Auditory and Vestibular Research

Effect of acute and chronic salicylate-induced tinnitus on social disorders and aggressive behaviors in animal model

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1- **Tinnitus sufferers report social issues, but nobody knows if this is a direct result**
2- **Salicylate induced tinnitus may have an effect on social behavior and aggression**
3- **Chronic tinnitus increased rats' aggressive behavior compared to acute tinnitus**

ABSTRACT

Background and aim: Many tinnitus sufferers have reported significant difficulties in social situations. Nobody knows for sure if this disability is a direct consequence of tinnitus or not. Due to the possibility of controlling longitudinal data in animal studies (pre and post tinnitus induction and its duration), this study set out to examine how acute and chronic salicylate-induced tinnitus influences on rat social interactions and aggressive behaviors.
Methods: Seven rats have been assigned to the acute salicylate group and seven to the chronic salicylate group with separately sham/salin peers (n=7) for each of them to examine the effects of duration of salicylate-induced tinnitus on social behaviors. Rats has been evaluated using the following measures: auditory brainstem response (ABR), pre-pulse inhibition (PPI), gap pre-pulse inhibition of acoustic startle (GPIAS), social interaction and aggressive behaviors tests. These assessments were conducted at baseline, 6 hours post salicylate injection in acute group and one day after salicylate injection in chronic group (400mg/kg, per day).

Results: GPIAS test was significantly decreased after acute and chronic salicylate injection, which confirmed the induction of tinnitus. Some social contact and aggressive behaviors after salicylate injection were significantly different in both acute and chronic groups. However, other social interactions and aggressive behaviors were significantly increased only after chronic salicylate injections.

Conclusions: According to the findings, salicylate induced tinnitus may have an effect on social behavior and aggression. Rats' aggressive behavior increased following chronic tinnitus induction, suggesting that the duration of tinnitus has an effect.

Keywords: Tinnitus; salicylate; pre-pulse inhibition; social interaction test; aggressive behavior

Introduction

Subjective tinnitus is a frequent auditory disorder that 11.9–30.3% of the general populations experience it, and 3–3.9% of those individuals describe persistent and bothersome tinnitus. Tinnitus is commonly associated with unpleasant emotions, including higher levels of stress, frustration, sleep difficulties, depression and nervousness (1-3). For some people, tinnitus is a huge source of emotional distress and social withdrawal, and it can significantly impact their quality of life (4). Whether tinnitus itself causes a negative response or whether certain personality qualities or psychological circumstances already predispose tinnitus sufferers to high degrees of tinnitus-related misery is not well understood. Animal models can facilitate the study of social interactions and aggressive behaviors associated with tinnitus, due to control over longitudinal data (before and after tinnitus induction, and its duration) and potential confounding factors (age, gender, and hearing loss) (2). So, it is more likely that only one subtype of the ailment is being investigated in animal studies (5).

The salicylate toxicity model has been used by scientists for several years to study potential biochemical and neurophysiological causes of tinnitus. Therapeutic doses due to its mild analgesic, anti-inflammatory, and antipyretic qualities, salicylate is commonly prescribed for moderate headache and has a long history of usage in treating arthritis with rheumatism (6,7). To confirm the occurrence of emotional reactions following tinnitus, it can examine the effect of tinnitus duration on emotional reactions. It can be perusing with acute and chronic models of salicylate-induced tinnitus. Moreover, the animal model of salicylate-induced tinnitus, due to the induction of slight hearing loss (8), can be helpful to distinguishing between social behavioral disorders of hearing loss and tinnitus.

Few studies have attempted to look at how tinnitus affects animal social interaction, despite the fact that tinnitus is linked to major increases in anxiety disorders and emotional distress in people and is therefore expected to affect social interactions and aggressive behaviors (2,4,9). Therefore, in this study, we examine the effect of salicylate induced tinnitus and its duration (acute versus chronic condition), on social and aggressive behavioral disorders in the animal model.

Methods

Animals and experimental design

The Experimental and Comparative Studies Center of the Iran University of Medical Sciences had been requested to acquire twenty-eight male Wistar rats, who were three months old and weighed between 260 and 300 g. All rats were kept in normal laboratory conditions (8, 10) and they were placed in small groups of 3 to 4 inside the cage to avoid social isolation stress. The cages were kept in a quiet room to reduce the disturbing effects of noise. The research project was approved by the Ethics Committee of the Iran University of Medical Sciences (IR.IUMS.REC.1400.842) and implemented the guidelines for handling and usage of animals for research purposes (National Institutes of Health Publication No. 80-23, revised 1978).

Five days before the tinnitus and social and aggressive behavior tests, the animals' hearing threshold was evaluated using the Auditory Brainstem Response (ABR) instrument.

Within five days of handling, normal hearing rats were divided into two groups: acute (saline, n=7; salicylate, n=7) and chronic (salin, n=7; salicylate, n=7). The ABR, tinnitus and social behaviors tests were performed before, 2 and 6 hours post salicylate injection in acute group and one day after salicylate injection (400 mg/kg per day) in chronic group between the hours of 9 a.m. and 4 p.m. (8,11). The study's experimental setup is schematically illustrated in Fig.1.

Drug administration

Sodium salicylate (Merck, CAS 54-21-7) was freshly dissolved in 0.9% saline at a concentration of 200 mg/ml and intraperitoneally injected at the dose of 400 mg/kg. In acute group, salicylate was injected at one time (at 8:00 a.m.); while in chronic group, in order to reduce the mortality rate of repeated injections of high doses of salicylate, a dosage of 200 mg/kg was injected twice daily (at 8:00 a.m. and 16:00 p.m.) for fourteen consecutive days (11). Rats in the saline group received the same volume of saline.

Auditory brainstem response

The auditory brainstem response (Biologic/Navigator Pro, USA) was conducted to evaluate hearing threshold under anesthesia with ketamine (10%, 80 mg/kg, intraperitoneally) and xylazine (2%, 10 mg/kg, intraperitoneally) (8, 10). Via a high-frequency loudspeaker placed 3 cm away from the right ear of the rat, acoustic stimuli were delivered. These stimuli included click and tone bursts of 4, 8, 12, and 16 kHz, with a duration of 5 ms and a rise/fall time of 2 ms. Evoked potentials were recorded from the intensity of 70 dBSPL to the threshold level by subcutaneous needle electrodes (on vertex, right ear and left ear). Wave II was monitored (8, 10).

Tinnitus evaluation

The SR-LAB system (San Diego Instrument, San Diego, CA) was utilized to administer the pre-pulse inhibition (PPI) and gap pre-pulse inhibition of the acoustic startle (GPIAS) tests, which are performed to evaluate tinnitus (8, 10). The stimuli were implemented in accordance with the research conducted by Turner and Parrish (12). Trials (10 startle trials and 10 gap trials in the GPIAS test or pre-pulse trials in the PPI test) have been conducted in a quasi-random fashion with inter stimulus intervals of 15 to 20 s. A brief burst of broadband noise (BBN)

lasting 20 ms at 115 dBSPL was used to trigger startle reactions. Half of the animals were evaluated using the PPI test first, while the other half were evaluated using the GPIAS test. The mean percentage of GPIAS and PPI before and after tinnitus induction was compared in acute and chronic salicylate and saline groups. Prior to social interaction evaluations, tinnitus assessments were conducted.

Social interaction and aggressive behavior tests

An open field equipment was used to administer the social interaction and aggressive behavior tests (SIT), while a recording device was positioned one hundred centimeters over the focal point of the setup to capture the results. Half an hour prior to the tests, the animal was transported into the testing room and put inside the box to allow it to adjust to the new environment. To begin, an un-manipulated male rat that matched the study rat's age and weight was placed in the open field apparatus. After that, the testing rat was set free in the far left corner of the arenas, and its every move was recorded for a duration of twenty minutes (10). Social interaction criteria were partner following number and time, struggling number and time, partner grooming number and time and sniffing number. Aggressive behaviors contained biting, fisting, wounding, attacking numbers with latency time to first attack (2). Mean of each parameter before and after tinnitus induction was compared in acute and chronic salicylate groups.

Statistical analysis

We used PRISM v9 software to do the statistical analysis. All four tests (ABR, PPI, GPIAS, and SIT) were analyzed using a two-way repeated measures ANOVA, and subgroups were analyzed using either Tukey's or Sidak's multiple comparisons test. Statistical significance was determined whenever $P<.05$.

We reported the data as mean ± standard error of the mean.

Results

Effect of salicylate administration on hearing threshold

Two-way repeated measures ANOVA analysis of hearing thresholds were significantly different before and after salicylate injection at all of stimuli (click and tone bursts) in acute group (400mg/kg, at one time, Fig. 2.A, $F(1,30)=80.44$, $p<0.0001$; $p=0.049$, CI95%=(-9.98_-0.02); $p=0.049$, CI95%=(-9.98_-0.02); $p<0.0001$, CI95%=(-14.27_-4.31); $p<0.0003$, CI95%=(-13.55_-3.59); $p<0.0003$, CI95%=(-13.55_-3.59), respectively) and 8, 12 and 16 kHz tones in chronic group (200 mg/kg, twice a day, and fourteen consecutive days, Fig. 2.B, $F(1,30)=100.3$, $p<0.0001$; $p=0.0003$, CI95%=(-12.49_-3.23); $p<0.0001$, CI95%=(-16.06_-6.79); $p<0.0001$, CI95%=(-16.78_-7.51), respectively); while there were not significant in saline groups (Fig. 2.C, D, $p>0.05$).

Effect of salicylate administration on tinnitus evaluation

According to Fig.3.B, two-way repeated measures ANOVA analysis of the GPIAS test results in acute group showed significant differences before and 6 hours after salicylate injection (400mg/kg, at one time, $F(2,24)=7.086$, $p=0.0038$; $p=0.0009$, CI 95%=(11.47_45.23); while it was not significant 2 hours' post ($p>0.05$). Moreover, the GPIAS test results in chronic group indicated significantly decreased after salicylate injection (200

mg/kg, twice a day, and fourteen consecutive days, F (1,12)=102.9, p<0.0001; p<0.0001, CI95%=(42.71_63.23), Fig. 3.D). As well as, results from the PPI test remained relatively consistent across the two groups (p>0.05, Fig.3.A,C, respectively). Also, in the saline groups, GPIAS and PPI tests results were not significant (p>0.05, Fig.3.A-D, respectively).

Effect of salicylate administration on social interaction test and aggressive behavior.

The two-way repeated measures ANOVA analysis of partner following number and time revealed significant reductions after salicylate injection in acute (400mg/kg, at one time) and chronic (200 mg/kg, twice a day, and fourteen consecutive days) groups (F(1.12)=21.95, p=0.0005; p=0.0378, CI95%=(0.23_8.05); p=0.0041, CI95%=(2.09_9.91); F(1.12)=28.92, p=0.0002; p=0.0218, CI95%=(5.31_65.26); p=0.0012, CI95%=(24.03_83.97), respectively); while there was not difference between groups after salicylate injection (p>0.05, table.1). The results of partner grooming number and time were not significant in both groups before and after salicylate injection (p>0.05, table.1). The struggling number and time were significantly incremented before and after acute and chronic induced tinnitus (F(1,12)=75.14, p<0.0001; p=0.0055, CI95%=(-15.36_-2.92); p<0.0001, CI95%=(-26.93_-14.5); F(1,12)=91.14, p<0.0001; p=0.0004, CI95%=(-74.2_-25.8); p<0.0001, CI95%=(-102.2_-53.8), respectively); as well as, there was significant difference between two groups after salicylate injection (F(1,12)=11.29, p=0.0057; p=0.0002, CI95%=(-17.38_-5.76); F(1,12)=4.361, p=0.0587; p=0.0138, CI95%=(-50.61_-5.39), respectively, table.1). Moreover, the results of sniffing number, wounding number, attacking number, latency time to first attack, fisting number and were significantly increased only after chronic (200 mg/kg, twice a day, and fourteen consecutive days) salicylate injection (F(1,12)=13.5, p=0.0032; p=0.002, CI95%=(-2.27_-0.59); F(1,12)=7.2, p=0.0199; p=0.0163,CI95%=(-1.29_-0.14); F(1,12)=47.3, p<0.0001;p<0.0001, CI95%=(-20.33_9.67); F(1,12)=11.11, p=0.006; p=0.0014, CI95%=(-427.9_-119.2); F(1,12)=27.84, p=0.0002; p<0.0001, CI95%=(-14.35_-5.94), respectively); also, there was significant difference between two groups after salicylate injection (F(1,12)=6, p=0.0306; p=0.004, CI95%= (-1.93_-0.36); F(1,12)=3.2, p=0.0989; p=0.0364, CI95%=(-1.11_-0.03); F(1,12)=10.85, p=0.0064; p=0.0002, CI95%=(-14.69_-4.74); F(1.12)=9.4, p=0.0098; p=0.0004, CI95%=(-406.3_-117.9); F(1,12)=11.8p=0.0049, p=0.0001, CI95%=(-11.93_-4.07), respectively, table.1). As well as, biting number was significantly increased only after salicylate induced tinnitus (F(1,12)= 6, p=0.0306); while there was not difference between groups after salicylate injection (p>.05, table.1). There was not significant difference before and after acute or chronic saline injection (p>0.05, table.1).

Discussions

In the present study, the ABR results showed a significant increase after salicylate injection in the acute (in all stimuli) and chronic (in 8, 12 and 16 kHz tones) groups. However, similar to others studies (8, 13), hearing thresholds increased nearly 0–20 dB in individual rats, which did not affect behavioral assessments. The PPI test results did not change significantly after salicylate injection, which, in accordance with previous studies (8, 12), showed the appropriate hearing sensitivity of rats during the behavioral evaluations. Furthermore, the GPIAS test

was significantly decreased after acute and chronic salicylate injection, which confirmed the induction of tinnitus. However, a significant decrease in GPIAS test was observed only 6 hours post salicylate injection, not 2 hours later. In addition, prior research has demonstrated that GPIAS test results significantly decline at intervals ranging from 30 minutes to 14 hours following an acute injection of salicylate (8, 14-16). Moreover, the rats' behavior changed after tinnitus induction. Findings of this research demonstrated that some social contact (partner following number and time, additionally struggling number and time) were significantly different in both acute and chronic groups. However, other social interaction (partner sniffing number) and all of the aggressive behaviors (biting, fisting, wounding and attacking number and latency time to first attack) were significantly increased only after chronic salicylate injections. Moreover, according to the findings, the rats' aggressive behavior significantly increased following chronic tinnitus compared to acute tinnitus, suggesting that the duration of tinnitus has an effect. As well as, the saline group did not show any significant behavioral changes, indicating that the injection had no effect on the test. When animals exhibit less social contacts and more aggressive behaviors, it messes with their ability to communicate, particularly with another gender, and it can lead to a lot of stressful situations. Despite the high prevalence of negative emotions associated with tinnitus, the underlying mechanism is still poorly understood. As well as it is challenging to separate the emotional implications of hearing loss from specific cases of tinnitus in listeners who experience both (2, 5). The inherent diversity of the tinnitus population is a major drawback of clinical investigations and, maybe, an advantage of an animal model. Since the causative condition can be more easily and precisely controlled in animal studies, it is more likely that only one subtype of the ailment is being investigated (5). Social interaction and aggressive behaviors are common measures of anxiety in rodents. Some studies showed that social interaction tests results were abnormal post acoustic overexposure or salicylate injection (2). Guitton showed that salicylate induced tinnitus (4-day injection) causes profound social abnormalities in mice. However, in contrast to our findings, salicylate treated mice had an irrational increase in the number of conspecific followings, even though their total social contacts were much lower. Moreover, in line with our study, tinnitus mice had greater frequency of grooming (4). Consistent with our research, Zheng et al. discovered that noise induced tinnitus animals are more aggressive when interacting with other animals (tinnitus or sham) and sham animals are also aggressive when approached with tinnitus animals. The findings imply that tinnitus rats are more likely to initiate interactions, but these interactions may be inappropriate, leading to more aggressive occurrences between tinnitus animals and their counterpart. Therefore, sham animals often escape from tinnitus animals. Evidence from their study suggests that tinnitus causes complicated alterations in social interaction in rats, beyond those explained by elevated anxiety levels alone (9). However, sound exposure may be the method of choice for producing hearing impairment in studies aiming to examine the mechanisms behind emotional distress, while tinnitus can cause distress in some (30-80%) (2, 5, 6, 8). Lauer et al. demonstrated that social interaction test was decreased in almost all animals noise exposed and acute salicylate-treated rats (2). However, they did not screen rats for induction tinnitus due to the limited duration of effect span of single dosages of salicylate, while the onset time of acute salicylate induced tinnitus was different in previous studies (between 30 min to 6 hours) (8, 14-16). Also, some articles did not found any behavior related signs of tinnitus post-acute salicylate administration (11). While

investigating potential mechanisms of salicylate-induced tinnitus, it is vital to keep in mind the differences between the effects of acute and chronic salicylate treatment (6). Most research has focused on acute salicylate-induced tinnitus; however, the result of various studies supports the idea that tinnitus develops over time, and they show that the auditory system reacts differently to a single dose of salicylate than it does to multiple doses (6, 11). Yi et al. demonstrated that the response to acute salicylate-treatment is more of a short-term stress reaction than a permanent one. Furthermore, the transition from acute to chronic tinnitus is not well understood, nor is the role of non-auditory areas during tinnitus (11).

Tinnitus, according to new research, develops as a result of maladaptive neuroplastic alterations in a dispersed network of neurons that includes parts of the auditory system as well as direct and indirect interconnections with other parts of the brain linked to alertness, nervousness, tension, and concentration (1, 2, 11). People with tinnitus may experience a wide range of behavioral changes, some of which will manifest at the individual level and others at the societal level (2). Although salicylate can cause tinnitus in both humans and animals, few studies have looked into whether or not it also causes social behavior disorders (especially in chronic injection). The utilization of animal models has frequently been employed to establish the perceptual auditory component of tinnitus, although its emotional ramifications have not been extensively explored. Our results suggest that salicylate induced tinnitus may have an effect on social behavior and aggression. Animal studies cannot be fully generalized to human studies due to inherent differences in the etiologies of tinnitus, variations in the duration of tinnitus (acute and chronic criteria) and other relevant factors. These findings lend support to the notion that the psychological and social difficulties experienced by tinnitus patients should not be disregarded, but rather treated as an integral aspect of the condition. Through the implantation of social behavioral tests, it is feasible to investigate the impact of different treatments or interventions on tinnitus associated behavior and mood disturbances, in addition to evaluating tinnitus itself. Given the potential impact of tinnitus on interpersonal communication with the opposite sex, it is recommended that this variable be explored in future research.

Conclusion

Research on animals is very helpful in understanding the mechanics of tinnitus development, since human research have methodological and ethical limitations in this area. This study examines how duration of salicylate induced tinnitus (acute versus chronic) influences rat social interactions and aggressive behaviors. Our findings show that both of acute and chronic salicylate induced tinnitus may have a negative effect on social behavior and aggression. However, aggressive behaviors increased following chronic tinnitus induction, suggesting that the duration of tinnitus has an effect.

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Author contribution

MR: Study design, acquisition of data, interpretation of the results, Statistical analysis, drafting and revision the manuscript, MA: Study design, interpretation of the results, Statistical analysis, revision the manuscript

MF: Acquisition of data

Reference

1. Chen YC, Chen GD, Auerbach BD, Manohar S, Radziwon K, Salvi R. Tinnitus and hyperacusis: Contributions of paraflocculus, reticular formation and stress. Hear Res. 2017;349:208-22. DOI: 10.1016/j.heares.2017.03.005
2. Lauer AM, Larkin G, Jones A, May BJ. Behavioral Animal Model of the Emotional Response to Tinnitus and Hearing Loss. J Assoc Res Otolaryngol. 2018;19(1):67-81. DOI: 10.1007/s10162-017-0642-8
3. Winne J, Boerner BC, Malfatti T, Brisa E, Doerl J, Nogueira I, et al. Anxiety-like behavior induced by salicylate depends on age and can be prevented by a single dose of 5-MeO-DMT. Exp Neurol. 2020;326:113175. DOI: 10.1016/j.expneurol.2020.113175
4. Guitton MJ. Tinnitus-provoking salicylate treatment triggers social impairments in mice. J Psychosom Res. 2009;67(3):273-6. DOI: 10.1016/j.jpsychores.2008.10.017
5. Bauer CA, Brozoski TJ. Assessing tinnitus and prospective tinnitus therapeutics using a psychophysical animal model. J Assoc Res Otolaryngol. 2001;2(1):54-64. DOI: 10.1007/s101620010030
6. Stolzberg D, Salvi RJ, Allman BL. Salicylate toxicity model of tinnitus. Front Syst Neurosci. 2012;6:28. DOI: 10.3389/fnsys.2012.00028
7. Samlan SR, Jordan MT, Chan SB, Wahl MS, Rubin RL. Tinnitus as a measure of salicylate toxicity in the overdose setting. West J Emerg Med. 2008;9(3):146-9.
8. Rezapour M, Akbari M, Dargahi L, Zibaii MI, Shahbazi A. The Auditory Brainstem Response (ABR) Test, Supplementary to Behavioral Tests for Evaluation of the Salicylate-Induced Tinnitus. Indian J Otolaryngol Head Neck Surg. 2023;75(Suppl 1):6-15. DOI: 10.1007/s12070-022-03117-x
9. Zheng Y, Hamilton E, McNamara E, Smith PF, Darlington CL. The effects of chronic tinnitus caused by acoustic trauma on social behaviour and anxiety in rats. Neuroscience. 2011;193:143-53. DOI: 10.1016/j.neuroscience.2011.07.026
10. Rezapour M, Farrahizadeh M, Akbari M. Effectiveness of transcutaneous vagus nerve stimulation (tVNS) on salicylate-induced tinnitus. Neurosci Lett. 2024;822:137639. [DOI:10.1016/j.neulet.2024.137639]
11. Yi B, Hu S, Zuo C, Jiao F, Lv J, Chen D, et al. Effects of long-term salicylate administration on synaptic ultrastructure and metabolic activity in the rat CNS. Sci Rep. 2016;6:24428. DOI: 10.1038/srep24428
12. Turner JG, Parrish J. Gap detection methods for assessing salicylate-induced tinnitus and hyperacusis in rats. Am J Audiol. 2008;17(2):S185-92. DOI: 10.1044/1059-0889(2008/08-0006)
13. Radziwon KE, Stolzberg DJ, Urban ME, Bowler RA, Salvi RJ. Salicylate-induced hearing loss and gap detection deficits in rats. Front Neurol. 2015;6:31. DOI: 10.3389/fneur.2015.00031
14. Berger JI, Coomber B, Shackleton TM, Palmer AR, Wallace MN. A novel behavioural approach to detecting tinnitus in the guinea pig. J Neurosci Methods. 2013;213(2):188-95. DOI: 10.1016/j.jneumeth.2012.12.023
15. Sun W, Doolittle L, Flowers E, Zhang C, Wang Q. High doses of salicylate causes prepulse facilitation of onset-gap induced acoustic startle response. Behav Brain Res. 2014;258:187-92. DOI: 10.1016/j.bbr.2013.10.024
16. Martel DT, Pardo-Garcia TR, Shore SE. Dorsal Cochlear Nucleus Fusiform-cell Plasticity is Altered in Salicylate-induced Tinnitus. Neuroscience. 2019;407:170-81. DOI: 10.1016/j.neuroscience.2018.08.035

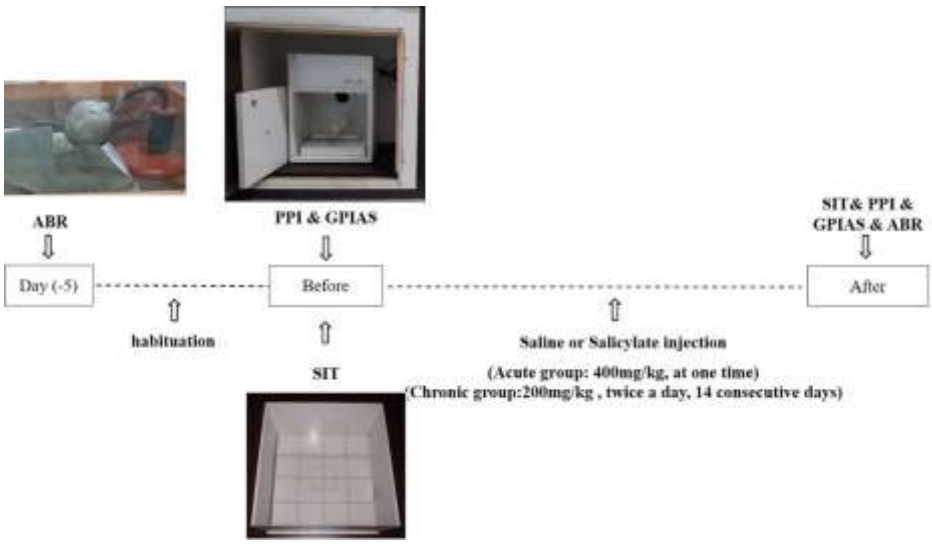

Fig.1. Schematic illustration of experimental design of study. ABR: auditory brainstem response, PPI: pre-pulse inhibition, GPIAS: gap pre-pulse inhibition of the acoustic startle, SIT: social interaction and aggressive behavior test.

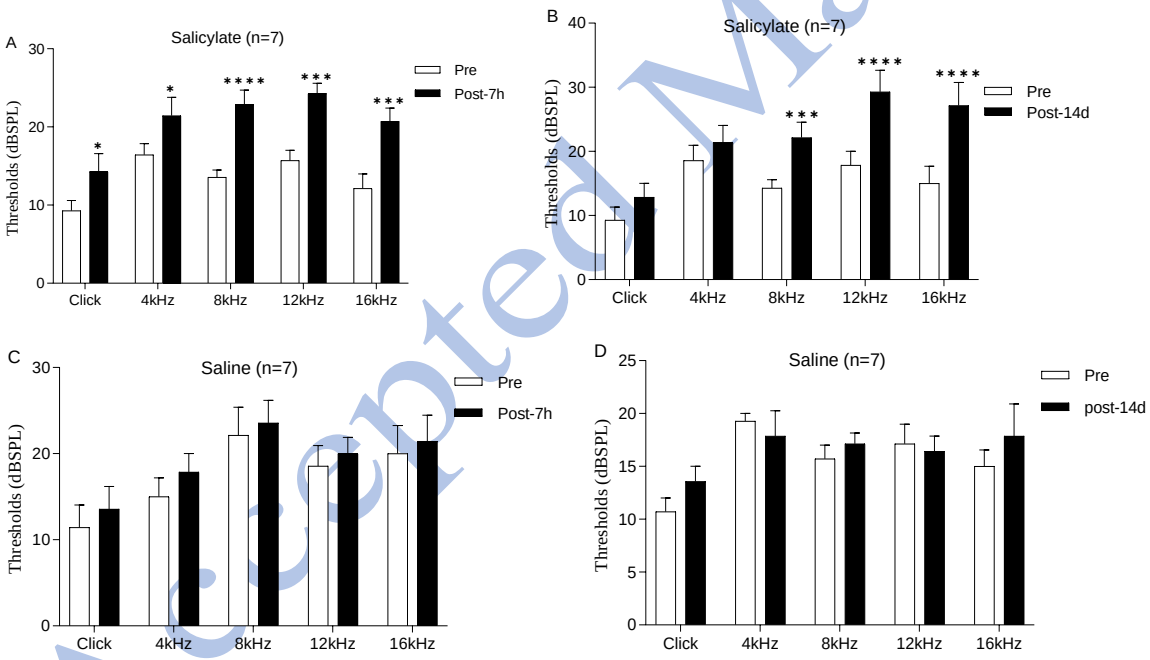

Fig.2. Effect of acute and chronic salicylate administration on hearing threshold of auditory brainstem response. The results are presented as mean±SEM. The differences between groups were determined by two way repeated measures ANOVA followed by Sidak test. * P<0.05, ***P<0.001and, ****p<0.0001.

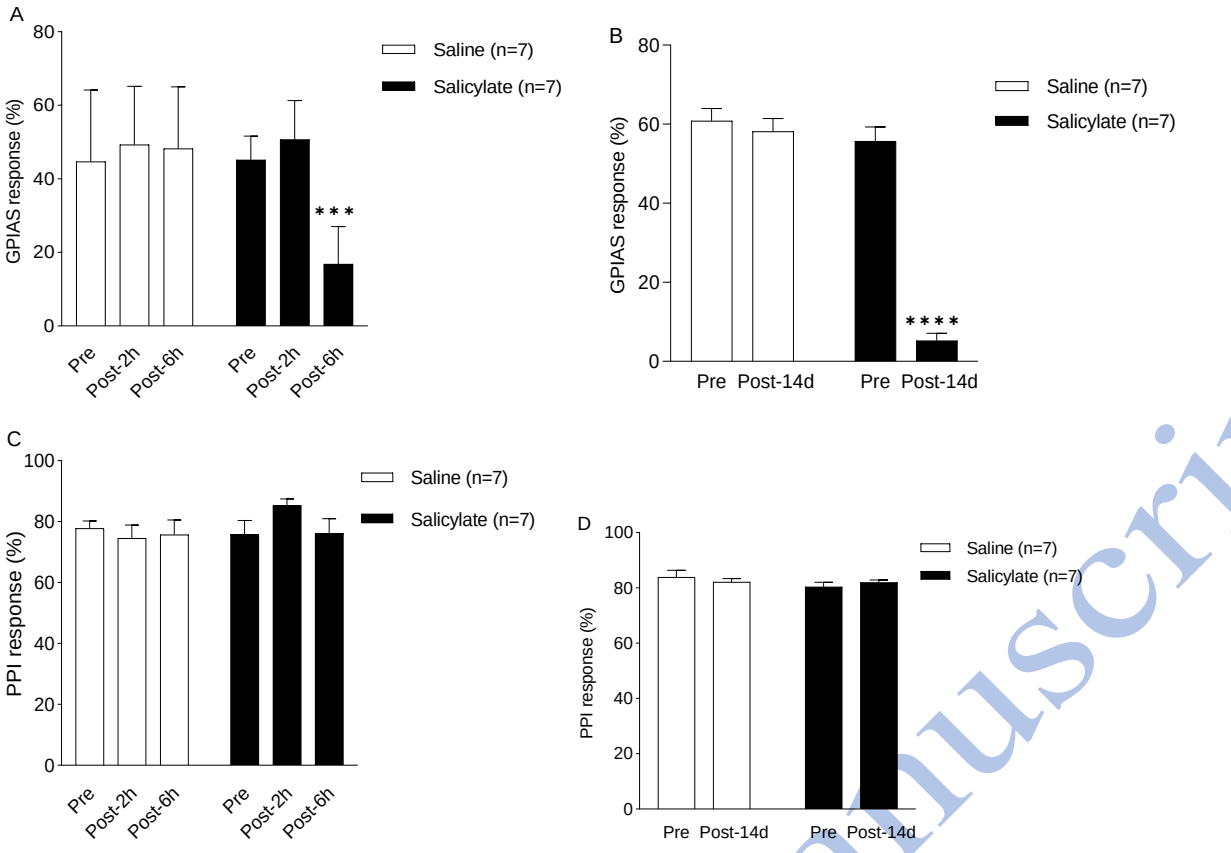

Fig.3. Effect of acute and chronic salicylate administration on gap pre-pulse inhibition of the acoustic startle and pre-pulse inhibition tests. The results are presented as mean±SEM. The differences between groups were determined by two-way repeated measures ANOVA followed by Tukey test. *** P<0.001and, ****p<0.0001.

Table.1. Effect of acute and chronic saline and salicylate injection on social interaction and aggressive behaviors tests.

Group/ Test			partner following number & time	partner grooming number & time	struggling number & time	partner sniffing number	wounding number	attacking number	latency time to first attack	fisting number	biting number
Acute	saline	Pre	7±1.4	0.43±0.3	0.28±0.18	0.14±0.14	0	0.14±0.14	4.29±4.28	0.29±0.18	0
			45.29±9.42	0.71±0.47	0.43±0.3						
		Post	7.71±1.21	0.57±0.2	0.29±0.18	0.28±0.18	0	0	0	0.14±0.14	0
			45.71±5.15	0.86±0.59	0.57±0.43						
	salicylate	Pre	7.71±1.27	0.71±0.47	0	0.14±0.14	0	0	0	0	0
			51.57±8.5	1.43±1.02	0						
		Post	3.57±0.92 *	0.71±0.18	9.14±1.47 **	0.43±0.2	0.14±0.14	5.58±0.94	11.43±5.03	2.14±0.63	0.29±0.18
			16.29±4.38 *	1.43±0.61	50±9.04***						
Chronic	saline	Pre	8.86±1.7	0.71±0.47	0.14±0.14	0.29±0.18	0	0.14±0.14	5.71±5.71	0.14±0.14	0
			51.71±14.54	1.14±0.77	0.57±0.57						
		Post	7.71±1.73	0.57±0.3	0.29±0.18	0.43±0.3	0	0.14±0.14	13.86±13.86	0.14±0.14	0
			57.14±16.31	0.57±0.57	0.71±0.47						
	salicylate	Pre	9±1.67	0.14±0.14	0	0.14±0.14	0	0	0	0	0
			62±14.52	0.57±0.57	0						
		post	3±0.62**	1±0.3	20.71±3.11****	1.57±0.37**	0.71±0.29*	15±2.8****	273.6±85.36**	10.14±2.24****	0.57±0.3
			8±1.59**	2.86±1.03	78±9.9 ****						
P					###	##	#	###	###	###	
					#						

* Pre vs post-acute or chronic saline or salicylate injection. *P<0.05, **P<0.01, ***p<0.001, ****p<0.0001.
# Post-acute vs chronic salicylate induced tinnitus. # P<0.05, ## P<0.01and, ### p<0.001.