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

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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

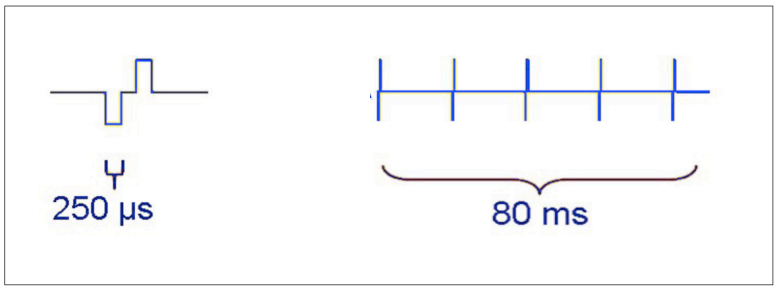
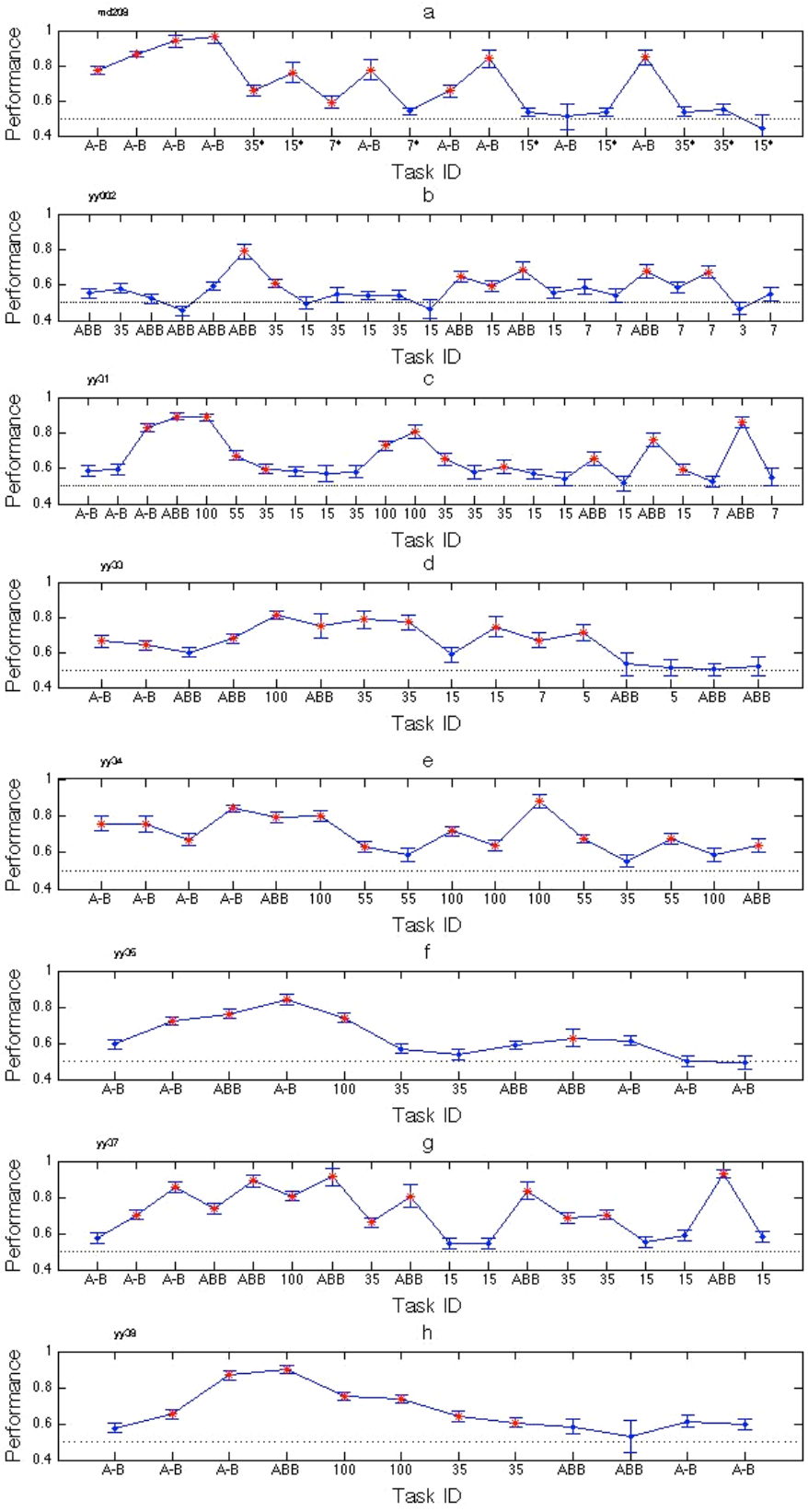


Fig. S2 Performance of all sessions of all rats in chronological order (left to right). Each panel represents data from one rat. Each data point represents the performance of one session. The error bars show s.e.m. The x-axis label indicates the stimulus type of each training session. All training sessions 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


a
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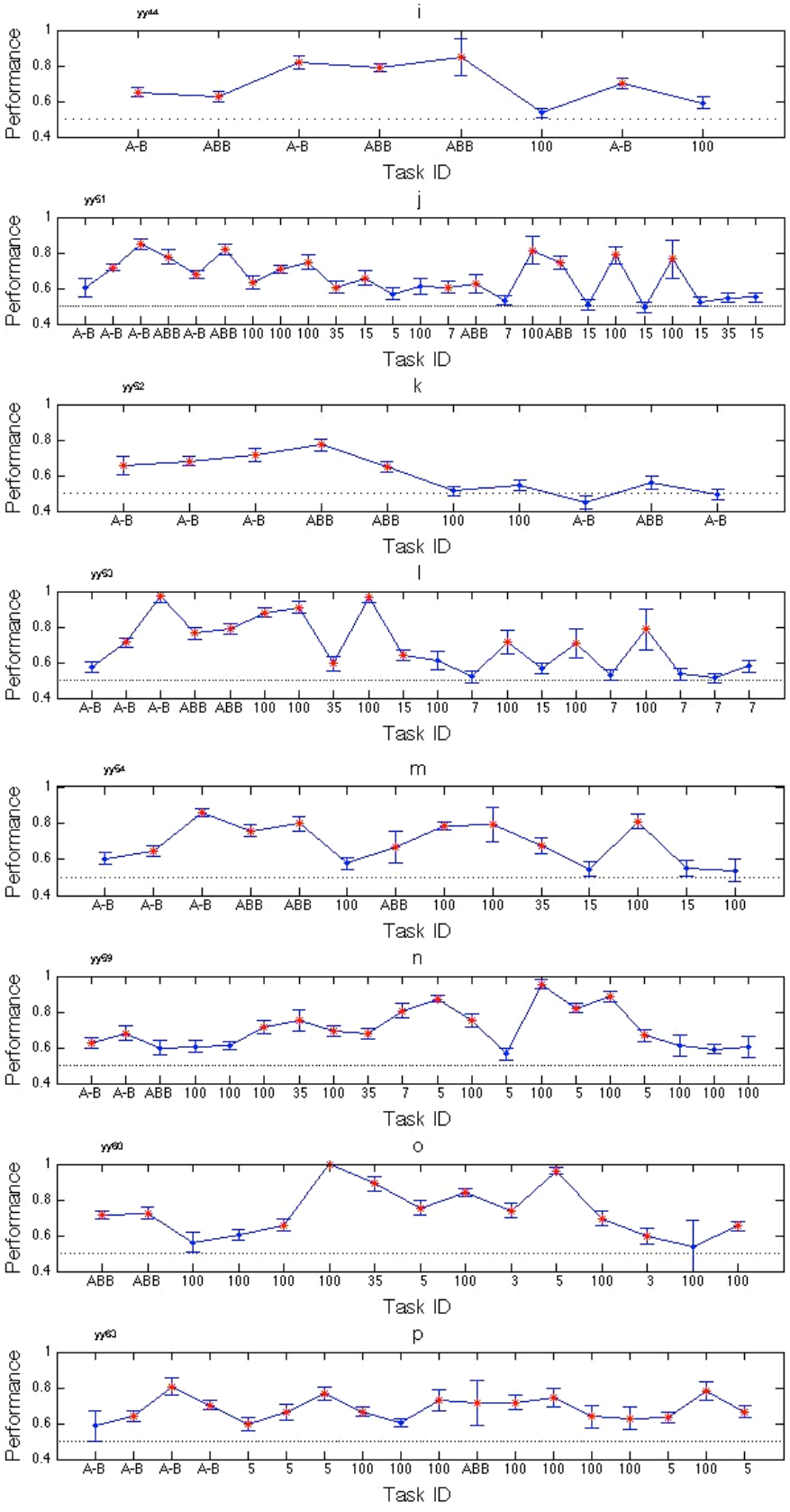
Performance
Task ID


i
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A-B ABB A-B ABB ABB 100 A-B 100
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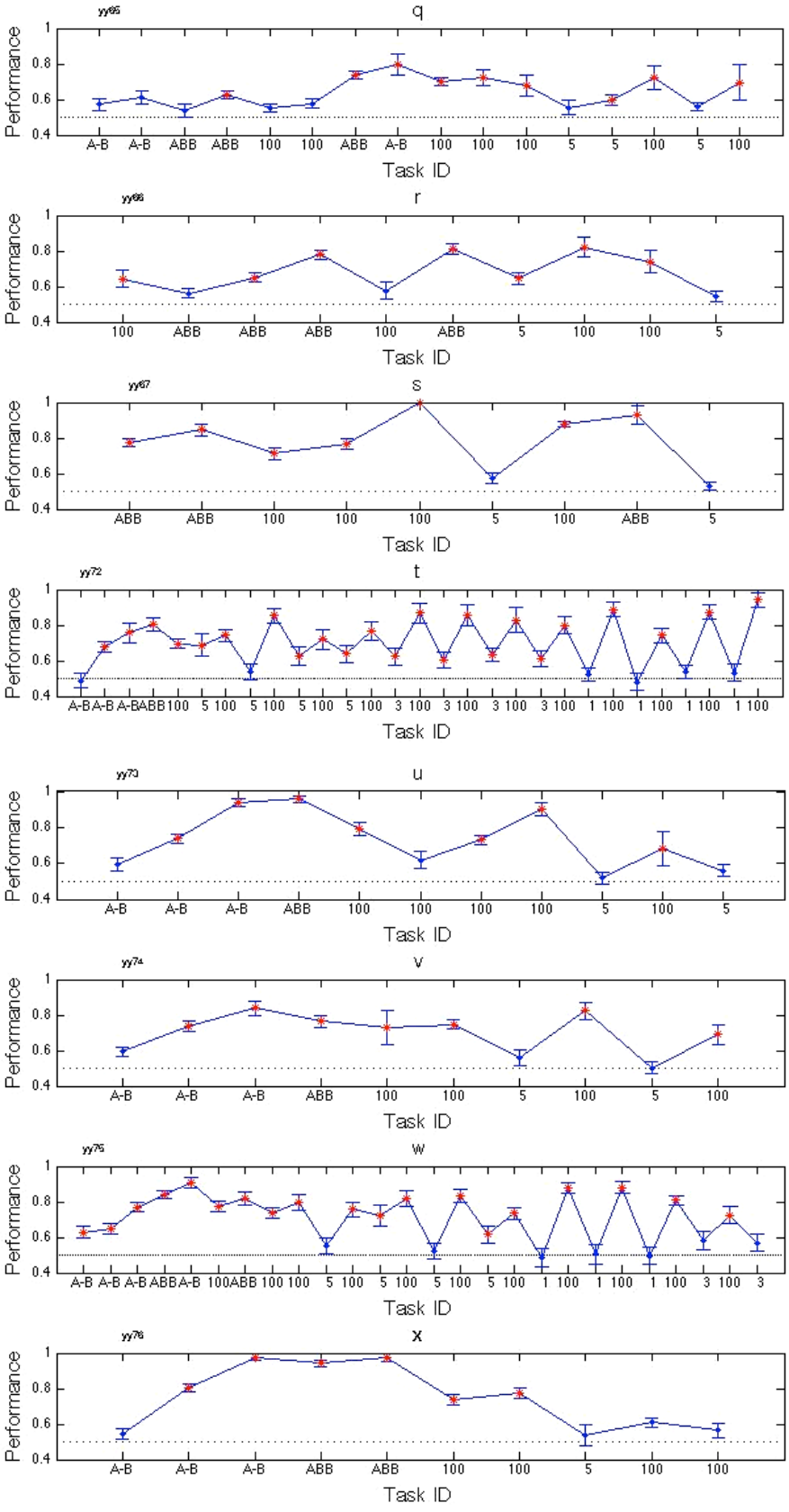
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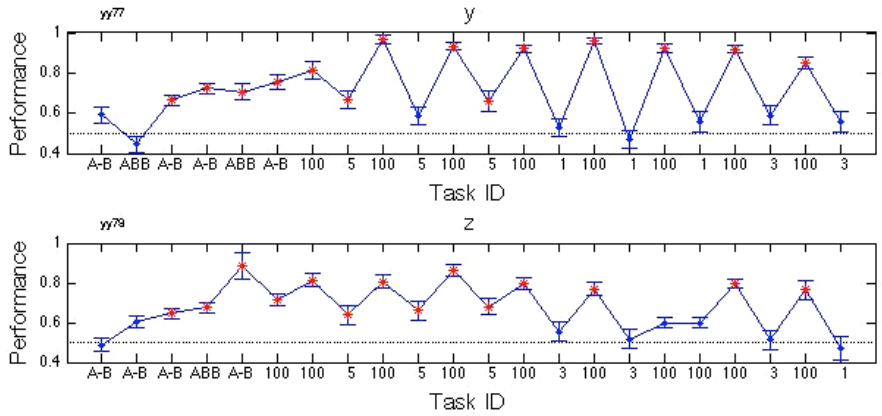
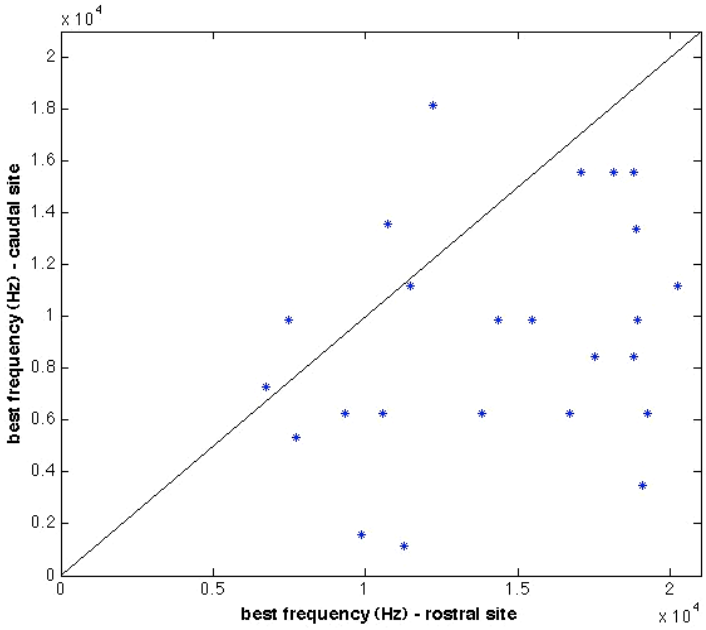


Figure S3 Best frequency of stimulation sites. Each data point shows the best frequency of two stimulation sites for each animal. V-shaped tuning curves were recorded at both sites for 24/26 subjects (all except for subjects k and r). To avoid overlapping points, we added 5% random jitter to the best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



References:

1. Stoney, S. D., Jr., Thompson, W. D. & Asanuma, H. J Neurophysiol 31, 659-69 (1968).