研究ノート

胎生後期におけるセロトニン5-HT_{1A}受容体作動薬暴露は成長後の仔マウスの聴覚性驚愕反応のプレパルス抑制に影響を及ぼした

Exposure to a 5-HT_{1A} agonist during late pregnancy affects prepulse inhibition in offspring C57BL/6J mice during adulthood

Abstract

Serotonin (5-hydroxytryptamine; 5-HT) is a monoamine neurotransmitter, and its effects are mediated *via* 5-HT receptors (5-HTRs). Of the several subtypes of 5-HTRs, the 5-HT_{1A} receptor is thought to be involved in emotional behaviors and psychiatric disorders. Previous studies have revealed that gene modification and the blockade of 5-HT_{1A} receptors during early developmental periods cause anxiety-like behavior later in adulthood. However, how abnormal 5-HT_{1A} receptor activation during early developmental stages affects emotional behavior remains unclear. In the present study, we examined the effect of transplacental exposure to a 5-HT_{1A} agonist [8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide; 8-OH-DPAT] on the behavior of offspring during adulthood. Mice exposed to 8-OH-DPAT from embryonic day 14 to parturition showed normal development, and showed no difference in locomotion and anxiety- or fear-related behaviors compared to saline-exposed mice. However, 8-OH-DPAT-exposed mice exhibited impaired prepulse inhibition in the acoustic startle response. These results suggest that excessive activation of 5-HT_{1A} receptors during late pregnancy may alter neural development and subsequent information processing.

***Key Words*:** serotonin, 5-HT_{1A} receptor, 8-OH-DPAT, prepulse inhibition, mouse, development

山　田　一　之*

*　本学経営学部　特任教授

Ⅰ. Introduction

Serotonin (5-hydroxytryptamine; 5-HT) is a monoamine neurotransmitter thought to be involved in mood- and anxiety-related behaviors, as well as in the etiology of psychiatric disorders, such as depression, obsessive compulsive disorder, and panic disorder [1]. The effects of 5-HT are mediated *via* 5-HT receptors (categorized into 5-HT_1 to 5-HT_7) [1]. Among the many 5-HT receptor subtypes, the 5-HT_{1A} receptor has been well studied [2]. During the early developmental stage, 5-HT acts as a morphogenic factor, exerting effects on neuronal development. For example, a delayed establishment of serotonergic projections to the midbrain and interbrain has been observed in both 5-HT_{1A} receptor knockout (5-HT_{1A} KO) and overexpressing (5-HT_{1A} OE) mice [3]. 5-HT_{1A} KO and 5-HT_{1A} OE mice also show behavioral abnormalities. 5-HT_{1A} KO mice show increased anxiety- and stress-like behaviors [4], decreased exploratory behavior, and increased fear-related behavior [5]. In contrast, 5-HT_{1A} OE mice exhibit decreased anxiety-like behavior [6].

As the expression of 5-HT_{1A} receptors starts on embryonic days 12 to 14 [2], the disturbance of neural transmission *via* the alteration of 5-HT_{1A} receptors during early maturational periods may affect neural development and have subsequent behavioral consequences. Indeed, blockade of 5-HT_{1A} receptors by the 5-HT_{1A} receptor antagonist WAY-100,653 during early postnatal periods exerts long-lasting effects on anxiety [7]. Moreover, knockdown of 5-HT_{1A} receptors in the raphe nucleus of mice during postnatal days (P)14 to 30 results in increased anxiety and decreased social investigation in adulthood [8]. These results strongly indicate that the intact expression and function of 5-HT_{1A} receptors is required for the normal development of emotional behavior. Although aberrant 5-HT levels during early developmental stages have been shown to affect the emotional behaviors of adults in both humans [9] and animals [10], it is unclear whether abnormal 5-HT_{1A} receptor activity during early development affects adult behavior.

In the present study, we examined how abnormal 5-HT_{1A} receptor activation during the embryonic period influenced adult behavior. For this purpose, we employed a mouse model wherein the embryos were continuously exposed to a full 5-HT_{1A} agonist [8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide; 8-OH-DPAT] *via* the placenta.

Ⅱ. Materials and methods

Animals: Six-week-old male (n=5) and female (n=10) C57BL/6J mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). Mice were acclimated to the breeding conditions for 1 week, and were then allowed to mate (one male and two females per cage). Every morning during the mating period, female mice were manually examined for signs of copulation. When copulation was confirmed, females were separated from males and individually housed; the day of separation was designated embryonic day 0 (E0). All newborn mice were weaned at 4 weeks of age, separated based on sex, and housed with 3–4 littermates per cage. In the present study, only male mice were used as subjects. At 10 weeks of age, mice were individually housed for 1 week prior to starting the behavioral experiments. The breeding and experiment rooms were air-conditioned (22 ± 1°C, 50–60% humidity), and the mice were kept on a 12-h light/dark cycle (lights on at 0800). Food and water were freely available in the breeding cages. All behavioral experiments, with the exception of home-cage activity measurements, were conducted during the light cycle (between 1300 and 1700). All animal experimental procedures in the present study were performed in strict accordance with the guidelines of the Institute of Physical and Chemical Research (RIKEN), and were approved by the institute's Animal Investigation Committee.

Drug treatment: (±)-8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide (8-OH-DPAT) (Cat. No: 0529: TOCRIS, MO, USA) was diluted in physiological saline at room temperature (22 ± 1°C). The final concentration

of the drug solution was 3mM. The drug solution was enclosed in an osmic pump (Alzet 1007D, Muromachi Kikai, Tokyo, Japan) and administered at a dosage of 1 mg/mouse/week (about 0.1 mg/day). Pregnant female mice were anesthetized by intraperitoneal infusion (i.p.) of 2,2,2-tribromoethanol (Avertin) (CAS-No: 75-80-9, Nacalai Tesque, Kyoto, Japan) solution (1.25%, 300 μl/mouse)[1)], and pumps were subcutaneously implanted into the lower part of the neck. Female mice were kept warm in their home cages until awakening. The time course of drug exposure and the experimental schedule is summarized in Figure 1.

Behavioral assessment: Behavioral assessment began when mice were 11 weeks of age. Behavioral examinations were conducted as previously described [10, 11], and consisted of the following procedures: 24-h home-cage activity measurement for 1 week, open field test [in a 50 (W) × 50 (D) × 40 (H) cm white plastic arena] for 15 min, light-dark box test [in a 20 (W) × 20 (D) × 20 (H) cm, white and black box with a 3 × 5 cm sliding door in the center panel] for 10 min, elevated plus maze test [closed arms: 25 (W) × 5 (D) × 15 (H) cm; open arms 25 (W) × 5 (D) × 0.3 (H) cm; center platform: 5 × 5 cm] for 5 min, acoustic startle response (ASR) and prepulse inhibition (PPI) measurement, and classical fear conditioning (consisting of conditioning, context, and cued testing phases).

For ASR and PPI measurements, each mouse was placed into a small plastic tube (30 or 35 mm in diameter and 12-cm-long) that was set on a vibration sensor block in a sound-proofed chamber [60 (W) × 50 (D) × 67 (H) cm]. A dim light was fixed on the ceiling of the chamber (10 Lux at the center of the sensor block), and continuous 65-dB white noise was provided as background noise. The mice were acclimatized to the plastic tube and experimental environment for 5 min before the experimental session began. The experimental session consisted of an initial startle measurement, a startle threshold measurement, a prepulse inhibition test, and a final startle measurement. During the initial startle measurement, a 120-dB startle stimulus (40 ms) was presented 10 times with a random inter-trial interval (10–20 s). During the startle threshold measurement, the startle responses to stimuli presented at various intensities were assessed. Each of the white noise stimuli (70, 75, 80, 85, 90, 95, 100, 110, and 120 dB; 40 ms) were presented five times in a quasi-random order, and with random inter-trial intervals (10–20 s). During the prepulse inhibition test, the mice experienced five types of trials: no stimulus, startle stimulus only (120 dB, 40 ms), prepulse 70 dB and pulse 120 dB, prepulse 75 dB and pulse 120 dB, and prepulse 80 dB and pulse 120 dB. The prepulse duration was 20 ms, and the lead time (duration between the beginning of the prepulse and the beginning of the pulse) was 100 ms. Each type of trial was presented 10 times in a quasi-random order with random inter-trial intervals (10–20 s). During the final startle measurement, only a 120-dB startle stimulus (40 ms) was presented 10 times with random inter-trial intervals (10–20 s). The total duration of the ASR and PPI test was approximately 40 to 45 min.

Data collection and analysis were conducted using Image J OF4 (open field test), Image J LD4 (light-dark box test), Image J EPM (elevated plus maze test), Mouse Startle (ASR and PPI), and Image J FZ2 (classical fear conditioning test). All apparatus and software used are commercially available (O'Hara, Tokyo, Japan). Each test was conducted after intervals of 1 to 7 days.

Statistical methods: Statistical analyses were conducted using SPSS™ version 19 statistical software (Japan IBM, Tokyo, Japan) and Excel Statistics version 5.0 statistical software for non-parametric statistics (Esumi, Tokyo, Japan). A Student's t-test was used to compare continuous data, and a Mann-Whitney U-test was used to analyze the ratio data. Repeated testing paradigms were analyzed using a repeated measures analysis of variance

1) The side effects of Avertin have been demonstrated, and Avertin is no longer recommended for use in anesthetizing animals. This research was conducted before these guidelines were set.

[ANOVA; general linear model (GLM)]. When Mauchly's hypothesis of sphericity was not supported, degrees of freedom were modified using the Greenhouse-Geisser method [12]. Bonferroni's method was used for multiple comparisons between groups. Differences with a $p < 0.05$ were deemed statistically significant.

Ⅲ. Results

Mice exposed to 8-OH-DPAT from embryonic day 14 to parturition (PD0) showed normal body weight and development throughout the experiment. Behavioral assessments revealed that both activity and anxiety levels were not altered by drug exposure: home-cage activity measurement (total: t=-0.880, p>0.05), open field test (distance traveled: t=0.303, p>0.05; total center time: t=-0.599, p>0.05), light-dark box test (distance traveled: t=-0.751, p>0.05; % time in light box: U=45, p>0.05), and elevated plus maze test (distance traveled: t=0.153, p>0.05; % time open arms: U=32.5, p>0.05). Drug-exposed mice also showed no abnormalities in contextual and cued fear memory function in a classical fear conditioning test (context test: U=49, p>0.05; cued test: U=30, p>0.05). Results are summarized in Table 1.

In the ASR and PPI test, no differences were observed in the initial and final startle measurement between drug- and saline-exposed mice (main effect of drug: F(1, 18)=0.001, p=0.976; main effect of repeat: F(1, 18)=0.363, p=0.554; *drug x repeat* interaction: F(1, 18)=1.454, p=0.244; data not shown). There were also no differences in the startle threshold measurement (main effect of drug: F(1, 18)=0.064, p=0.803; main effect of repeat: F(2.539, 45.708)=96.609, p=0.000; *drug x repeat* interaction: F(2.539, 45.708)=0.735, p=0.515; Figure 2A). On the other hand, there were statistically significant differences in the PPI between drug- and saline-exposed mice (main effect of drug: F(1, 18)=4.933, p<0.05; main effect of repeat: F(2, 36)=37.142, p=0.000; *drug x repeat* interaction: F(2, 36)=2.869, p=0.070; Figure 2B). Drug-exposed mice exhibited significantly lower percent (%)PPI in 70 dB (F(1, 18)=5.401, p<0.05) and 75 dB (F(1, 18)=5.365, p<0.05) prepulse (PP) conditions, but showed almost identical %PPI in the 80 dB condition (F(1, 18)=0.123, p=0.73, Figure 2B).

Ⅳ. Discussion

In the present study, we examined the effect of 5-HT_{1A} activation during the late embryonic stage on behavior during adulthood. For this purpose, we continuously exposed embryos to the 5-HT_{1A} agonist 8-OH-DPAT *via* an osmotic pump implanted in the dam. The drug was infused at a constant rate from embryonic day 14 to parturition. However, unlike previous studies that have used 5-HT_{1A}-gene-modified mice or early 5-HT_{1A} blockade, our results revealed that drug-exposed mice did not show any alterations in anxiety- or fear-related behaviors on examination with standard home-cage activity measurements, or open field, light-dark box, elevated plus maze, and classical fear conditioning tests (Table 1). Although drug-exposed mice exhibited normal startle responses and startle thresholds, they showed largely impaired prepulse inhibition at both low (70 dB) and intermediate (75 dB) prepulse intensities (Figure 2). The C57BL/6J strain of mice has been reported to display hearing loss with advancing age [13]. However, the mice used in the present study were not old enough to have hearing loss, and mice exposed to drugs showed normal fear responses to auditory stimuli in the classical fear conditioning test, as well as normal startle responses and startle thresholds in the ASR test. Therefore, as the hearing ability was thought to be intact, hearing loss could not explain the PPI deficiency observed in drug-exposed mice.

PPI of the auditory startle response, which is defined as a reduction in the ASR after a weak prepulse stimulus, provides an operational measure of sensorimotor gating. PPI has been observed in both rodents and humans [14, 15]. Moreover, as impaired PPI has been observed in patients as well as in pre-symptomatic individuals with schizophrenia and other mental disorders, it is recognized as an important experimental model for the etiology and treatment of schizophrenia [16]. Previous studies have suggested a relationship between PPI and 5-HT1

receptors using both pharmacological and genetic models [17]. For example, pharmacological studies have shown that administration of the 5-HT_{1A} agonist 8-OH-DPAT increases PPI in mice, while the 5-HT_{1B} agonist anpirtoline induces PPI deficits. However, 5-HT_{1A}-KO mice do not show alterations in PPI, whereas 5-HT_{1B}-KOs show some PPI impairment [17, 18]. Inconsistency among these results may, at least in part, be attributable to strain-related differences in the levels of 5-HT and/or 5-HT1 receptors [19]. As the 5-HT_{1A} KO mice used in the previous studies had 129Sv [18] and Swiss-Webster [4] backgrounds, studies using genetically modified mice with a C57BL/6J background are required.

The present study is preliminary, and therefore, only limited interpretations can be derived. Nevertheless, our findings are meaningful, and suggest that 5-HT_{1A} activation during the late embryonic period could influence behavior and information processing in adulthood. Furthermore, the drug-treated mice used in this study have the potential to provide a new model to study the prevention of schizophrenia and other mental disorders. Previously reported animal models of schizophrenia (e.g., mice treated with the non-competitive NMDAR antagonist MK-801 [20] and Zic2 knockdown mice [21]) show abnormal behavior (e.g., hyperlocomotion) that is representative of patients with the disorder, and provide useful models for treatment. Although these models mimic diagnostic symptoms well, they are not useful models of pre-symptomatic schizophrenia. As the drug-exposed mice in the present study showed no behavioral abnormalities except for PPI impairment, they could prove useful in predicting the onset of mental diseases, as well as for identifying other potential behavioral abnormalities. Detailed behavioral analyses as well as anatomical and histochemical evaluations are required to establish a new effective model.

Ⅴ. Conclusions

In the present study, we found that sustained activation of the 5-HT_{1A} receptor in embryos during late pregnancy altered PPI during adulthood without altering other behaviors. These results suggest that transmission *via* 5-HT_{1A} receptors may affect the development of neural circuits related to information processing. Moreover, our findings suggest that these mice may provide a new experimental model that can be used for further studies on the prevention and treatment of mental diseases.

Acknowledgements

I would like to thank Dr. Kanno J. and Dr. Kitajima S. at the National Institute of Health Sciences and Professor Tanemura K. at Tohoku University for inviting me to work on this research project. I would also like to thank Ms. Homma C. for her technical assistance, and Editage (www. Editage.jp) for English language editing. This study was supported in part by Health Sciences Research Grants from the Ministry of Health, Labor, and Welfare, Japan (FY 2008-2010).

References

[1] Murphy DL, Andrews AM, Wichems CH, Li Q, Tohda M, Greenberg B. Brain serotonin neurotransmission: an overview and update with an emphasis on serotonin subsystem heterogeneity, multiple receptors, interactions with other neurotransmitter systems, and consequent implications for understanding the actions of serotonergic drugs. J Clin Psychiatry. 1998;59 Suppl 15:4-12.

[2] Altieri SC, Garcia-Garcia AL, Leonardo ED, Andrews AM. Rethinking 5-HT_{1A} receptors: emerging modes of inhibitory feedback of relevance to emotion-related behavior. ACS Chem Neurosci. 2013;4(1):72-83. doi: 10.1021/cn3002174. Epub 2012 Dec 20.

[3] Deng DR, Djalali S, Höltje M, Grosse G, Stroh T, Voigt I, Kusserow H, Theuring F, Ahnert-Hilger G, Hörtnagl H. Embryonic and postnatal development of the serotonergic raphe system and its target regions in 5-HT_{1A} receptor deletion or overexpressing mouse mutants. Neuroscience. 2007;147(2):388-402. Epub 2007 Jun 1.

[4] Parks CL, Robinson PS, Sibille E, Shenk T, Toth M. Increased anxiety of mice lacking the serotonin1A receptor. Proc Natl Acad Sci U S A. 1998;95(18):10734-9.
[5] Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D, Hen R. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci U S A. 1998;95(24):14476-81.
[6] Kusserow H, Davies B, Hörtnagl H, Voigt I, Stroh T, Bert B, Deng DR, Fink H, Veh RW, Theuring F. Reduced anxiety-related behaviour in transgenic mice overexpressing serotonin 1A receptors. Brain Res Mol Brain Res. 2004;129(1-2):104-16.
[7] Vinkers CH, Oosting RS, van Bogaert MJ, Olivier B, Groenink L. Early-life blockade of 5-HT(1A) receptors alters adult anxiety behavior and benzodiazepine sensitivity. Biol Psychiatry. 2010;67(4):309-16. doi: 10.1016/j.biopsych.2009.08.013. Epub 2009 Oct 6.
[8] Donaldson ZR, Piel DA, Santos TL, Richardson-Jones J, Leonardo ED, Beck SG, Champagne FA, Hen R. Developmental effects of serotonin 1A autoreceptors on anxiety and social behavior. Neuropsychopharmacology. 2014;39(2):291-302. doi: 10.1038/npp.2013.185. Epub 2013 Aug 2.
[9] Oberlander TF, Gingrich JA, Ansorge MS. Sustained neurobehavioral effects of exposure to SSRI antidepressants during development: molecular to clinical evidence. Clin Pharmacol Ther. 2009;86(6):672-7. doi: 10.1038/clpt.2009.201. Epub 2009 Nov 4.
[10] Yamada K. Single exposure to antidepressants during infancy is associated with delayed behavioral changes in C57BL/6 mice. WJNS. 2016;6:151-64. dx.doi.org/10.4236/wjns.2016.62019.
[11] Yamada K. Temporal alterations in monoamine metabolism during early development induce emotional behavior disturbances in adult C57BL/6J mice. Environment and Management (Journal of Shizuoka Sangyo University). 2017;23(1):41-55.
[12] Chino, N. A critical review on and around sphericity tests. Bulletin of Faculty of Humanities of Aichigakuin University. 1994;24:103-19.
[13] Ison JR, Allen PD, O'Neill WE. Age-related hearing loss in C57BL/6J mice has both frequency-specific and non-frequency-specific components that produce a hyperacusis-like exaggeration of the acoustic startle reflex. J Assoc Res Otolaryngol. 2007;8(4):539-50. doi: 10.1007/s10162-007-0098-3. Epub 2007 Oct 19.
[14] Swerdlow NR, Caine SB, Braff DL, Geyer MA. The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. J Psychopharmacol. 1992;6(2):176-90. doi: 10.1177/026988119200600210.
[15] Swerdlow NR, Braff DL, Geyer MA. Sensorimotor gating of the startle reflex: what we said 25 years ago, what has happened since then, and what comes next. J Psychopharmacol. 2016;30(11):1072-1081. Epub 2016 Aug 18.
[16] Cadenhead KS, Geyer MA, Braff DL. Impaired startle prepulse inhibition and habituation in patients with schizotypal personality disorder. Am J Psychiatry. 1993;150(12):1862-7.
[17] van den Buuse M. Exploring the role of 5-HT1A receptors in the regulation of prepulse inhibition in mice: implications for cross-species comparisons. ACS Chem Neurosci. 2013;4(1):149-60. doi: 10.1021/cn300118t. Epub 2012 Dec 18.
[18] Dulawa SC, Gross C, Stark KL, Hen R, Geyer MA. Knockout mice reveal opposite roles for serotonin 1A and 1B receptors in prepulse inhibition. Neuropsychopharmacology. 2000;22(6):650-9.
[19] Popova NK, Naumenko VS, Tibeikina MA, Kulikov AV. Serotonin transporter, 5-HT1A receptor, and behavior in DBA/2J mice in comparison with four inbred mouse strains. J Neurosci Res. 2009;87(16):3649-57. doi: 10.1002/jnr.22155.
[20] Torrisi SA, Salomone S, Geraci F, Caraci F, Bucolo C, Drago F, Leggio GM. Buspirone counteracts MK-801-induced schizophrenia-like phenotypes through dopamine D(3) receptor blockade. Front Pharmacol. 2017;8:710. doi:10.3389/fphar.2017.00710.
[21] Hatayama M, Ishiguro A, Iwayama Y, Takashima N, Sakoori K, Toyota T, Nozaki Y, Odaka YS, Yamada K, Yoshikawa T, Aruga J. Zic2 hypomorphic mutant mice as a schizophrenia model and ZIC2 mutations identified in schizophrenia patients. Sci Rep. 2011;1:16. doi: 10.1038/srep00016. Epub 2011 Jun 17.

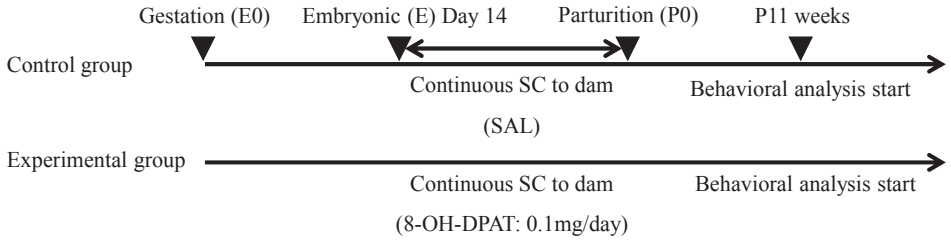

Figure 1 Summary of the time course to the experiment.
P: postnatal Day/Week, SC: subcutaneous administration, SAL: physiological saline

Table 1 Summary of the behavioral assessment

	saline	8-OH-DPAT	statistics
HC activity (mean of 6 days) (count[*1])	744.3	810.1	t=-0.880, p>0.05
Open field			
distance traveled (cm)	7261.6	7135.13	t=0.303, p>0.05
total center time (sec)	191.1	202.3	t=-0.599, p>0.05
Light-dark box			
distance traveled (cm)	2495.0	2589.7	t=-0.751, p>0.05
% time (light box)	297.9	291.2	U=45, p>0.05
Elevated plus maze			
distance traveled (cm)	967.5	948.1	t=0.153, p>0.05
% time (open arms)	30.1	17.0	U=32.5, p>0.05
Classical fear conditioning			
(% time freezing)			
context test	30.1	27.6	U=49, p>0.05
cued test (with tone cue)	20.7	34.3	U=30, p>0.05

*1) arbitrary unit: count 4 – 5 times per sec.

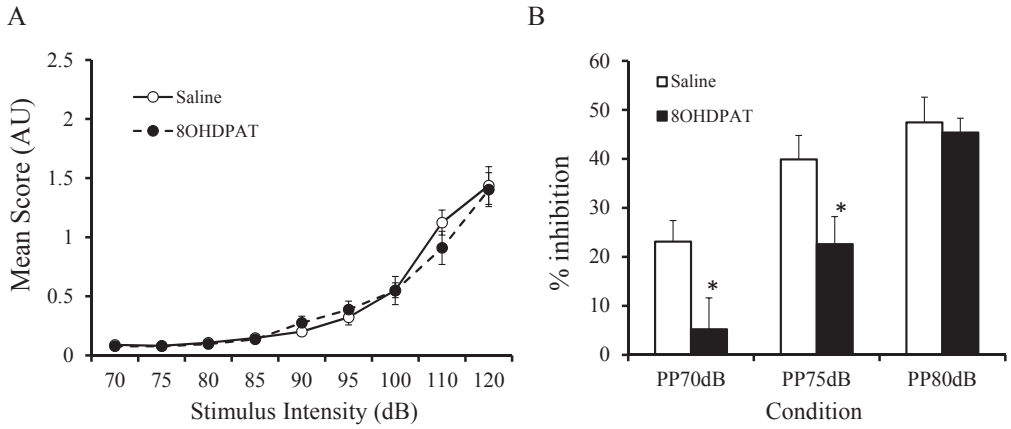

Figure 2 Results of the startle threshold measurement (A) and prepulse inhibition test (B).
AU: arbitrary unit, %inhibition = {1- (startle response with prepulse/startle response to 120dB stimulus)}*100,
Data represent mean ± S.E.M. (A), Data represent mean + S.E.M. , *: p<0.05 (B)