.

2.4 Implantation Surgery

All procedures were approved by the Cold Spring Harbor Laboratory Animal Committee. Animals were anesthetized with an intraperitoneal injection of a mixture of ketamine (60mg/kg) and medetomidine (0.51 mg/kg). Wounds were infiltrated with lidocaine.

For the animals that were implanted in the auditory cortex: During the surgery, temporal muscle over the left auditory cortex was recessed and a craniotomy and a duratomy were performed. Electrodes were implanted 4.5 and 5.6 mm posterior to bregma and 6.4 mm left from the midline. After surgery, animals were left to recover for several days before resuming water deprivation.

For the animals that were implanted in the visual cortex: During the surgery, a craniotomy and a duratomy were performed. Electrodes were implanted 6.0 mm and 7.1 mm posterior to bregma and 4.0 mm left from the midline. After surgery, animals were left to recover for several days before resuming water deprivation.

For the animals that were implanted in the barrel cortex: During the surgery, temporal muscle over the left barrel cortex was recessed and a craniotomy and a duratomy were performed. Electrodes were implanted 1.5 and

2.6 mm posterior to bregma and 5.6 mm left from the midline. After surgery, animals were left to recover for several days before resuming water deprivation.

For the animals used in the sensory deprivation experiment:

1) Experimental group

Temporal muscle over the contralateral (to the deprived whisker side) barrel cortex was recessed and a craniotomy and a duratomy were performed. Electrodes were implanted 1.5 and 2.6 mm posterior to bregma and 5.6 mm lateral from the midline. After surgery, animals were left to recover for several days before resuming water deprivation.

2) Control group 1

Temporal muscle over the ipsilateral (to the deprived whisker side) barrel cortex was recessed and a craniotomy and a duratomy were performed. Electrodes were implanted 1.5 and 2.6 mm posterior to bregma and 5.6 mm lateral from the midline. After surgery, animals were left to recover for several days before resuming water deprivation.

3) Control group 2

Animals were not sensory deprived. During the surgery, temporal muscle over the left barrel cortex was recessed and a craniotomy and a duratomy were performed. Electrodes were implanted 1.5 and 2.6 mm posterior to bregma and 5.6 mm left from the midline. After surgery, animals were left to recover for several days before resuming water deprivation.

2.5 Electrical Stimulation

We used tetrodes for intracortical electrical stimulation. Tetrodes were made of polyimide-coated nichrome wires (H.P. Reid, Inc., FL; wire diameter12.5 μm), and were all gold plated with impedance ranging from 250 to 350 kΩ at 1 kHz. Two tetrodes were loaded into a custom made tetrode drive designed by Gonzalo Otazu (Otazu, Tai et al. 2009). The tetrodes could move independently, and the distance between the two tetrodes was approximately 1.1 mm.

Each electrical stimulus consisted of a train of 5 biphasic 4-volt voltage pulses (RP2, Real-time processor, TDT; see Fig. 2.2) which were passed through a 1:2.2 transformer (SP-21, Triad Magnetics). We used a scull screw (P64, Small Parts) on the contralateral temporal bone as ground. The impedance from the electrode to the ground ranged from 400K to 1M, so that stimulation currents ranged from about 8 uA to 22 uA. The diameter of the stimulated area was estimated to be ~75 mm.

2.6 Optical Stimulation

We used 70um optical fibers for light delivery into the brain (Schott). Fibers were glued to polyimide tubes (SWPT-0031, 0.0031 ID, 0.0075WL, Small Parts), and the polyimide tubes were glued to metal tubes (30G XTW; 23G RW, Small Parts). Then the fiber and the metal tubes were glued to a side-mount miniature LED (KingBright) using clear EPOXY (Epotec) which cures quickly under UV (see Figure 2.3, top). Then we cover the LED and clear EPOXY with black EPOXY (Epotec) which cures overnight.

We cut the fibers and load two fibers into one head stage before implantation. (Fig 2.3, bottom)

The stimulation was controlled using 4-channel LED controllers (MITEX).

2.7 Multiunit Recording

We used 1M Ohm tungsten electrodes. Each electrode is glued to an LED-fiber assembly, with the tip sticking out about 0.2 mm, so that the light from the fiber could cover the area that the electrode records from. (Figure 2.3, bottom)

The software we used was Exper developed by Tomas Hromadka. The amplifier we used was Cyberamp 380 and the pre-amp was smartprobe AI 401 10X (Molecular Devices/Automate Scientific). We recorded LFP using 1-300Hz band pass and multiunit using 300Hz high pass, set using Exper software.

In order to minimize the stimulus artifact caused by passing current through the LED which is very close to the recording electrode and the preamp, we shielded the LED assembly with colloidal silver liquid (Electron Microscopy Sciences) and grounded the shielding. The shielding effectively reduced the noise level.

2.8 Behavioral Setup

Animal training was performed in a 30 cm cubic box containing 3 nose cones (38 mm inner diameter, 38 mm depth). The floor was composed of 17

stainless rod grids. A pair of infrared photodiode and phototransistor was placed on either side of the nose poke at 15 mm depth from the surface to measure the occurrence and timing of nose pokes (Tai and Zador 2008). A cartoon showing the behavioral setup is shown in figure

The training box with water delivery valves are maintained in a single walled sound booth (Industrial Acoustics Company, NY).

A custom Matlab program (The MathWorks, Inc., MA) developed by Mainen and Uchida, adapted by Otazu controls two RP2 real time processors units (Tucker-Davis Technologies, FL).

One RP2 was used for monitoring the nose poke signals and control the water delivery system. A second RP2 was used for delivering electrical or optical stimulation pulses.

2.9 Behavioral Training Procedure

Animals were water deprived under a protocol approved by the Cold Spring Harbor Laboratory Animal Committee.

2.9.1 Auditory cortex stimulation

Naïve animals were first trained on an auditory 2-alternative-forced choice (2-AFC) task. The animal introduced its nose into the center port, which triggered the presentation of the acoustic stimulus (a 0.3 second chord) from a speaker located either on either the left or right side of the soundbooth. The chord was composed of 16 tones between 1 and 16 kHz, uniformly distributed on a

logarithmic axis. The intensity of the chord was 69dB RMS SPL. The chord indicated the location on that trial of the reward port for which a poke would be rewarded with water.

On the first day of training, the right port was blocked, and the animals had to poke into the center to trigger a left chord. At the same time the left water port was open and a drop of water was delivered ("direct" mode). After about 50 trials the animals would poke into the left port immediately after the center port. At this point we changed the water delivery from direct to "next poke correct", and the animals had to poke into the left port to trigger the water delivery, within 3 seconds of the center poke.

On the second day of training, the left port was blocked, and the animals had to learn to get water from the right port. On the second or the third day, all three ports were open, and the animals could choose freely from the left port or the right port depending on the stimulus. Most animals could perform above 80% on the first try.

After animals reached criterion performance (80%), we implanted electrodes at two sites (A and B, ~1.1 mm apart) into the rat's left primary auditory cortex (area A1). The electrodes were made of Nichrome wire 12.5 um in diameter. Four wires were bound together and used as one conductor. A skull screw in the right parietal bone served as ground for the stimulation.

Two days after implanting the tetrode drive, we started to train the implanted animals on a simple stimulation task. To ensure that the animals implanted with the electrodes could detect the intracortically delivered electrical

stimuli, we first trained them to go left for stimulation of site A and right for stimulation of site B.

Before the first session of electrical stimulation, we trained the animals again on the sound localization task and let them perform up to 100 trials, to make sure they still remember the 2AFC task. Then we plug the animals in and again block the right port, have the animals go to the center to trigger the A stimulation and go to the left port to collect water reward for 30-50 trials, and then block the left port for 30-50 trials. Then we open all three ports and let the animals choose freely.

If they could perform the task above chance, we trained them to go left for simultaneous stimulation of A and B, and to go right for stimulation in B only. After they could perform this task above chance, we introduced an inter-stimulus interval (ISI) into the task by adding stimulation in site A to the right stimulus. We started with ISI = 100 ms, and reduced it to probe the behavioral threshold. For rats m-z, we began each day of training with a few trials of the easier (ISI=100 ms) task to confirm that the animal was still able to detect stimulation from both sites before challenging with shorter ISIs.

In early experiments, we reduced the ISI gradually with multiple intermediate steps to obtain an estimate of the timing threshold. The intermediate ISIs included 55 ms, 35 ms, 15 ms, 7 ms, and 5 ms. If an animal could perform a task at a certain ISI above chance, we probed with a shorter ISI until the animal failed for two consecutive sessions, after which we trained the animal again on the ISI = 100 ms task. Training was terminated if the animal also failed to perform

above chance in this task for two consecutive sessions. For example, if a rat performed above chance at ISI = 15 ms, we next trained it on 7 ms. Not until it could perform above chance when ISI = 7 ms would we start training it on 5 ms. If it failed on the ISI = 5 ms task for two consecutive sessions and also failed on ISI = 100 ms task for two sessions, we terminated the training.

In later experiments, after we found that some animals could perform the task when the ISI was as short as 5 ms, we adopted a different training strategy in which we dispensed with the intermediate ISIs and reduced the ISI abruptly from 100 ms to shorter ISIs (e.g. 5 ms). Our reasoning was that since the performance appeared to decline over time (possibly as the result of deterioration of the electrode and/or damage to the cortical neurons by the chronically implanted electrodes), this procedure would allow us to train the animals more extensively at the shortest ISI. In this way we were able to train some animals on ISI=5 ms and ISI=3 ms. To see if rats can learn ISI=1 ms, we trained one rat (z) on ISI=1 ms even after it failed on the ISI = 3 ms task.

2.9.2 Visual cortex stimulation

Naïve animals were first trained on a visual 2-alternative-forced choice (2-AFC) task. The animal introduced its nose into the center port, which triggered the presentation of the visual stimulus (a white LED turned on) on top of the left or the right port. The left LED is associated with water reward from the left port, and the right LED is associated with water reward from the right port.

Training procedures of the electrical stimulation experiments in visual cortex were similar to that of the auditory cortex.

2.9.3 Barrel cortex stimulation

Naïve animals were first trained on the sound localization task described in 2.9.1. Training procedures of the electrical stimulation experiments in barrel cortex were similar to that of the auditory cortex.

In order to obtain psychometric curve for these animals, we went back to 15 ms, 35 ms and 55 ms ISI's after training them on shorter timing tasks (3 ms, 5 ms, 1 ms).

2.9.4 Optical stimulation

Naïve animals were first trained on the sound localization task described in 2.9.1.

Similar to the electrical stimulation experiments, we first trained the animals to detect the intracortical stimuli. For the first few animals, we found that they could discriminate stimulus vs. no stimulus, but not stimulus A vs. stimulus B. Then we found that they were using the visual cue: the blue light inside their auditory cortex was visible.

To avoid training animals on a visual task, we combined several strategies. One is to turn on a bright white LED in the center port when the animal poked into the center to trigger the light pulse. The other is to have a blue LED flashing at the same frequency as the light pulse inside their head when the stimulus was triggered.

Training procedures of the optical stimulation experiments in barrel cortex were similar to that of the electrical stimulation experiments. Behavior setup is shown in Figure 2.5.

2.9.5 Sensory deprivation experiments

Naïve animals were first trained on the sound localization task described in 2.9.1.

Training procedures for both the control group and the sensory deprived group were the same. Also similar to the stimulation experiments done in auditory cortex, as described in detail in 2.9.1.

2.10 Data Analysis

We used standard errors across trials for the error bars of each data point in Figures 3.1, 4.1, 5.1, 6.1, 7.1. Better performance and greater numbers of trials yield smaller error bars.

We computed the significance for each session assuming a binomial distribution, the null hypothesis being equal probability of obtaining correct trial and incorrect trial, since chance performance is 0.5 on this task. We set the threshold for significance at $p=0.01$. Thus for each session, $p<0.01$ means that by chance the probability of obtaining this performance or better was below 1%. For a session to be "significantly above chance", p value has to be below 0.01.

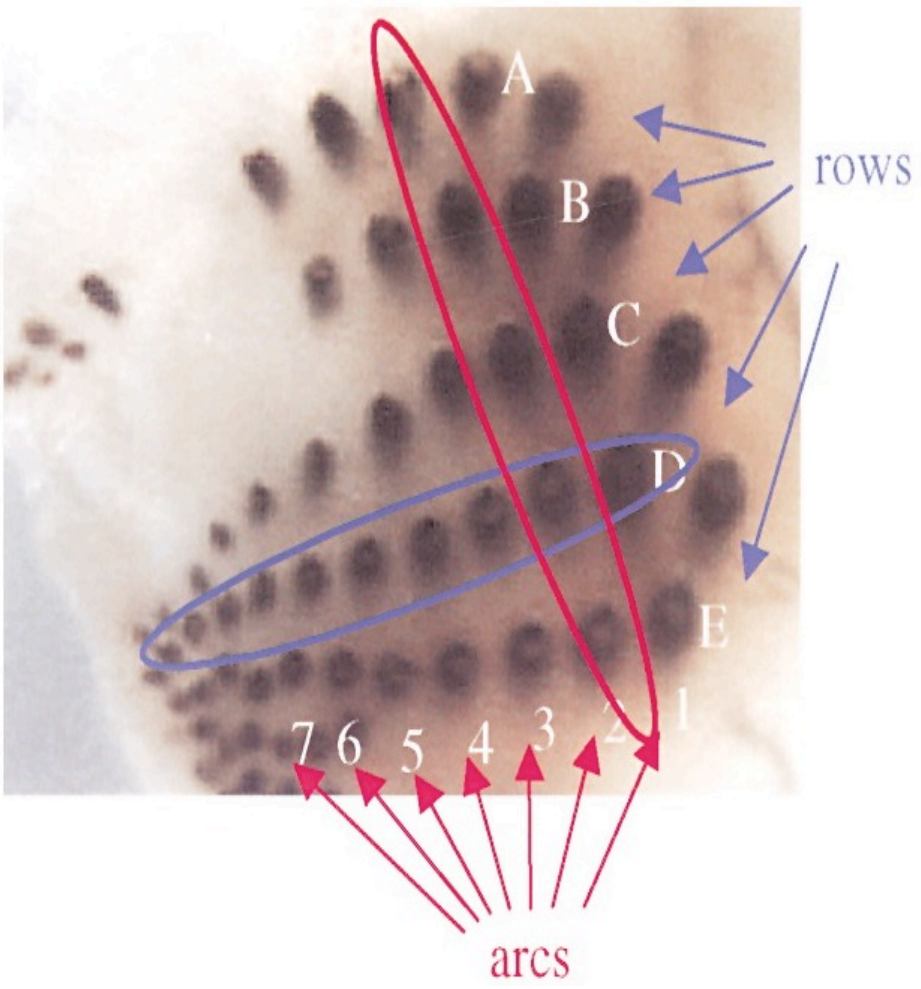


Figure 2.1 Rat whisker pattern

Rows A-E, arcs 1-4 (for row A and B), arcs 1-8 (for row C-E) can be easily identified, as is shown in this figure. (Ahissar and Arieli 2001)

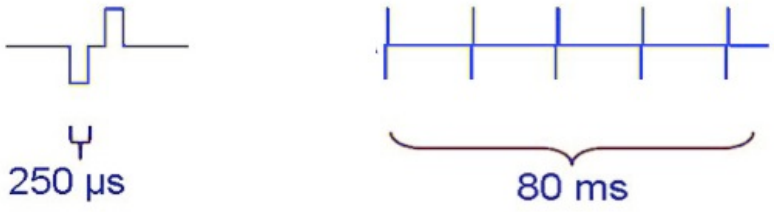


Figure 2.2 Electrical stimulus structure.

Each stimulation consisted of 5 pairs of 250 microseconds cathode-leading square pulses at 50 Hz, lasting 80 ms total.

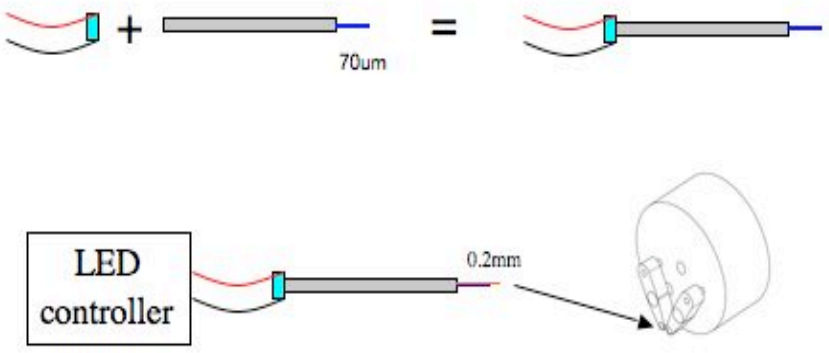


Figure 2.3 Fiber optic drive for optical stimulation.

Top: LED-fiber assembly. Each fiber optic is 70 um in diameter, protected by polyimide tube and metal tubes. The LED end of the fiber is flattened, polished, and glued to the LED using clear EPOXY and covered with black EPOXY. If the assembly is used for recording, the LED is also covered with silver paint after the black EPOXY.

Bottom: drive design

Each drive was loaded with 2 LED-fiber assemblies which could move independently. For recording, an electrode was glued to the fiber.

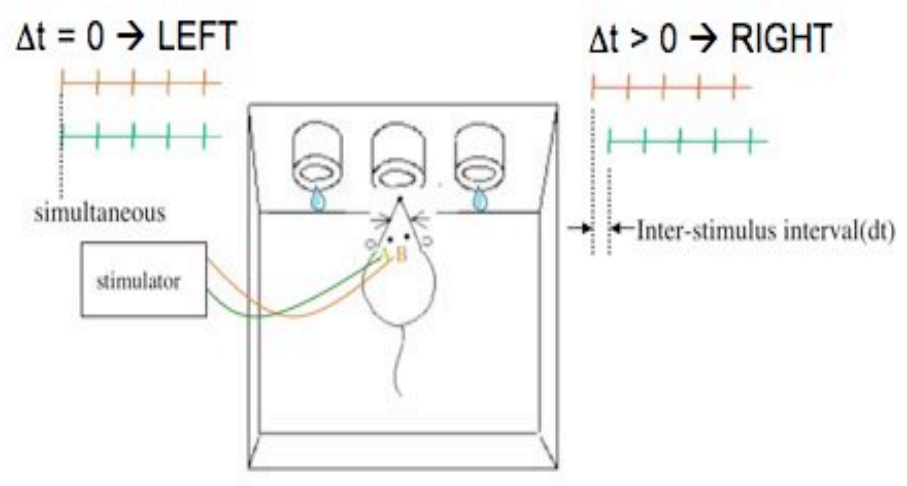


Figure 2.4 Behavior Setup: electrical stimulation

Two tetrodes were implanted into the cortex at two sites, A and B. If A and B were stimulated with simultaneous electrical pulses, the rat had to choose the left port to collect reward. If A and B were stimulated with sequential electrical pulses with a time difference dt, the rat had to choose the right port to collect reward. If the rat could perform above chance level, then we knew that the cortex could distinguish dt from 0.

Electrical pulses were controlled by RP2.

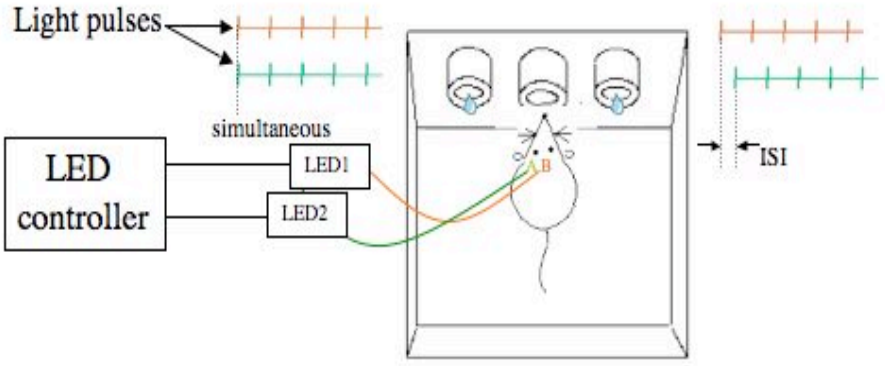


Figure 2.5 Behavior Setup: optical stimulation

Two optical fibers, each glued to a miniature LED, were implanted into the left auditory cortex at two sites, A and B. If A and B were stimulated with simultaneous blue light pulses, the rat had to choose the left port to collect reward. If A and B were stimulated with sequential blue light pulses with a time

difference dt, the rat has to choose the right port to collect reward. If the rat could perform above chance level, then we knew that the cortex could distinguish dt from 0.

Light was controlled using MITEX LED controller.

Chapter 3

Auditory Cortex Stimulation

It is known that neurons in auditory cortex can respond with high temporal precision to acoustic stimuli (DeWeese, Wehr et al. 2003), and it is also known that inter-aural time difference (ITD) of a few microseconds can be computed in subcortical structures to determine the spatial localization of sound. But it's not known if fine timing information in auditory cortex can be used in a behavioral context.

We use intracortical microstimulation in a behavioral task to study the ability of auditory cortex to exploit fine timing differences for behavior.

3.1 Sound localization task

For the first 5 animals we trained, we implanted naïve animals with two tetrode and trained them directly on the electrical stimulation task. We wanted to avoid losing trained animals in the implantation surgery. However, 3 out of 5 animals failed to learn the basic stimulation task (A vs. B, see 2.9.1). We reasoned that the animals failed not because they couldn't detect the stimulations, but because they were confused by the unnatural stimuli (cortical stimulation) and failed to understand the 2AFC task.

We then changed our training procedure and trained naïve animals first on a simple 2AFC task, the sound localization task (L vs. R, see 2.9.1), to make sure that they understand the task before we trained them on the electrical stimulation tasks.

It takes approximately 2-3 days for the animals to fully understand the task and perform above the threshold level of 80%.

3.2 Basic Stimulation Task (A vs. B)

Two days after the implantation surgery, we deprived the animals of drinking water and started to train them on the basic microstimulation task of A vs. B. Before the first session of electrical stimulation, we trained the animals again on the sound localization task to make sure they still remember the 2AFC task. All animals remembered the task and immediately performed above threshold (80%) in the sound localization task (data not shown).

We successfully trained 26 rats on the basic A vs. B task. Most rats learned A vs. B within 3 sessions of training.

Rats that failed to learn the A vs. B task were discontinued from training and were not included in the data analysis. The failures could be due to failed surgeries or broken electrodes.

3.3 Fine Timing Task

After animals perform above chance ($p<0.01$) on the A vs. B task, we trained them next on AB vs. B. Most animals learned AB vs. B within one session. Then we started to introduce timing information into the task. We started with 100 ms. Of the 24 rats trained on the AB vs. B-100ms-A task, 22 were able to perform the task significantly above chance ($p<0.01$, see Figure 3.2). Most

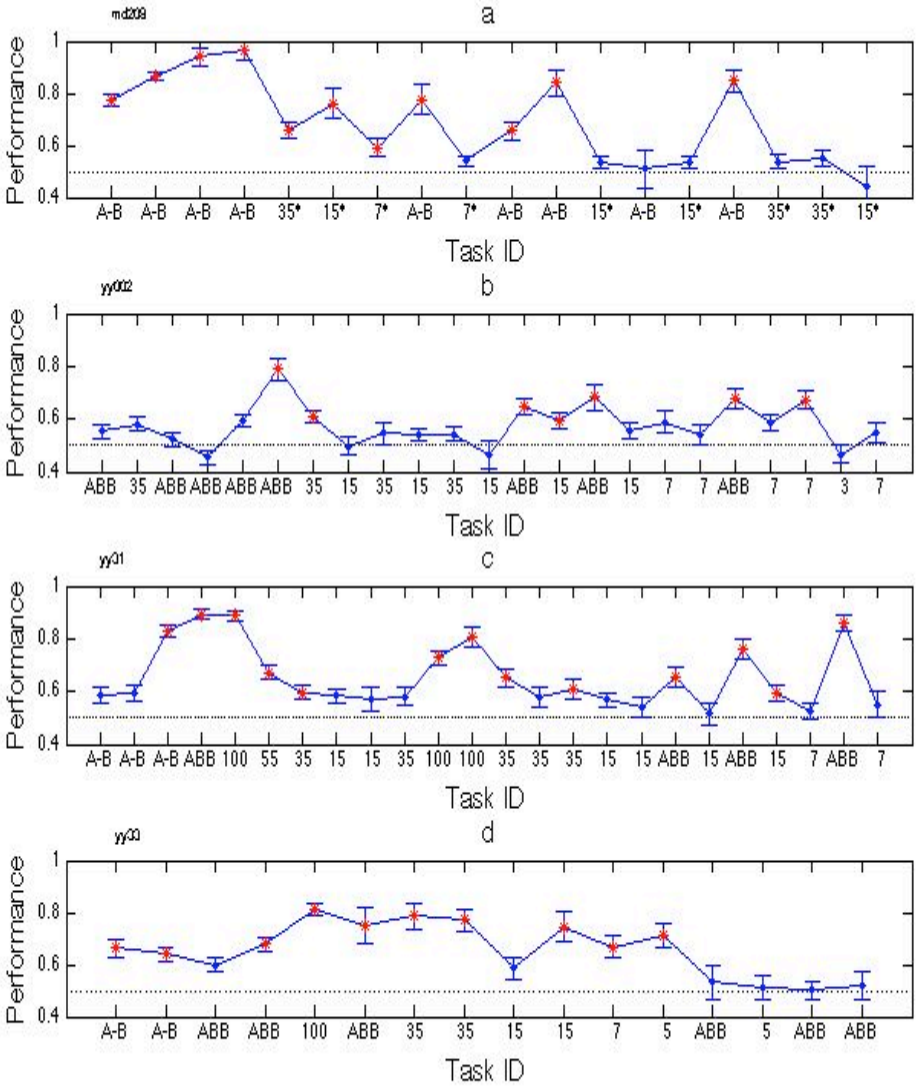
animals were able to perform 100ms task immediately above chance.

The animals that could perform 100ms tasks were later trained on shorter timing tasks. At the beginning we reduced the inter-stimulus intervals (ISI) gradually, from 100ms to 35 ms, 15 ms, 7ms, and 5 ms. After we had successfully trained animals on 5 ms tasks, we jumped from 100ms directly to 5 ms. During the training procedure, the electrodes could go bad, the areas of stimulation could be damaged, and animals could get confused by change of tasks. In order to collect more data on shorter ISI's, we skipped the intermediate ISI's.

Eleven out of 13 were able to perform the task for ISI = 35 ms, 6/8 for ISI =15 ms, 5/7 for ISI = 7ms, 10/15 for ISI = 5 ms, 2/7 for ISI= 3 ms and 0/4 for ISI=1 ms. performance of each rat is shown in Figure 3.1.

From our experiments, we concluded that auditory cortex was able to make use of fine timing information as short as 3 ms. And using our protocol, the threshold for auditory cortex is likely to be between 1 and 3 ms. (Figure 3.2)

We also plotted the performance of each rats on each ISI (all trials from all sessions pooled together) and the median performance of all rats. (Figure 3.3)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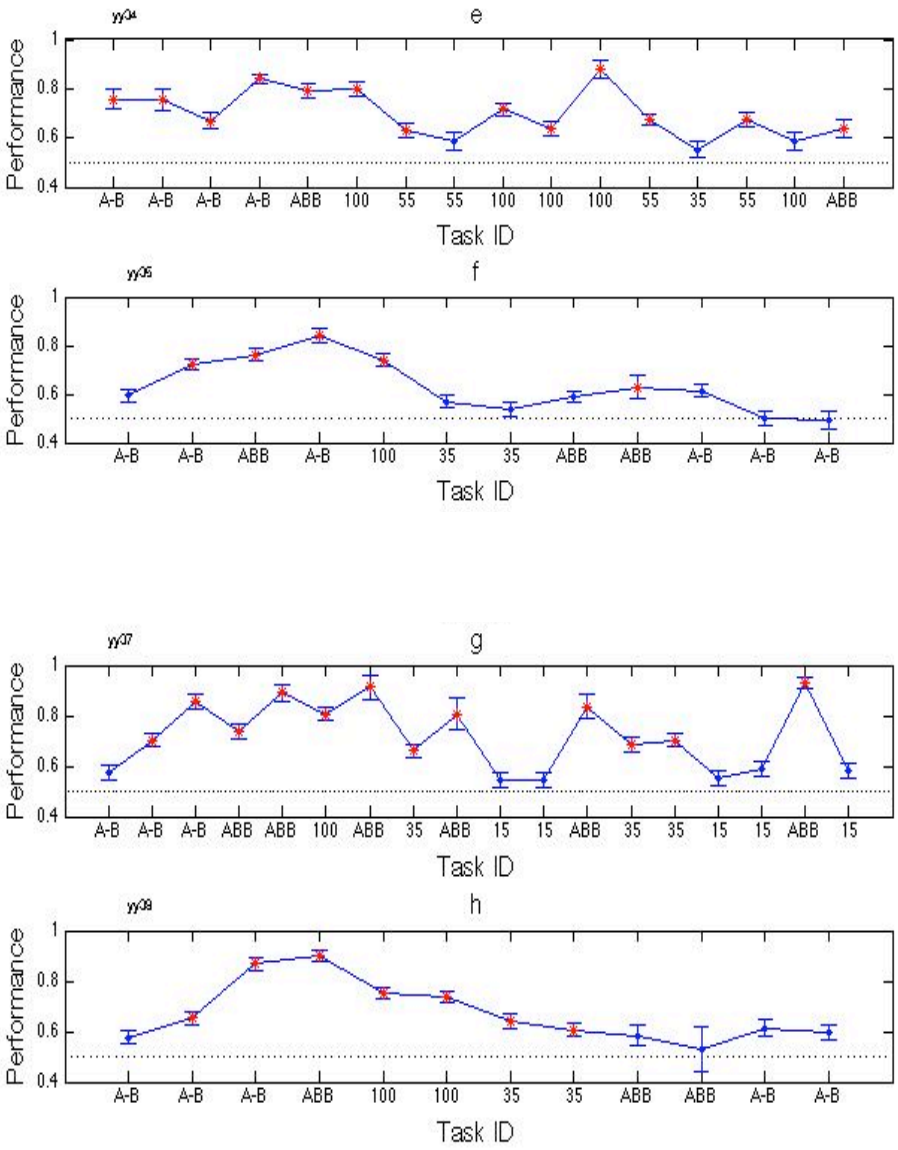
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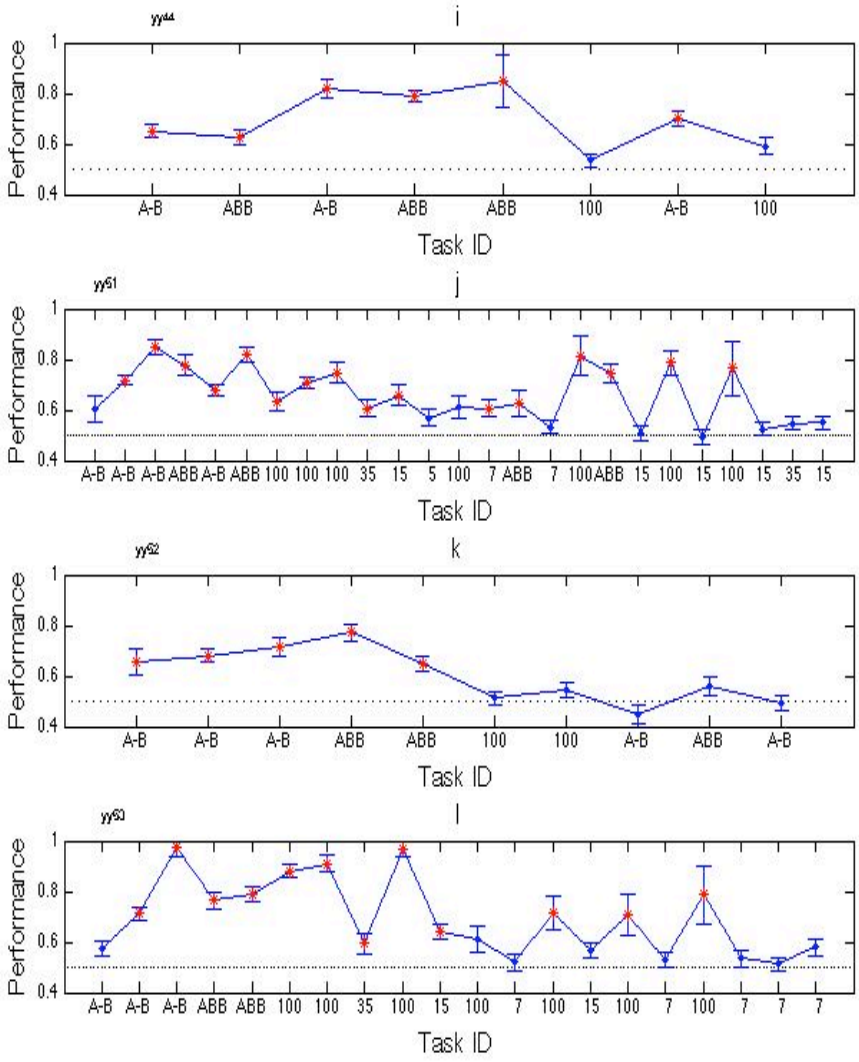
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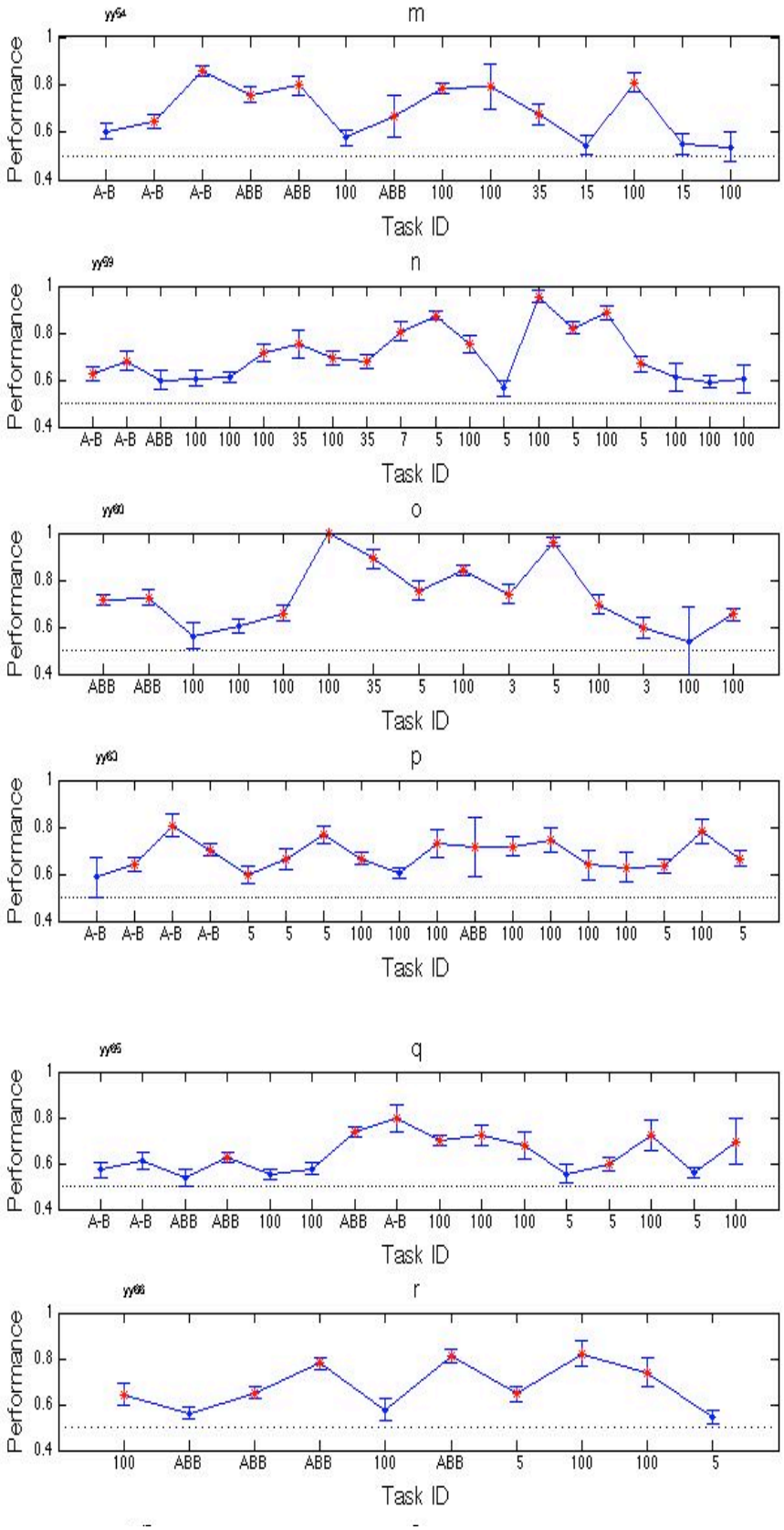
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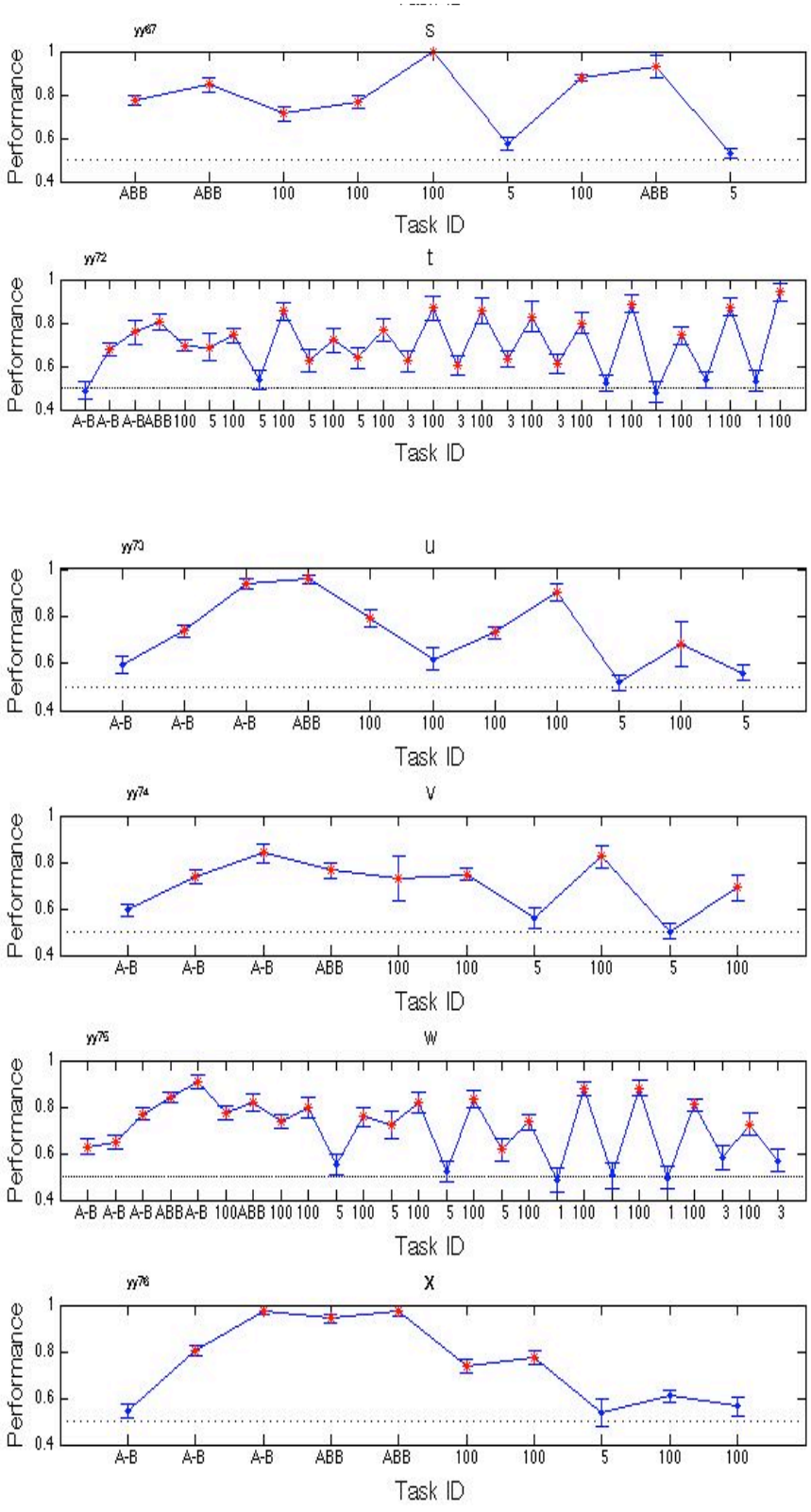
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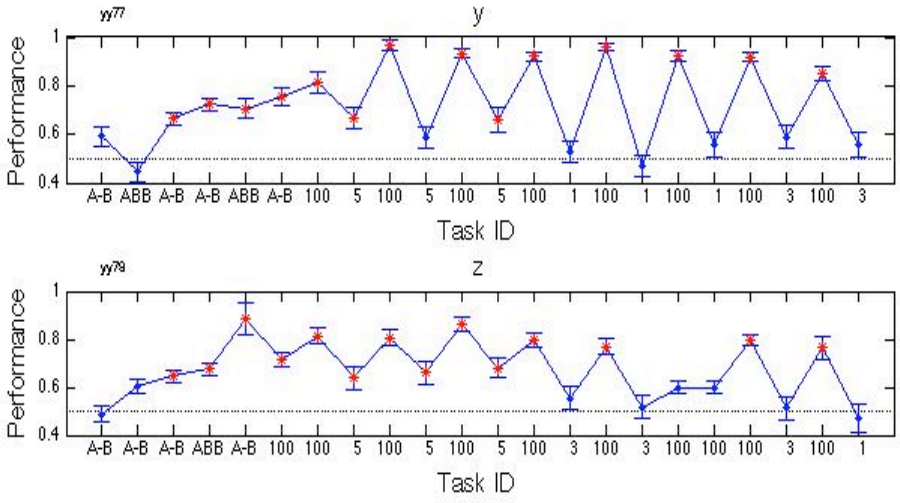


Figure 3.1 The performance of all rats on all electrical stimulation tasks plotted in chronological order.

Each data point represents one behavioral session. The error bar is the standard error across trials in each session.

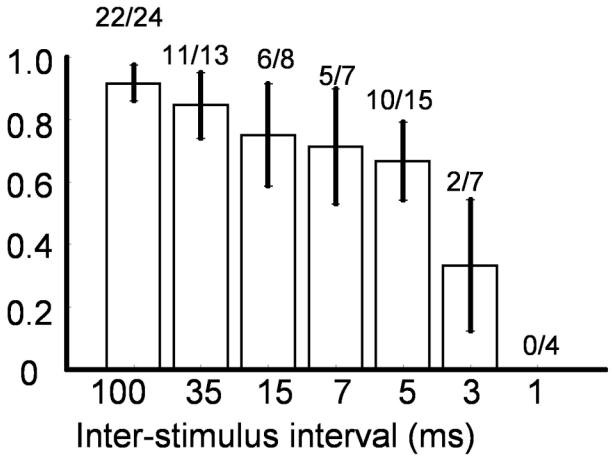


Figure 3.2 Population plot of all animals trained on all dt tasks.

22 out of 24 rats were able to perform 100 ms tasks above chance. Eleven out of 13 rats were able to perform the task for ISI = 35 ms, 6/8 for ISI =15 ms, 5/7 for ISI = 7 ms, 10/15 for ISI = 5 ms, 2/7 for ISI= 3 ms and 0/4 for ISI=1 ms. The lower behavioral limit for auditory cortex was likely to be between 1 ms and 3 ms.

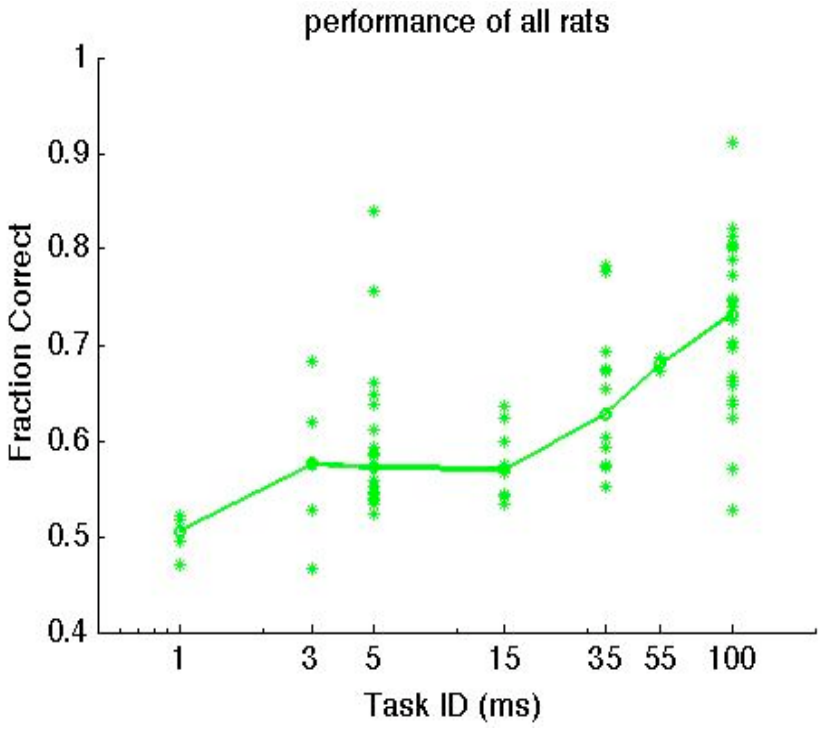


Figure 3.3 Performance of all rats on all Inter-stimulus Intervals

Each data point represents the performance from all all sessions for one rat. The line represents the median performance of all rats.

Chapter 4

Visual cortex stimulation

We found that auditory cortex can make use of fine timing information as short as 3 ms. Auditory cortex was known to produce precisely timed spikes in response to acoustic stimuli (Barbour and Wang 2003; DeWeese, Wehr et al. 2003), and audition was considered to be a fast sense. However, for visual cortex, there seems to be a conflict. On one hand, visual cortex could also produce precisely timed spikes in response to stimuli that contains timing components (Buracas, Zador et al. 1998). One the other hand, vision was considered to be a slow sense. So, is visual cortex able to resolve fine timing differences in neural activity as well as auditory cortex?

4.1 visual 2AFC task

We trained naïve animals to first perform a simple visual 2AFC task, also a localization task, to make sure that they understand the task before we trained them on the electrical stimulation (see 2.9.2).

The animal introduced its nose into the center port, which triggered the presentation of the visual stimulus (LED) on top of either the left or the right port. The left LED was associated with a left water reward from the left port, and the right LED was associated with water reward from the right.

It normally took 3-4 days, one more day than the auditory task, for the naïve animals to learn the visual task and perform above chance. The reason why the animals were a little slower could be that the visual stimulus was not ideal for

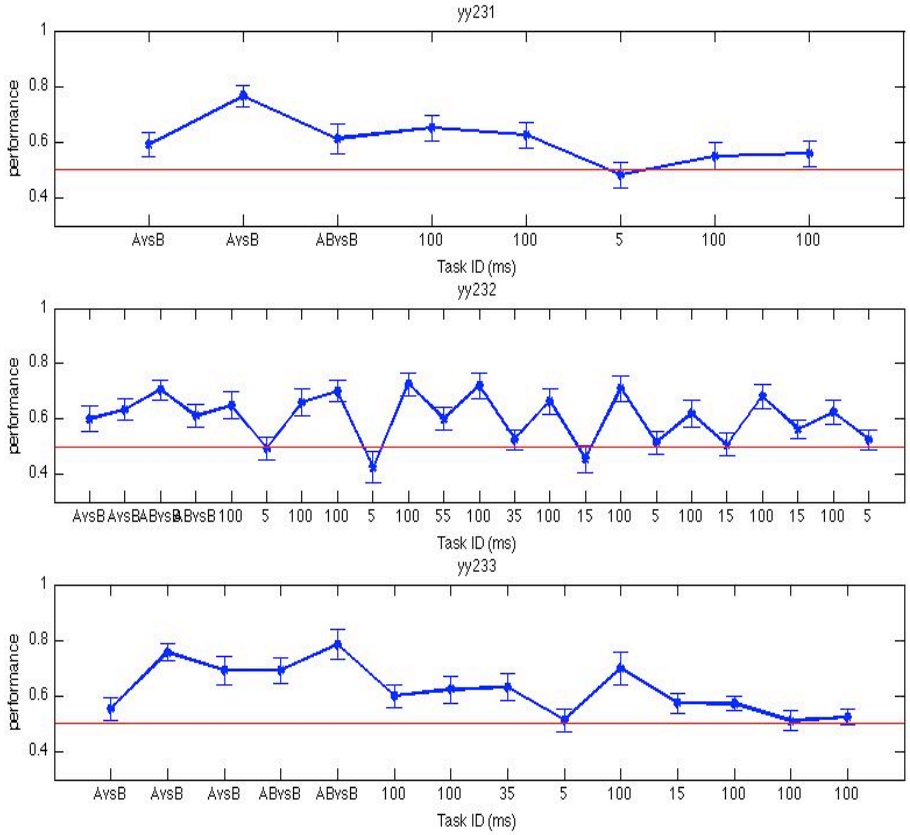
the animals (light from the LEDs might be partially blocked by the center port when the animals were poking in to trigger the stimulus), while the auditory stimuli had been optimized for training. Another reason might be that rats don't depend on their vision as much as they depend on their audition.

4.2 visual stimulation and timing

We successfully trained 10 rats on the basic A vs. B microstimulation task. 7 out of 10 rats trained on the AB vs. B-100ms-A task were able to perform the task significantly above chance ($p<0.01$). 4 out of 4 were able to perform above chance for ISI = 55 ms, 5/5 for ISI =35 ms, 2/6 for ISI = 15 ms, 0/7 for ISI = 5 ms.

The performance of each rat is shown in Figure 4.1. Performance of all rats on all Inter-stimulus Intervals is shown in Figure 4.2.

From our experiments, we concluded that visual cortex is not as good as auditory cortex in extracting fine timing information (Figure 4.3). Using our protocol, the threshold for visual cortex is likely to be between 5 and 15 ms. (Figure 4.4)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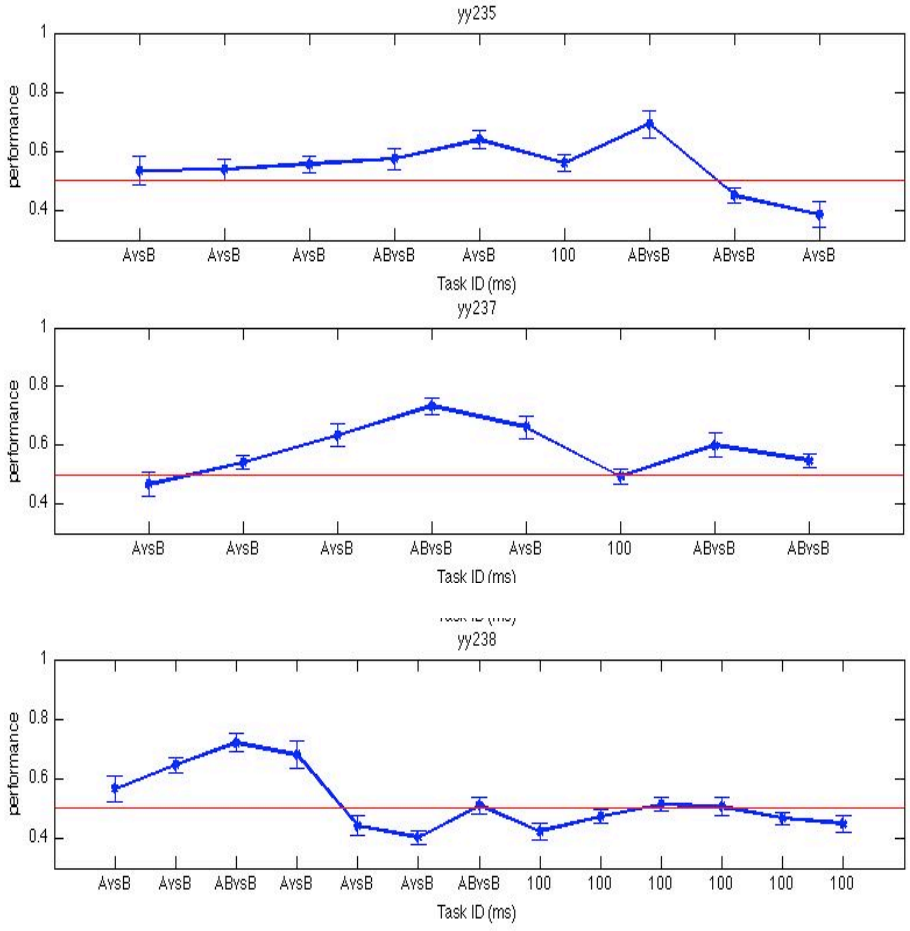
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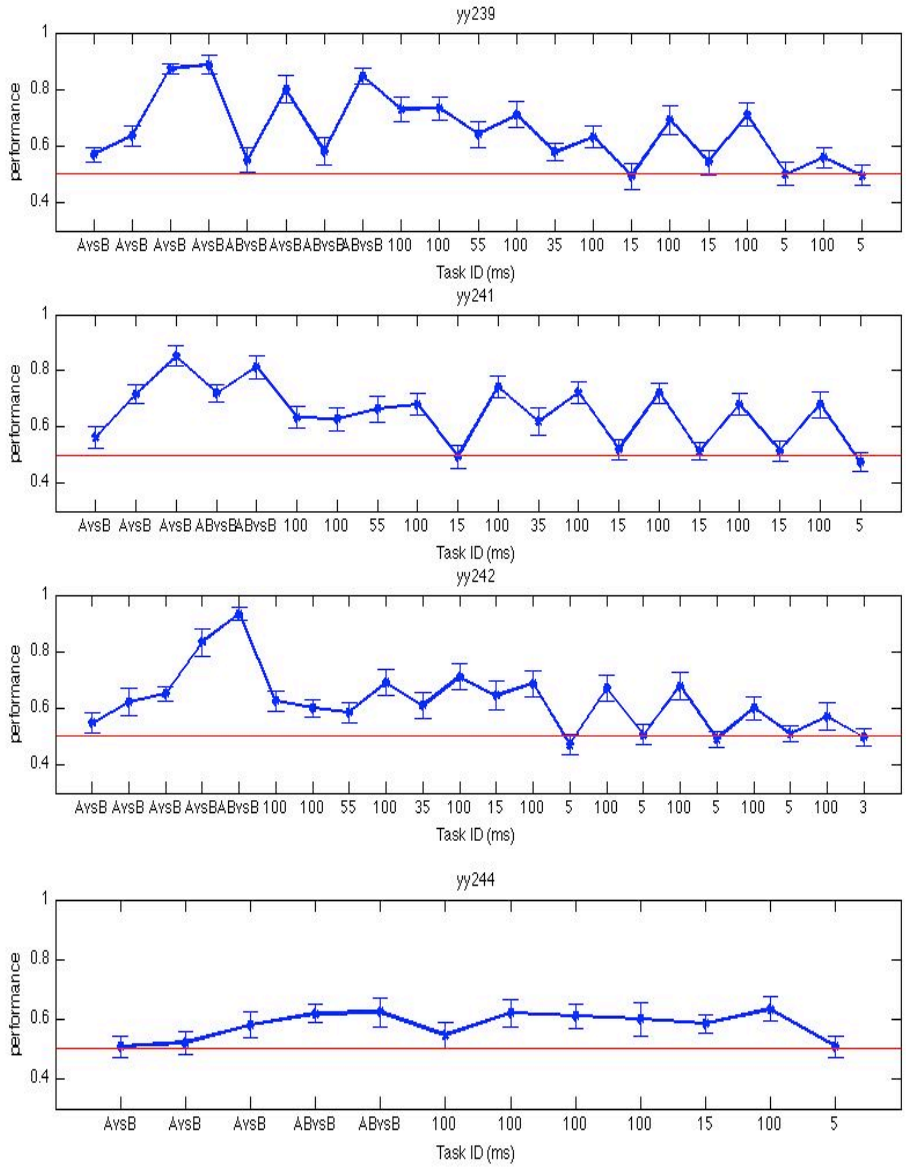


Figure 4.1 Performance of all rats: visual cortex

The performance of all rats on all electrical stimulation tasks plotted in chronological order. Each data point represents one behavioral session. The error bar is the standard error across trials in each session.

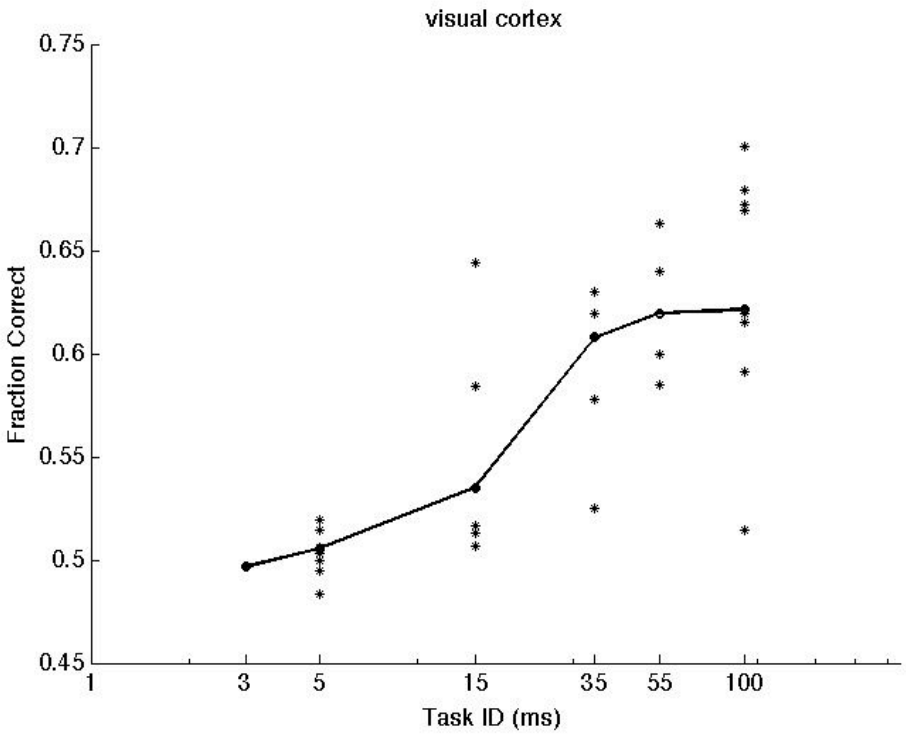


Figure 4.2 Performance of all rats on all Inter-stimulus Intervals

Each data point represents the performance on each ISI for one rat. For each ISI, all trials from all sessions were pooled together.

The line represents the median performance of all rats.

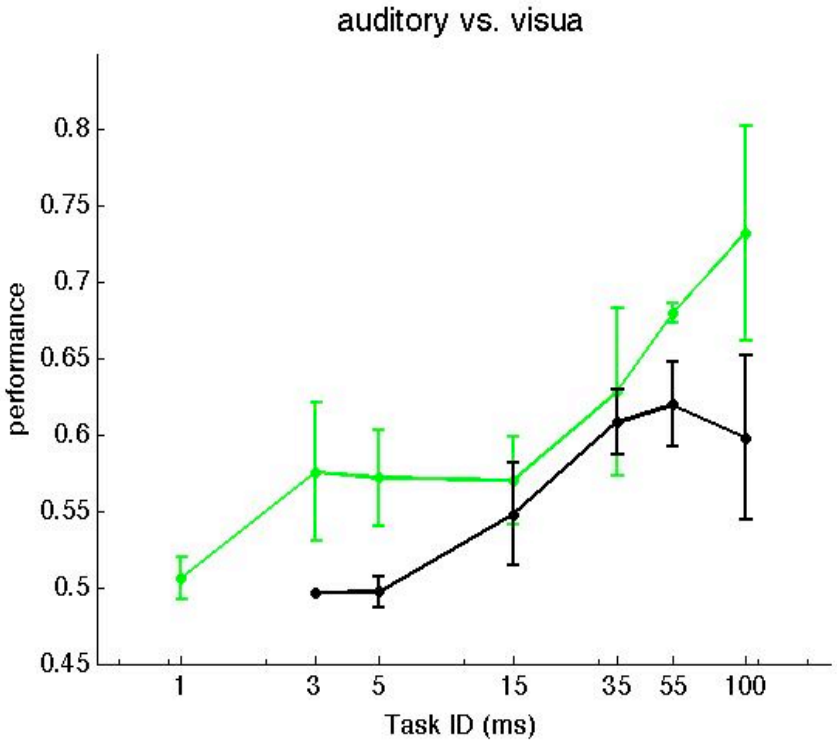


Figure 4.3 Comparing the performances for Auditory cortex and visual cortex animals

Each data point represents the median performance of all rats on each ISI. The error bar is the median absolute deviation from the median.

Green: auditory. Black: visual.

Performances on ISI = 3 ms, 5 ms and 100 ms are significantly different for auditory and visual cortex animals ($p<0.05$).

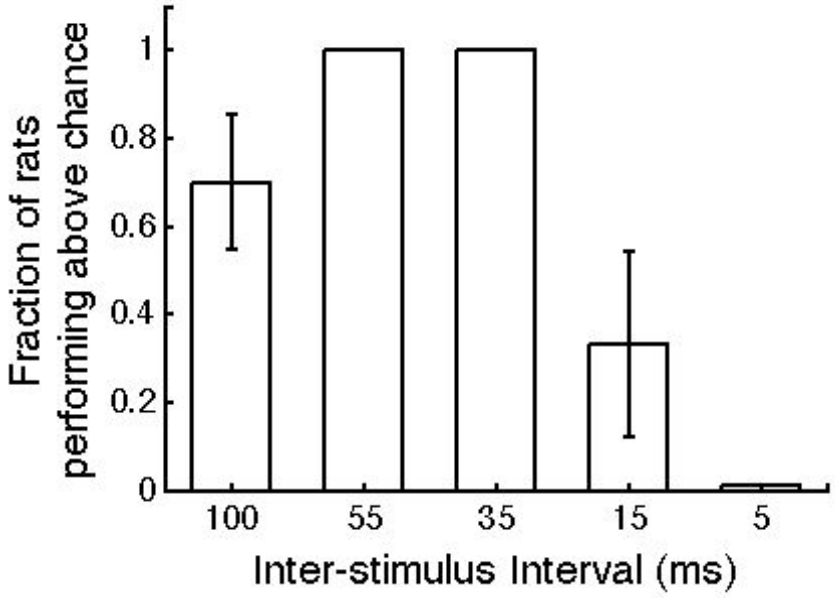


Figure 4.4 Population plot of all rats on all dt tasks

7 out of 10 rats trained on the 100 ms task were able to perform the task significantly above chance ($p<0.01$). 4 out of 4 were able to perform above chance for ISI = 55 ms, 5/5 for ISI =35 ms, 2/6 for ISI = 15 ms, 0/7 for ISI = 5 ms.

The lower behavioral limit for visual cortex was likely to be between 5 ms and 15 ms.

Chapter 5

Barrel Cortex Stimulation

Having had 2 different results from 2 different cortices, we would like to test whether the fastness of the sensory modality was correlated with the ability of the corresponding cortical region to decode fine timing information. Barrel cortex and the rat whisker system were known to be involved in processing fast information (the tactile information). We therefore decided to study barrel cortex, and find out if barrel cortex was indeed good at extracting fine timing information.

5.1 pre-training and training

We didn't have a setup for training animals to detect whisker stimulation. But because the purpose of our 2AFC training prior to the surgery was simply to teach the animals the structure of the 2AFC task, not to prime them for the detection of any cortical stimulation, we decided to use auditory 2AFC task to pre-train the naïve animals for barrel cortex stimulation. The procedure was the same as the procedure for pre-training animals for the auditory cortex stimulation.

We then implanted the animals and trained them first on A vs. B, then AB vs. B, and then timing tasks (see 2.9.3). We implanted and trained 6 rats on barrel cortex stimulation. All 6 rats could perform 100ms, 5 ms and 3 ms tasks well above chance. 4 out of 6 could even perform 1 ms above chance. 0 out of 6 could perform 0.3 ms.

Performance of each rat is shown in Figure 5.1.

From our experiments, we concluded that barrel cortex was even better than auditory cortex in extracting fine timing information. Using our protocol, the threshold for barrel cortex was likely to be between 0.3 and 1 ms. (Figure 5.2)

Performance of all rats on all Inter-stimulus Intervals was shown in Figure 5.3.

5.2 Symmetric stimulation

Most animals we trained (except for one auditory cortex animal) were trained with asymmetric stimulation tasks: simultaneous stimulation of A and B vs. sequential stimulation of A and B (A&B vs. A-dt-B).

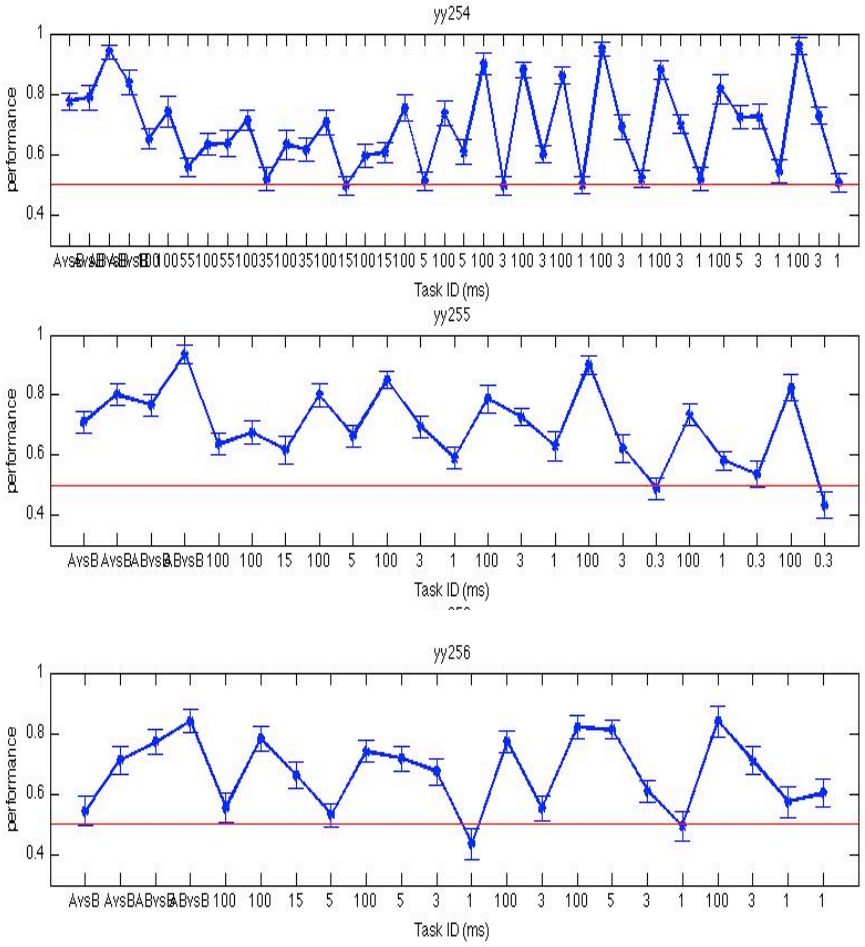
To address the concern that simultaneous stimulation may cause some stimulation artifact, we trained 2 animals on symmetric stimulations (A-dt-B vs. B-dt-A). We didn't see any significant difference behaviorally. Both rats learned to perform 5 ms task above chance, and one learned 3 ms and 1 ms, but not 0.3 ms. The results are similar to the asymmetric stimulation.

Performances were shown in Figure 5.4.

5.3 Comparing barrel cortex, visual cortex and auditory cortex stimulations

The thresholds for barrel cortex, visual cortex and auditory cortex were all different. For barrel cortex, the threshold was 0.3 -1 ms, for visual cortex 5 -15 ms, and for auditory cortex 1 -3 ms. Barrel cortex was the fastest among the three

cortical regions, and visual cortex was much slower than auditory cortex and barrel cortex.



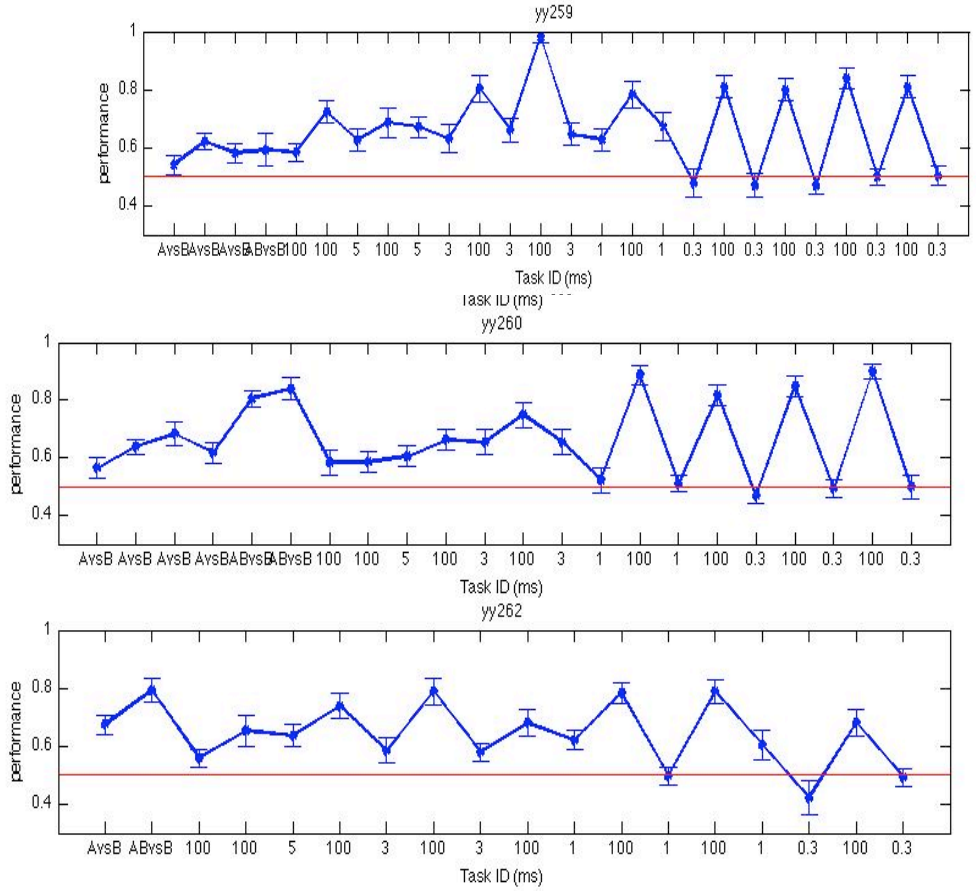


Figure 5.1 Performance of all rats: barrel cortex

The performance of all rats on all electrical stimulation tasks plotted in chronological order.

Each data point represents one behavioral session. The error bar is the standard error across trials in each session.

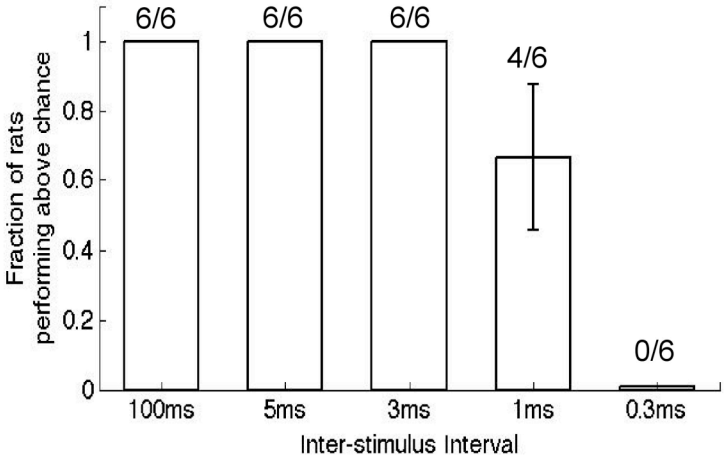


Figure 5.2 Population plot of all rats on all dt tasks

All 6 could perform 100ms, 5 ms and 3 ms tasks above chance. 2 out of 6 could perform 1 ms above chance. 0 out of 6 could perform 0.3 ms.

The lower behavioral limit for barrel cortex was likely to be below 1 ms.

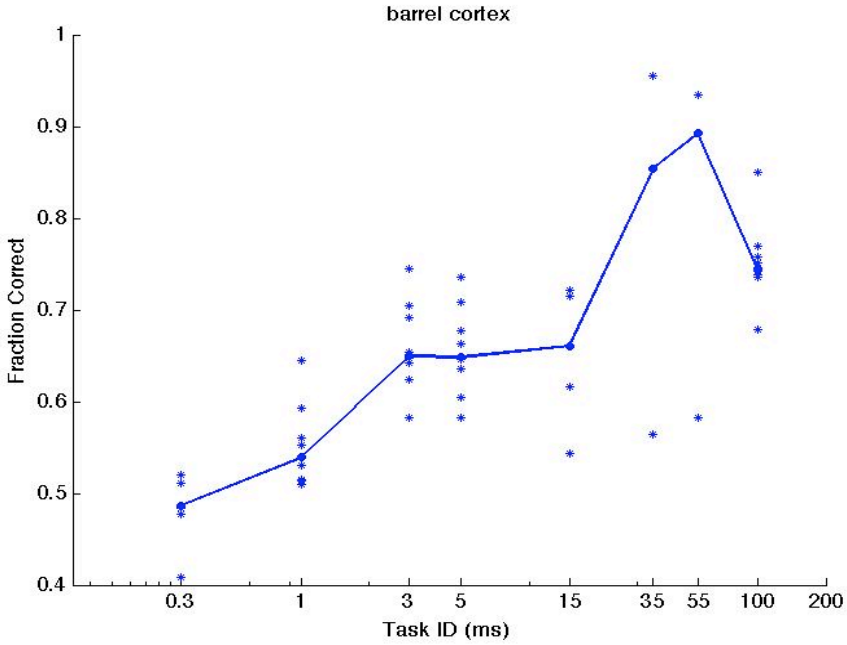


Figure 5.3 Performance of all rats on all Inter-stimulus Intervals

Each data point represents the performance from all all sessions for one rat. The line represents the median performance of all rats.

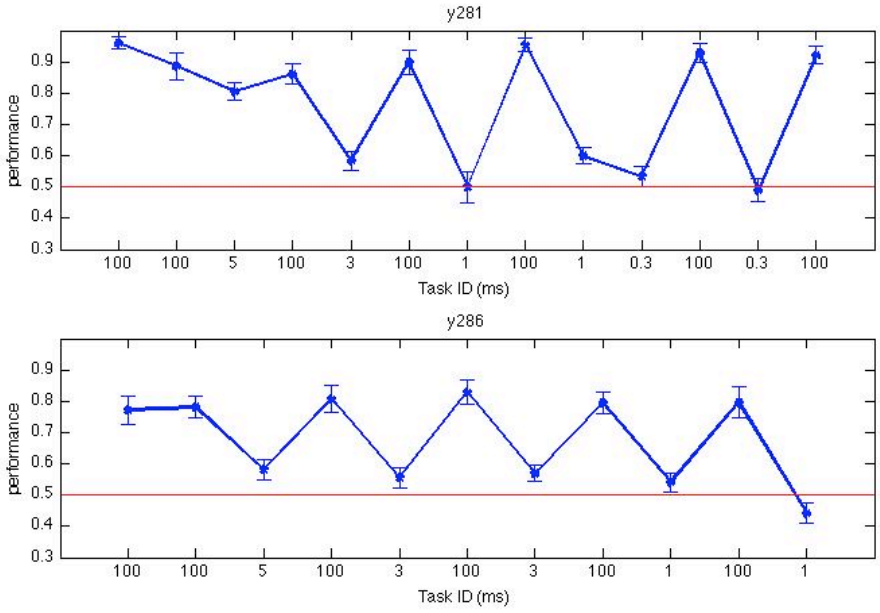


Figure 5.4 Performances on symmetric (A-dt-B vs. B-dt-A) tasks

Both rats could perform A-5 ms-B vs. B- 5 ms-A and A-3 ms-B vs. B- 3 ms-A tasks significantly above chance (on the first try).

y281 could also perform A-1 ms-B vs. B- 1 ms-A. The threshold is similar to the asymmetric stimulation protocol.

Chapter 6

Stimulation in barrel cortex of Sensory deprived animals

We found that different cortical areas are differentially able to derive behaviorally relevant information from the fine timing of neural activity. For those cortical areas, the sensory information has different temporal properties: auditory stimuli are often fast-paced, while visual scenes don't change rapidly. The vibrissa system also deals with time-varying tactile information. Is the ability of a certain sensory cortex to make use of fine timing information hard-wired, or does it depend on sensory experience?

The sensory deprivation of the vibrissa system is well established. It's known that sensory deprivation of the facial whiskers could affect the physiological properties (Fox 1992), temporal coding (Ahissar and Arieli 2001) and anatomy (Micheva and Beaulieu 1995) of the barrel cortex, as well as tactile behavior of the sensory deprived animals (Carvell and Simons 1996). We decided to use the vibrissa system as our model system. We tested whether depriving rats of sensory input into the barrel cortex by trimming one side of their whiskers from birth till adulthood could affect the their ability to resolve fine timing differences in the barrel cortex.

6.1 Sensory Deprivation

One side of the whiskers were all trimmed within 12 hours of birth of the animals, and then trimmed every 24 hours till adulthood (50-60 days, see

methods). 2 control rats were raised alongside their littermates, going through the same anesthetic and handling procedures everyday, only without being sensory deprived.

6.2 sensory deprived animals and control animals

We trained 11 rats total. 1 was non-deprived control, 2 were implanted in the ipsilateral barrel cortex of the trimmed whiskers, and 8 were implanted in the contralateral barrel cortex of the trimmed whiskers.

6.2.1 Sensory deprived animals were severely impaired in cortical timing discrimination

The three controls all performed well above chance on tasks with timing differences down to 3 ms. We grouped them with the 6 animals that were implanted in the barrel cortex for data analysis.

All the sensory deprived animals showed significant defect in the timing tasks.

Performances were shown in Figure 6.1.1 and Figure 6.1.2.

For each animal, we pooled all trials for each ISI, and plotted the performance for each animal. Performances of the sensory deprived animals on all ISIs were generally lower than those of the control animals. (Figure 6.2.1, showing the mean performance of all animals and s.e.m, Figure 6.2.2, showing the median performance of all animals and median absolute deviation)

Comparing auditory cortex with visual cortex:

At ISI = 3 ms, 5 ms and 100 ms, the performances were significantly different between the two groups ($p<0.05$).

Comparing barrel cortex and auditory cortex:

At ISI = 3 ms, 5 ms and 15 ms, the performances were significantly different between the two groups ($p<0.05$).

Comparing barrel cortex control group and barrel cortex sensory deprived group:

At ISI = 3 ms, 5 ms, 15 ms, 35 ms and 100 ms, the performances were significantly different between the two groups ($p<0.05$).

We also compared the best session performance (Figure 6.3.1 and Figure 6.3.2) for the control and the sensory deprived animals, as well as the first session performance (Figure 6.4.1 and Figure 6.4.2).

There is one data point at 3 ms that looks like an outlier in those figures. One sensory deprived rat (yy284) performed even better than all the control animals on ISI = 3 ms. This particular rat improved with training on all ISI's, and the one session of ISI = 3 ms was obtained after it was able to perform 5 ms task well above chance.

6.2.2 Sensory deprived barrel cortex animals were similar to the visual cortex group

When we compare the median performance for each ISI for different groups of animals, we found that sensory deprived barrel cortex was most similar to visual cortex, which was quite different from auditory cortex and barrel cortex.

(Figure 6.5)

Comparing the barrel cortex sensory deprived animals with the visual cortex animals, the performances were not significantly different in all dt tasks. ($p>0.05$)

6.2.3 Sensory deprived animals showed more improvement with training than the control animals

The control animals learned the timing tasks faster and the performance plateaued mostly within the first three sessions. But for the sensory deprived animals, they show gruadual improvement in performance with training. The sensory deprived animals were deprived of the sensory input from whiskers to the barrel cortex, but later received input from cortical electrical stimulation. The results suggest that the ability to discriminate timing information in the cortex is use-dependent.

We plotted the learning curve for 100ms tasks for all four groups (Figure 6.6.1-4). For the animals that were never deprived of sensory input, their performances don't show much improvement with training. However, for the sensory deprived animals, with training, performance improved substantially.

We also tried to fit an exponential function to the learning curve of the control and deprived barrel cortex animals. (Figure 6.7.1-2)

Function: $y = 0.5+(k-0.5)*(1-\exp(-t/tau))$, k is the asymptote performance, tau is the time constant.

Comparing the time constant of the two groups, we found that the deprived group are improving over a longer period of time. (Figure 6.8)

6.3 Whisker trimming after adulthood

We trimmed whiskers of 2 adult rats that were able to perform 3 ms task above chance. The performance on the timing tasks (100ms, 5 ms and 3 ms) were not affected, suggesting that sensory deprivation plays an essential role during the critical period of development. After the cortex is fully developed, sensory deprivation doesn't have an effect on fine timing discrimination behavior. (Figure 6.9)

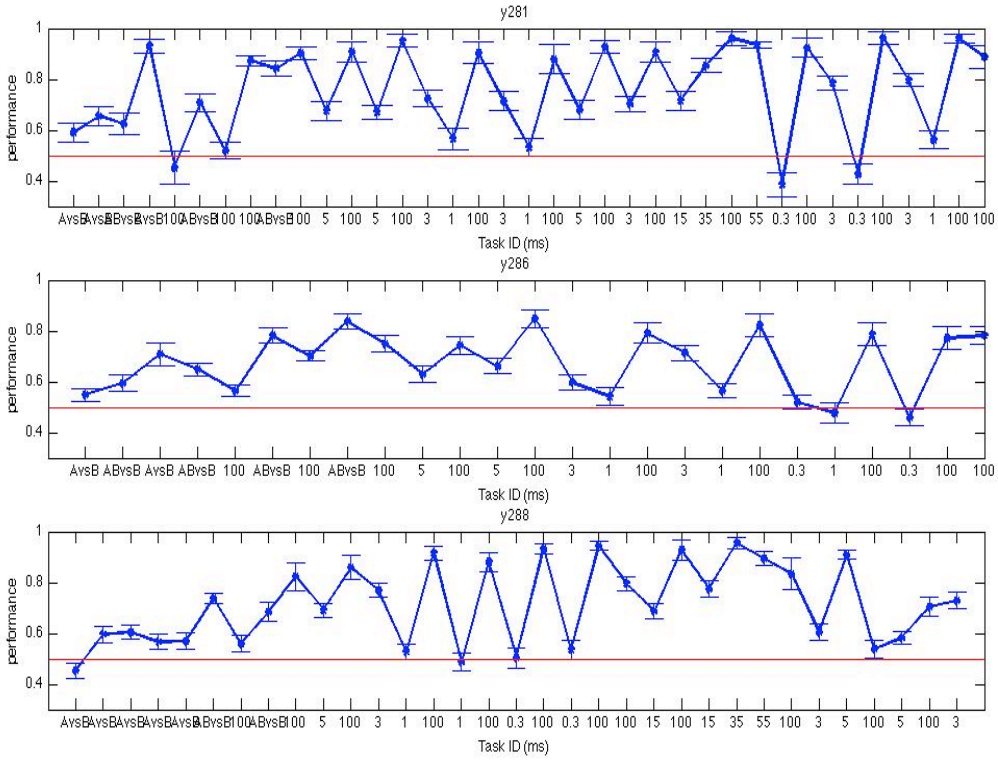
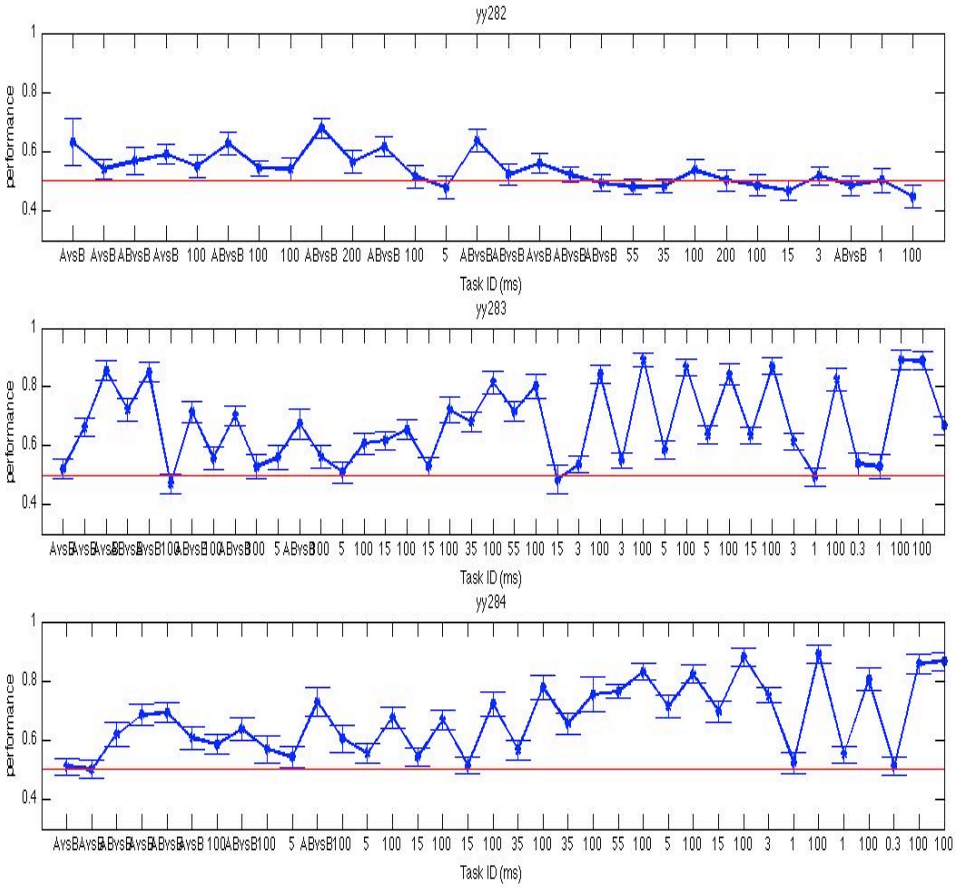


Figure 6.1.1 Performance plots of all control animals on all electrical stimulation tasks

y281 was raised with the other sensory deprived littermates but was not sensory deprived. It was able to perform 5 ms and 3 ms tasks significantly above chance over several sessions.

y286 and y288 were both sensory deprived. They were implanted on the ipsilateral side of the trimmed whiskers. They could both perform 5 ms and 3 ms tasks above chance, just like y281 and other non-deprived animals trained to discriminate fine timing in barrel cortex.



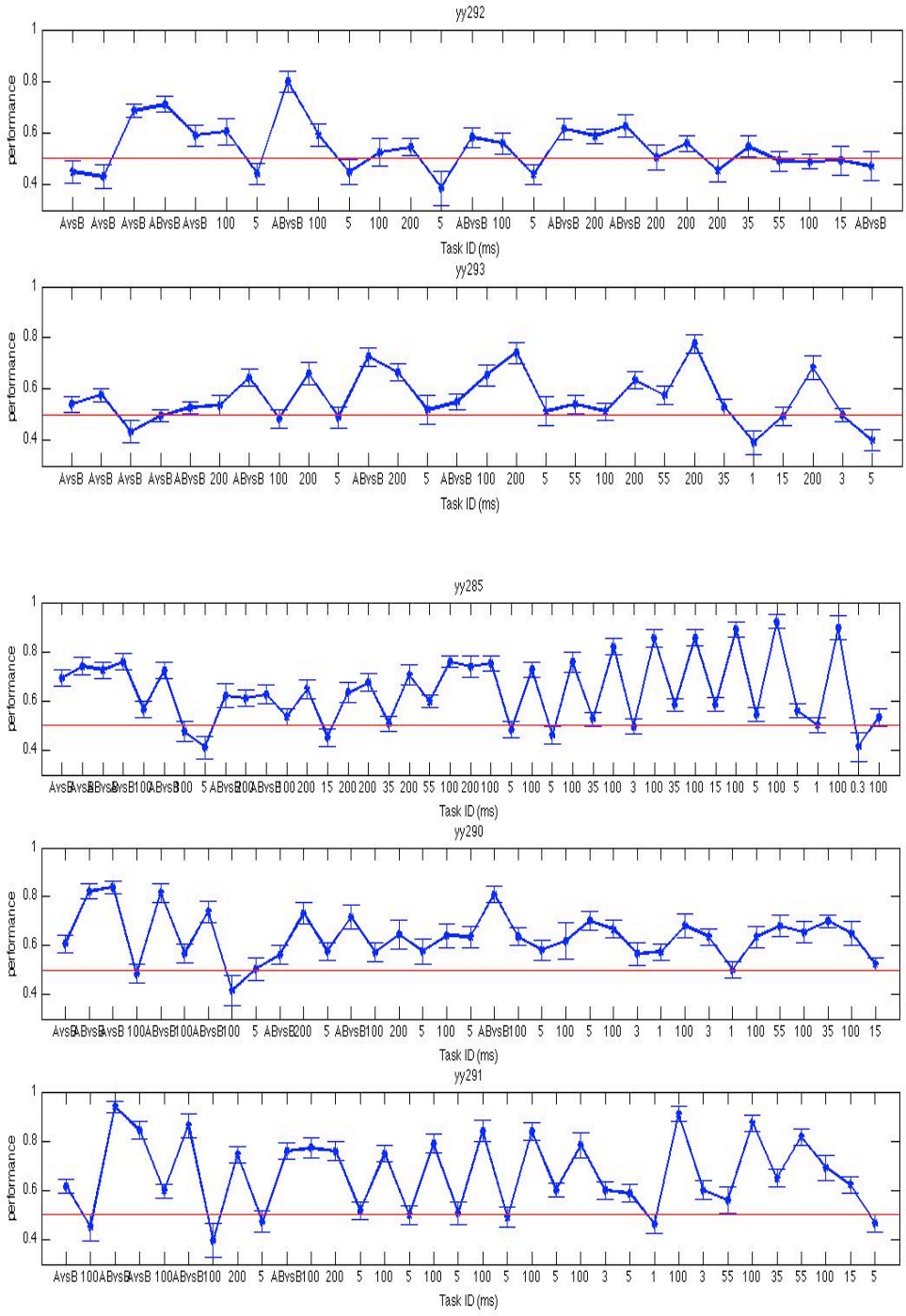


Figure 6.1.2 Performance of all sensory deprived animals

These animals were sensory deprived on one side from P0 to P60 and were implanted in the contralateral barrel cortex. All rats showed impairment on the timing tasks (both long and short) initially. Three rats (yy283, yy284, yy290) were able to perform 5 ms and 3 ms tasks above chance after days of training. All

the other sensory deprived rats couldn't perform the fine timing tasks even after weeks of training.

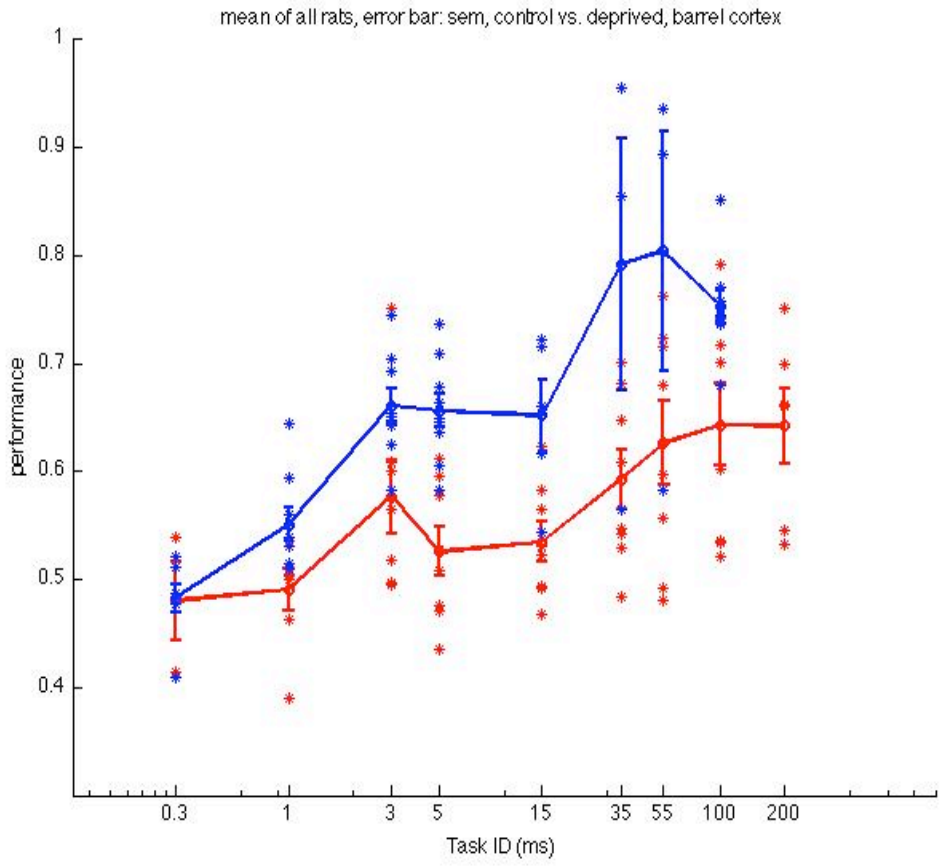


Figure 6.2.1 Comparison of barrel cortex control and deprived animals: all trials

Each data point represents the performance of all trials on the ISI for one rat. The thick line represents the mean performance of all animals and standard error of the mean. Blue: control. Red: sensory deprived.

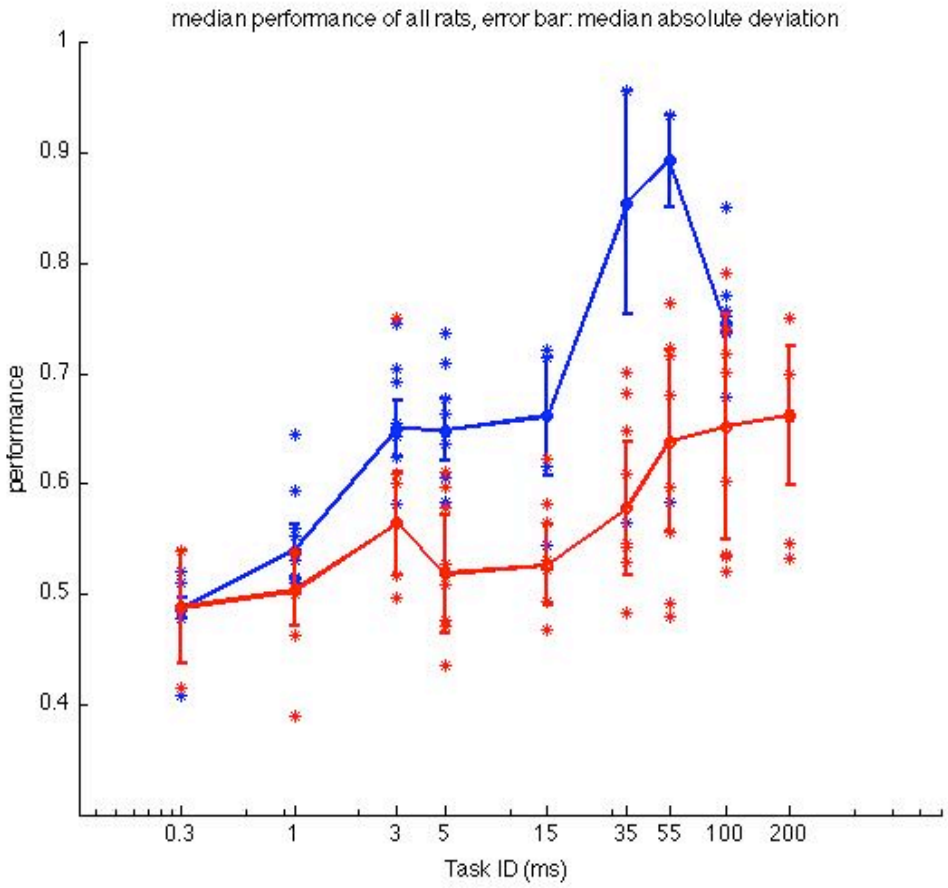


Figure 6.2.2 Comparison of barrel cortex control and deprived animals: all trials

Each data point represents the performance of all trials on the ISI for one rat. The thick line represents the median performance of all animals and median absolute deviation of the median. Blue: control. Red: sensory deprived.

For ISI=3 ms, 5 ms 15 ms, 35 ms and 100 ms, the performances were significantly different between two groups.

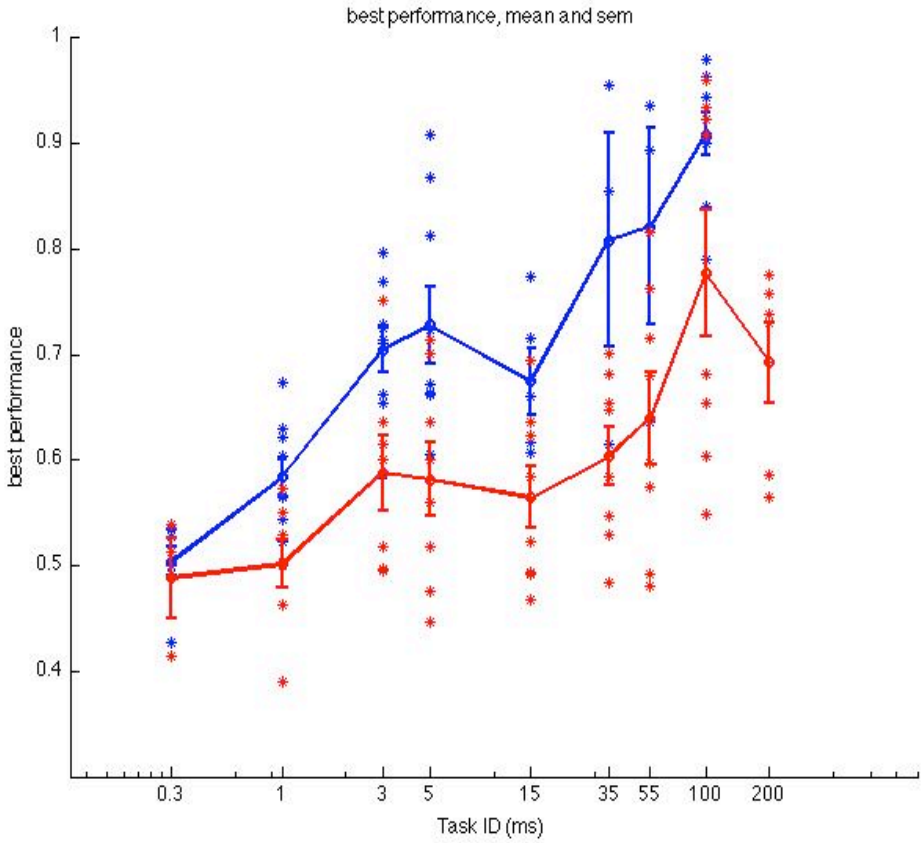


Figure 6.3.1 Comparison of barrel cortex control and deprived animals: best session

Each data point represents the best performance session on the ISI for one rat. The thick line represents the mean performance of all animals and standard error of the mean. Blue: control. Red: sensory deprived.

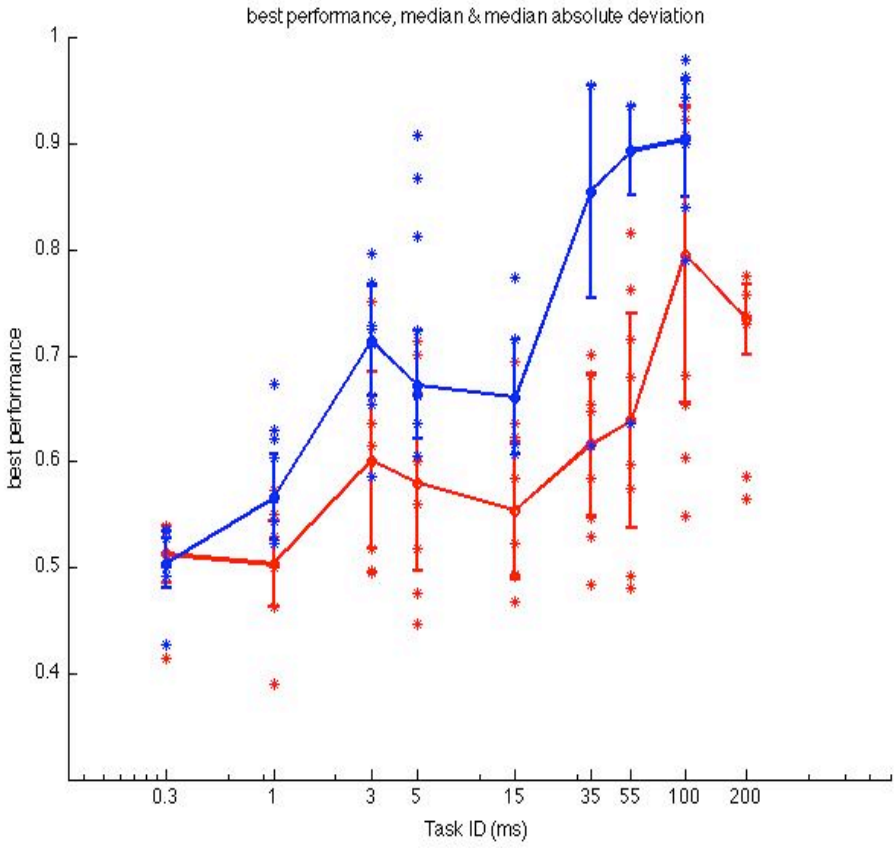


Figure 6.3.2 Comparison of barrel cortex control and deprived animals: best session

Each data point represents the best performance session on the ISI for one rat. The thick line represents the median performance of all animals and median absolute deviation of the median. Blue: control. Red: sensory deprived.

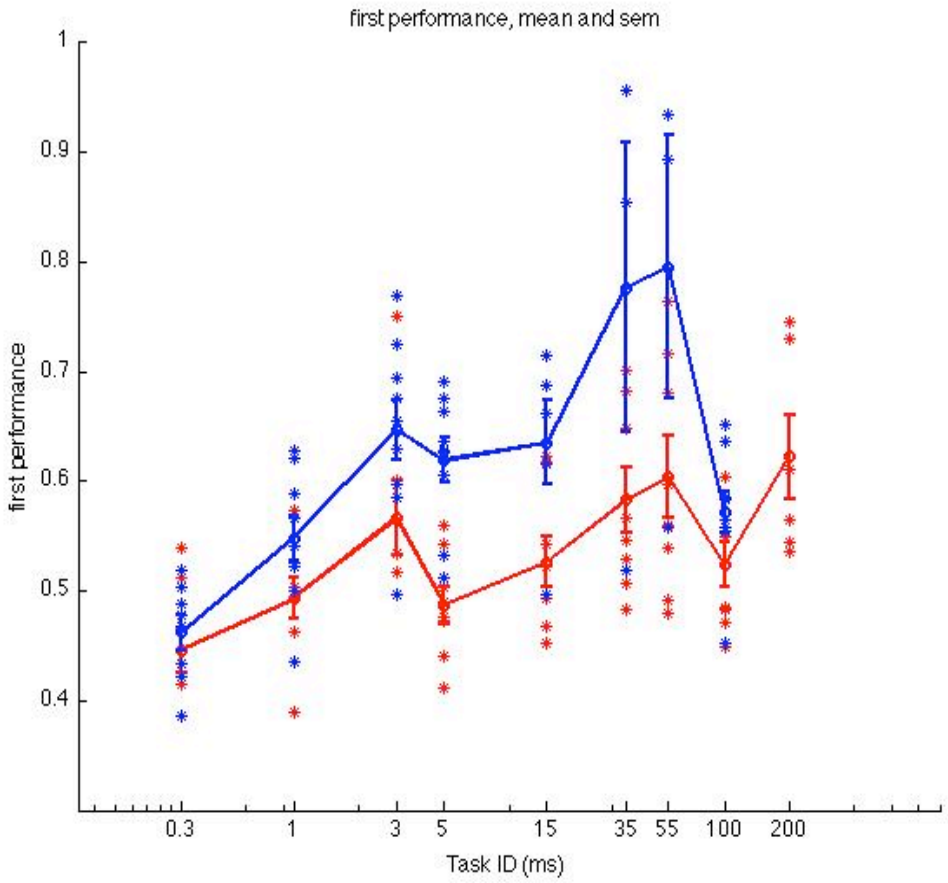


Figure 6.4.1 Comparison of barrel cortex control and deprived animals: first session

Each data point represents the first performance session on the ISI for one rat. The thick line represents the mean performance of all animals and standard error of the mean. Blue: control. Red: sensory deprived.

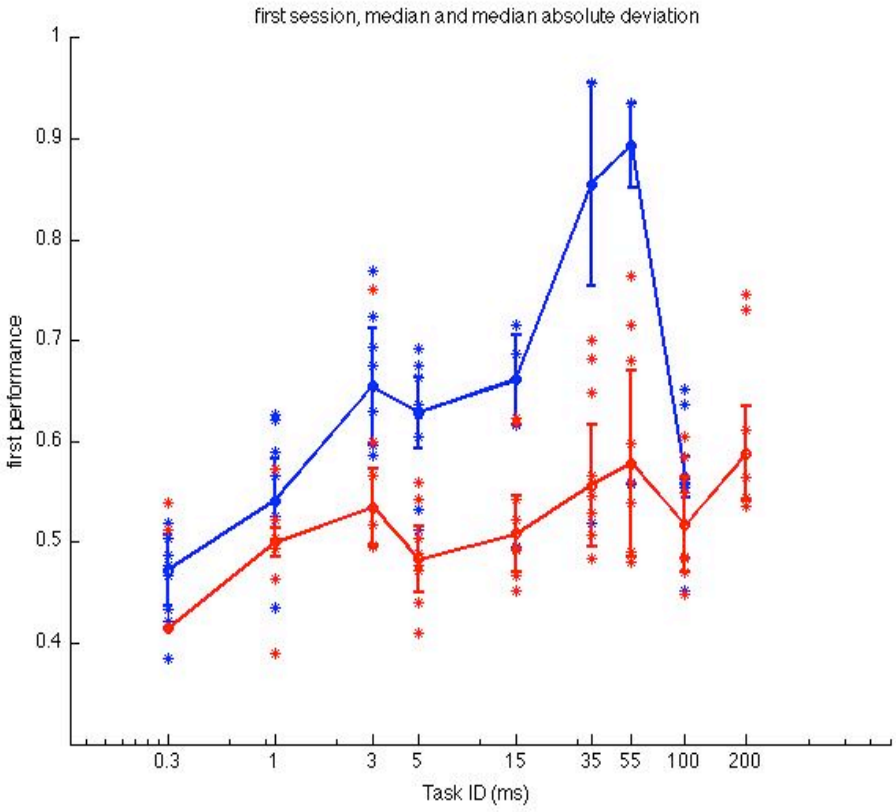


Figure 6.4.2 Comparison of barrel cortex control and deprived animals: first session

Each data point represents the first performance session on the ISI for one rat. The thick line represents the median performance of all animals and median absolute deviation of the median.

Blue: barrel cortex control. Red: barrel cortex sensory deprived.

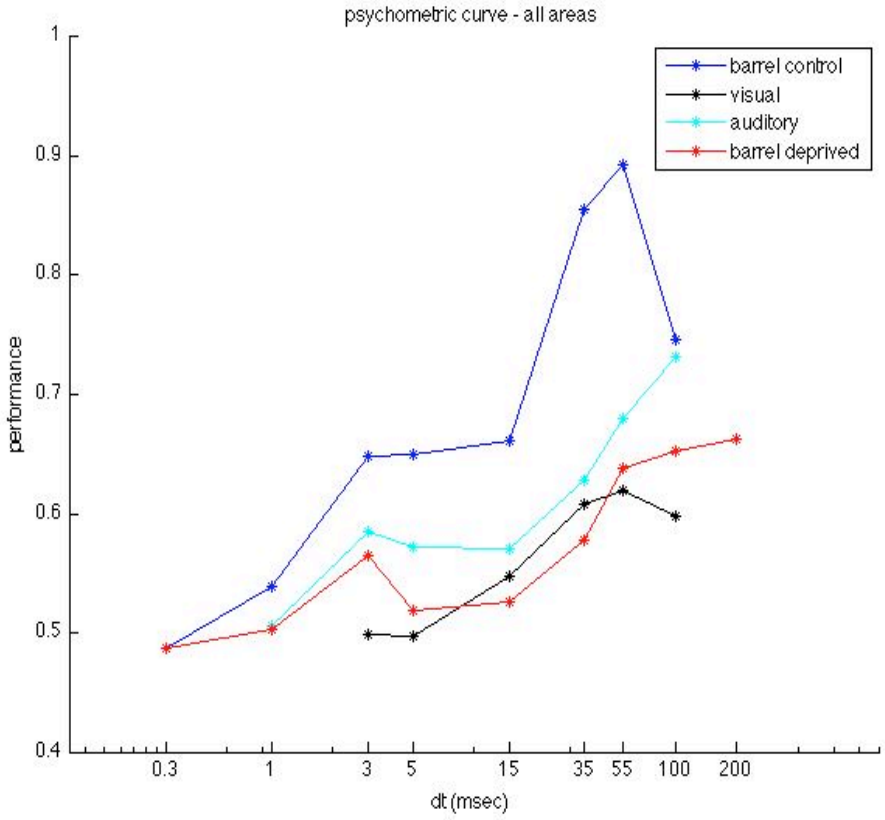


Figure 6.5 Comparison of visual cortex, barrel cortex, auditory cortex and barrel (deprived) animals

Plotted were median performances on all timing tasks of all animals in each experimental group. Each data point was the median performance of all animals trained on the task, and for each animal, all trials were pooled for each dt. (The plot of all trials pooled from all animals was similar to this plot; plot not shown)

The barrel cortex control animals were better at almost all dt's than the other groups. Auditory cortex animals were not as good as the barrel cortex controls, but were better than both the barrel cortex sensory deprived group and the visual cortex group. The barrel cortex deprived group and the visual cortex group were surprisingly similar.

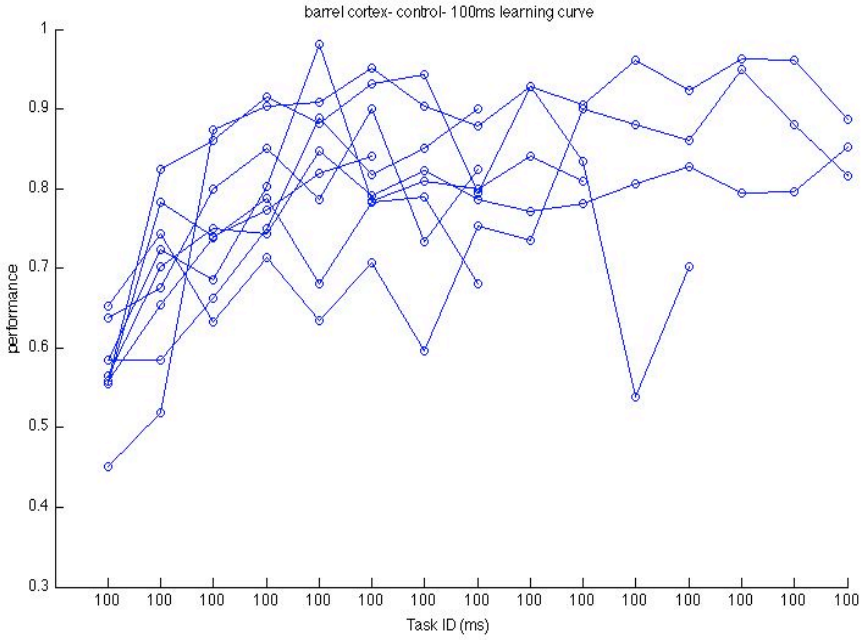


Figure 6.6.1 Barrel cortex control, learning curve for 100 ms task

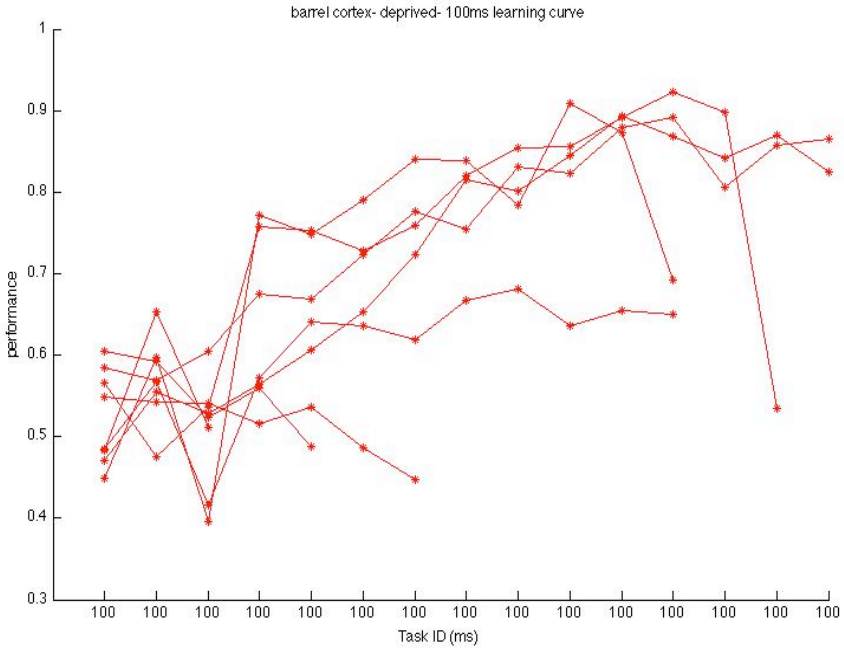


Figure 6.6.2 Barrel cortex, sensory deprived, learning curve for 100 ms task

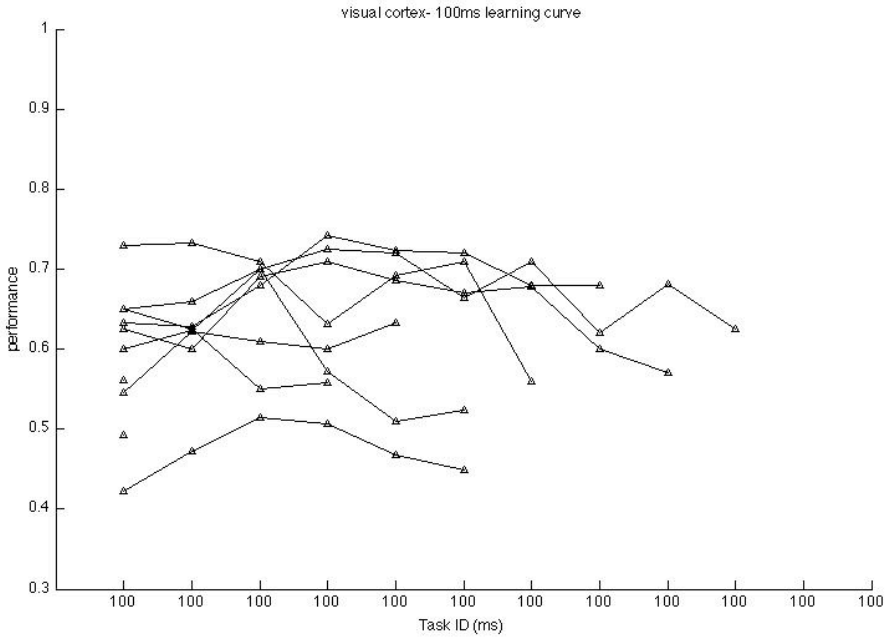


Figure 6.6.3 Visual cortex, learning curve for 100 ms task

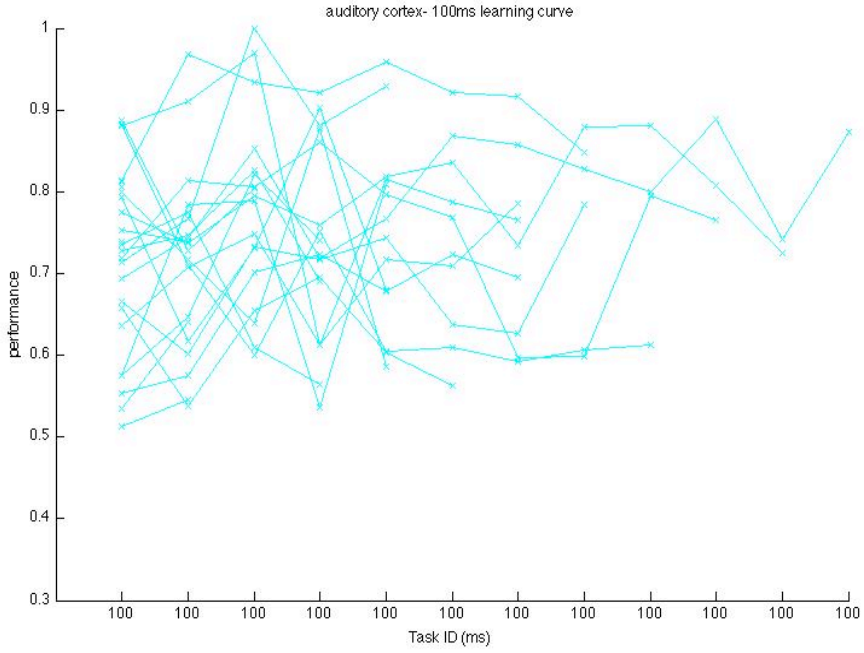
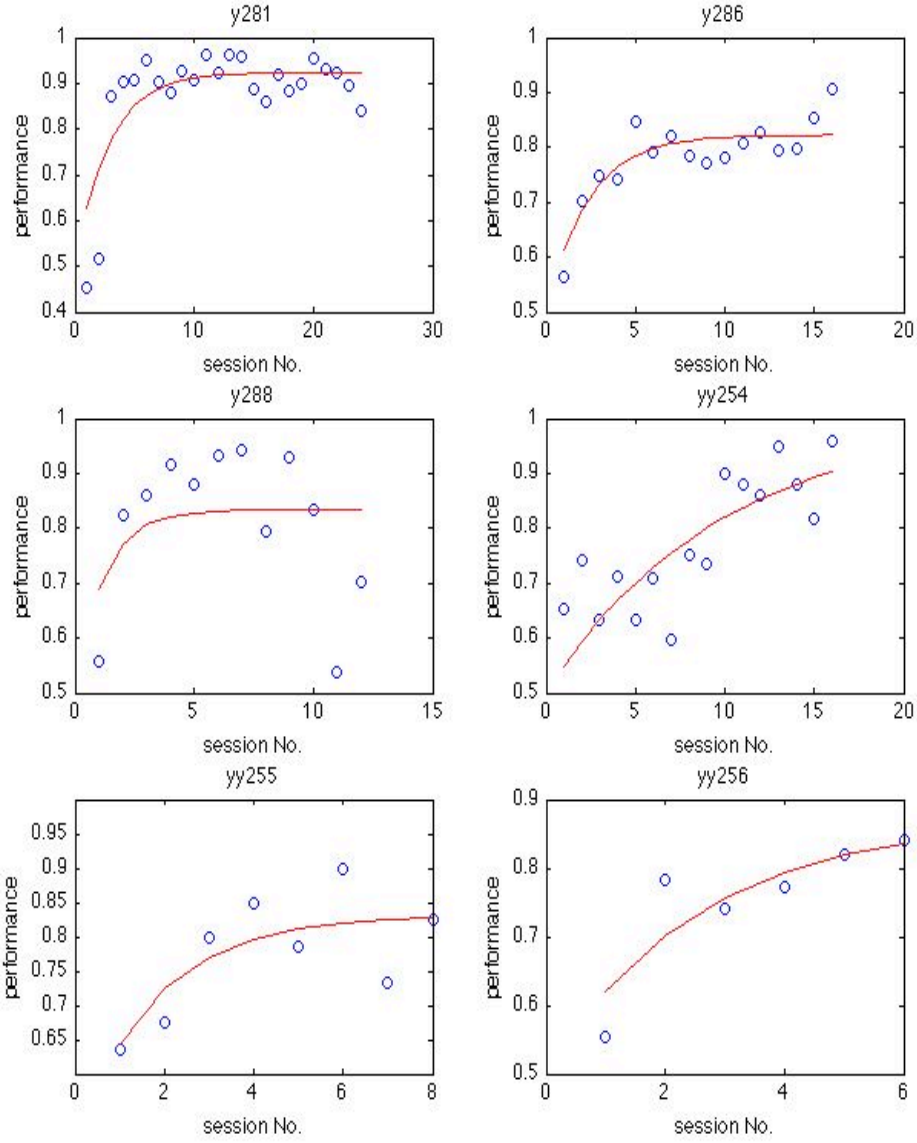


Figure 6.6.4 Auditory cortex, learning curve for 100 ms task


y281
performance
session No.
y286
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y288
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yy254
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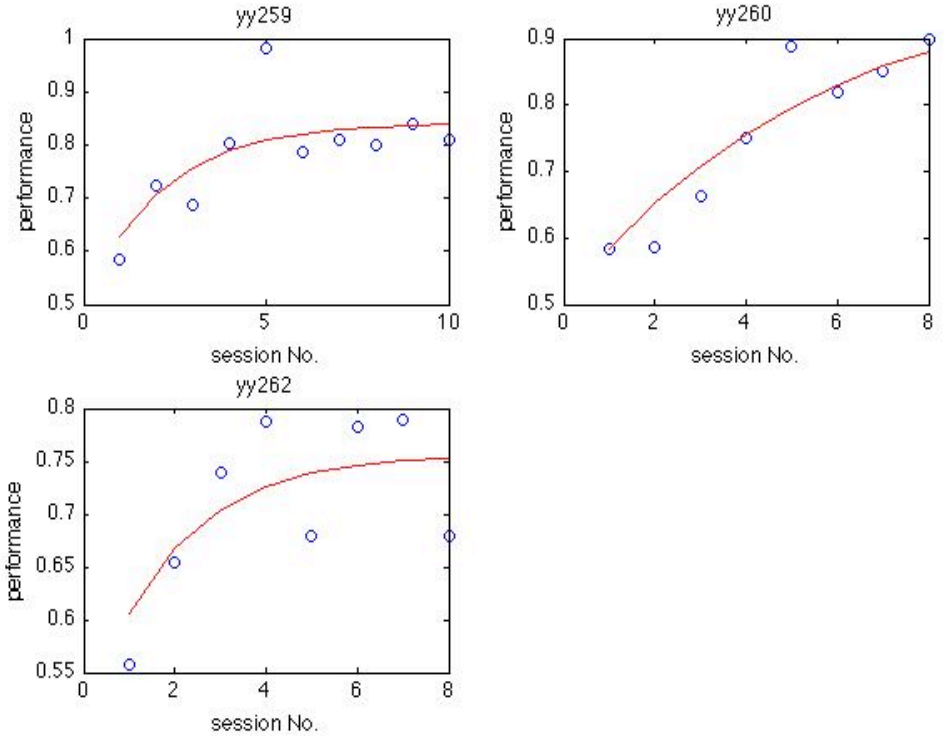
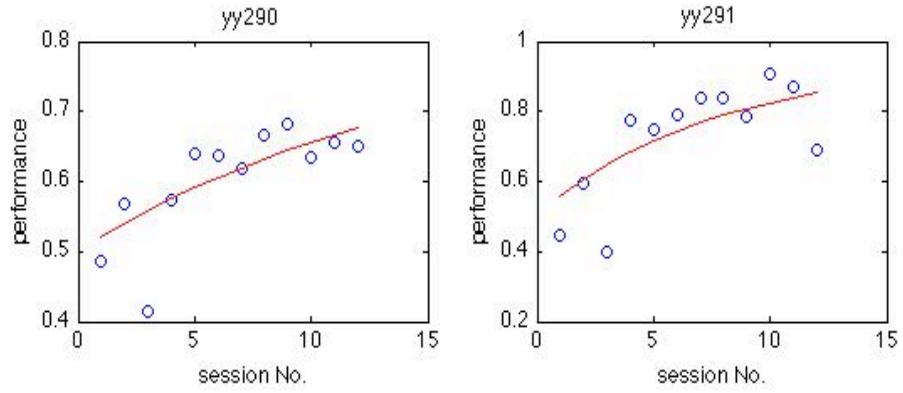


Figure 6.7.1 Fit 100 ms learning curve of barrel cortex control animals to exponential function

$y = 0.5+(k-0.5)*(1-\exp(-t/tau))$



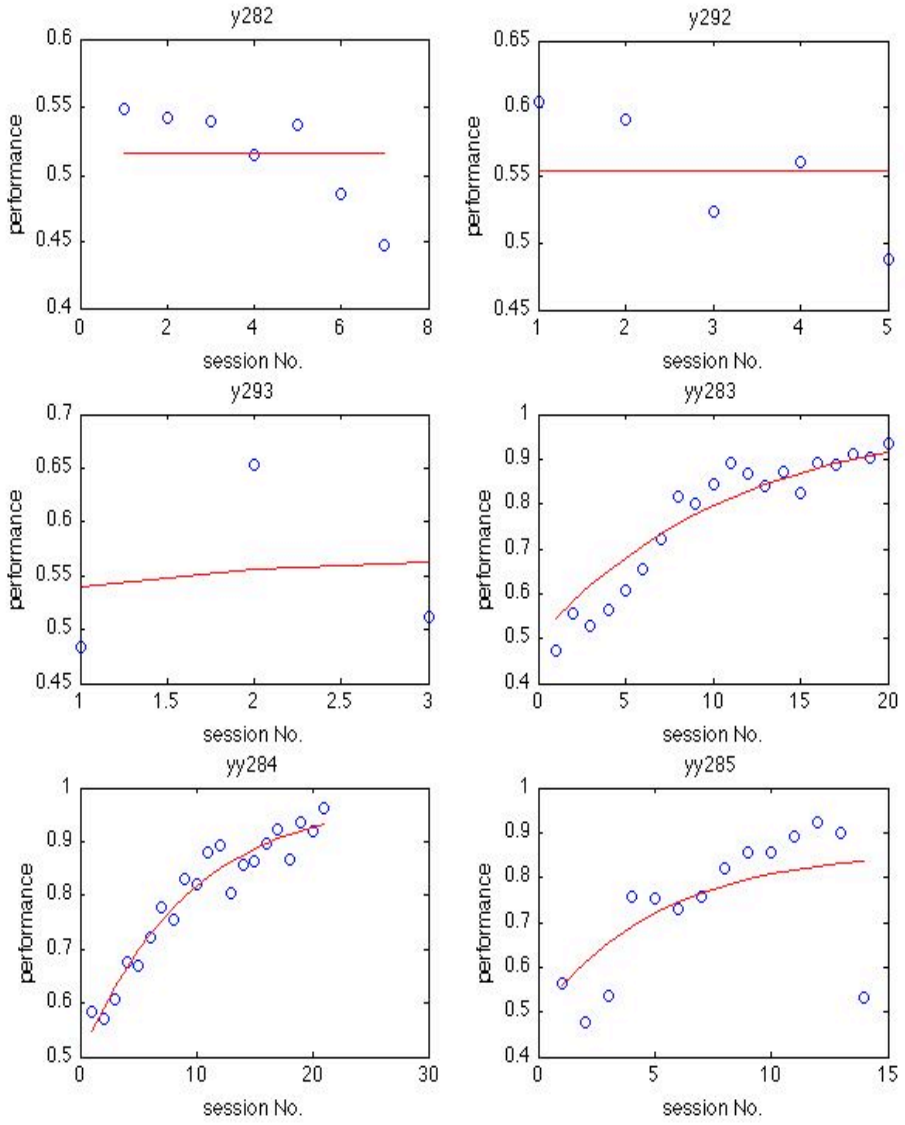


Figure 6.7.2 Fit 100 ms learning curve of barrel cortex deprived animals to exponential function

$y = 0.5+(k-0.5)*(1-\exp(-t/tau))$

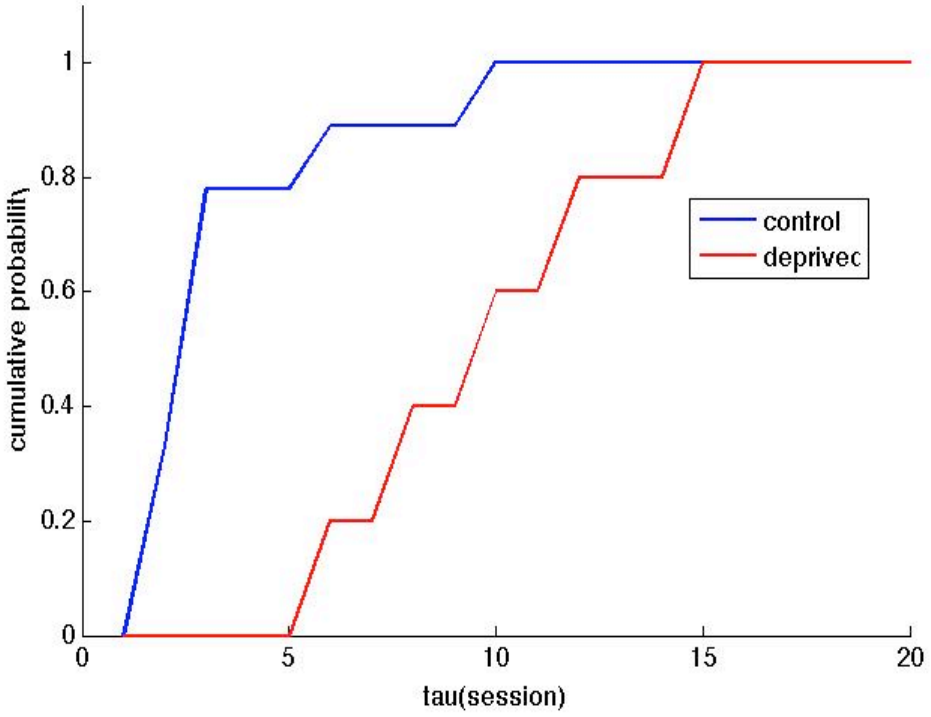
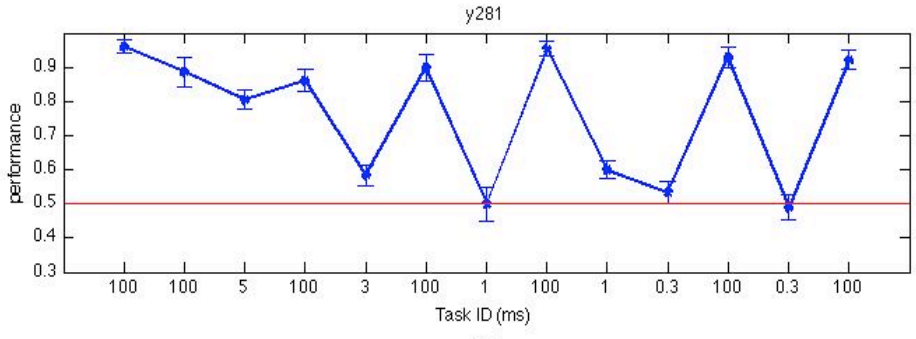


Figure 6.8 Comparing the timing constant of 100 ms session learning curve for control and deprived animals.

Blue: control. Red: deprived.

With the exception of one animal that didn't improve at all with training (excluded from this plot), the deprived animals have a longer time constant compared to the control. (p=0.0028)



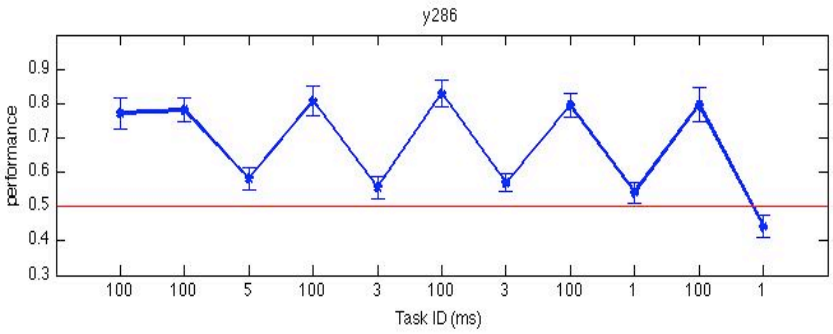


Figure 6.9 Performance after adult whisker trimming

Trimming whiskers after adulthood doesn't affect fine timing behavior. Both rats trained could still perform the timing tasks (long and short).

After trimming, y281 could still perform 100 ms, 5 ms, 3 ms and 1 ms above chance, but not 0.3 ms. y286 could still perform 100 ms, 5 ms and 3 ms above chance, but not 1 ms.

Chapter 7

Optical Stimulation in Auditory Cortex

We previously used electrical stimulation to probe the precision with which cortical timing information can be used to guide behavior in various cortical areas. Electrical microstimulation with chronically implanted electrodes could sometimes cause damage to the brain. Also, electrical pulses stimulate all types of cells plus passing fibers in the vicinity of the stimulation electrode, and therefore it was not an appropriate tool for stimulating certain types of neurons.

We developed a new paradigm using channelrhodopsin-2 (ChR2) and optical stimulation, to first validate the results we obtained using electrical microstimulation, and to provide a basis for pathway and/or cell type specific stimulations in the future.

7.1 Virus expression

In order to repeat the electrical stimulation results, we used an AAV virus with a CAGS promoter which expresses at a high level in all cell types. The virus we used was AAV-CAGS-ChR2-Venus. We directly injected the virus into the left auditory cortex of 4-week-old young rats, and wait about 4 weeks for the virus to express, and for the young rats to grow. The virus expression could cover most of auditory cortex. (Figure 7.1)

7.2 Optical stimulation and physiology

To make sure the virus expression was high enough, and the fiber optic we used could deliver enough light to trigger spikes in vivo, we recorded from anesthetized animals injected with ChR2 using an optotrode we made (Figure 2.3, bottom). The optotrode was made of a 70-um fiber glued to a 1-M Ohm tungsten electrode. We were able to record light evoked local field potential and multiunit activity with the tungsten electrode. (Figure 7.2)

7.3 Behavior

We implanted two 70-um optical fibers glued to two miniature LEDs into the auditory cortex of the animals that were injected with AAV-CAGS-ChR2-Venus. We managed to repeat the microstimulation experiments and get comparable results: animals could be trained to perform the cortical timing task above chance at a timing difference of only 5 ms (figure 7.3).

The first batch of animals we trained learned to detect the blue light very quickly, but later we found that they were using the visual cue. If we use a mask bright light when they are performing the optical stimulation task, the animals all failed.

To avoid training animals on a visual task, we combined several strategies. One was to turn on a bright white LED in the center port when the animal poked into the center to trigger the cortical optical stimulation. The other is to have a blue LED flashing at the same frequency as the light pulse inside their head when the stimulus was triggered. This way we made sure that the animals were using

the cortical cue to guide their behavior, not the visual cue.

A B

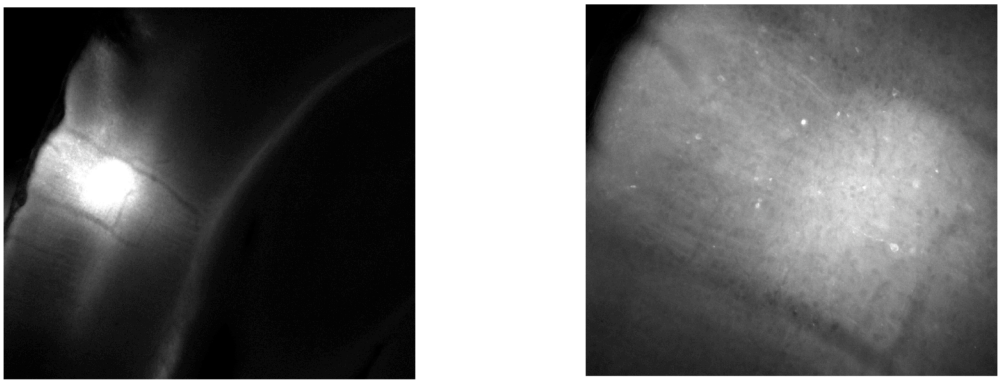


Figure 7.1 Virus expression

A: the bright region was auditory cortex injected with AAV-CAGS-ChR2-Venus.

B: a blowup showing the cell bodies in the injected cortex

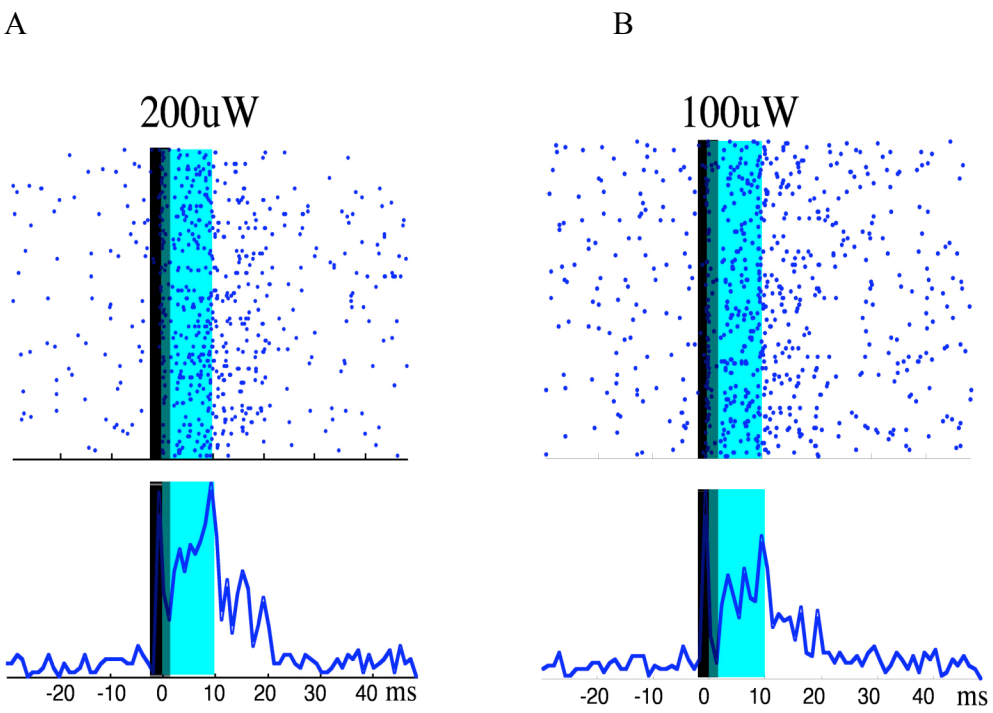


Figure 7.2 light evoked response in vivo

A: top: dots represent individual spikes recorded with tungsten electrodes. Bottom: peri-stimulus time histogram (PSTH). The blue area represents the blue light duration. The dark grey area is the transient stimulus artifact. The light intensity at the tip of the fiber optic was 200uW.

B: same as A. The light intensity was 100uW at the tip of the fiber optic. The response was smaller at lower light intensity.

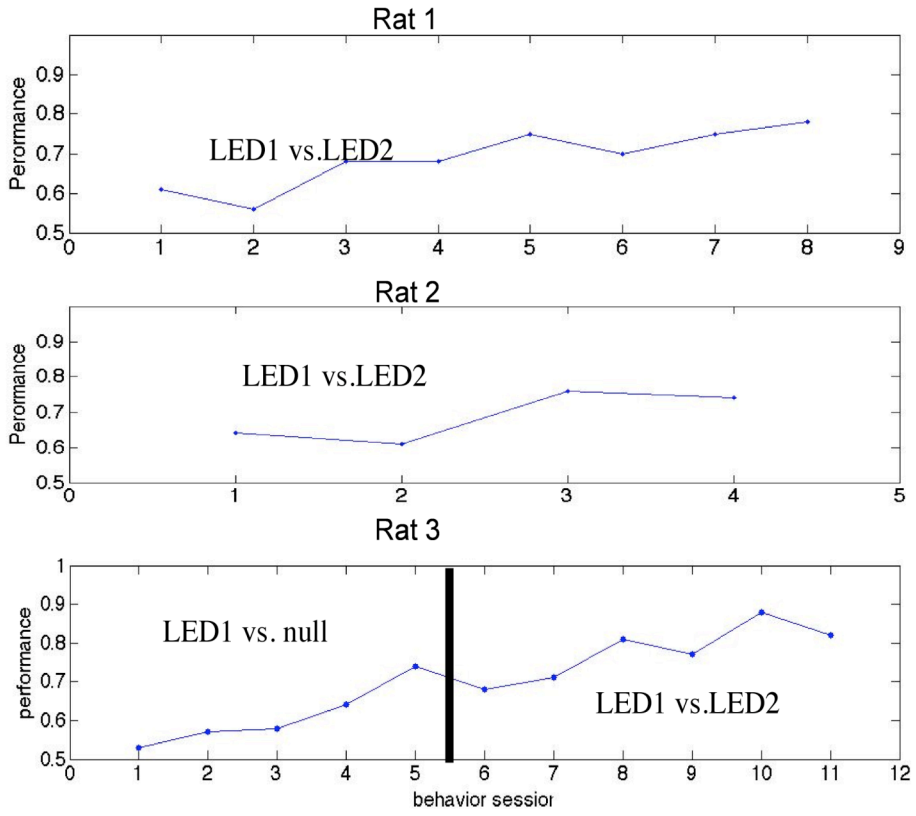


Figure 7.3.1 Animals were trained to detect ChR2 stimulation

Rats 1, 2 and 3 can all be trained to perform the basic stimulation task of LED1 vs. LED2 above chance. Rat 3 was first trained to discriminate LED1 from no light. Soon after switching the task from LED1 vs. null to LED1 vs. LED2, rat 3 was able to perform above chance.

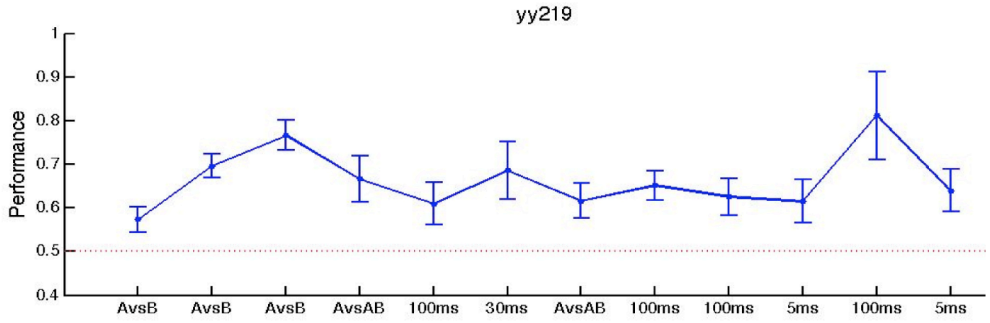


Figure 7.3.2 Animals could perform 5 ms task above chance

yy291 was trained first to perform the basic optical stimulation task (A vs. B, or LED1 vs. LED2). After it could perform significantly above chance, it was

trained on timing tasks. This rat could perform 100 ms, 30 ms, and even 5 ms tasks significantly above chance.

Chapter 8

Conclusions and Perspectives

We used a century-old technique (electrical microstimulation) to study a novel question: can fine timing information in different areas of the cerebral cortex be used to drive behavior? We found that fine timing information in certain cortical regions can be read out and used by the animals in a behavioral context, and different areas of the cortex are differentially capable of extracting fine timing information. The ability of the sensory cortices to exploit timing information behaviorally is not hard-wired but depends on sensory experience. We also used a new technique (ChR2 combined with optical stimulation) to confirm the electrical stimulation results.

Fine timing information as short as 3 milliseconds delivered directly into auditory cortex can be used to guide animal's behavior (chapter 3). We implanted two electrodes into the auditory cortex of rats, and trained the rats to distinguish simultaneous stimulation from sequential stimulation with a time delay. We found that a majority of the animals we trained (10 out of 15) could tell the time difference of 5 milliseconds, and 2 animals out of 6 could even tell 3 milliseconds from 0. None of the animals could distinguish 1 millisecond from 0, which suggests that in auditory cortex, the lower limit was likely to be between 1 millisecond and 3 milliseconds.

Vision is believed to be a slow sense. Although visual cortex contains neurons that could respond with millisecond precision to certain visual stimuli (Buracas, Zador et al. 1998) visual cortex does not specialize in extracting fine

timing information. We found that using the same stimulation protocol and experimental procedure, animals that were trained to discriminate timing differences in visual cortex could not perform the timing tasks equally well compared to auditory cortex. Only a small proportion of the animals could perform the 15 ms task above chance (2 out of 7), and none of the animals trained could tell the difference between 5 ms and 0. The threshold for visual cortex is likely to be between 5 ms and 15 ms. The performances of the visual cortex animals on 3 ms, 5 ms and 100 ms tasks are significantly different from the performances of the auditory cortex animals. Our conclusion is that visual cortex is not as good as auditory cortex in terms of making use of fine timing information.

Is auditory cortex special, or is visual cortex special? To find out, we did the same experiment in barrel cortex. To our surprise, barrel cortex was even better than auditory cortex in using fine timing information to guide behavior. Of all the animals we trained, all could perform well above chance in 5 ms and 3 ms tasks. Some could even do 1 ms. Only when we reduced the timing difference to 0.3 ms did all the rats fail to perform the task.

For the three cortical areas, the sensory input has different temporal properties: auditory stimuli are often fast-paced, visual scenes don't change rapidly, and the vibrissa system deals with fast time-varying tactile information. There seems to be a correlation between the pace of the sensory stimuli a certain sensory cortex receives and the quickness of the sensory cortex. Is the ability of the sensory cortices to decode fine timing information hard-wired, or does it

depend on sensory experience? We trimmed all whiskers on one side of the rats' face from P0 till P60, and then trained them to discriminate fine timing in barrel cortex. All 8 rats trained in the timing task showed significant defect. 3 of the 8 animals improved with training and could eventually perform the 5 ms and 3 ms task, though with lower performance compared to the control group. All the other sensory deprived animals were a lot worse than their littermate controls. The performance on cortical timing tasks in the sensory deprived group was similar to the performance of animals implanted in the visual cortex.

We also used ChR2 combined with optical stimulation to confirm the results. We expressed ChR2 in auditory cortex using AAV virus (AAV-CAGS-ChR2-Venus), which expresses in all cell types. Then we used fiber optics glued to miniature LEDs to deliver light directly into the cortex. We successfully trained animals to detect the stimulation of the ChR2 positive cells, and even trained them to perform the cortical timing task. One animal was able to perform the task when timing information was 5 ms. The result was consistent with the electrical stimulation experiments.

We showed that fine timing information in certain cortical regions can be read out and utilized by the animals in a behavioral context, and different areas of the cortex are differentially able to extract fine timing information. However, it's not clear which cells and which pathways are responsible for utilizing the fine cortical timing information. Using electrical stimulation method, it's hard to solve the problem. However, with the new optogenetic approaches, it is possible to stimulate certain cell types and certain pathways. Therefore, with careful design

of the experiments, it is possible not just to find out whether certain brain regions can make use of fine timing information, but also how they make use of the timing information.

With our current experimental design, we can only speculate about how and where the computation is done. One possibility is that different cortical areas converge to the same "computational center" for extracting fine timing information, and the difference we see is due to the difference in connection strength between each sensory cortex and the computational center. Another possibility is that different cortices project to different areas of the brain for reading out fine timing, and the difference in the discrimination ability is simply due to the difference among those areas. My belief is that because the electrical stimulations are artificial, the animals probably could learn to make use of the most direct or efficient pathway to make the discrimination. The connectivity strength between different sensory cortices and downstream areas is vastly different, so it is more likely that the computation is done in different regions for different sensory cortices.

To find the downstream areas responsible for the fine timing computation, one could either lesion specific areas or use muscimol or other drugs to inactivate the candidate areas. It is also possible to combine viral and optogenetic tools to trace down the target regions.

Another question one might ask is, how does the finest timing extractable vary with the distance between the two electrodes? For barrel cortex, because the interconnections are strong within each barrel, and because when computing

sensory input from whiskers, each barrel serves as one unit, it could be hard for the animals to discriminate timing differences when the two electrodes were placed in the same barrel. However, it may not make a big difference whether the two electrodes are one or more barrels apart, as projections from and to different barrels are likely to be similar, and when solving real-life problems like texture discrimination, input from more than one whiskers are often required.

For Visual cortex and auditory cortex, the relationship between behavioral threshold and distance may be more complicated. On one hand, when the distance is very small, stimulations with the two electrodes may elicit spikes from the same group of neurons, which would make it harder for the animals to discriminate the timing difference, and even if the animals could discriminate, it may simply reflect the absolute refractory period of the stimulated neurons. On the other hand, when the distance gets longer, possibility of the two groups of neurons connecting to the same downstream area decreases, and the strength of the common connection may also decrease, making the computation more difficult.

Because the temporal properties of the sensory input to different sensory cortices are different, the cortices might have evolved to adapt to the pace of the sensory stimuli. Though we found that sensory deprivation could severely impair the ability of barrel cortex to solve fine timing information, and therefore there is an experience-dependent component in the timing discrimination ability, we couldn't exclude the possibility that the fundamental differences between different sensory cortices, e.g., different anatomical structures, different ascending and descending projection patterns, etc., are what lead to the vast differences in

the ability to behaviorally make use of cortical fine timing.

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