

Role of the caudate-putamen nucleus in sensory gating in induced tinnitus in rats

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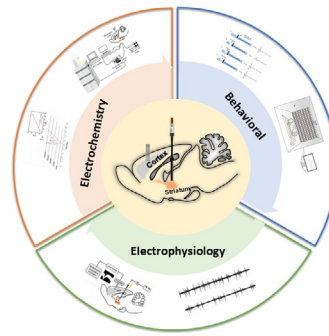
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Graphical Abstract Caudate-putamen nucleus plays a key role as a sensory gating area in sodium salicylate-induced tinnitus



Abstract

Tinnitus can be described as the conscious perception of sound without external stimulation, and it is often accompanied by anxiety, depression, and insomnia. Current clinical treatments for tinnitus are ineffective. Although recent studies have indicated that the caudate-putamen nucleus may be a sensory gating area involved in noise elimination in tinnitus, the underlying mechanisms of this disorder are yet to be determined. To investigate the potential role of the caudate-putamen nucleus in experimentally induced tinnitus, we created a rat model of tinnitus induced by intraperitoneal administration of 350 mg/kg sodium salicylate. Our results revealed that the mean spontaneous firing rate of the caudate-putamen nucleus was increased by sodium salicylate treatment, while dopamine levels were decreased. In addition, electrical stimulation of the caudate-putamen nucleus markedly reduced the spontaneous firing rate of neurons in the primary auditory cortex. These findings suggest that the caudate-putamen nucleus plays a sensory gating role in sodium salicylate-induced tinnitus. This study was approved by the Institutional Animal Care and Use Committee of Peking University Health Science Center (approval No. A2010031) on December 6, 2017.

Key Words: caudate-putamen nucleus; deep brain stimulation; dopamine; primary auditory cortex; sodium salicylate; sound; striatum; tinnitus

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Introduction

Tinnitus refers to the sensation of hearing sound without any external acoustic stimulation (Lanting et al., 2009). Epidemiological studies have reported that the global prevalence of tinnitus among adults is as high as 10–25% (Henry et al., 2005; Shargorodsky et al., 2010; Kim et al., 2015), and increases with age (Sindhusake et al., 2003; Rauschecker et al., 2010; Shargorodsky et al., 2010; Manche et al., 2016). Moreover, 1.5–7% of patients are severely affected by tinnitus, which has negative affect on their life quality (Nondahl et al., 2002; Bhatt et al., 2016), while 97% of patients present varying degrees of hearing loss (Manche et al., 2016). Tinnitus is often accompanied by anxiety, depression, insomnia, and even suicide in severe cases (Trevi

et al., 2016, 2018; Bhatt et al., 2017; Martz et al., 2018; Chai et al., 2019). It is difficult to clinically treat tinnitus because the underlying neurophysiological and pathological mechanisms remain unclear.

Previous animal studies have suggested that cochlear lesions may cause central auditory gain (Eggermont, 2005; Salvi et al., 2016), which might explain the decreased tolerance to loudness and increased sensitivity to sound in patients with tinnitus. However, surgical removal of the auditory nerve does not alleviate tinnitus symptoms (Kameda et al., 2010). Increasing evidence indicates that tinnitus is a central plasticity disorder caused by peripheral lesions that are difficult to treat (Shore et al., 2016; Wu et al., 2016; Roberts, 2018). The underlying pathological process involves multiple neural

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networks, including the classical auditory (Sedley et al., 2015; Leaver et al., 2016), limbic (Kraus and Canlon, 2012; Leaver et al., 2016), cerebellar (Chen et al., 2017; Du et al., 2017), and basal ganglia (Ahsan et al., 2018; Perez et al., 2019) systems.

The striatum is the largest nucleus in the basal ganglia. It receives fiber projections from all cortices, including the auditory, motor, and sensory cortices (Hunnicutt et al., 2016; Miyamoto et al., 2018). The dorsal striatum, which comprises the caudate-putamen nucleus (CPu), serves as a sensory gating region in information transmission to the cerebral cortex and is suggested to play a crucial role in tinnitus (Lowry et al., 2004; Cheung and Larson, 2010; Larson and Cheung, 2013; Ahsan et al., 2018; Perez et al., 2019). Lowry et al. (2004) reported a case of chronic tinnitus that was cured after a cerebrovascular accident in the left corona radiata, including the caudate body and caudodorsal putamen. Subsequently, several studies have successfully used deep brain stimulation (DBS) to treat patients with Parkinson's disease or essential tremor and tinnitus (Cheung and Larson, 2010; Larson and Cheung, 2013). These studies reported significant suppression or even complete alleviation of tinnitus and suggest that the caudate nucleus may be a neuroregulatory target for the inhibition of tinnitus. DBS of the caudate nucleus interferes with tinnitus information integration in the central auditory system, thus suppressing tinnitus noise. Ahsan et al. (2018) were the first to confirm the therapeutic effects of DBS of the caudate nucleus on tinnitus suppression in animals with noise-induced tinnitus. They observed that electrical stimulation of the caudate nucleus reduced cluster discharge in neurons in the auditory cortex. Moreover, the assessment of startle reflex behavior indicated tinnitus suppression. Together, these studies provide strong evidence for the involvement of the CPu in tinnitus; however, it remains unclear how this nucleus regulates tinnitus.

Dopamine, an important monoamine neurotransmitter, is involved in somatic movement, psychological activity, psychological dependence, and other body regulation functions (Puopolo, 2019; Thomas Broome et al., 2020). Dopaminergic neurons are mainly located in the substantia nigra zona compacta and ventral tegmental area (Wu et al., 2017), and the CPu is dominated by long axial ascending neurons from the substantia nigra pars compacta. Moreover, previous clinical studies have indicated that dopaminergic agents may have a therapeutic effect on tinnitus (de Azevedo et al., 2009; Sziklai et al., 2011). We therefore hypothesized that the CPu might be involved in the underlying mechanisms of tinnitus through dopaminergic regulation of the indirect basal ganglia pathway.

Sodium salicylate (SS), the main ingredient in aspirin, is an effective anti-inflammatory drug for fever and chronic pain. Because large SS doses can cause tinnitus as a side effect, it is now used as a standard tool for establishing animal models of tinnitus (Yang et al., 2007; Lu et al., 2011). To investigate the potential role of the CPu in sensory gating, we first verified that SS induced tinnitus behavior in animals by measuring gap-prepulse inhibition of the acoustic startle reflex (GPIAS). Next, we explored the spontaneous firing rate (SFR) of neurons in the CPu in a rat model of SS-induced tinnitus. Given the association between neuronal electrical activity and neurotransmitter release, extracellular dopamine levels were also measured before and after SS treatment. Furthermore, to investigate regulatory mechanisms between the CPu and primary auditory cortex (Au1), we recorded SFR changes in the Au1 after electrical stimulation of the CPu.

Materials and Methods

Animals

Healthy adult male Sprague-Dawley rats (specific-pathogen-free level, 8 weeks old, 280–350 g) were obtained from the Department of Laboratory Animal Science, Peking University

Health Science Center (PUHSC), Beijing, China (license No. SYXK (Jing) 2016-0041). All experiments were designed and reported according to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines. All experimental procedures were approved by the Institutional Animal Care and Use Committee of PUHSC (approval No. A2010031) on December 6, 2017. Rats were individually housed under standard conditions with free access to food and water. A 12-hour light/dark cycle (lights on 7:00 to 19:00), room temperature of $24 \pm 1^\circ\text{C}$, and air humidity of 50–60% was maintained in the housing environment. Eight rats underwent tinnitus evaluation using GPIAS, 12 rats were used to determine the effects of SS on neural SFRs in the CPu, 12 rats were used to detect SS-induced changes in extracellular dopamine levels in the CPu, and eight rats were used to determine SFRs in the Au1 by electrical stimulation of the CPu.

Behavioral assessment of tinnitus

Eight rats underwent behavioral testing for tinnitus assessment using GPIAS. This experiment was conducted in a noise-shielded box (ZS-ZJT, ZS Dichuang Co., Beijing, China). Each rat was restrained in a transparent polycarbonate breathable acoustic holder installed on a plexiglass base that contained a sensitive piezoelectric sensor with an output connected to an A/D converter on an RP2 real-time processor (ZS Dichuang Co., Beijing, China). Sound stimuli were generated using a custom RP2 software and presented by a loudspeaker placed approximately 20 cm above the startle platform (**Figure 1**). The background noise was centered at 6, 12, and 16 kHz, and broadband noise intensity of 65 dB sound pressure was used for each trial. A startle stimulus (115 dB sound pressure level, 20 ms duration) was embedded in the background noise of each trial. Intense startle reflexes were suppressed by inserting a 50-ms silent gap in a continuous background noise burst before the startle stimulus. The inhibitory effect of the silent gap was indicated by the GPIAS ratio and the absolute startle amplitude between the non-gap and gap protocols. We defined a GPIAS ratio of less than 30% as an indicator of tinnitus behavior (Kraus et al., 2010; Longenecker and Galazyuk, 2011; Longenecker et al., 2014). We measured gap detection deficits using a specific acoustic startle reflex hardware and software (ZS-ZJT, ZS Dichuang Co.). Similarity between the background noise and the tinnitus frequency indicated impaired gap inhibition in rats with tinnitus. The GPIAS values were recorded before and 2 hours after the SS injection.

Single-neuron recordings in the CPu

All animals ($N = 12$) were anesthetized using isoflurane (RWD Life Science, Shenzhen, China) (anesthesia induction: 3–5% for 3 minutes; anesthesia maintenance: 1–2%; flow rate 0.2–0.3 L/min) and placed in a stereotaxic head frame on a heating blanket. Anesthesia adequacy was confirmed by the absence of a hind-paw withdrawal reflex. A craniotomy was performed over the right CPu (anteroposterior (AP) = 0 mm, mediolateral (ML) = 3 mm, dorsoventral (DV) = 3 mm), conforming to rat brain stereotaxic coordinates (Paxinos and Watson, 2007). Three stainless steel screws were drilled into the skull as reference electrodes, with the tip making slight contact with the dura. Using tweezers, the dura mater was carefully removed under a surgical microscope (YZ20P5, Suzhou Liuliu Vision Technology, Suzhou, China) to expose the brain tissue above the CPu. Subsequently, recording electrodes (Institute of Semiconductors, the Chinese Academy of Sciences, Beijing, China) were implanted along the dorsoventral axis of the micromanipulator. Next, multiple single neurons were recorded using a 16-channel silicon electrode (4×4 array; Plexon Inc., Dallas, TX, USA), as previously described (Song et al., 2016; Du et al., 2017; Xiong et al., 2019). The baseline SFR was recorded 5 minutes before the SS (350 mg/kg, 10%, intraperitoneal; Sigma-Aldrich, St. Louis, MO, USA) or equivalent saline injection. Subsequently, SFR recordings

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were performed for at least 5 minutes at the following post-injection time points: 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 hours. At the end of the experiment, the rats were euthanized with a lethal dose of urethane solution (Sigma-Aldrich).

Measurement of dopamine levels using high-performance liquid chromatography

Given the association between neuronal electrical activity and neurotransmitter release, we hypothesized that SS treatment might change neurotransmitter levels in the CPU. The CPU is rich in dopamine, which is an important neurotransmitter that modulates various physiological responses in the central nervous system (Grillner et al., 2020). To assess the effects of SS on dopamine levels, a microdialysis guide cannula was implanted in the CPU to measure the extracellular dopamine levels.

We randomly divided 12 rats into the SS ($N = 6$; 350 mg/kg, 10%, intraperitoneal) and saline ($N = 6$; equivalent dose of SS) groups. We performed microdialysis for the extracellular solution in the right CPU and analyzed the dopamine levels using a high-performance liquid chromatography system combined with electrochemical detection (**Figure 2**). Under isoflurane anesthesia, a microdialysis guide cannula (MAB.6.14.2ss, MBA, Stockholm, Sweden) was implanted into the vertical dural surface of the right CPU (AP = 0 mm, ML = 3 mm, DV = 3 mm) and permanently secured by supporting screws (Misumi, Shanghai, China) and dental cement (Tianjin Ruierdeyuan Medical Biomaterials, Tianjin, China). The rats were allowed at least 2 postoperative days to recover prior to the subsequent experiments. Next, the stylet was replaced by a concentric microdialysis probe with a 4-mm semipermeable membrane (Bioanalytical Systems Inc., West Lafayette, IN, USA) and inserted through the guide cannula into the CPU. The concentric microdialysis probe was connected to a perfusion pump (CMA100, CMA, Stockholm, Sweden) that maintained a flow rate of 2 $\mu\text{L}/\text{min}$ with artificial cerebrospinal fluid (Beijing Chemical Works, Beijing, China). After a 90-minute stabilization period, three sequential dialysate samples were obtained at 20-minute intervals to establish pre-treatment baseline values. Next, dialysate samples were collected for the extracellular dopamine measurements at 30-minute intervals until 4 hours after the SS or saline injection. All dialysate samples were then analyzed using a reverse-phase high-performance liquid chromatography system (Shimadzu Corporation, Kyoto, Japan) with an analytical C18 column (150 mm \times 4.6 mm, 5 mm particle size). A 20 μL injection of methanol/acetonitrile (98:2, v/v) was used as the mobile phase at a flow rate of 1.0 mL/min and a column temperature of 25°C. The mobile phase was filtered through a 0.45-mm nylon filter and degassed for 30 minutes by ultrasonication. The obtained brain dialysates were delivered into the thin-layer electrochemical flow cell through tubing. The electrochemical system had three components: a glassy carbon working electrode (6 mm in diameter), an Ag/AgCl reference electrode, and a stainless steel counter electrode.

In order to get the dopamine standard solution electrochemical detection linear equation, 1000 nM dopamine solution was prepared by dissolving 0.95 mg dopamine (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) in 5 mL ultra-pure water then the dopamine concentration was diluted to five standard dopamine concentrations (1, 10, 25, 50 and 100 nM). From low to high solution, the standard was successively passed through the high performance liquid liquid-online electrochemical detection system to obtain the concentration-voltage dopamine standard linear equation.

CPu electrical stimulation

Eight rats were anesthetized using isoflurane (anesthesia induction: 3–5% for 3 minutes; anesthesia maintenance: 1–2%, flow rate 0.2–0.3 L/min) and were each mounted onto

a stereotaxic head frame with hollow ear bars on a heating blanket. The right CPU was exposed as described as for the CPU single-neuron recordings, and a stimulating electrode was inserted along the dorsoventral axis for electrical stimulation. We obtained recordings from the right Au1 region (AP = -5.2 mm, ML = 6.8 mm, DV = -2.5 mm) (Paxinos and Watson, 2007), which was exposed from the bregma as previously described (Song et al., 2016; Du et al., 2017; Ding et al., 2018; Xiong et al., 2019). The skull and relevant dura mater were removed, and the recording electrode was implanted into the Au1 along the dorsoventral axis of the micromanipulator. A stainless steel ground electrode was placed on the skull surface. An electrical stimulator (Marster-9, A.M.P.I., Jerusalem, Israel) delivered shock trains (pulse duration, 2 ms; train duration, 16.7 ms; rate, 60 Hz; and intensity, 250 μA) to the CPU through a custom-made bipolar tungsten electrode. The SFR in the Au1 was recorded for 3 minutes before and after CPU stimulation (**Figure 3**).

Statistical analysis

Statistical analyses were performed using SPSS version 20.0 (IBM, Armonk, NY, USA) and the results are presented as the mean \pm standard error of the mean (SEM). The paired-samples *t*-test was used to analyze GPIAS suppression and absolute startle amplitude with different background noises and groups, as well as the pre- and post-stimulation mean SFR in the Au1. Between-group comparisons of neuronal SFR and extracellular dopamine levels in the CPU were performed using two-way analysis of variance (ANOVA) with the least significant difference (LSD) *post hoc* test. One-way ANOVA with the LSD *post hoc* test was used to compare changes in the SFR and dopamine levels from baseline in different groups and at different time periods. A linear regression model was used to explore the linear response to the dopamine standard solution. All graphical presentations were obtained using GraphPad Prism 6.01 (GraphPad Prism, San Diego, CA, USA) and Origin 2017 (OriginLab Corporation, Northampton, MA, USA). We considered statistical significance at $P < 0.05$. N and n represent the number of animals and number of samples (neurons), respectively.

Results

SS treatment to induce tinnitus in rats

Eight rats underwent GPIAS testing before and after SS treatment to obtain behavioral evidence of tinnitus. Before the SS injection, the animals showed strong GPIAS suppression at background frequencies of 6 kHz ($46.5 \pm 5.6\%$), 12 kHz ($54.1 \pm 3.4\%$), and 16 kHz ($55.6 \pm 20.3\%$). However, 2 hours after the SS treatment, the GPIAS decreased to $38.7 \pm 5.3\%$, $32.1 \pm 6.1\%$, and $20.3 \pm 6.3\%$ at 6, 12, and 16 kHz, respectively. This decrease in the GPIAS was significant at 12 kHz ($t = 3.649$, $P = 0.008$) and 16 kHz ($t = 5.214$, $P = 0.001$), but not at 6 kHz ($t = 1.467$, $P = 0.186$).

The absolute amplitude of startle responses was also measured pre- and post-SS treatment in both gap and no-gap conditions. The startle response amplitude in both gap and no-gap conditions was significantly different at 6 kHz ($t = -3.258$, $P = 0.014$), 12 kHz ($t = -3.569$, $P = 0.009$), and 16 kHz ($t = -4.119$, $P = 0.004$) before SS treatment; however, after SS treatment, a significant difference was only observed at 16 kHz ($t = -2.953$, $P = 0.022$), but not at 6 kHz ($t = -2.285$, $P = 0.056$) or 12 kHz ($t = -2.280$, $P = 0.057$; **Figures 4–6**).

Effects of SS on the neuronal SFR of the CPU

To determine the effects of SS on the neuronal SFR in the rat CPU, the rats were injected with 350 mg/kg SS or an equivalent volume of saline. We recorded the neural firing activity of single units in the CPU both before and at 0.5-hour intervals after SS or saline treatment. We recorded the spontaneous activity of 49 and 57 CPU neurons in the SS and

saline groups, respectively. Two-way ANOVA with LSD *post hoc* tests revealed significant between-group differences ($F = 2.300$, $P = 0.019$). One-way ANOVA with LSD *post hoc* tests demonstrated a significant increase in the mean SFR of the CPU at 2 hours ($P = 0.012$) and 2.5 hours ($P = 0.016$) after the SS treatment; the SFR increased from 4.8 ± 0.5 Hz to 7.4 ± 0.8 Hz and 7.2 ± 0.9 Hz, respectively. Conversely, saline treatment did not significantly affect the mean SFR of CPU neurons ($F = 0.682$, $P = 0.707$; **Figure 7**).

Dopamine levels in the CPU

High-performance liquid chromatography analysis demonstrated a good linear response to the dopamine standard solution from 1 to 100 nM. The linear equation was $U (V) = 0.5054 C_{DA} (nM) + 0.8588$ (U : the mathematical symbol for voltago; C_{DA} : dopamine concentration) with a linear coefficient of 0.9934 (**Figure 8A**). Two-way ANOVA revealed a significant difference in dopamine levels between the SS and saline groups ($F = 6.358$, $P = 0.013$). In the SS group, there was a gradual decrease in extracellular dopamine levels in the CPU, reaching $78.9 \pm 2.3\%$ of the baseline level after 4 hours ($t = 6.298$, $P = 0.000$). Conversely, extracellular dopamine levels remained stable in the saline group ($F = 1.090$, $P = 0.387$; **Figure 8B**).

Effects of CPU stimulation on the SFR of Au1 neurons

We recorded the SFR of neurons in the Au1 after CPU electrical stimulation. Eighty-seven Au1 neurons were recorded from eight rats, and the mean basal SFR was 7.2 ± 0.8 spikes/s. The paired-samples *t*-test revealed a significant decrease in the SFR of Au1 neurons after CPU electrical stimulation, from 7.2 ± 0.8 spikes/s to 4.1 ± 0.5 spikes/s (drop rate of 43.5%; $t = 6.541$, $P < 0.01$; **Figures 3 and 9**).

Discussion

This study investigated whether the CPU plays a potential regulatory role in tinnitus. To characterize the role of the CPU in SS-induced tinnitus in rats, we examined acoustic startle reflex behavior and performed electrophysiological and neurochemical tests. We obtained the following findings: (1) SS treatment significantly reduced the GPIAS with background noise at 12 and 16 kHz; (2) SS treatment significantly increased the SFR and decreased extracellular dopamine levels in the CPU, and (3) electrical stimulation of the CPU inhibited Au1 excitability. Overall, our findings indicate that the CPU plays an important role in tinnitus and may have a key role in sensory gating.

GPIAS is a behavioral screening method that can be used to validate the presence of tinnitus in SS-injected rats. In the present study, SS treatment significantly decreased the GPIAS at 12 and 16 kHz; this is consistent with previous findings (Yang et al., 2007; Wu et al., 2019). Conversely, there was no significant between-group difference in the GPIAS at 6 kHz. Moreover, the absolute startle amplitude did not differ significantly between the gap and no-gap conditions at 6 and 12 kHz after SS treatment. Tinnitus is a phantom auditory perception that can mask the gap in the acoustic startle reflex. Thus, the failure to detect a gap in background noise is considered evidence of tinnitus. We revealed that the tinnitus pitch in SS-treated rats was close to the frequency range of 12 to 16 kHz. Notably, in the GPIAS behavioral test, non-auditory systems (including the midbrain reticular formation, cuneiform nucleus, superior colliculus, striatum, and medial prefrontal cortex) as well as the auditory pathway are involved in startle inhibition (Azzopardi et al., 2018; Fulcher et al., 2020).

Several studies have suggested that tinnitus is caused by altered central structural and functional plasticity, and many studies have reported that the CPU is involved in tinnitus (Cheung and Larson, 2010; Larson and Cheung, 2012, 2013;

Chen et al., 2017; Ahsan et al., 2018; Perez et al., 2019). In the present study, SS treatment significantly increased the neuronal SFR and decreased extracellular dopamine levels in the CPU, which indicates the involvement of the CPU in tinnitus. SS can cross the blood-brain barrier after intraperitoneal injection and suppress GABAergic inhibition, leading neural hyperactivity. However, SS also had an effect on dopaminergic regulation of the indirect pathway of basal ganglia, which may make the neuronal SFR recover after 2.5 hours. Further studies should investigate whether the increased SFR of CPU neurons is related to the release of dopamine and other neurotransmitters, including the excitatory neurotransmitter glutamate, the inhibitory neurotransmitter gamma aminobutyric acid, and several neuromodulators (such as ascorbic acid), and ions (such as Ca^{2+} , Mg^{2+}). These neurotransmitters may be involved in the relevant electrophysiological processes and should therefore be further assessed.

Dopamine is an important neurotransmitter that modulates various physiological responses in the central nervous system. We revealed that SS treatment significantly decreased extracellular dopamine levels in the CPU. This suggests that SS-induced acute tinnitus causes dopamine suppression in this nucleus. In keeping with these findings, previous clinical studies have shown that dopaminergic agents may have a therapeutic effect on tinnitus (de Azevedo et al., 2009; Sziklai et al., 2011). The dopamine agonists piribedil (de Azevedo et al., 2009) and pramipexole (Sziklai et al., 2011) may alleviate tinnitus by regulating neuronal activity in the auditory pathway. In addition, the extrapyramidal system is regulated by neural circuits of the basal ganglia, and the substantia nigra/striatum dopaminergic system is an important link, with the striatum playing a central regulatory role. Decreased dopamine levels in the CPU may therefore affect the indirect basal ganglia pathway and decrease inhibition in the globus pallidus externa. Consequently, this decreased inhibition may enhance baseline subthalamic nucleus inhibition, reducing the inhibitory effect of the globus pallidus internus on the thalamus, in turn leading to increased Au1 excitability (**Figure 10**). However, this potential, complex mechanism requires further validation.

The CPU receives comparable afferent fiber projections from the Au1 and anterior auditory field (Nakata et al., 2020). Increased SFR in the auditory cortex is a biomarker for tinnitus (Ochi and Eggermont, 1996; Kimura and Eggermont, 1999; Song et al., 2016; Ahsan et al., 2018). Furthermore, functional magnetic resonance imaging studies have shown that both auditory and non-auditory systems, including the CPU, are involved in tinnitus (Chen et al., 2015). We demonstrated that electrical stimulation of the CPU led to decreased SFR in Au1 neurons. This result is consistent with the findings of Ahsan et al. (2018), wherein DBS of non-auditory pathway structures (i.e., the anterior caudate) was able to reduce tinnitus through modulation of the auditory cortex. Therefore, tinnitus inhibition may be dependent on the mechanisms underlying the excitatory and inhibitory effects of DBS (McIntyre et al., 2004). Electrical stimulation of the CPU activates the indirect basal ganglia pathway, which increases the inhibitory effects on the globus pallidus externa. This, in turn, decreases suppression of the subthalamic nucleus, which in turn increases excitation of the globus pallidus internus. This leads to further inhibition of the thalamus and auditory cortex, which results in the observed decrease in the SFR of the Au1 neurons (Yamamoto et al., 2006; Graybiel, 2008; Ahsan et al., 2018; **Figure 10**).

To the best of our knowledge, this is the first study to report SFR and dopamine level changes in the CPU in a rat model of tinnitus. Our findings suggest the potential role of DBS of the CPU in the treatment of tinnitus. The major strength of the

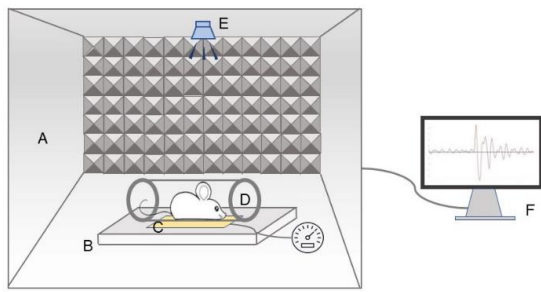


Figure 1 | Schematic diagram of the behavioral experimental setup. The device included a noise-shielded box (A), startle platform (B), sensitive piezoelectric sensor (C), holder (D), loudspeaker (E), and workstation (F).

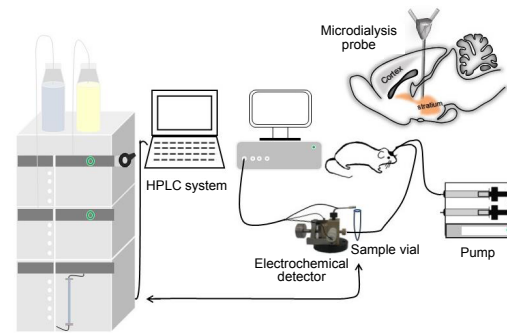


Figure 2 | Schematic diagram of the experimental apparatus for *in vivo* dopamine analysis by microdialysis and high-performance liquid chromatography (HPLC) with electrochemical detection.

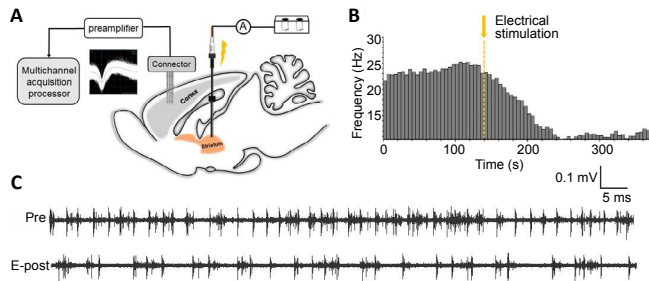


Figure 3 | Effects of caudate-putamen nucleus (CPu) electrical stimulation on primary auditory cortex (Au1) neurons.

(A) Schematic diagram of the electrical stimulation system and multi-electrode array recording system. (B) Representative firing rate histogram of Au1 neurons showing an inhibitory response to CPu electrical stimulation. The arrow and dashed line denote the shock artifact. (C) Neuron spike discharges recorded in the Au1 before and after CPu electrical stimulation. E-post: After CPu electrical stimulation; Pre: before CPu electrical stimulation.

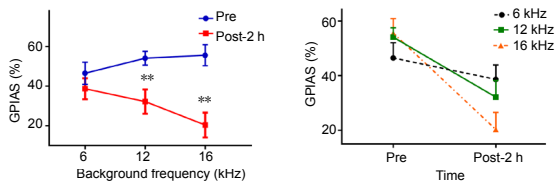


Figure 5 | Gap detection performance at different frequencies measured before and after sodium salicylate (SS) treatment.

Injection of 350 mg/kg SS decreased the gap-prepulse inhibition of the acoustic startle reflex (GPIAS) at 12 kHz ($t = 3.649$, $P = 0.008$) and 16 kHz ($t = 5.214$, $P = 0.001$). Data are presented as the mean \pm SEM ($N = 8$ rats). $**P < 0.01$, vs. pre-SS treatment (paired-samples t -test).

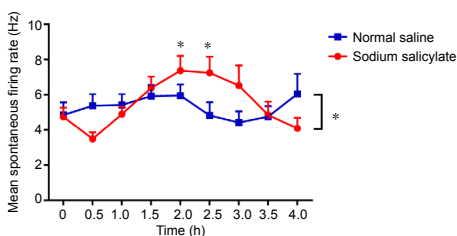


Figure 7 | Effects of sodium salicylate (SS) on the neuronal firing rate of caudate-putamen neurons.

There was a significant between-group difference in the mean spontaneous firing rate (SFR; SS group: $N = 6$, $n = 49$; saline group: $N = 6$ rats, $n = 57$ neurons; $*P < 0.05$, two-way analysis of variance followed by the least significant difference *post hoc* test). SS treatment, but not saline treatment, significantly affected the mean SFR at 2 and 2.5 hours ($*P < 0.05$, one-way analysis of variance followed by the least significant difference *post hoc* test). Data are presented as the mean \pm SEM.

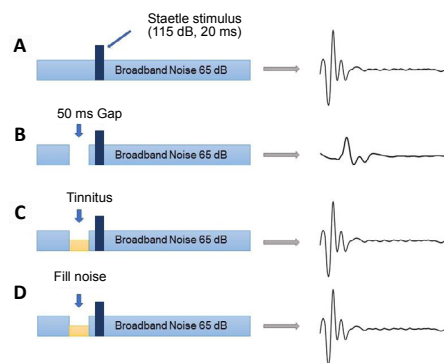


Figure 4 | Schematic view of gap-prepulse inhibition of the acoustic startle reflex.

(A) Normal rats can detect the acoustic startle stimulus in continuous background noise. (B) The acoustic startle reflex in normal rats is forcefully inhibited when a silent gap of 50 ms is inserted into continuous background noise. (C) The tinnitus noise frequency fills the silent gap and there is no inhibition of the acoustic startle reflex. (D) The background noise frequency is near or as high as the putative tinnitus frequency.

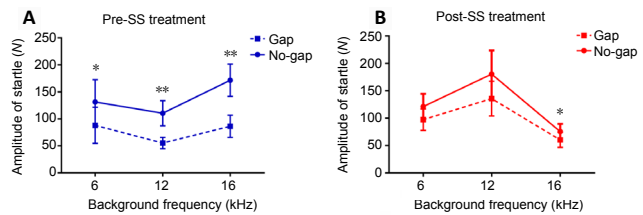


Figure 6 | Effects of sodium salicylate (SS) on the startle response amplitude at different frequencies.

(A) The startle response amplitudes in both gap and no-gap conditions were significantly different at 6 kHz ($t = -3.258$, $P = 0.014$), 12 kHz ($t = -3.569$, $P = 0.009$), and 16 kHz ($t = -4.119$, $P = 0.004$) before the SS treatment. (B) After the SS treatment, there was a significant difference at 16 kHz ($t = -2.953$, $P = 0.022$) only. Data are presented as the mean \pm SEM ($N = 8$ rats). $*P < 0.05$, $**P < 0.01$, vs. gap condition (paired-samples t -test).

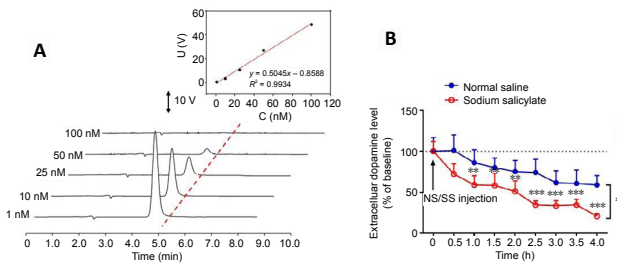


Figure 8 | Effects of sodium salicylate (SS) on extracellular dopamine levels in the caudate-putamen nucleus (CPu).

(A) Typical linear response to the dopamine standard solution. The high-performance liquid chromatography analysis showed a good linear response to the dopamine standard solution from 1 to 100 nM. A linear regression model was used to explore correlations and the linear equation was $U (V) = 0.5054 C_{DA} (nM) + 0.8588$ with a linear coefficient of 0.9934. (B) Between-group comparison of dopamine levels in the CPu at different time points. Statistical results of extracellular dopamine levels based on their baseline levels in the CPu in both groups as a function of time, with each point representing the mean dopamine percentage of the basal level. There was a significant between-group difference in the dopamine levels (SS group: $N = 6$ rats; saline group: $N = 6$ rats; $*P < 0.05$, two-way analysis of variance followed by the least significant difference *post hoc* test). SS treatment significantly decreased the dopamine levels in the CPu between 1 and 4 hours after treatment ($**P < 0.01$, $***P < 0.001$, vs. normal saline group, one-way analysis of variance followed by the least significant difference *post hoc* test). Data are presented as the mean \pm SEM.

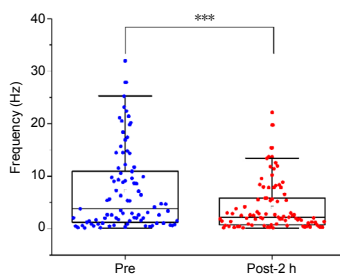


Figure 9 | Effects of caudate-putamen nucleus (CPu) electrical stimulation on primary auditory cortex (Au1) neurons.

CPu electrical stimulation decreased the spontaneous firing rate of the Au1 from 7.2 ± 0.8 spikes/s to 4.1 ± 0.5 spikes/s. Data are presented as the mean \pm SEM ($N = 8$ rats, $n = 87$ neurons). $***P < 0.001$ (paired-samples *t*-test). E-post: After CPu electrical stimulation; Pre: before CPu electrical stimulation.

current study is that it provides accumulating evidence of the role of the basal ganglia in tinnitus and suggests a potential therapeutic target. Moreover, dopaminergic agents may be potential therapeutic agents for tinnitus. Given that a previous study suggested that improper DBS of the CPu increases the risk of tinnitus development and aggravation, it is crucial to determine the appropriate stimulus parameters and accurate stimulus location for this therapy (Larson and Cheung, 2012).

The present study has several limitations. First, the sample size was small; therefore, our findings are preliminary and must be confirmed by large-scale studies. Second, the electrophysiological and neurochemical experiments were performed under anesthesia. Although we tried to ensure a consistent anesthesia status across all animals, the anesthetic itself is likely to have affected the neuronal firing activity. Future studies should therefore be performed on neuronal firing activity in awake animals.

In conclusion, our findings support the premise that the CPu plays a key role in sensory gating in SS-induced tinnitus, and that dopamine receptor agonists may serve as a potential tinnitus treatment. This study is beneficial to explore the mechanism of tinnitus outside the classical auditory pathway. Abnormal changes in CPu electrophysiology and dopamine level provide animal experimental basis for the etiology diagnosis and treatment of tinnitus and indicate the need to explore the neural mechanistic role of the basal ganglia in

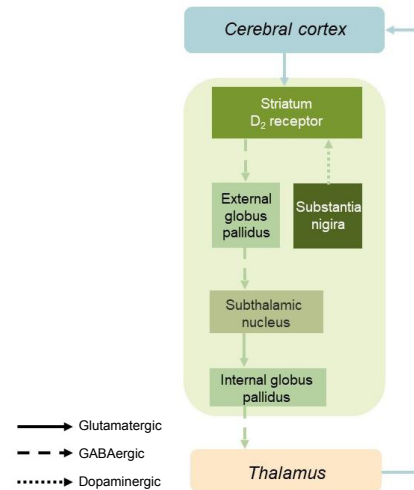


Figure 10 | Neuroregulatory mechanism of the striatum-auditory cortex loop in the sodium salicylate-related tinnitus rat model.

GABA: γ -Aminobutyric acid.

tinnitus.

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Additional file:

Additional file 1: Original data of the experiment.

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