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Effect of Auditory Stimulation on Parasympathetic Nerve Activity in Urethane-anesthetized Rats

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Abstract. *Previously, it has been demonstrated that auditory stimulation with music (Traeumerei [TM] by Schumann) decreased renal sympathetic nerve activity (RSNA) and blood pressure (BP) with a central mechanism, while it is unknown whether TM affects parasympathetic nerve activity. Here, the effects of auditory stimulation with TM on gastric vagal nerve activity (GVNA) in urethane-anesthetized rats were investigated. Auditory stimulation with TM, but not with white noise (WN) caused a significant elevation of GVNA. In addition, exposure to TM increased the number of c-Fos-immunoreactive cells in the auditory cortex (AuC). These findings suggest that exposure to music can increase GVNA through the auditory pathway.*

In the field of music therapy, application of music to the patient is known to elicit various psychological responses (1, 2). In order to alleviate the mental stress related diseases of cardiovascular functions, a pharmacological approach is generally used (3), however, the effectivity of music therapy on hypertensive patients has been recently reported (4). Moreover, auditory stimulation with music lowers heart rate and blood pressure (BP) in human beings (5) or spontaneously hypertensive rats (6), suggesting that music can affect autonomic and cardiovascular function. In regard to autonomic responses, using an electrophysiological technique, it was recently found that auditory stimulation with music (Träumerei [TM] composed by Schumann) decreased renal sympathetic nerve activity (RSNA) and BP in urethane-anesthetized rats (7). Thus, it is suggested that music exposure might affect sympathetic nerve activities and regulate cardiovascular functions.

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Autonomic nerves are composed of sympathetic and parasympathetic nerves, and the parasympathetic nerve innervating the stomach plays an important role in digestion and absorption. Recently, it has been confirmed that vagotomy of the stomach branch reduced ghrelin levels (8), an appetite regulator (9), suggesting that autonomic nerves of the stomach branch regulate the release of ghrelin for appetite control. However, the effects of auditory stimulation with TM have not been examined on the vagal nerves innervating the stomach.

To address the auditory-autonomic pathway, previous studies observed that animals with bilateral lesions of the cochleae or the auditory cortex (AuC) eliminated TM-induced changes in the RSNA and BP, indicating that the changes to RSNA and BP did depend on signaling through the auditory system (7). On the other hand, responses of the auditory system to music exposure with TM have not yet been investigated.

From the above facts, it was speculated that TM stimulation is converted into a neural signal by the cochleae and is sent to the auditory cortex to cause changes in autonomic function. Therefore, to verify this idea, the effect of auditory stimulation with TM on gastric vagal nerve activity (GVNA) was investigated along with c-Fos induction, a neural activity marker, in the AuC of urethane-anesthetized rats.

Materials and Methods

Animals. Male Wistar rats weighing 300-400 g were used. Animals were housed in a room maintained at 24±1˚C and illuminated for 12 h every day. Food (MF; Oriental Yeast Co., Tokyo) and water were freely available. Rats were adapted to the environment for at least 1 week prior to the experiment. All animal care and handling procedures were approved by the Institutional Animal Care and Use Committee of the Institute for Protein Research, Osaka University.

Electro-physiological experiment. General preparations were performed as described previously (10). Briefly, under anesthesia (1 g/kg urethane, given intraperitoneally), a polyethylene catheter was inserted into the right femoral vein for intravenous injection. The rat was then cannulated intratracheally, while body temperature was maintained at 37.0±0.5˚C using a heating pad. To record GVNA, the distal ends of the gastric branch of the ventral subdiaphragmatic vagal nerve was ligated and hooked to a pair of silver wire electrodes. The

recording electrodes were immersed in a mixture of warm petroleum jelly and liquid paraffin oil to prevent dehydration and for electrical insulation. The rat was allowed to stabilize for 30-60 min after the nerve was placed on the recording electrodes. Electrical changes in GVNA were amplified 2000-5000 times with a band path of 100 to 1000 kHz, and monitored by an oscilloscope. Raw data of the nerve activity was converted to standard pulses by a window discriminator, which separated discharge from electrical background noise which remained post mortem. Both the discharge rates and the neurogram were sampled with a Power-Lab analog-to-digital converter for recording and data analysis on a computer. Data were obtained as described previously (10). Two needle electrodes were placed under the skin at the right arm and left leg to record an electrocardiogram (ECG). The ECG signal was amplified with a bioelectric amplifier (AB-620G, Nihon Kohden, Japan). The ECG was monitored with an oscilloscope, sampled with the Power-Lab, and stored on a hard disk for off-line analysis to calculate heart rate (HR).

For auditory stimulation, white noise (WN) and music (TM: "Träumerei" from Kinderszenen Op.15-7, R. Schumann) were each adjusted to average sound levels of 50 dB. Because WN at 60 dB but not 50 dB elevated RSNA, BP and HR in preliminary experiments, 50 dB was used as the loudness for WN and music in the experiments. The stimulation was presented for 60 min by repeating WN or TM through the earphones, which were fixed to both ears before the stabilization period. At the end of the experiment, hexamethonium chloride (10 mg/kg) was administered intravenously to ensure that postganglionic efferent parasympathetic nerve activity had been recorded.

Immunohistochemistry. Rats were randomly divided into three groups: no stimulation (NST) and auditory stimulation with WN or TM. After the experiment, the rats were perfused transcardially with 4% paraformaldehyde. Their brains were soaked in the same fixative for 24 h and in 20% sucrose for 48 h, and then frozen in OCT compound and cut in 20-μm sections using a cryostat (total of 15 sections [1 of every 6 sections]/rat) referring the atlas of Paxisons and Watsons (11). Coronal sections through the auditory cortex (AuC) were treated with an anti–c-Fos polyclonal rabbit antibody (Abcam plc, Cambridge, UK) and then with biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA). Sections were then treated with ABC kits (Vector Laboratories) according to the manufacturer's instructions. The number of cells immunoreactive (IR) for c-Fos in the stained sections was quantified using Image J (NIH Image).

Data analysis. The GVNA data measured during each 5-min period after the start of auditory stimulation were analyzed by digital signal processing and appropriate statistical analyses. All data were expressed as means±SEM. Because of the inter-individual variability in the pre-stimulation state, percent changes from the baseline values were calculated for the GVNA. The Mann–Whitney *U*-test was used to compare the baseline values in the groups. Two-way ANOVA was used to compare group responses of the GVNA to auditory stimulation. To perform statistical analysis of the c-Fos data, ANOVA, followed by multiple comparisons using Dunnett's multiple range tests, was used. $P<0.05$ was considered statistically significant.

Results

Effects of auditory stimulation with TM on GVNA. Typical examples of the effects of no stimulation (NST) or auditory stimulation with WN or TM on GVNA for 60 min are presented in Figure 1A. None or auditory stimulation with 50 dB WN for 60 min induced no remarkable change in the GVNA, whereas auditory stimulation with TM at 50 dB significantly increased GVNA. Auditory stimulation with TM gradually increased GVNA, which reached a maximum of 154.9±18.5% at 50 min (Figure 1B). However, NST or auditory stimulation with WN did not affect GVNA within the 60 min of stimulation. The significances of differences between groups analyzed by ANOVA for values obtained from 5-60 min with NST or auditory stimulation with WN or TM are as follows: GVNA, NST *vs.* WN, NS (F=1.8); NST *vs.* TM, $p<0.05$ (F=7.8); TM *vs.* WN, $p<0.05$ (F=10.5). Basal values (at 0 min) of GVNA for all groups are summarized in Table I. No significant differences were detected in the respective basal values among the three groups.

Table I. *Basal levels (0 min) of GVNA in experimental group.*

Groups	GVNA (spikes/5 s)
NST	174.2±4.2 (5)
WN	175.8±31.0 (5)
TM	102.4±45.2 (5)

The number of rats is in the parenthesis. Data are presented as means±SEM.

Effects of auditory stimulation on the expression of c-Fos in the AuC. To further test whether the AuC is involved in the effects of TM on GVNA, c-Fos expression was examined using immunohistochemistry. Auditory stimulation with either WN or TM significantly increased the number of c-Fos–IR cells in the AuC when compared with the number of c-Fos–IR cells in the AuC of NST group (Figure 2).

Discussion

Animal studies have demonstrated that music exposure affects the central nervous system and changes hypothalamic levels of neurotrophins (12) or lowers BP in spontaneously hypertensive rats (13). Previously, it has been shown that auditory stimulation with Schumann's Träumerei reduced sympathetic nerve activity innervating the kidney and BP in urethane-anesthetized rats (7). Although autonomic nerves are composed of sympathetic and parasympathetic nerves, it is still unclear whether music exposure affects parasympathetic nerve activity. Here, in this study, it has been observed that auditory stimulation with Schumann's Träumerei increased GVNA in urethane-anesthetized rats (Figure 1). To the best of author's knowledge, this is the first study to demonstrate the parasympathetic response to auditory stimulation with TM.

With respect to the sensation pathway of sound, it is generally known that transmission route form the cochleae to the AuC is a major pathway, but whether a response of neural

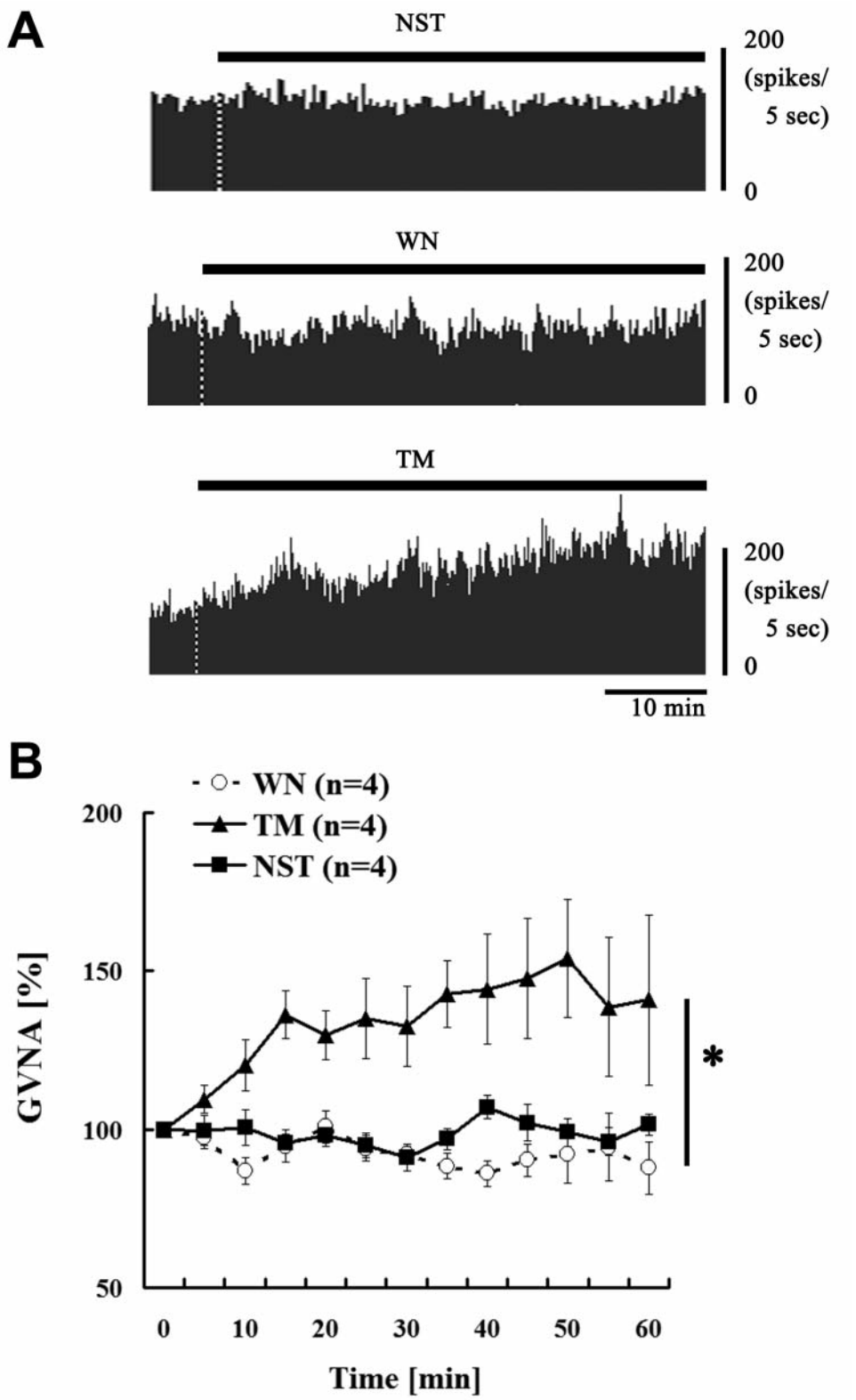

Figure 1. *Effects of auditory stimulation with TM on GVNA. (A) Representative recordings of GVNA in rats 60 min after NST or auditory stimulation with white noise (WN, 50 db) or Träumerei (TM; 50 db). Bars over traces indicate the stimulation period, lower bars indicate 10 min. Changes in GVNA (B) are expressed as means±SEM of the percentage of the values at 0 min. The number of animals in each group is shown in parentheses. *p<0.05 compare between NST group and TM group.*

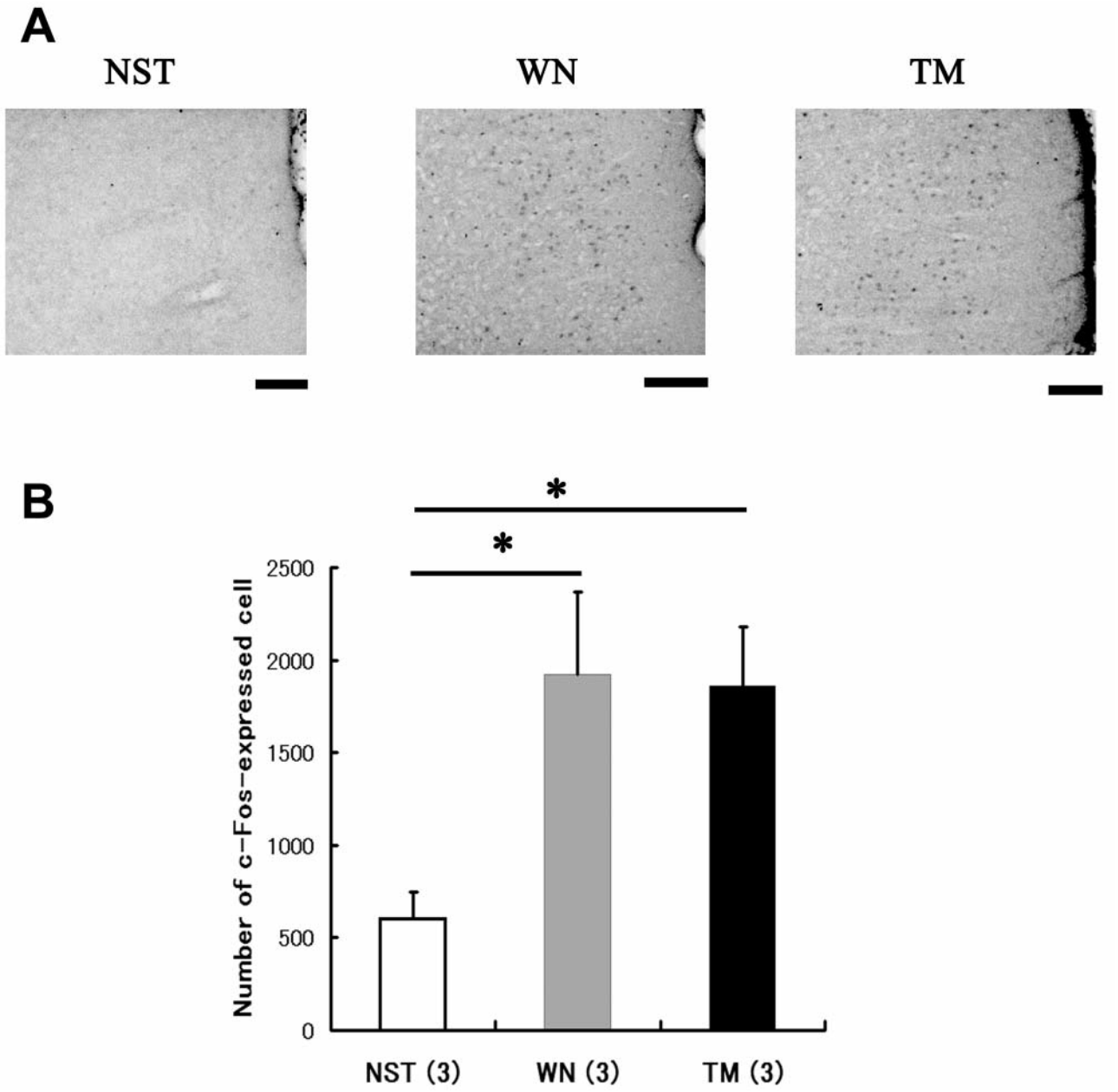

Figure 2. *c-Fos expression in the AuC 60 min after auditory stimulation with TM. Representative photomicrographs of c-Fos-IR in the AuC (A) 60 min after no stimulation (NST; left panels) or auditory stimulation with WN (middle panels) or TM (right panels). The length of the scale bars is 100 μm. Quantification of c-Fos–positive cells in the AuC (B). The number of animals in each group is shown in parentheses. The values are expressed as means±SEM. *p<0.05 compare with NST group.*

activity in the AuC to music exposure with TM is evoked has not been tested. To clear this issue, the present study showed that auditory stimulation with either WN or TM significantly increased the number of c-Fos–IR cells in the AuC when compared with those of non-stimulated rats (Figure 2). Moreover, a previous study demonstrated that bilateral lesions of the cochleae or the AuC eliminated TM-induced changes in the BP (7). Thus, these lines of evidences suggested that the music signal of TM is processed through the AuC, inducing changes in autonomic nerve activities.

Therefore, here is speculation on the physiological significance of the GVNA activation by auditory stimulation with TM. Previous studies reported that there is close relation between autonomic function and appetite modulation (14,15). With regards to the relation between GVNA and feeding behavior, a previous study demonstrated that both suppression

of food intake and GVNA are caused simultaneously (16), suggesting the parasympathetic nerve innervating the stomach might be implicated in appetite modulation. Moreover, previous studies confirmed that music exposure increases central dopamine levels in rats (13) which are involved in feeding behavior (17). This suggests that auditory stimulation might act on the brain and alter food intake. Preliminary studies obtained data indicating that long-term auditory stimulation with TM increased food intake in rats (unpublished data), supporting this idea and suggesting that auditory stimulation with TM might affect autonomic nerves through the central nervous system for appetite control.

With regards to the central mechanism in TM-induced GVNA change, it has been previously observed that bilateral lesions of the hypothalamic suprachiasmatic nucleus (SCN) or central pretreatment with thioperamide, a histamine H3-receptor, abolished sympathetic and cardiovascular responses to TM (7), suggesting that this effect is mediated by histamine H3-receptor and the SCN. An anatomical study using pseudorabies virus showed that the SCN connects with the dorsal motor nucleus of the vagus (18) which innervates the stomach *via* autonomic nerve, and an electrophysiological study showed that an SCN-lesion eliminated acceleration of the GVNA by olfactory stimulation with lavender oil (19). On the other hand, with respect to the histaminenergic system, response of GVNA to odor exposure with lavender oil was blocked by thioperamide injection (19). In addition, a preliminary study confirmed that central administration of small dose of histamine increased the GVNA (unpublished data). These data suggest that the SCN or histaminergic system might be involved in GVNA control. Thus, it is possible that auditory stimulation with TM might reduce the GVNA *via* a central mechanism containing the hypothalamus-histaminergic route. Of course, this is not certain and further research will be needed in the future.

In conclusion, auditory stimulation with TM can elevate GVNA. This finding is consistent with the idea that music exposure affects not only the sympathetic nerves but also the parasympathetic nerves. Since TM increased the number of c-Fos–IR cells in the AuC, autonomic effects of TM are mediated by an auditory transmission route containing the AuC.

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