

# The Amplitude Sensitivity of Mouse Inferior Collicular Neurons in the Presence of Weak Noise

Jia Tang<sup>1</sup>, Fei-Jian Wu<sup>1</sup>, Dan Wang<sup>1</sup>, Philip H.-S. Jen<sup>2</sup>, and Qi-Cai Chen<sup>1</sup>

<sup>1</sup>*College of Life Sciences, Central China Normal University  
Wuhan 430079, Hubei, PRC*

and

<sup>2</sup>*Division of Biological Sciences and Interdisciplinary Neuroscience Program  
University of Missouri  
Columbia, MO 65211, USA*

## Abstract

Natural auditory environment consists of multiple sound sources that are embedded in ambient strong and weak noise. For effective sound communication and signal analysis, animals must somehow extract biologically relevant signals from the inevitable interference of ambient noise. The present study examined how a weak noise may affect the amplitude sensitivity of neurons in the mouse central nucleus of the inferior colliculus (IC) which receives convergent excitatory and inhibitory inputs from both lower and higher auditory centers. Specifically, we studied the amplitude sensitivity of IC neurons using a probe (best frequency pulse) and a masker (weak noise) under simultaneous masking paradigm. For most IC neurons, weak noise masking increases the minimum threshold and decreases the number of impulses. Noise masking also increased the slope and decreased the dynamic range of the rate amplitude function of these IC neurons. The strength of this noise masking was greater at low than at high sound amplitudes. This variation in the amplitude sensitivity of IC neurons in the presence of the weak noise was mostly mediated through GABAergic inhibition. These data indicate that in the real world the ambient weak noise improves amplitude sensitivity of IC neurons through GABAergic inhibition while inevitably decreases the range of overall auditory sensitivity of IC neurons.

**Key Words:** amplitude sensitivity, bicuculline, GABA-mediated inhibition, inferior colliculus, mouse, simultaneous masking, weak noise

## Introduction

In acoustic communication including human speech, acoustic signals are often transmitted in noisy environment. Yet, animals and humans are able to extract biologically relevant signals that are embedded in ambient noise although the latter must inevitably affect effective auditory signal processing. Indeed, previous studies have shown that strong or broadband noise decreases the auditory sensitivity of neurons in the central nucleus of the inferior colliculus (IC) and auditory cortex (AC) of mammals (5, 12, 21, 32, 34).

These studies show that auditory neurons increase minimum threshold (MT), lengthen the response latency, decrease the number of impulses and become poor in detection of stimulus interval and interaural phase difference in the presence of moderate or strong noise. Opposite from these observations, other studies have shown that ambient noise improves auditory signal detection (8, 10, 30, 33, 40, 45). For examples, stochastic resonance enhances signal detection in humans and the ambient noise can act as a selective force to shape the audiograms and sound spectra in fish (8, 30). In addition, presentation of moderate

Corresponding authors: Dr. Qi-Cai Chen, College of Life Sciences, Central China Normal University, Wuhan 430079, Hubei, PRC. Tel: +86-27-67867229, Fax: +86-27-67861936, E-mail: qcchen2003@yahoo.com.cn and Dr. Philip H.-S. Jen, Division of Biological Sciences and Interdisciplinary Neuroscience Program, University of Missouri, Columbia, MO 65211, USA. Tel: 573-882-7479, Fax: 573-884-5020, E-mail: jenp@missouri.edu

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noise enhances human hearing (45). However, the neural mechanism underlying these opposite observations has not been studied. Furthermore, whether the sensitivity of auditory neurons is modulated as well in the presence of weak noise as that often embedded in the natural environment has not been examined.

In the ascending auditory pathway, the IC receives and integrates excitatory and inhibitory inputs from many lower auditory nuclei as well as from the auditory cortex (1, 2, 14, 15, 35, 37, 41). In the IC, GABA-mediated inhibition is one of the major inhibitory inputs (11, 35, 36). Many studies have shown that the interaction between excitation and GABA-mediated inhibition shapes auditory response properties and multi-parametric selectivity of IC neurons (*e.g.* duration, frequency, amplitude, direction, *etc.*) using iontophoretic application of bicuculline (7, 13, 17, 18, 20, 22-26, 39, 42, 44, 47), an antagonist for GABA<sub>A</sub> receptors (4). Conceivably, this GABA-mediated inhibition may also be one of the neural mechanisms underlying the noise masking of the amplitude sensitivity of IC neurons.

In the real world, acoustic environment consists of multiple sound sources that are embedded in both strong and weak noises. The main objective of the present study is to show that the ambient weak noise also plays a role in shaping the amplitude sensitivity of IC neurons through GABA-mediated inhibition. To achieve this objective, we specifically study the rate-amplitude functions (RAF) of IC neurons using a best frequency (BF) pulse as a probe and a sub-threshold noise as a masker under simultaneous masking paradigm with and without iontophoretic application of bicuculline.

## Materials and Methods

Nine adult mice (*Mus musculus* Km) (20-25 g, b. wt.) with a positive Preyer's pinna reflex were used for this study. The surgical procedures were basically the same as previous studies (19, 43). Briefly, the flat head of 1.8-cm nail was glued onto the exposed skull of each anesthetized (pentobarbital sodium, 60 ~ 90 mg/kg b. wt.) mouse with acrylic glue (502) and dental cement. The mouse was then secured to an aluminum plate with a plastic band inside a sound-proof room (temperature 28-30°C). The ceiling and walls of the room were covered with a layer of foam (thickness: 8 cm) to reduce echoes.

The mouse's head was immobilized by fixing the shank of the nail into a brass rod with a set of screw during recording session. The head was oriented with the eye-nostril line pointed to 0° in azimuth and 0° in elevation with respect to the frontal auditory space. A small hole (diameter: 200-500 μm) was

drilled in the skull above the IC for inserting 2 M NaCl glass pipette electrodes (impedance: 5-10Ω). A silver wire indifferent electrode was placed at the nearby temporal muscles. Each recording electrode was inserted as orthogonally as possible and the recording depth of each neuron was read from the scale of a hydraulic drive (Model 640, David-Kopf Instruments, Tujunga, CA, USA). Additional doses of pentobarbital were administered during later phases of recording when the mouse showed signs of restlessness. A local anesthetic (Lidocaine) was applied to the open wound area to alleviate any possible pain. The experiments were conducted with the approval of the Institutional Animal Care and Use Committee of Central China Normal University, Wuhan, Hubei, PRC.

Two sound generation systems were used for this study. To generate sound stimuli, continuous sine waves from a function generator (GFG-8016G, Good Will Inst Co., Ltd, Bayan Lepas, Penang, Malaysia) were formed into tone pulses (40 ms with 2 ms rise-decay times), delivered at 2 pulses per second) by custom-made tone burst generator (electronic switch) driven by a stimulator (Model SEN-7203, Nihon Kohden Co, Shinjuku, Tokyo, Japan). The tone pulses were then amplified (custom-made amplifier) after passing a decade attenuator (LAT-45, Leader, Kohoku, Yokohama, Japan) before they were fed into a small loudspeaker (AKG model CK 50, 1.5 cm in diameter, 1.2 g, frequency response 1 ~ 100 kHz). The loudspeaker was placed 30 cm away from the mouse and at 30° contralateral to the recording site. Calibration of the loudspeaker was conducted with a 1/4 inch microphone (4939, B & K, Nærum Denmark) placed at the mouse's ear using a measuring amplifier (2610, B & K, Nærum, Denmark). The output of the loudspeaker was expressed in dB SPL referred to 20 μPa root mean square. A white noise generator (ND-502, Nanjing University, Nanjing, Jiangsu, PRC) was used to generate noise stimuli from the second sound generation system.

Upon isolation of an IC neuron with 40 ms pure tone pulses, the neuron's BF was audio-visually determined by changing the frequency and amplitude of sound stimuli. The MT at the BF was defined as the sound amplitude that on average elicited 50% response probability from the neuron. The neuron's RAF was then plotted using the number of impulses in response to BF pulses delivered at the MT and 10 dB step increments above the MT.

To determine how the amplitude sensitivity of IC neurons may be modulated in the presence of the weak noise, a 40 ms white noise, which served as the masker, was specifically delivered at 5 dB below the neuron's MT and simultaneously with a 40 ms BF tone, which served as the probe. The neuron's RAF

was then plotted before and during the presence of the weak noise with and without bicuculline application. Variation in the MT, the best amplitude (BA), the dynamic range (DR) and slope of the RAF of IC neurons was then quantitatively studied and statistically compared using mean  $\pm$  standard deviation ( $m \pm sd$ ), linear regression analyses and one-way ANOVA at  $P < 0.05$ . Statistical analysis and charting of the data were performed with the software of SPSS 13.0 and SigmaPlot 2000.

Iontophoretic application of bicuculline to recorded IC neurons has been described in previous studies (29). Briefly, a three-barrel electrode (tip: 10–15  $\mu\text{m}$ ) was piggybacked to a 2 M NaCl single-barrel electrode (tip: less than 1  $\mu\text{m}$ ; impedance: 5–10 M $\Omega$ ) whose tip was extended about 10  $\mu\text{m}$  from the tip of the three-barrel electrode. The 2 M NaCl single-barrel recording electrode was used to record neural responses. One of the barrels of the three-barrel electrode was filled with bicuculline methiodide (10 mM in 0.16 M NaCl, pH 3.0; Sigma). The bicuculline was prepared just prior to each experiment and the electrode was filled immediately before use. The drug channel was connected *via* silver-silver chloride wire to a microiontophoresis constant current generator (Dual Current Generator 260, WPI, Sarasota, FL, USA) that was used to generate and monitor iontophoretic currents. During drug application, a positive 10 ~ 80 nA current was applied until the effect of bicuculline reached a steady state. The application current was then changed to 10 nA during data acquisition. The other two barrels were filled with 1 M NaCl (pH 7.4), one of which was used as the ground and the other as the balanced barrel. The balance electrode was connected to a balance module. The retaining current was negative 8–10 nA.

Recorded action potentials were amplified and band-pass filtered (ISO-DAM, WPI, USA) before being sent to an oscilloscope (PM3084, FLUKE, Avenel, NJ, USA) and an audio monitor (Grass AM9, Warwick, RI, USA). They were then sent to a computer for acquisition of peri-stimulus-time (PST) histograms (bin width: 0.5 ms; sampling period: 100 ms) of the neuron's response to 32 stimulus presentations. The PST histograms show the neuron's temporal discharge pattern to sound stimulus. The total number of impulses in each histogram was used to quantify the neuron's response under each stimulus condition.

## Results

In this study, we examined the amplitude sensitivity of 86 IC neurons in the presence of weak noise. These neurons, which discharged phasically (3–7 impulses) to presented 40 ms sound pulses, were recorded at the depths between 299 and 2034  $\mu\text{m}$

(average:  $1293.8 \pm 359.7 \mu\text{m}$ ) with BFs of 7.8–38.4 kHz (average:  $19.4 \pm 12.1 \text{ kHz}$ ) and MTs of 13–63 dB SPL (average:  $42.8 \pm 13.9 \text{ dB SPL}$ ). The first-spike latency of these neurons determined with BF sounds at 10 dB above the MT was between 5 and 37 ms (average:  $16.9 \pm 7.9 \text{ ms}$ ).

In the following, we first describe the RAF of these IC neurons in BF sound. We then describe the variation in the BA, DR, slope of the RAF and MT of these IC neurons during noise masking with and without bicuculline application.

### *Type of RAF of IC Neurons*

The discharge pattern, number of impulses and RAF of a representative IC neuron in response to selected BF stimulus amplitudes before and during weak noise masking with and without bicuculline application are shown in Fig. 1. The neuron's number of impulses in response to each stimulus amplitude decreased to varying degree during weak noise masking (Fig. 1A-1 vs. B-1). Bicuculline application during noise masking greatly increased the neuron's discharge duration and the number of impulses at all selected stimulus amplitudes (Fig. 1A-1, B-1 vs. C-1).

The neuron's RAFs plotted under these three stimulus conditions can be described as monotonic, non-monotonic and saturated. The number of impulses of the monotonic RAF progressively increases up to the strongest amplitude available by the stimulus system (Fig. 1A-2). The number of impulses of the non-monotonic RAF increases with sound amplitude from the minimum to the maximum and then decreases more than 25% at still stronger sound amplitude (Fig. 1B-2). The number of impulses of the saturated RAF increases with sound amplitude from the minimum to the maximum and then begins to level off at less than 10% thereafter at still stronger sound amplitude (Fig. 1C-2).

We used three parameters of a RAF to express a neuron's amplitude sensitivity. The first parameter was the DR which is defined as the amplitude range corresponding to the number of impulses that is 10% below the maximum and 10% above the minimum (DR in Figs. 1A-2, B-2, C-2, double arrowhead). The second parameter was the slope of the ascending limb of the RAF. The slope was obtained by dividing the difference in the normalized response of impulses obtained at 10% below the maximum and 10% above the minimum by the DR. A RAF with a smaller DR had a sharper slope than a RAF with a larger DR. As such, the former had a greater sensitivity to the change in sound amplitude than the latter. The third parameter was the BA which is the sound amplitude that elicited the maximal number of impulses from the neuron (Figs. 1A-2, B-2, C-2, indicated by a star).

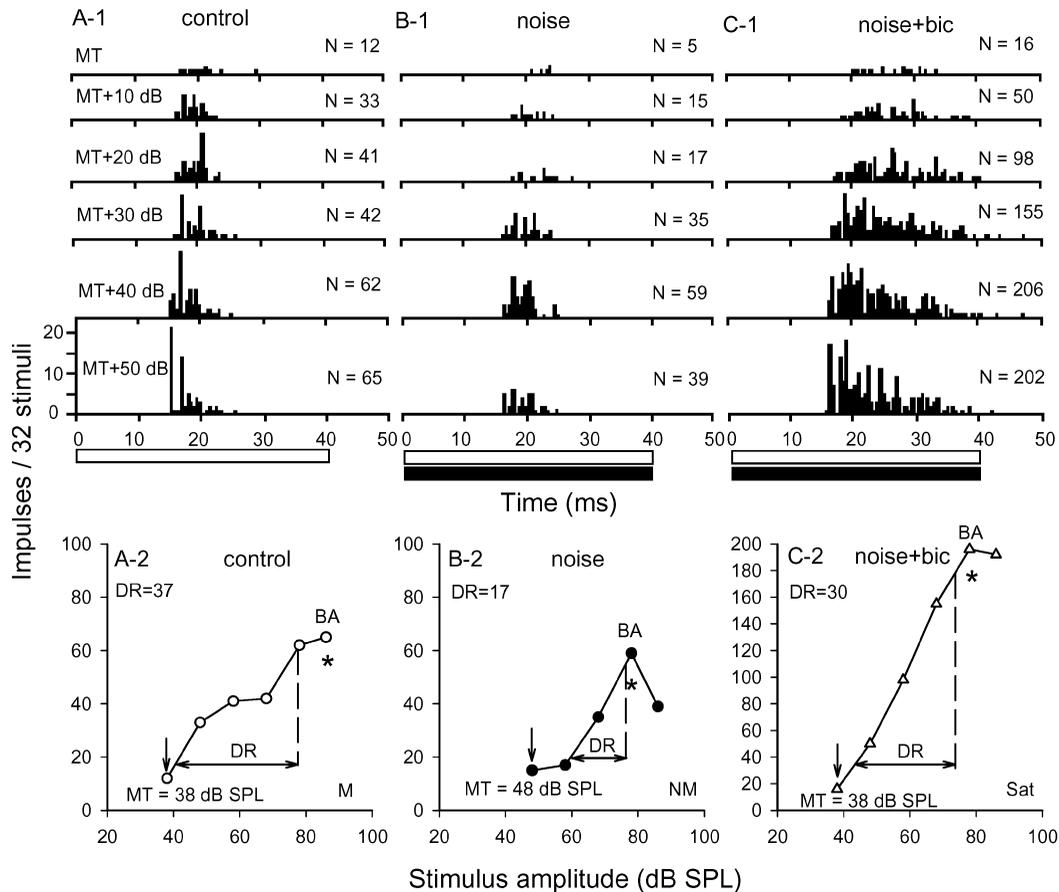


Fig. 1. A-1, B-1, C-1: The peri-stimulus-histogram (PST) showing the discharge pattern of a representative inferior collicular (IC) neuron in response to selected stimulus amplitudes under three stimulation conditions. A-1: the neuron's response to 32 presentations of best frequency (BF) sounds delivered at the minimum threshold (MT) and at 10-dB step increment above the MT; B-1: the neuron's response to BF sounds delivered at the same selected stimulus amplitudes in the presence of a weak noise delivered at 5 dB below the MT; C-1: the neuron's response to BF sounds delivered at the same selected stimulus amplitudes in the presence of a weak noise during bicuculline application. The duration of the BF sound and noise are shown below the PST histograms in unfilled and filled bars. Note that the neuron's number of impulses, which decreased in the presence of the weak noise, greatly increased during bicuculline application. A-2, B-2, C-2: The neuron's rate-amplitude function (RAF) plotted with the number of impulses obtained at selected sound amplitudes under the three stimulation conditions. The ordinate and abscissa represent the number of impulses per 32 stimulus presentations and amplitude in dB SPL. The MT and the best amplitude (BA) are denoted with an arrow and asterisk. The dynamic range (DR) of the RAF is indicated with horizontal double arrow. The neuron's recording depth ( $\mu\text{m}$ ), BF (kHz) and MT (dB SPL) were 881, 19.8 and 38.

Among 86 IC neurons studied, the number of impulses was either decreased (68, 79%), increased (9, 10.5%) or not affected (9, 10.5%) in the presence of weak noise. The RAF of three representative IC neurons plotted before and during weak noise masking is shown in Fig. 2. It is clear that in the presence of weak noise the RAF was lowered in one neuron (Fig. 2A), elevated in another neuron (Fig. 2B) and hardly changed in the third neuron (Fig. 2C).

#### Amplitude-Dependent Noise Masking of IC Response

Because the number of impulses of most (68) IC neurons was decreased in the presence of weak noise,

we studied how this noise masking of responses of IC neurons is related to stimulus amplitude. As shown in Fig. 3, the discharge duration of a representative neuron varied with stimulus amplitude and its number of impulses progressively increased from 14 impulses at 57 dB SPL to a maximum of 88 impulses at 87 dB SPL before decreasing to 84 at 92 dB SPL (Fig. 3A-1, control). In the presence of the weak noise, the neuron's number of impulses decreased at a greater degree at low than at high sound amplitudes (Fig. 3A-1, noise, 78.5% at 57 dB SPL vs. 20.5% at 87 dB SPL). The neuron's RAF became lower when plotted during than before noise masking (Fig. 3A-2 a vs. b).

Since the decrease in the number of impulses

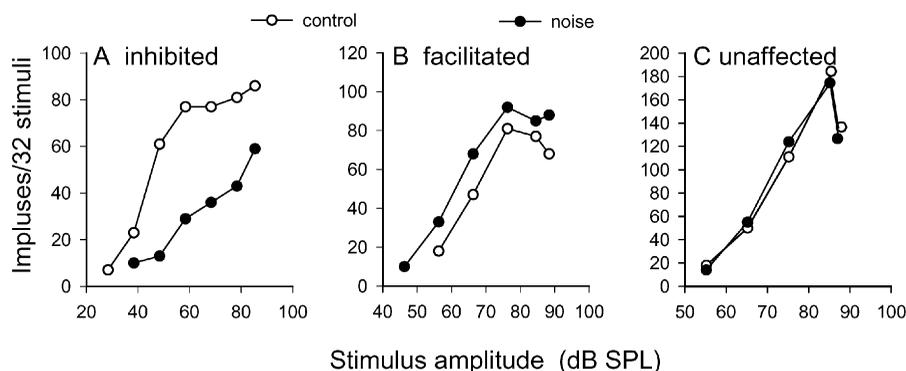


Fig. 2. The RAF of three representative IC neurons plotted before (unfilled circles) and during (solid circles) noise masking. Note the noise masking lowered (A, inhibition), elevated (B, facilitation) and did not affect (C, unaffected) the RAF of these three neurons. The recording depth ( $\mu\text{m}$ ), BF (kHz) and MT (dB SPL) of these three neurons were 1135, 12.2, 28 (A); 929, 17.1, 56 (B); 1074, 14.3, 55 (C).

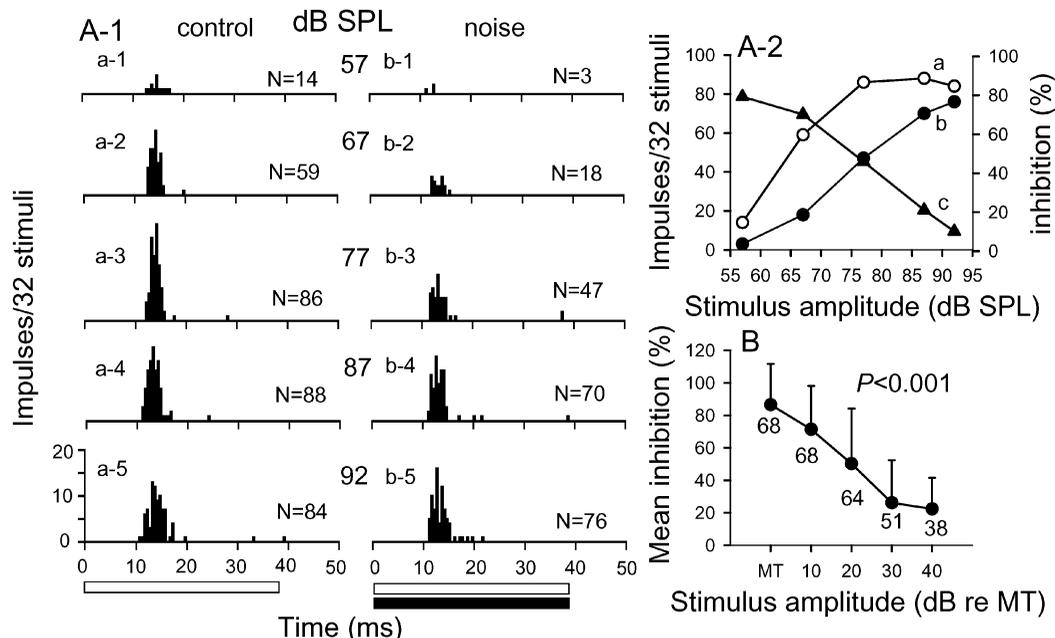


Fig. 3. A-1: The PST histograms showing the discharge pattern of a representative IC neuron in response to selected stimulus amplitudes obtained before (control) and during weak noise masking. N: number of impulses per 32 stimuli. The stimulus amplitude (dB SPL) is shown between two panels. A-2a,b: The neuron's RAF plotted before (a) and during (b) weak noise masking (refers to left ordinate). A-2c: Percent inhibition curve showing the decrease in the number of impulses at selected stimulus amplitudes during weak noise masking (refers to right ordinate). B: Average percent inhibition curve of IC neurons obtained during weak noise masking. The number of neurons and half a standard deviation is shown at each data point. The neuron's recording depth ( $\mu\text{m}$ ), BF (kHz), and MT (dB SPL) were 1669, 15.8 and 57.

during noise masking is most likely mediated by GABAergic inhibition (*e.g.* Fig. 1), we calculated the percent inhibition by dividing the decrease in the number of impulses by the control number of impulses obtained before noise masking for all sound amplitudes tested. Clearly, the maximal percent inhibition at low sound amplitude progressively decreases with sound amplitude (Fig. 3A-2c). As shown in Fig. 3B, the average percent

inhibition in the number of impulses of all IC neurons in the presence of weak noise significantly decreases with sound amplitude (One-way ANOVA,  $P < 0.001$ ).

#### Variation in the BA and RAF of IC Neurons in the Presence of Weak Noise

In this study, we studied the RAF of all 68 IC

**Table 1. Variation of best amplitude (BA) and type of rate-amplitude function (RAF) of IC neurons during simultaneous noise masking with and without bicuculline application**

Change in RAF	n	Change in BA					
		noise masking (N = 68)			noise masking plus bic (N = 32)		
		decrease	increase	no change	decrease	increase	no change
M → M	n	0	1	13	0	0	3
M → NM	n	1	0	0	0	0	0
M → Sat	n	1	1	2	0	0	1
NM → NM	n	5	7	8	1	4	3
NM → Sat	n	2	8	2	1	1	2
NM → M	n	1	1	1	0	7	0
Sat → Sat	n	2	2	3	2	0	3
Sat → M	n	0	4	1	0	2	1
Sat → NM	n	2	0	0	1	0	0
N		14 (21%)	24 (35%)	30 (44%)	5 (16%)	14 (44%)	13 (40%)

M, NM and Sat: monotonic, non-monotonic and saturated RAF, n: number of neurons, N: total number of neurons

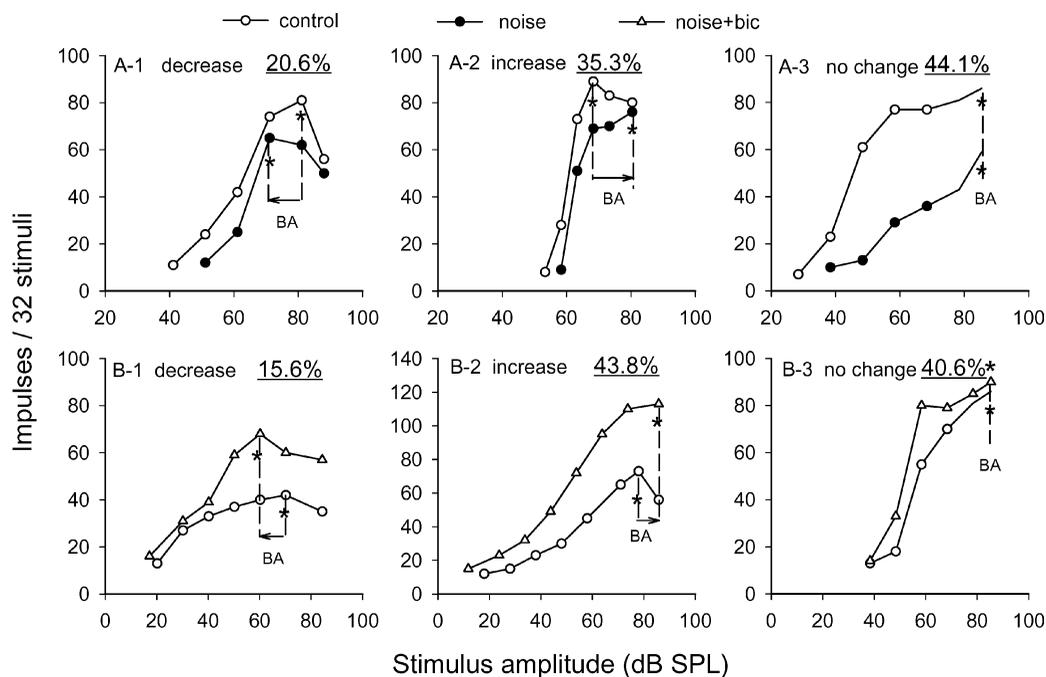


Fig. 4. Variation in the BA (shown with star) of the RAF of IC neurons during noise masking without (A-1, A-2, A-3) and with bicuculline application (B-1, B-2, B-3). The RAF plotted before and during noise masking with and without bicuculline application is shown in unfilled circles, filled circles and unfilled triangles, respectively. The percent of neurons of each type of variation in BA is shown within each panel. The recording depth ( $\mu\text{m}$ ), BF (kHz) and MT (dB SPL) of these six neurons were 1263, 14.2, 41(A-1); 1060, 20.2, 53(A-2); 1135, 12.2, 28 (A-3); 906, 16.6, 20 (B-1); 1272, 10.7, 18 (B-2); 1494, 17.5, 38 (B-3).

neurons whose number of impulses was decreased during noise masking. However, we only studied the RAF of 32 neurons during noise masking with bicuculline application because of the time constrain and loss of neurons through the course of study.

Noise masking lowered the RAF of all 68 IC neurons to varying degree and the BA decreased in 14 (20.6%, Fig. 4A-1) neurons, increased in 24 (35.3%,

Fig. 4A-2) neurons and did not change in the remaining 30 (44.1%, Fig. 4A-3) neurons. Opposite to this observation, bicuculline application during noise masking elevated the RAF of all 32 neurons to varying degree and the BA decreased in 5 (15.6%, Fig. 4B-1) neurons, increased in 14 (43.8%, Fig. 4B-2) neurons and did not change in the remaining 13 (40.6%, Fig. 4B-3) neurons.

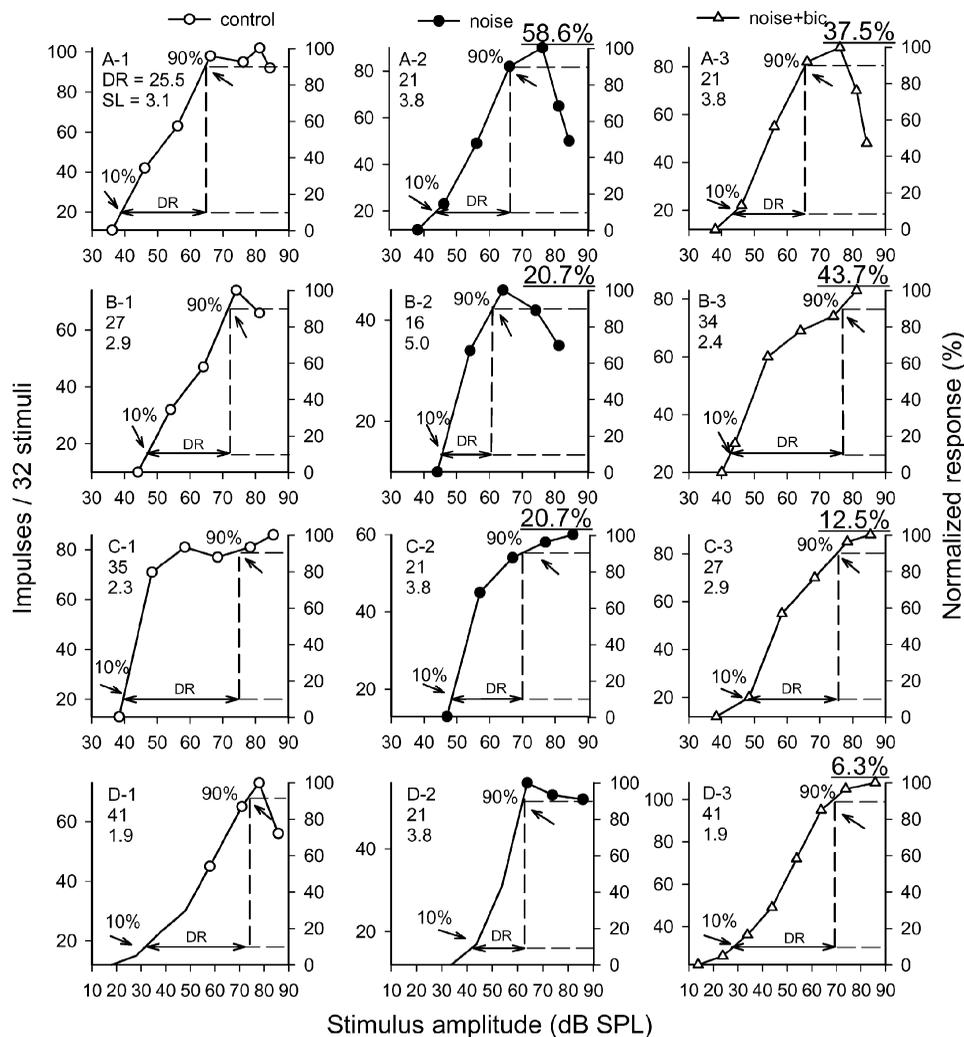


Fig. 5. The RAF of IC neurons determined under different stimulus conditions. The DR (dB) and slope (%/dB) of each RAF are shown within each panel. Note that the decrease in the DR during noise masking occurred either at the low amplitude end (A-1, A-2), at the high amplitude end (B-1, B-2), or at both low and high amplitude ends (C-1, C-2; D-1, D-2). Bicuculline application during noise masking either did not change the DR (A-2 vs. A-3) or increased the DR that was longer (B-2 vs. B-3 vs. B-1), shorter (C-2 vs. C-3 vs. C-1) or equal (D-2 vs. D-3 vs. D-1) to the control DR. Note also that the slope of the RAF increased when the DR decreased and vice versa. The recording depth ( $\mu\text{m}$ ), BF (kHz) and MT (dB SPL) of these four IC neurons were 1305, 16.2, 36 (A-1); 1296, 12.2, 43 (B-1); 1047, 11.6, 38 (C-1); 1272, 10.7, 18 (D-1).

Table 1 summarizes the variation in the BA and RAF of IC neurons during noise masking with and without bicuculline application. This table shows that in the presence of noise the RAF of 27 (39.7%) IC neurons changed from one type to another while the RAF of 41 (60.3%) IC neurons remained unchanged. During noise masking plus bicuculline application, the RAF of 16 (50%) of IC neurons changed from one type to another while the RAF of other half IC neurons remained unchanged.

#### *Variation in the DR and Slope of the RAF of IC Neurons in the Presence of Weak Noise*

Among 68 IC neurons studied, noise masking

did not change the DR of 10 (14.7%) neurons but shortened the DR of 58 (85.3%) neurons to varying degree in three ways. Shortening of the DR only occurred at the low amplitude end in 34 neurons (58.6%, Fig. 5A-1 vs. A-2), at the high amplitude end in 12 neurons (20.7%, Fig. 5B-1 vs. B-2) and at both low and high amplitude ends in another 12 neurons (20.7%, Fig. 5C-1 vs. C-2, D-1 vs. D-2). Noise masking increased the slope of the RAF of all IC neurons from  $3.9 \pm 1.7$  %/dB (range: 1.3-10%/dB) to  $5.5 \pm 3.3$  %/dB (range: 1.6-20%/dB) (Fig. 5A-1 vs. A-2, B-1 vs. B-2, C-1 vs. C-2, D-1 vs. D-2).

Among 32 IC neurons studied, bicuculline application during noise masking either did not change the DR (12/32, 37.5%, Fig. 5A-2 vs. A-3), on increased

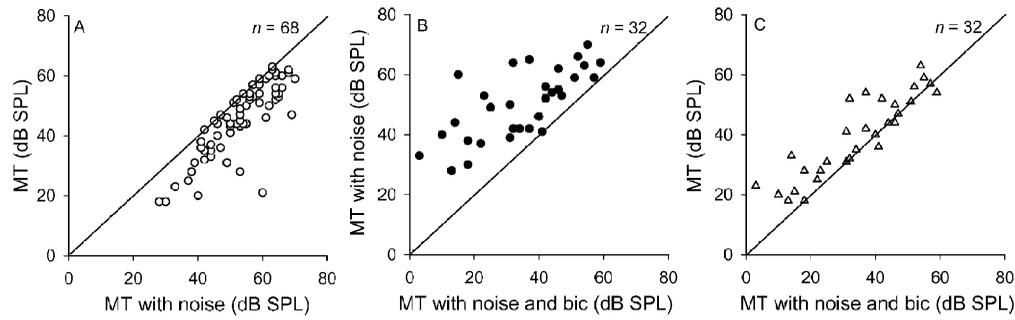


Fig. 6. Scatter plots showing the comparisons of the MT of IC neurons determined before and during noise masking without (A, unfilled circles) and with bicuculline application (B, filled circles and C, unfilled triangles). Note that the scatter plot in B shows the comparison of MT of IC neurons obtained during noise masking with the MT obtained during noise masking plus bicuculline application. The diagonal solid line indicates equal value of MT. *n*: number of IC neurons.

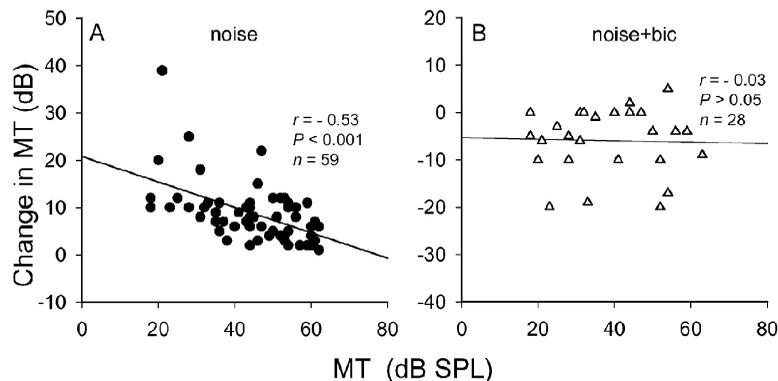


Fig. 7. Scatter plots showing the change in the MT of IC neurons relative to the MT during noise masking without (A) and with (B) bicuculline application. The linear regression line and the correlation coefficient of each plot are shown with a solid line and *r*. *P*: significance level.

the DR that was longer (14/32, 43.7%, Fig. 5B-2 vs. B-3 vs. B-1), shorter (4/32, 12.5%, C-2 vs. C-3 vs. C-1) or equal (2/32, 6.3%, Fig. 5D-2 vs. D-3 vs. D-1) to the control DR. Bicuculline application during noise masking decreased the slope of the RAF of IC neurons from  $5.5 \pm 3.3$  %/dB (range: 1.6-20%/dB) to  $3.2 \pm 1.6$  %/dB (range: 1.7-13%/dB) (Fig. 5A-2 vs. A-3, B-2 vs. B-3, C-2 vs. C-3, D-2 vs. D-3).

#### Variation in the MT in the Presence of Weak Noise

Among 68 neurons studied, noise masking did not affect the MT of 9 (13.2%) neurons but increased the MT of all remaining 59 (86.8%) neurons to varying degree (range: 28-70, average:  $53.3 \pm 10.6$  dB). When bicuculline was applied during noise masking, the MT of 31 of 32 neurons studied decreased to varying degree (range: 3-59, average:  $34.3 \pm 15.2$  dB) while the MT of one neuron remained unchanged.

Fig. 6 compares the MT of IC neurons determined before and during noise masking with or without bicuculline application. During noise masking, all

data points of increased MT of 59 neurons are distributed below the diagonal equal value line while the data points of 9 neurons with unaffected MT are distributed at the diagonal equal value line (Fig. 6A). When bicuculline was applied during noise masking, all but one data points of decreased MT are distributed above the diagonal equal value line when compared with the MT obtained during noise masking (Fig. 6B). Most (20/32, 62.5%) of these data points of decreased MT are still distributed above the diagonal equal value line when compared with the control MT obtained before noise masking (Fig. 6C).

To further examine the variation in the MT of IC neurons during noise masking with and without bicuculline application, we perform linear regression analysis of the scatter plot of MT change in relation to MT. As shown in Fig. 7, the increase in the MT due to noise masking is greater for IC neurons with low MT than with high MT. As such, the MT change due to noise masking significantly decreases with MT of IC neurons (Fig. 7A,  $P < 0.001$ ). However, the MT change during bicuculline application plus noise

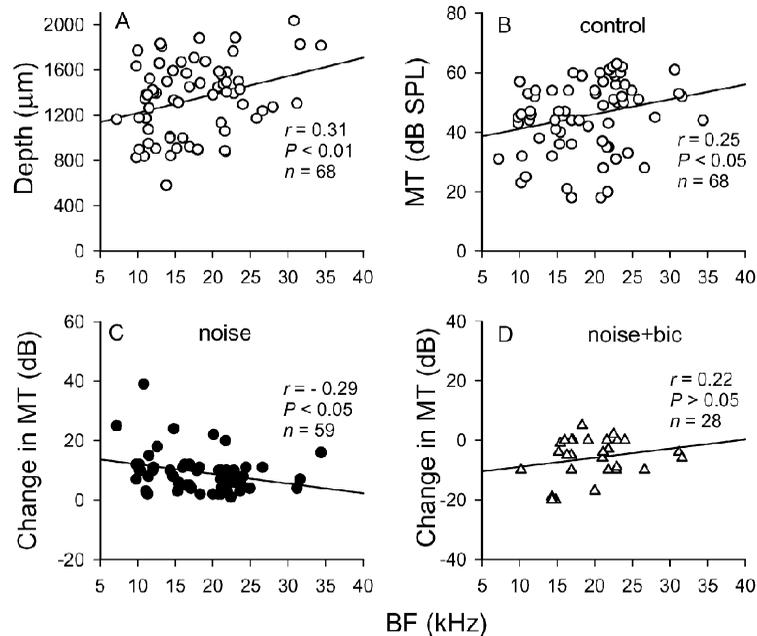


Fig. 8. Scatter plots showing the recording depth (A), MT (B) and change in MT of IC neurons during noise masking without (C) and with bicuculline application (D) in relation to BF. The linear regression line and the correlation coefficient of each plot are shown with a solid line and  $r$ .  $P$ : significance level.

masking is not significantly correlated with the MT of IC neurons (Fig. 7B,  $P > 0.05$ ).

To determine if the BF of these neurons is correlated with recording depth and MT, we plotted the distribution of recording depth and MT of these neurons against the BF (Fig. 8, A and B). Although the recording depth and MT of these neurons are scattered over a wide range, linear regression analyses indicate that high BF neurons tend to be recorded at deeper IC with high MT than low BF neurons at upper IC with low MT ( $P < 0.05$ ).

We also plotted the distribution of MT change against the BF of these neurons and determine if the MT change during weak noise masking with and without bicuculline application is correlated with BF (Fig. 8, C and D). A linear regression analysis reveals that the increase in MT during noise masking is significantly greater for low than for high BF neurons (Fig. 8C,  $P < 0.05$ ).

However, the MT change by bicuculline application during weak noise masking is not correlated with BF (Fig. 8D,  $P > 0.05$ ). To summarize the variation in the RAF of IC neurons determined in the presence of noise with and without bicuculline application, the average MT, DR, slope and BA of the RAF of IC neurons obtained under different stimulation conditions are shown in Table 2. It is clear that noise masking increased the MT, slope and BA but decreased the DR of the RAF of IC neurons while bicuculline application during noise masking produced

opposite effects. Excluding the variation in the BA, all average MT, DR, slope of RAF of IC neurons obtained under three stimulation conditions differed significantly (Repeated measure one-way ANOVA,  $P < 0.05$ ).

## Discussion

### *GABA-Mediated Inhibition Underlying Weak Noise Masking of Amplitude Sensitivity of IC Neurons*

Previous studies showed that presentation of above-threshold noise significantly increased the MT and slope but decreased the DR of the RAF of auditory nerve fibers (9), AC neurons (12) and most IC neurons (32, 34). In the present study, we observed similar change in these measurements of most IC neurons even in the presence a sub-threshold weak noise (Figs. 1-8, Table 2). Furthermore, we observed that the change in these measurements of IC neurons due to weak noise masking was mostly cancelled during bicuculline application (Figs. 5-8, Table 2). These observations suggest that GABA-mediated inhibition is also one of the neural mechanisms underlying the sub-threshold noise masking of amplitude sensitivity of IC neurons observed in the present study. In addition, our observations also suggest that GABAergic inputs activated during the presence of the sub-threshold noise must have lower response threshold than that of the excitatory inputs.

**Table 2. Comparisons of the minimum threshold (MT), dynamic range (DR), slope (SL) and best amplitude (BA) of IC neurons obtained under three different stimulation conditions**

		Stimulation conditions			One-way ANOVA	*	
		Control (1)	Noise (2)	Noise+bic (3)			
MT (dB SPL)	n	68	68	32			
	range	18~63	28~70	3~59			
	m $\pm$ sd	45.5 $\pm$ 12.1	53.2 $\pm$ 10.1	34.5 $\pm$ 15.0	$P < 0.001$	1 vs. 2 1 vs. 3 2 vs. 3	$P < 0.01$ $P < 0.001$ $P < 0.001$
DR (dB)	range	8~62	4~49	6~46			
	m $\pm$ sd	23.6 $\pm$ 11.4	18.8 $\pm$ 10.6	28.8 $\pm$ 11.5	$P < 0.001$	1 vs. 2 1 vs. 3 2 vs. 3	$P < 0.05$ $P < 0.05$ $P < 0.001$
	SL (%/dB)	range m $\pm$ sd	1.3~10 3.9 $\pm$ 1.7	1.6~20 5.5 $\pm$ 3.3	1.7~13.3 3.2 $\pm$ 1.6	$P < 0.05$	1 vs. 2 1 vs. 3 2 vs. 3
BA (dB SPL)	range	38~91	40~91	58~89			
	m $\pm$ sd	74.3 $\pm$ 11.7	76.6 $\pm$ 10.9	74.5 $\pm$ 10.9	$P > 0.05$		

N: number of IC neurons, Control: obtained with BF sounds; Noise: obtained with noise masker; Noise+bic: obtained with noise masker and bicuculline application. \*: Significance level of a post-test with the Student-Newman-Keuls Multiple Comparison test between each paired group is shown in the far right column.

In agreement with previous studies (16, 46), we observed that the strength of weak noise masking greatly decreases with stimulus amplitude (Fig. 3). Why did this weak noise masking of IC amplitude sensitivity response become poor at high sound amplitude? Previous studies have shown that stronger inhibition is more effective than weak inhibition in shaping response properties of IC neurons (27, 28). It is therefore conceivable that the strength of GABA-mediated inhibition activated by the sub-threshold noise used in this study becomes less effective when the strength of excitation is strong at high sound amplitude. In other words, the effectiveness of weak noise masking becomes poor at high sound amplitudes when neurons are strongly excited. For example, the sound amplitude that excites the high MT neurons is presumably so strong that GABA-mediated weak noise masking becomes less effective. This is evident by the finding that the increase in MT due to noise masking is significantly smaller for high than for low MT neurons (Fig. 7A).

In this study, we observed that the data points of the MT of most IC neurons determined during bicuculline plus noise masking are distributed above the diagonal equal value line when compared with the control MT obtained before noise masking (Fig. 6C). This observation indicates that the MT of most IC neurons obtained during bicuculline application plus noise masking is lower than the control MT. This observation is also an indication that the sub-threshold

noise used in the present study only partly activated the GABA-mediated inhibition to increase the MT of most IC neurons. Bicuculline application removed the full extent of GABA-mediated inhibition of most IC neurons to produce a decrease in the MT that was far greater than the amount of MT increase in the presence of this sub-threshold noise. As such, the average MT obtained during bicuculline application plus the sub-threshold noise masking is significantly lower than the average control MT (Table 2).

We observed that IC neurons obtained in this study are tonotopically organized such that low BF neurons at upper IC have lower MT than high BF neurons at deeper IC have (Fig. 8A,B). This finding is in agreement with previous studies (19, 31, 35, 43). We found that noise masking was more effective for low than for high BF neurons (Fig. 8C). What might be the reason for this observation? Conceivably, this is due to that fact that the increase in MT due to noise masking was greater for IC neurons with low than with high MT (Fig. 7A) and the former have lower BF than the latter have (Fig. 8B). In other words, noise masking was more effective for low BF and MT neurons at upper IC than for high BF and MT neurons at deeper IC. We showed that bicuculline application appeared to produce a greater degree of change in the MT for high than for low BF IC neurons although this correlation between the MT change and BF of IC neurons was not significant, perhaps due to small sample size (Fig. 8D,  $P > 0.05$ ).

### *Facilitation or no Effect on Amplitude Sensitivity during Weak Noise Masking*

We observed that noise masking produced facilitation or no effect on the response of 18 (21%) neurons (Fig. 2, B and C), similar to previous reports (3, 8, 30, 40, 45). Conceivably, facilitation of responses of IC neurons in the presence of a weak noise may be the result of recruitment of stronger excitatory inputs or removal of GABA-mediated inhibition through the activation of an inhibitory interneuron (Fig. 2B). Alternatively, weak noise might be too weak to activate GABA-mediated inhibition to produce any noticeable effect on the response of some IC neurons (Fig. 2C).

### *Possible Biological Relevance of the Present Study*

In the real world, acoustic environment consists of multiple sound sources that are embedded in strong and weak noises. For effective sound communication, animals must somehow extract biologically relevant signals from the inevitable interference of these ambient noises. One way to overcome this noise interference is to increase the signal to noise ratio as shown by songbirds which regulate the song amplitude to overcome ambient noise interference during acoustic communication (6). Alternatively, the ambient noise may somehow improve signal processing in a certain parameter at the expenses of others. This is shown in many previous studies in which ambient noise improves auditory signal detection and enhances human hearing (10, 30, 33, 40, 45).

Consonant with these studies, we showed that even sub-threshold noise decreased the number of impulses and increased the MT of most IC neurons as well as increased the slope and shortened the DR of their RAFs (Figs. 1, 5, 6, 7, Table 2). As such, the range of overall amplitude sensitivity of these IC neurons is decreased while the sensitivity of IC neurons to amplitude variation is greatly improved. Conceivably, this increase of sensitivity to amplitude variation in the presence of sub-threshold noise may serve as one of the underlying neural mechanism that enhances modulation sensitivity in cochlear implant listeners (8).

On the other hand, we showed the sub-threshold noise facilitated the response of other IC neurons indicating an increase in amplitude sensitivity (Fig. 2B). All these data suggest that in the real world the ambient noise may serve as a modulating force to improve the sensitivity of most IC neurons to small amplitude fluctuation and to increase the sensitivity of other neurons to a wide amplitude spectrum as well. Although our present study was conducted on anesthetized preparations, we believe that the same finding can also be obtained from awaken animal preparations. As such, this dynamic modulation of amplitude sensitivity by ambient noise

may conceivably serve as one of the underlying neural mechanisms that facilitate an animal to extract biologically significant signals in the seemingly chaotic noise environment such as the cock-tail party phenomenon during human communication (38).

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