Millisecond-scale differences in neural activity in auditory cortex can drive decisions

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Neurons in the auditory cortex can lock with millisecond precision to the fine timing of acoustic stimuli, but it is not known whether this precise spike timing can be used to guide decisions. We used chronically implanted microelectrode pairs to stimulate neurons in the rat auditory cortex directly. Here we demonstrate that rats can exploit differences in the timing of cortical activity as short as three milliseconds to guide decisions.

Animals can detect the fine timing of some stimuli. For example, interaural time differences of less than one millisecond are used for the spatial localization of sound[1]. It is also clear that cortical neurons can lock with millisecond precision to the fine timing of some stimuli in the auditory cortex[2, 3], the visual cortex[4], somatosensory cortex [5, 6] and *in vitro*[7]. Furthermore, spike generation in the auditory cortex is controlled by a stereotyped and precisely timed sequence of excitatory input followed approximately three milliseconds later by inhibitory input[8]. However, although it has recently been established that even a few cortical spikes are sufficient to drive decisions[9, 10], it has been difficult to establish whether the fine timing of cortical activity can suffice.

We therefore set out to probe the precision with which the fine timing of neural activity in the auditory cortex could guide behaviour in the rat. For the spatial localization of sound, the relevant sub-millisecond interaural time difference cues are extracted by specialized subcortical structures. To ensure that we were probing cortical rather than subcortical mechanisms, we bypassed subcortical auditory pathways and trained animals to respond to direct intracortical electrical stimulation. We used transient biphasic current trains delivered via two chronically implanted intracortical microelectrodes[11-13] to stimulate two populations of neurons in primary auditory cortex (area A1; Fig. 1a). We designed the stimulation patterns so that the only cue available to guide behaviour was the relative timing of the activity elicited in the two cortical populations.

We first trained adult male Long Evans rats to perform a simple auditory two alternative choice task (2-AC)[14]. The animal initiated a trial by inserting its nose into the centre port of a three port operant chamber, triggering one of two acoustic stimuli. These stimuli indicated whether the left or right goal port would be rewarded with water (Fig. 1A; for details, see *Supplemental material*). Chance performance was 50%. After animals reached criterion performance (> 90%), we implanted electrodes at two sites (A and B; Fig. 1A) about 1.1 mm apart along the rostro-caudal axis in the rat's left primary auditory cortex (Fig. S3). We then substituted electrical stimulation (< 30 μA) through these

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electrodes for the acoustic cue on the 2-AC task. Stimulus 1 (associated with the left reward port) consisted of simultaneous (AB) stimulation of the two intracortical sites, whereas stimulus 2 (associated with the right reward port) consisted of sequential (B-ISI-A) stimulation of the two sites. The two stimuli were separated by a variable interstimulus interval (ISI) which ranged from 1 to 100 msec. After animals reached criterion performance for long ISIs (100 or 35 msec), we reduced the ISI to probe the psychophysical limit for discrimination in the timing of the activity of the two cortical populations.

Twenty-six rats successfully learned the easy discrimination (A vs. B) and were trained on more challenging tasks. The training history of one subject is illustrated in Fig. 1b. Like most subjects, it rapidly learned to perform the discrimination for long ISIs (24/26 subjects trained on ISI=100 or ISI=35 msec performed above chance, $p<0.01$; *see* Fig. 1c and *Supplemental material*). Surprisingly, this subject also performed above chance ($p<0.01$) when challenged with the finer temporal discriminations of ISI=5 and even ISI=3 msec, but not ISI=1 msec. Over the population, most subjects (10/15) challenged with ISI=5 msec performed above chance ($p<0.01$; Fig. 1c); in some sessions performance exceeded 90% even at this short interval (Fig. 1d). Two subjects (Fig. 1b, Fig. S2-o) performed above chance even for ISI=3msec, but none of four subjects trained on ISI=1msec performed above chance. Performance declined with task difficulty both among (Fig. 1c) and within (Fig. 1e) subjects, but performance was variable; differences in performance could also be due to variability in the location of the electrodes, the effectiveness of the electrical stimulation, or other experimental factors.

Our results demonstrate that even fine differences as short as three milliseconds in the timing of artificially induced neuronal activity in the auditory cortex can be used to guide behaviour. Although artificial cortical microstimulation can be perceptually indistinguishable from natural stimulation[15], this need not always be the case; our results do not reveal the conditions under which such fine temporal differences are important for the readout of acoustically-evoked (*i.e.* natural) stimuli. Nevertheless, the ability of the animal to read out such subtle differences in timing raises the possibility that for some stimuli, the timing of cortical spikes can be behaviourally relevant.

Our experiments were conducted in the primary auditory cortex. Experiments in the somatosensory cortex have failed to find a correlation between spike timing and behaviour[16]. Audition is often considered to be a "fast" modality, and it is clearly one in which subtle differences in temporal structure can be behaviourally relevant. However, no special biophysical or circuit mechanisms need be posited to account for our result; many simple candidate neural circuits could mediate the readout of the fine timing differences we have described. Indeed, in auditory cortex the stereotyped sequence of excitatory activity followed 3 msec later by inhibitory activity[8] suggests one possible mechanism for the present results. Further experiments will be needed to resolve whether the capacity to exploit fine temporal differences is unique to the auditory cortex, or if it is a general strategy for cortical function (but see ref[16]).

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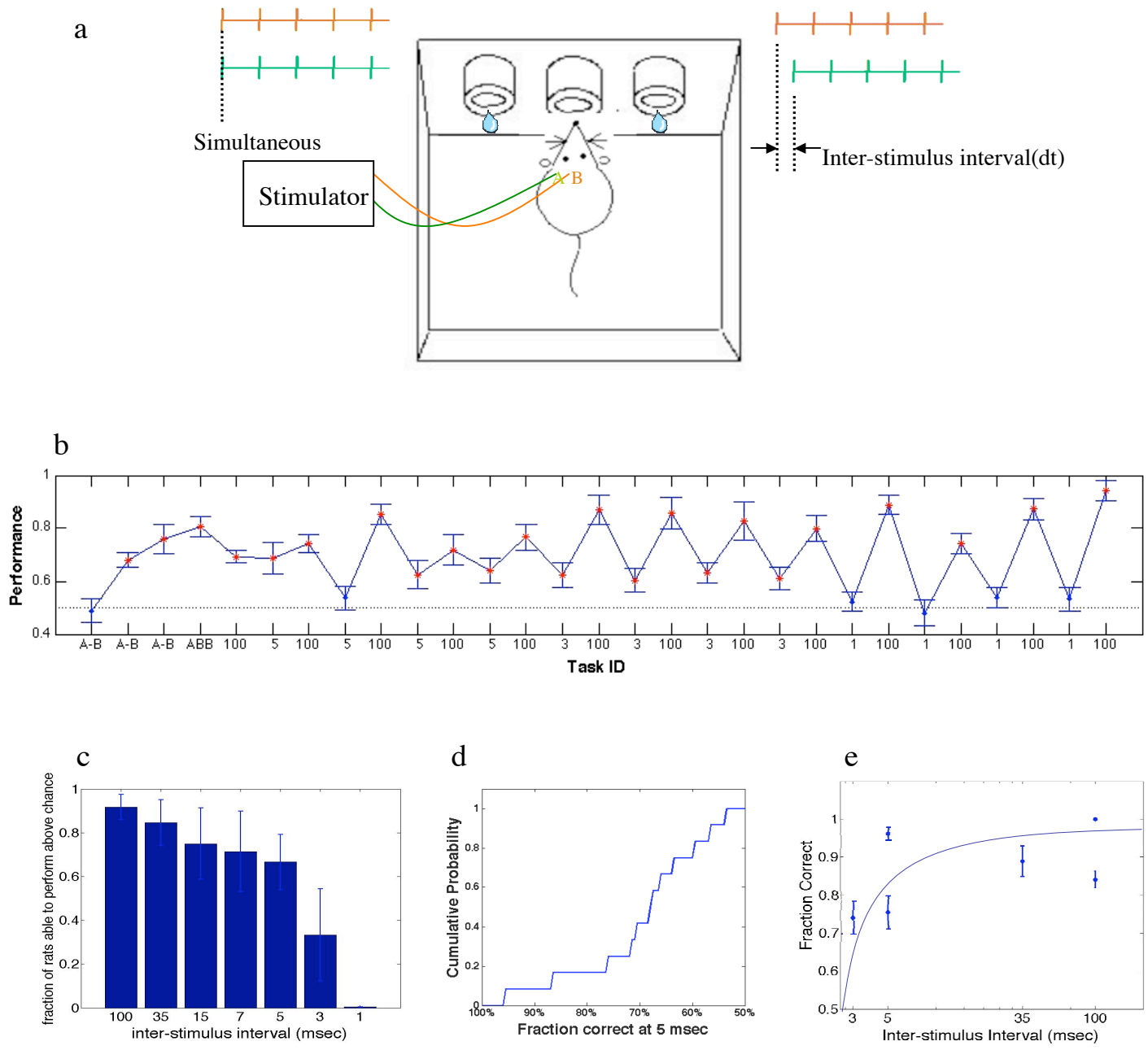
Figure 1 Finely timed cortical microstimulation can drive behaviour.
(**A**)Task design. The rat initiated a trial by poking its nose into the central poke hole. This poke triggered a stimulus, which was associated with a water reward at either the left goal port or the right goal port. In the microstimulation task, the left stimulus consisted of simultaneous stimulation of sites A and B in the primary auditory cortex (area A1), whereas the right stimulus consisted of sequential stimulation of B-ISI-A. Each stimulus consisted of a 50 Hz train of 5 biphasic cathode-leading current pulses. In one animal (Fig. S2-a) we used a symmetric discrimination A-ISI-B vs. B-ISI-A, rather than AB vs. B-ISI-A; the results were comparable and were therefore grouped together. (**B**) The training history of one subject. Each data point represents the performance of one session. The error bars show standard error of the mean (s.e.m). The x-axis label indicates the stimulus ID (A vs. B or AB vs. B) or ISI (in msec) for each training session. All training sessions are plotted, including sessions when animals perform above chance ($p<0.01$; red points) and at chance (blue points). The performance varies with ISI, i.e. with task difficulty. (**C**) Rats learned to perform above chance at most ISIs on which they were trained. For each ISI, the bar represents the following ratio: (number of rats able to perform the task defined by this ISI above chance on at least one session)/(number of rats tested at this ISI during at least one session). The error bars show s.e.m. (**D**) Cumulative histogram showing the best performance session of all 15 rats trained on the ISI=5 msec task. Ten rats were able to perform the ISI=5 msec task significantly above chance ($p<0.01$) for 1 or more sessions, each session containing 100-300 trials. (**E**) Performance declined with task difficulty within a subject. Performance is shown for sessions 5-10 for subject o (see also Fig. S2-o). The error bars show s.e.m.

Figure 1



Supplementary Material

Experimental Procedures

Behaviour All experiments were conducted in a single-walled soundbooth (Industrial Acoustics Company, Bronx, New York, USA). Animals were water deprived under a protocol approved by the Cold Spring Harbor Laboratory Animal Committee.

We used adult male Long Evans rats (250-300g). Naïve animals were first trained on an auditory 2-alternative choice (2-AC) 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.

After animals reached criterion performance (90%), we implanted electrodes at two sites (A and B, ~1.1 mm apart) in 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. After the surgery, while the animal was still anesthetized, we recorded from the two electrodes to confirm that they were in the auditory cortex.

Each electrical stimulus consisted of a train of 5 biphasic 4-volt voltage pulses (RP2, Real-time processor, TDT; see Fig. S1) which were passed through a 1:2.2 transformer (SP-21, Triad Magnetics). The impedance from the electrode to the ground ranged from 400K to 1M, so that stimulation currents ranged from about 8 μA to 22 μA. The diameter of the stimulated area was estimated to be ~75 μm. [1]

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. 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 msec, 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 msec) task to confirm that the animal was still able to detect stimulation from both sites before challenging with shorter ISIs.

In initial experiments (subjects a-o), we reduced the ISI gradually with multiple intermediate steps to obtain an estimate of the timing threshold. The intermediate ISIs included 55 msec, 35 msec, 15 msec, 7 msec, and 5 msec. 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 msec

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 msec, we next trained it on 7 msec. Not until it could perform above chance when ISI = 7 msec would we start training it on 5 msec. If it failed on the ISI = 5 msec task for two consecutive sessions and also failed on ISI = 100 msec task for two sessions, we terminated the training.

In later experiments (subjects p-z), after we found that some animals could perform the task when the ISI was as short as 5 msec, we adopted a different training strategy in which we dispensed with the intermediate ISIs and reduced the ISI abruptly from 100 msec to shorter ISIs (e.g. 5 msec). 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 msec and ISI=3 msec. To see if rats can learn ISI=1 msec, we trained one rat (z) on ISI=1 even after it failed on the ISI = 3 msec task,

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. 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.

All Results We successfully trained 26 rats on the basic A vs. B microstimulation task. Of the 24 rats trained on the AB vs. B-100msec-A task, 22 were able to perform the task significantly above chance ($p<0.01$). Eleven out of 13 were able to perform the task for ISI = 35msec, 6/8 for ISI =15msec, 5/7 for ISI = 7msec, 10/15 for ISI = 5msec, 2/7 for ISI= 3msec and 0/4 for ISI=1 msec. One rat (Fig. S2-a) was trained on a symmetric task (A-ISI-B vs. B-ISI-A); results from this animal were included in the summary (Fig 1c). Training results are shown in Fig S2 a-z.

Tuning Curve Analysis After surgery, while the animal was still anaesthetized, we played a series of pure tones (frequency ranging from 500Hz to 20kHz, intensity 45dB-70dB) and recorded from the two electrodes implanted into the primary auditory cortex. Figure S3 compares the best frequency at the rostral and caudal sites. Of the 26 rats we trained and recorded from, we could see V-shaped tuning curve in both sites for 24 rats. The separation in best frequency of the two sites was not correlated with behavioral performance.

Statistics We used standard errors across trials for the error bars of each data point in Fig. 1b, e and Fig S2. 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%.

Supplementary Figures

Fig. S1 Stimulus structure. Each stimulation consisted of 5 pairs of 250 microseconds cathode-leading square pulses at 50 Hz

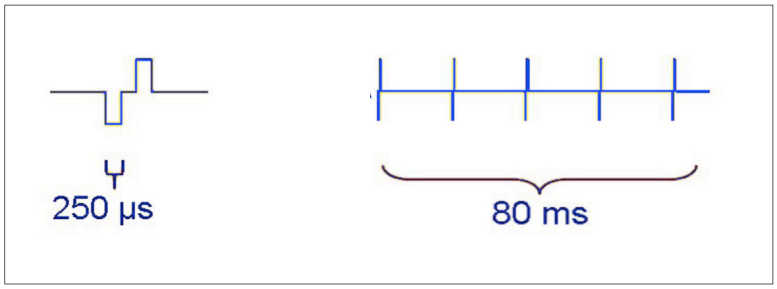
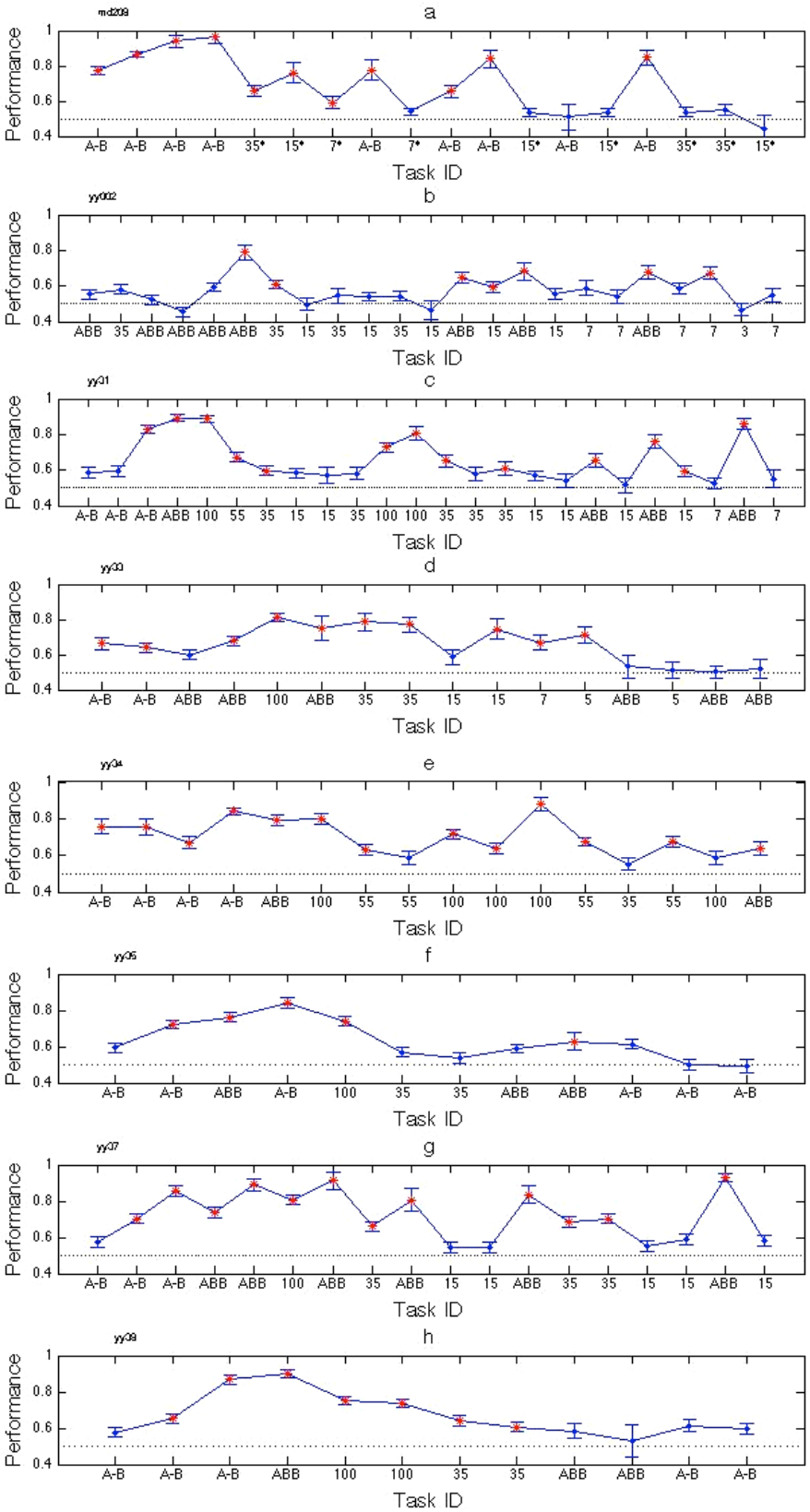
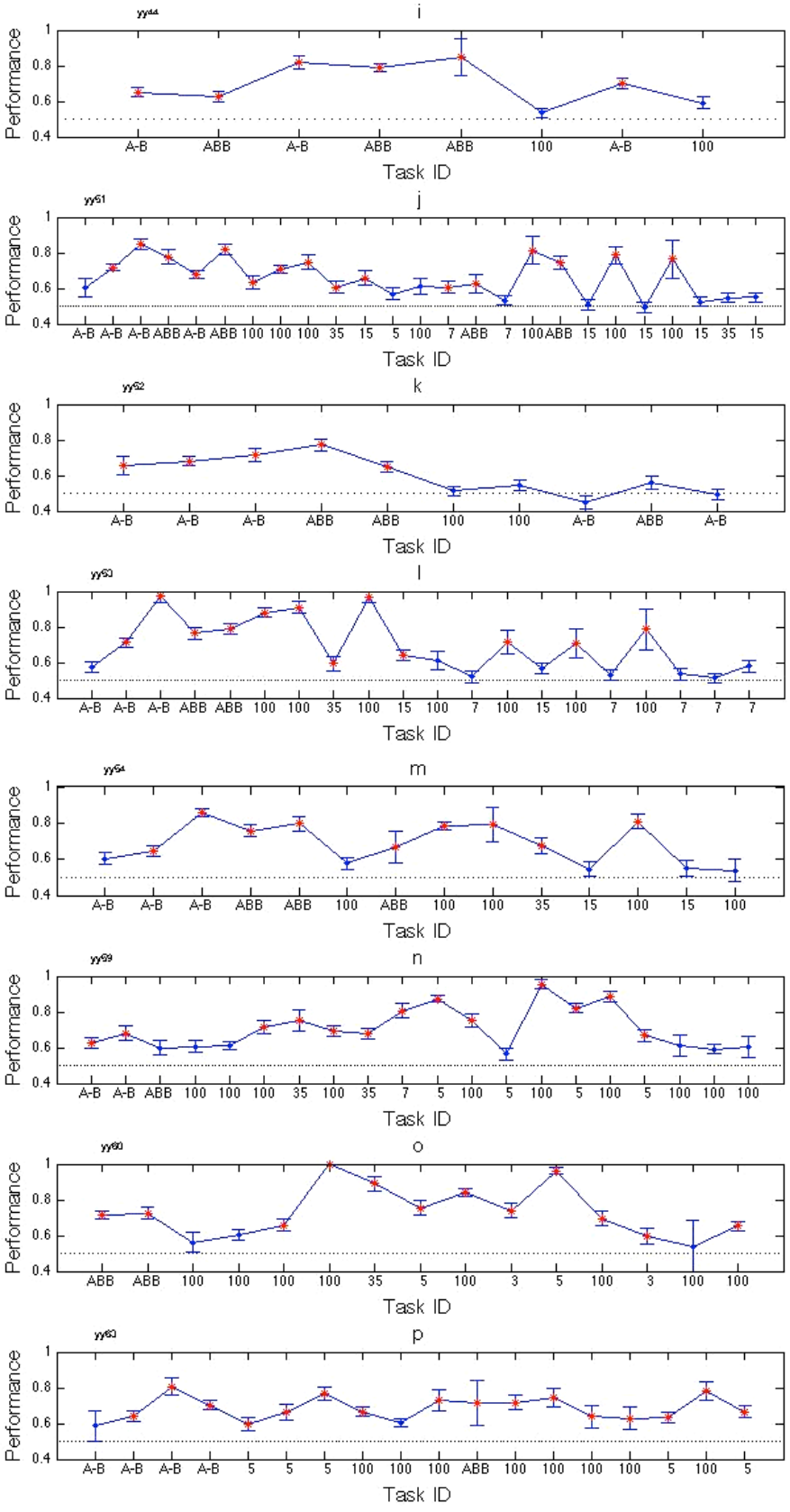
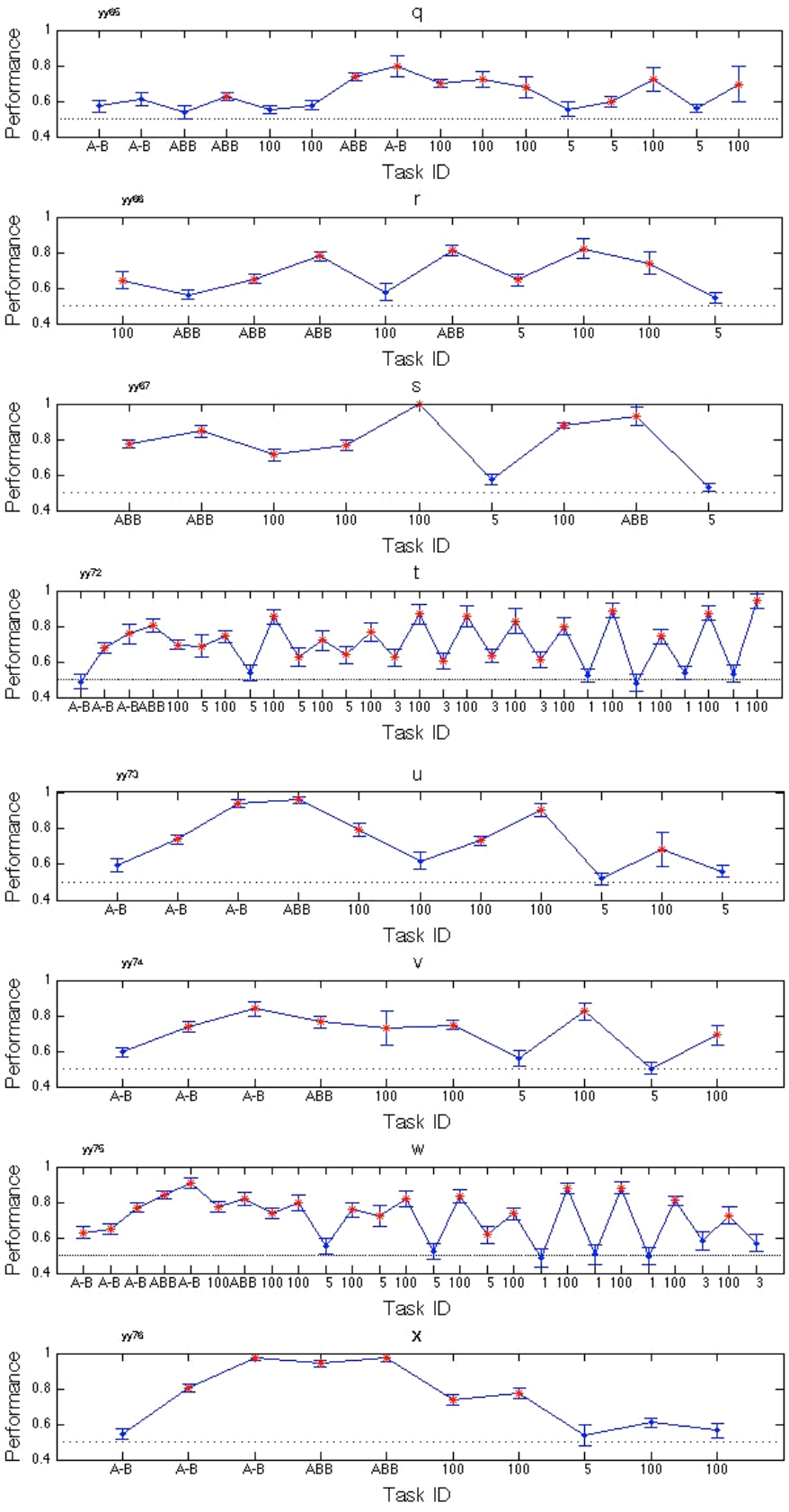


Fig. S2 Performance of all sessions of all rats in chronological order (left to right). Each panel represents data from one rat. Each data point represents the performance of one session. The error bars show s.e.m. The x-axis label indicates the stimulus type of each training session. All training sessions are plotted, including sessions when animals perform above chance (*red points*) and at chance (*blue points*).

Task ID	Task description
A-B	Stimulation at A only vs. stimulation at B only
ABB	Simultaneous A & B stimulations vs. B only
100	Simultaneous A & B stimulations vs. B-100msec-A
35	Simultaneous A & B stimulations vs. B-35msec-A
35*	A-35msec-B vs. B-35msec-A
15	Simultaneous A & B stimulations vs. B-15msec-A
15*	A-15msec-B vs. B-15msec-A
7	Simultaneous A & B stimulations vs. B-7msec-A
7*	A-7msec-B vs. B-7msec-A
5	Simultaneous A & B stimulations vs. B-5msec-A
3	Simultaneous A & B stimulations vs. B-3msec-A
1	Simultaneous A & B stimulations vs. B-1msec-A







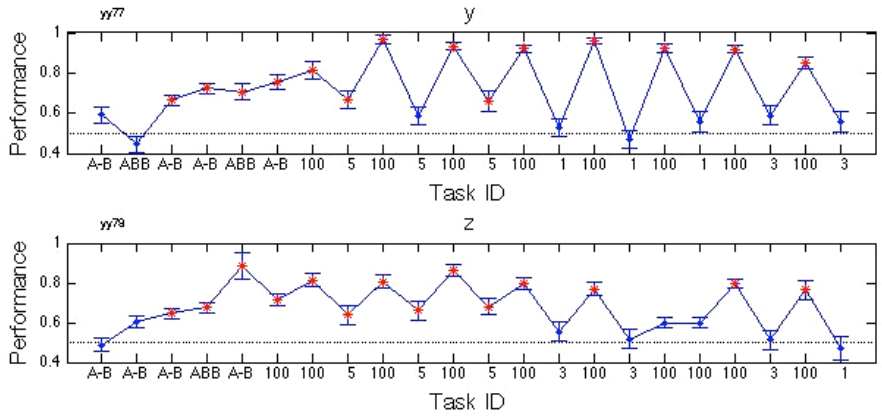
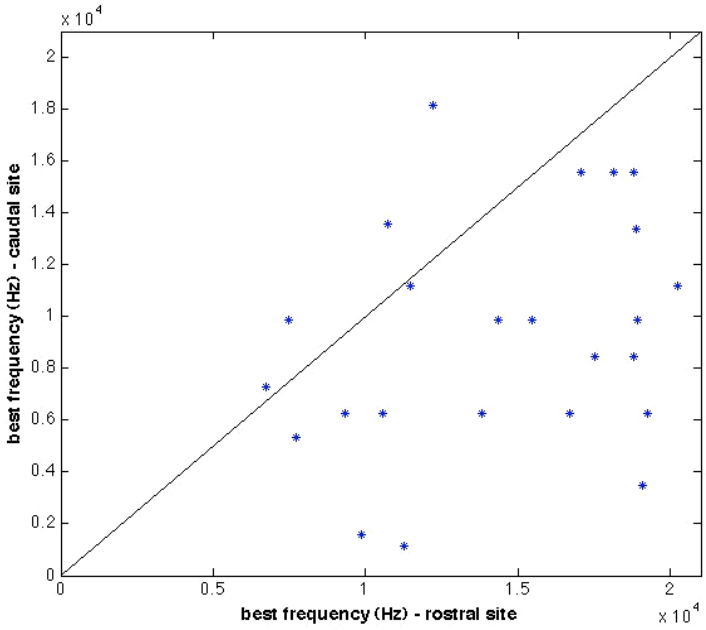


Figure S3 Best frequency of stimulation sites. Each data point shows the best frequency of two stimulation sites for each animal. V-shaped tuning curves were recorded at both sites for 24/26 subjects (all except for subjects k and r). To avoid overlapping points, we added 5% random jitter to the best frequency of each rostral site.



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