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Original Research

High Salt Intake Induces Active Coping Behaviors by Enhancing the Resilience against Psychological Stress in Mice

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Abstract

Background: High salt intake increases the active coping behavior during psychological stress. Acute fear-related severe stress enhances passive coping behavior during subsequent inescapable stress. **Methods**: We investigated the effect of high salt intake (2%) for 5 consecutive days on the coping behavior in C57BL6 mice which employing the tail suspension test (TST) at 1 h after the exposure to inescapable innate fear using 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), a synthetic component of fox feces. By using a different mouse group, to investigated whether anxiety-like behavior was correlated with coping behavior during the TST, we performed the elevated-plus maze (EPM) test at 1 h before the TST without TMT. **Results**: Both the distance traveled and the number of entries in the central zone of test box during TMT were negatively correlated with freezing time in both sodium- and water-intake mice. Sodium-intake increased the preference for central zone during TMT exposure, but did not change fear sensitivity and locomotor activity. Sodium-intake also prevented that TMT-induced increase in the immobility time during TST. The immobility time during TST was positively correlated with freezing time during TMT exposure in sodium-intake, but not in water-intake mice. Furthermore, the immobility time during TST in sodium-intake mice correlated with the distance traveled and with the number of entries in the central zone during TMT. Sodium intake also increased the number of entries and the time spent in the open arm of the EPM, indicating that high salt intake had an anxiolytic effect. However, neither the number of entries nor the time spent in the open arm of the EPM were correlated with immobility time during TST in sodium-intake mice. **Conclusions**: We conclude that a high salt intake induces active coping behavior after experiencing fear stress by enhancing stress resilience rather than by reducing the anxiety level.

Keywords: active coping behavior; anxiety; high salt intake; inescapable innate fear stress; stress resilience

1. Introduction

Excessive salt intake, particularly of sodium, is thought to increase the risk of developing disease, e.g., hypertension [1], stroke [2] and renal disease [3], those are due to an increase in the sympathetic nerve activity by the physiological osmotic stress [4]. In addition, high salt intake also increases the risk of gastric cancer by shifting mucin production from glandular to surface mucous cells [5]. There is also disagreement regarding the role of high salt intake on psychiatric diseases, such as depression and anxiety, which are frequently comorbid with cardiovascular diseases [6,7]. Moreover, high dietary salt intake promotes neuroinflammation in the stress-associated brain regions and increases behavioral hyperresponsivity to acute psychological stress in mice [8]. In consistent, high salt intake has been suggested to increase the stress responsivity and contributes to the development of stress-related psychiatric disorders in humans [9]. However, it has also been suggested that restricted salt intake in patients with cardiovascular disease might be associated with mental distress [10]. High dietary sodium intake and depression show a consistent inverse relation [11]. In humans, dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis is induced by excessive secretion of cortisol and this is thought to be a risk for major depressive disorders [12,13]. Because antidepressants have been shown to improve HPA axis activity [14,15], HPA axis is a target for therapy of major depressive disorders [16,17]. A 5-day salt loading period in mice reduced the expression of corticotropin-releasing hormone (CRH) in the paraventricular nucleus of the hypothalamus (PVN) and decreased the plasma corticosterone level during the recovery period of restrained stress [18]. In addition, excess sodium causes hypernatremia, promotes anxiolysis, and attenuates stress-induced activation of the HPA axis in mice [19]. Therefore, the role of high salt intake on the mental disease is still controversial.

Accumulating evidence has shown that high salt intake increases active coping behavior against acute stress in rodents, by promoting neuronal inflammation [8,20]. Resilience to stress-promoted helplessness is associated with an active coping style [21]. Mice that exhibit active coping behaviors against stressors have lower glucocorticoid responses than those with more passive coping responses [22]. Stress exposure is an independent risk factor for psychiatric disorders such as depression [23] and posttraumatic stress disorder (PTSD) [24,25]. One factor that may be re-

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lated to differential resilience or vulnerability to stress is the type of strategy used to cope with the stressor, i.e., active or passive coping [26]. Active coping is related to resiliency to stress and is defined as a behavioral response that minimizes the physical, psychological or social harm of a situation [26]. Resilience is defined as the dynamic ability to adapt successfully in the face of adversity, trauma, or significant threat. It is a complex psychobiological process that protects individuals from developing PTSD or from experiencing other negative consequences of exposure to extreme stress, including depression [27,28].

It has been demonstrated that a moderate level of electrical shock induced the shorter immobility time in untreated male rat. However, it has also been demonstrated that the severe level of fear induced by electrical shocks induced the longer immobility time during forced swimming test (FST), followed by a surge in plasma corticosterone level, thereby affecting their coping behavior against subsequent inescapable stress [29]. Reduced CRH expression in the PVN and increased the corticosterone level have been observed during recovery from exposure to restraint stress. Discordant results have been reported for plasma osmolality following intake of the same sodium concentration. Krause *et al*. [18] found that 2% sodium intake for 5 consecutive days significantly increased the plasma sodium concentration, whereas Mitchell *et al*. [20] showed that 2% sodium intake for 7 consecutive days did not alter the plasma sodium concentration. According to these reports, we hypothesized that subjecting the mice to fear-related stress before the aversive stress may clarify whether the high salt intake could affect their hypersensitivity or resilience by enhancing active coping with acute stress. Predator odor stress inoculation can be used as an effective training method to improve the active response to subsequent severe stress [30]. The immobility induced by exposure to an inescapable aversive situation such as a tail suspension test (TST), serves as an indicator of resignation of escape behavior from the aversive environment [31,32]. Therefore, in the present study, we investigated the effect of 2% sodium intake for 5 consecutive days on the coping behavior of mice during the TST as a subsequent aversive situation which was performed 1 h after exposure to an inescapable innate fear by 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), a synthetic component of fox feces used as a preceding severe fear-related stress.

Sex hormones such as estradiol and oxytocin have been suggested to contribute to affective illness [33,34]. The most consistent sex difference reported for affective disorders is the two-fold higher lifetime prevalence of major depression (MDD) in women compared with men [35,36]. It has been suggested that oxytocin is highly expressed within a subset of hypothalamic neurons and is anxiolytic and promotes resilience to stress [37]. In addition, estrogen has been suggested to influence body fluid homeostasis [38]. In order to focus on the effect of hypernatremia on coping behavior, only male mice were used in this study, thus excluding gender-related factors that might affect behavior during aversive and fear stress.

2. Materials and Methods

2.1 Animals and Ethics

Adult male C57BL/6J mice (8–12 weeks old) were supplied by Charles River Laboratories (Yokohama, Japan) and CLEA Japan (Tokyo, Japan). All mice were housed at 25 $\pm$ 2 °C on a 12-h light (08:00 to 20:00)/12-h dark (20:00 to 08:00) cycle and were allowed *ad libitum* access to food (0.3% sodium containing diet for rodents, #CE-2, CLEA Japan, Inc., Tokyo, Japan) and water. Mice were housed in groups of four to five per cage. This study was approved by the Animal Care Committee of Ohu University (Nos. 2016-29, 2017-34, and 2018-29). All animal procedures were performed in accordance with the guidelines of the Animal Care Committee of Ohu University. Principles of laboratory animal care were followed, with special care taken to minimize animal distress and to use the minimum number of animals needed for all experiments. This study was carried out in compliance with the ARRIVE guidelines. All behavioral tests were performed between 13:00 and 16:00 and were conducted and analyzed by investigators who were blinded to the group assignments. Data acquisition and analysis of all animal behavioral tests results were performed using ANY-maze software (version 6.35, Muromachi Kikai Co. Ltd., Tokyo, Japan).

2.2 2,5-Dihydro-2,4,5-Trimethylthiazoline (TMT) Odor Exposure

A total of 47 male C57BL/6J mice (8–12 weeks old) were used for the TMT-induced inescapable innate fear test (26 mice), with mineral oil (MO) used as the control (21 mice). All mouse were habituated to the arena of the TMT test box for 15 min one day prior to the test. Each mouse was placed in the center of an acrylic plastic open field box arena (W: 294 mm $\times$ D: 294 mm $\times$ H: 297 mm). The bottom and four inner walls of the box were covered by non-reflective paper and the top of the box was made of clear plexiglass to allow video recording with a web camera. A circular filter paper (D: 2 cm, Cat#; 1001-025; Whatman GE Healthcare Life Science, Little Chalfont, UK) infused with 20 μL of TMT was placed directly in the corner of the odor exposure box. The TMT was introduced 5 min after the mice were placed in the test box, and fear-related behaviors were observed throughout the test (15 min). To measure the avoidance response to TMT odor, the floor was divided into two areas by a diagonal line. Five test boxes were prepared and 3 to 5 mice per day were tested for TMT odor exposure. The TMT odor in each box was removed between experiments by treatment with 5% bleaching agent overnight and then carefully washing with detergent and water, and wiping clean with 70% ethanol.

2.3 Avoidance Response and Freezing Behavior

To evaluate the avoidance response to TMT, the mean time an individual mouse spent in the non-TMT diagonal side of the arena was compared with the time it spent in the TMT side [39]. The TMT stress-elicited freezing behavior was used as a measure of fear and was defined as the absence of any movement, except for those necessary for breathing [40]. The percentage of time spent in freezing was calculated for each mouse during the 10 min period after TMT was added to the filter paper in the corner of the box. Freezing time was analyzed every min (1 min/bin) or in two 5-min blocks (the first at 5–10 min and the second at 10–15 min).

2.4 Tail Suspension Test (TST)

All TSTs were performed during the light phase (12:00–16:00) of the light–dark cycle. Each mouse was individually suspended by its tail using adhesive tape placed approximately 2 cm from the tip of the tail and via two hanging hooks connected to the ceiling of the test box, which was located 42 cm above the bench top. Each mouse was suspended for 10 min and recorded with a digital video camera in the absence of an investigator. Another investigator, who did not perform the experiment measured the immobility time during the TST. This was performed after completion of the experiments and using ANY-maze software. The investigators were thus blinded to the allocated groupings. Mobility was defined as movement of the hind legs and/or other escape-oriented behaviors, including twisting of the body, but not breathing Mouse immobility was considered to represent the immobility time in this paradigm. Mice with a 2% NaCl intake that spent a shorter time immobile compared to those with water intake without escape-oriented behaviors were considered to demonstrate active coping behavior. The duration of immobility was calculated as the sum of the time periods during which the mouse was motionless for at least 2 s. Immobile behavior sensitivity was set to 70%, and the mouse needed to be immobile for 1 s to initiate scoring of immobility. Mice were returned to their home cages at the completion of the TST [41,42]. To provide an index of learned despair, 10 min of the test session was divided into two periods, the initial 5 min (0–5 min) and the final 5 min (5–10 min) [41,43], in addition, the immobility time for 5 min from 1 to 6 min during TST were also analyzed for evaluating the level of the behavioral despair [44].

2.5 Elevated Plus Maze (EPM)

A total of 21 male C57BL/6J mice (8–12 weeks old) were used for elevated plus maze (EPM). EPM is a reliable measurement instrument for anxiety-like behavior in animals and is used to evaluate emotionality in rodents [45]. The apparatus is comprised of two open arms (300 $\times$ 50 mm) without walls, two enclosed arms (300 $\times$ 50 mm) with walls that are 250 mm in height, and a central square (50 $\times$ 50 mm) that connects the arms. The four grey crossed arms form a plus sign with the central platform (Brain Science idea. Co., Ltd., Osaka, Japan). The apparatus was placed 400 mm above the ground, with the mice able to move freely in all directions. The EPM test was conducted in a quiet room, and the illumination level of the central area was 100 lux [46]. Each tested mouse faced the open arm and was positioned in the center of the apparatus. Their behavior was recorded for 5 min. The apparatus was cleaned with 70% ethanol to prevent the mice from being affected by other odors. The distance traveled, the number of entries into the open arms, and the time spent in the open arms were recorded by a video camera.

2.6 Statistical Analysis

The Student's *t*-test was used for comparisons between two groups. Two-way repeated measures analysis of variance (ANOVA) was followed by one- or two-way ANOVA to analyze each time block. This was followed by the Bonferroni post-hoc test for comparison of the groups. The correlation coefficient was analyzed by Pearson's correlation test, and Spearman's rank correlation coefficient analysis was performed to determine whether two variables were significantly correlated. Fisher *z*-transformation was performed to test the difference between two Pearson's correlation coefficients. Statistical analyses were performed using EZR (Easy R) software [47] (version 1.38; Saitama Medical Center, Jichi Medical University, Saitama, Japan) and BellCurve software (version 3.20, Social Survey Research Information Co., Ltd., Tokyo, Japan) [48]. All data in the bars indicate the mean $\pm$ standard error of the mean (SEM). Statistical significance was set at $*p < 0.05$ or $**p < 0.01$. NS signifies no significant difference.

3. Results

3.1 Effect of 2% NaCl Intake on Mineral Oil (MO) or TMT Exposure–Induced Mouse Freezing Behavior and the Correlation between Freezing Time and Distance Traveled

To investigate whether the 2% NaCl intake for 5 days affected the mice's innate fear sensitivity against TMT, we measured the percentage of freezing time before and during TMT exposure for 5 and 10 min, respectively. Two-way repeated measures ANOVA (NaCl $\times$ TMT) revealed that the TMT affected the time course of freezing behaviors ($F(3, 749) = 360.59$, $p = 5.94 \times 10^{-32}$, Fig. 1E). Bonferroni post hoc test indicated that both the water- and sodium-intake mice exhibited freezing behavior upon TMT exposure compared to for MO (TMT–H_2O: $p = 4.14 \times 10^{-27}$ vs. MO–H_2O; TMT–NaCl: $p = 3.03 \times 10^{-25}$ vs. MO–NaCl, Fig. 1E). However, sodium-intake did not affect the freezing behavior (MO–NaCl: $p = 1.000$ vs. MO–H_2O; TMT–NaCl: $p = 1.000$ vs. TMT– H_2O; Fig. 1E). Two-way repeated measures ANOVA revealed that freezing time for 5 min bin before and after the TMT exposure were affected ($F(3, 146) = 344.96$, $p = 4.71 \times 10^{-31}$, Fig. 1F). Although

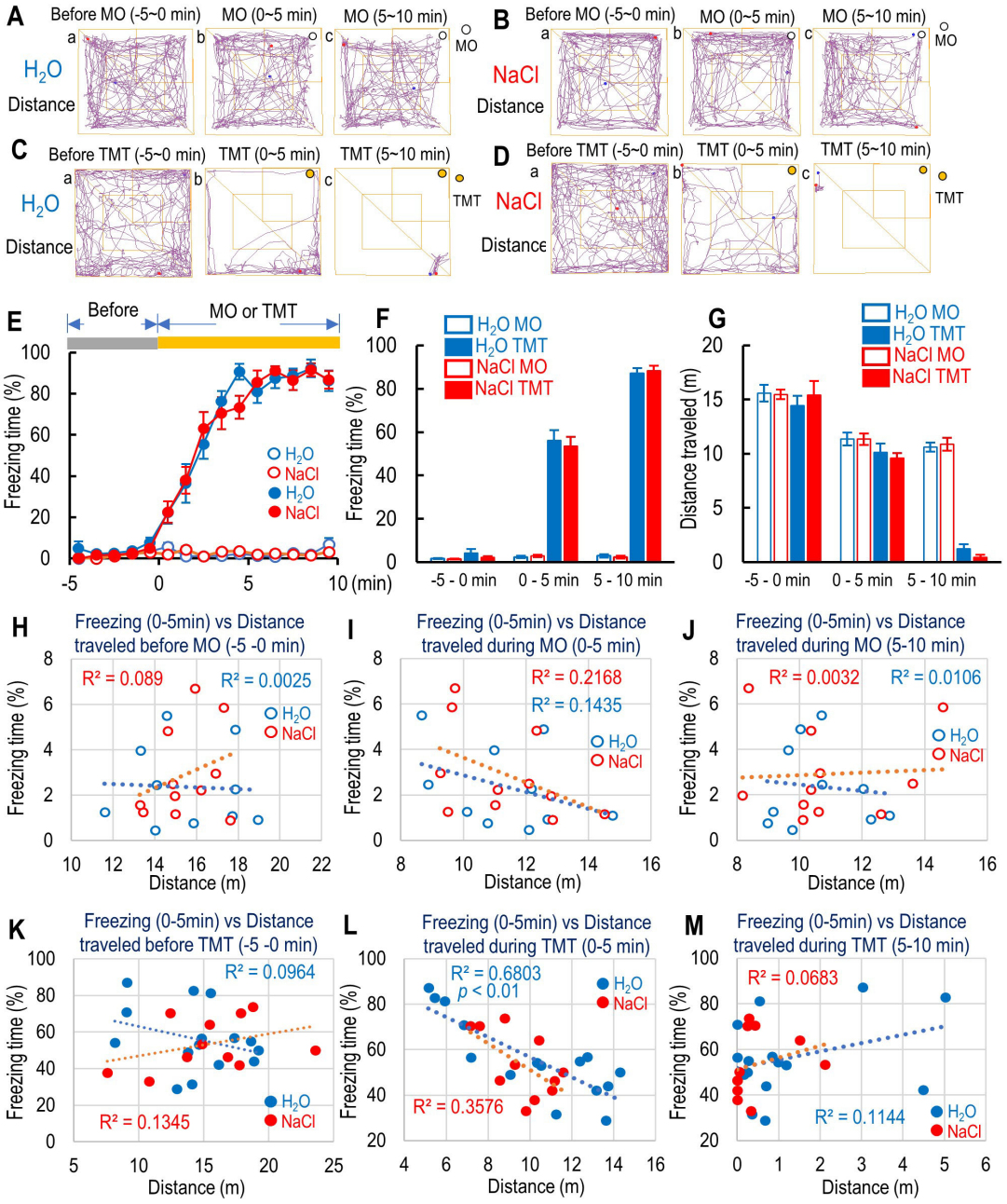

Fig. 1. Effect of 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) on the freezing behavior and the distance traveled in the test box. (A–D) Traces of mice moving in the box during the test under the mineral oil (MO) as a control. Traces of mice moving in the box for 5 min before (a: –5–0 min), the first 5 min (b: 0–5 min), and the second 5 min (c: 5–10 min) during MO exposure for H_2O-intake mice (A), MO exposure for NaCl-intake mice (B), TMT exposure for H_2O-intake mice (C), and TMT exposure for NaCl-intake mice (D). (E) Time course of the freezing behaviors before and during TMT exposure. The graph shows the percent of freezing time per min for 15 min (H_2O-intake mice, n = 11; NaCl-intake mice, n = 15). (F) The percent of freezing time for 5 min (–5–0 min) before MO and TMT exposure and the first (0–5 min) and second 5 min (5–10 min) under the presence of MO or TMT (H_2O–intake mice, n = 11; NaCl–intake mice, n = 15). (G) Distance traveled in the test box for 5 min (–5–0 min) before MO and TMT exposure and the first (0–5 min) and second 5 min (5–10 min) under the presence of MO or TMT (H_2O-intake mice, n = 11; NaCl-intake mice, n = 15). (H–M) Correlation between freezing behavior and the distance traveled in the test box before MO (H), during the first 5 min of MO exposure (I), during the second 5 min of MO exposure (J), before TMT exposure (K), during the first 5 min of TMT exposure (L), and during the second 5 min of TMT exposure (M) (H_2O-intake mice, n = 11; NaCl-intake mice, n = 15). All the data are mean ± SEM. R^2 is Pearson's correlation coefficient.

One-way ANOVA indicated that there was no significant difference during 5 min before and after the MO or TMT (before: $F(3, 18) = 1.566$, $p = 0.2937$, Fig. 1F), TMT affected the freezing time during both of first and last 5 min of TMT exposure (1st 5 min; $F(3, 48) = 79.251$, $p = 5.50 \times 10^{-18}$; last 5 min: $F(3, 48) = 712.27$, $p = 6.40 \times 10^{-38}$, Fig. 1F). Bonferroni post hoc test indicated that the TMT significantly induced the freezing behaviors in both water and sodium-intake mice during first and last 5 min (first 5 min: TMT-H_2O: $p = 1.11 \times 10^{-13}$ vs. MO-H_2O; TMT-NaCl: $p = 8.87 \times 10^{-13}$ vs. MO-NaCl; last 5 min: TMT-H_2O: $p = 7.35 \times 10^{-32}$ vs. MO-H_2O; TMT-NaCl: $p = 3.23 \times 10^{-32}$ vs. MO-NaCl; Fig. 1F). Two-way repeated measures ANOVA revealed that the total distance traveled in the whole area of the test box for the 5-min bin before exposure to TMT and for 10 min during exposure was also affected ($F(3, 140) = 22.81$, $p = 5.45 \times 10^{-9}$, Fig. 1G). One-way ANOVA for each 5-min bin indicated that the last 5 min of TMT exposure affected the total distance traveled in whole area of the test box (before TMT for 5 min: $F(3, 121) = 0.642$, $p = 0.589$; TMT exposure for the first 5 min: $F(3, 121) = 1.493$, $p = 0.2198$; TMT exposure for the last 5 min: $F(3, 121) = 63.689$, $p = 8.88 \times 10^{-25}$; Fig. 1G). Bonferroni post-hoc test indicated that distance traveled was significantly different in the last 5 min of TMT exposure for MO and TMT exposed-mice, but not water- or sodium-intake mice (TMT–H_2O: $p = 0.985$ vs. TMT–NaCl; MO–H_2O: $p = 1.000$ vs. MO–NaCl; $p = 1.000$ vs. non-TMT–H_2O; TMT–NaCl: $p = 1.000$ vs. TMT–H_2O, Fig. 1G). In the next, we investigated the correlation between the freezing level and the distance traveled in water- and sodium-intake mice. Spearman's rank correlation coefficient analysis indicated that only the percent of freezing time during the first 5 min and the distance traveled during the first 5 min of TMT exposure exhibited significant correlations in only water-intake mice (H_2O: $R^2 = 0.6803$, $p = 0.000907$; NaCl: $R^2 = 0.358$, $p = 0.1025$, Fig. 1L). This indicates that the water-intake mice exhibited a correlation between freezing level and the distance traveled during the first 5 min of exposure to TMT, but sodium-intake mice did not.

3.2 Effect of 2% NaCl Intake on the Correlation between Central Preference during TMT and Freezing Time during the First 5 min of TMT Exposure

During the TMT exposure, sodium-intake mice exhibited preference for the central zone in the test box compared to water-intake mice, although the preference of the central zone in sodium-intake mice were similar level with water-intake mice. Two-way repeated measures ANOVA revealed that the distance traveled, the time spent, and the number of entries in the central zone were affected by TMT exposure (distance traveled in the central zone: $F(3, 140) = 31.19$, $p = 7.20 \times 10^{-11}$, Fig. 2C; number of entries in central zone: $F(3, 140) = 30.78$, $p = 8.72 \times 10^{-11}$, Fig. 2D; time spent in the central zone: $F(3, 140) = 14.53$, $p = 1.12 \times 10^{-6}$; Fig. 2E). One-way ANOVA was followed by Bonferroni comparison test, which indicated that the distance traveled, the time spent, and the number of entries in the central zone were affected by sodium intake during the first 5 min of TMT exposure (one-way ANOVA: distance traveled in the central zone: $F(3, 46) = 34.93$, $p = 1.34 \times 10^{-11}$, Fig. 2C; number of entries in the central zone: $F(3, 46) = 36.83$, $p = 6.00 \times 10^{-12}$, Fig. 2D; time spent in the central zone: $F(3, 46) = 5.10$, $p = 0.0042$, Fig. 2E; Bonferroni post hoc test: distance traveled: NaCl-intake mice, $p = 0.0444$ vs. H_2O-intake mice, Fig. 2C; number of entries in the central zone: NaCl-intake mice, $p = 0.0411$ vs. H_2O-intake mice, Fig. 2D; time spent: NaCl-intake mice, $p = 0.0384$ vs. H_2O-intake mice; Fig. 2E). Spearman's rank correlation coefficient revealed that the distance traveled in the central zone in both the water- and sodium-intake mice was correlated with freezing time during the first 5 min of exposure to TMT (Pearson's correlation coefficient: H_2O-intake mice: $R^2 = 0.5625$, NaCl-intake mice: $R^2 = 0.4927$; Spearman's rank correlation: H_2O-intake mice: $p = 0.000153$; NaCl-intake mice: $p = 0.0146$, Fig. 2I). In addition, the number of entries in the central zone during the first 5 min of exposure to TMT was also correlated with freezing time in both water- and sodium-intake mice (number of entries: Pearson's correlation coefficient: H_2O-intake mice: $R^2 = 0.6392$, NaCl-intake mice: $R^2 = 0.4889$; Spearman's rank correlation: H_2O-intake mice: $p = 5.74 \times 10^{-6}$; NaCl-intake mice: $p = 0.0099$; Fig. 2J). However, the time that water-intake mice spent in the central zone was not correlated with freezing time during the first 5 min of exposure to TMT, and the freezing time in sodium-intake mice during the first 5 min of exposure to TMT was not correlated with the time spent in the central zone during the first 5 min of exposure to TMT (Pearson's correlation coefficient: H_2O-intake mice: $R^2 = 0.0213$, NaCl-intake mice: $R^2 = 0.5716$; Spearman's rank correlation: H_2O-intake mice: $p = 0.2443$; NaCl–intake mice: $p = 0.0028$; Fig. 2K). These results indicated that mice with a higher preference of the central zone during the first 5 min of exposure to TMT exhibited shorter freezing times.

3.3 Effect of 2% NaCl Intake on the Immobility Time during TST and the Correlation between the Freezing Time and the Immobility Time

We next investigated whether the NaCl intake affected mice's active coping behavior during the TST as an aversive situation for the mice. The immobility time during TST in each group gradually increased, and the two-way repeated measures ANOVA revealed that the TMT and NaCl affected the immobility time during TST ($F(3, 469) = 10.65$, $p = 2.31 \times 10^{-5}$, Fig. 3B). One-way ANOVA suggested that the immobility time for 1-min bins from 1–2 to 9–10 min were also affected, and the immobility time in mice with sodium intake and exposure to TMT were longer than those of mice with water intake and TMT exposure (0–1

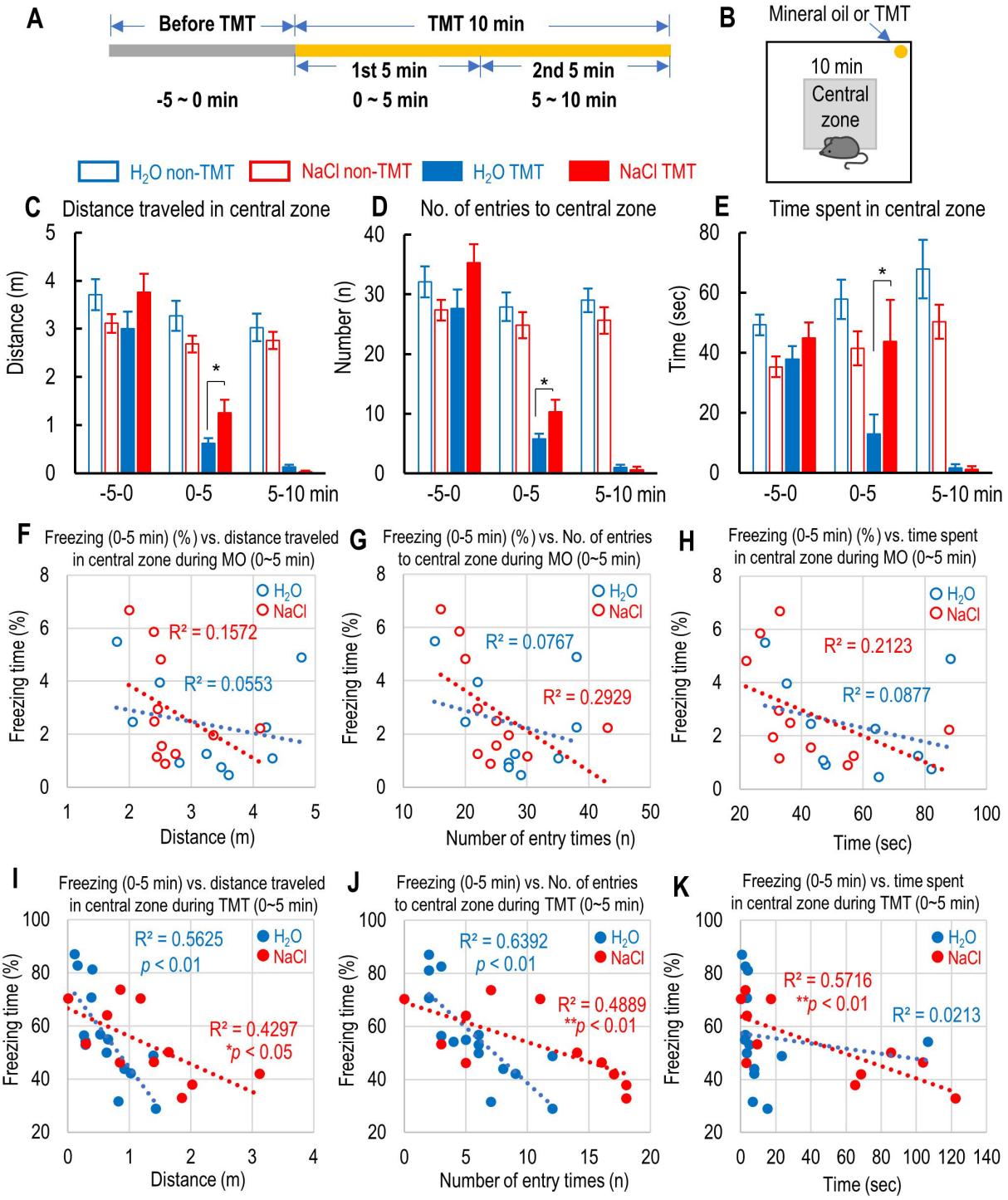

Fig. 2. The effect of 2% NaCl intake on the central preference and the correlation between freezing time and the central preference during inescapable innate fear caused by 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) exposure. (A) Schedule of the time course before and during the first and second 5 min of TMT exposure. (B) The location of the central zone and mineral oil (MO) or TMT in the test box. (C–E) Distance traveled (C), number of entries (D), and time spent (E) in the central zone in the test box before and during the first and second 5 min of MO or TMT exposure. (F–H) Correlation between the percent of freezing time during the first 5 min of MO exposure and the distance traveled (F), number of entries (G), and time spent (H) in the central zone in the test box for both water- and sodium-intake mice. (I–K) Correlation between the percent of freezing time during the first 5 min of TMT exposure and the distance traveled (I), number of entries (J), and time spent (K) in the central zone in the test box for both water- and sodium-intake mice. All the data are mean ± SEM. Statistical significance (*$p < 0.05$, **$p < 0.01$) is noted. R^2 is Pearson's correlation coefficient.

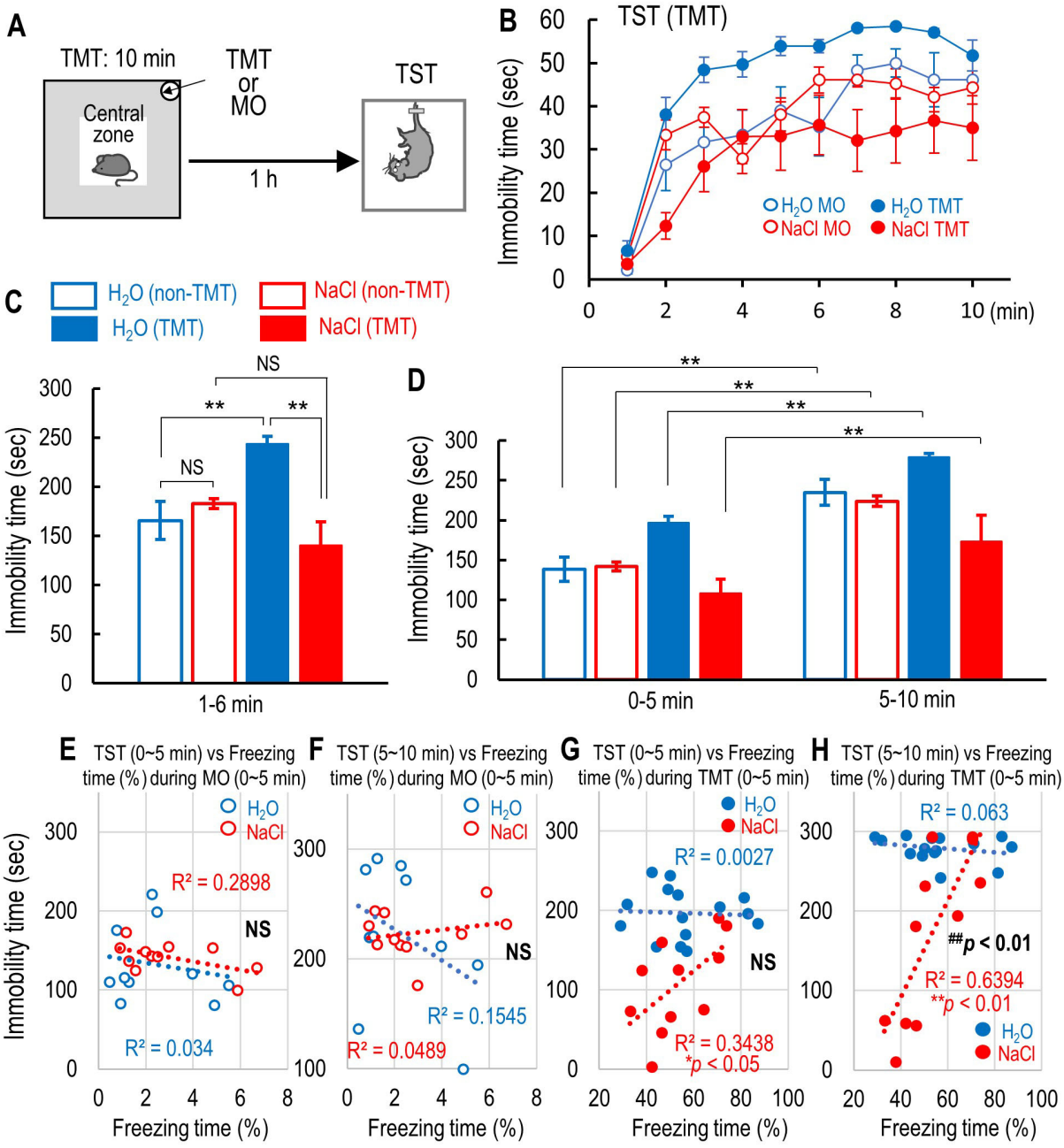

Fig. 3. The effect of 2% NaCl intake on the immobility time during tail suspension test (TST) and the correlation between the immobility time during TST and the freezing time during the first 5 min of mineral oil (MO) or 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) exposure. (A) Schematic drawings indicate the location of the central zone and the MO or TMT and the schedule of TST. (B) The time course of immobility time for 10 min of the tail suspension test (TST) in H_2O–MO (n = 9), H_2O–TMT (n = 15), NaCl–MO (n = 11), and NaCl–TMT mice (n = 11). (C) Immobility time during the first 6 min, except the first 1 min, of TST exposure in H_2O–MO (n = 9), H_2O–TMT (n = 15), NaCl–MO (n = 11), and NaCl–TMT mice (n = 11). (D) Summary of cumulative immobility time during the first (0–5 min) and last 5 min (5–10 min) 1 h after TMT exposure in H_2O–MO (n = 9), H_2O–TMT (n = 15), NaCl–MO (n = 11), and NaCl–TMT mice (n = 11). (E–D) Correlation between percent of freezing time during the first 5 min and the immobility time during the first 5 min of TMT under MO exposure (E), during the second 5 min of TST under MO exposure (F), during the first 5 min of TST under TMT exposure (G), and during the second 5 min of TST under TMT exposure (H) for both water- and sodium-intake mice. All the data are mean ± SEM. Statistical significance (**$p < 0.01$, *$p < 0.05$) is noted. Statistically significant differences between two Pearson's correlation coefficients by Fisher's r-to-z transformation($^{\#\#}p < 0.01$) are indicated. NS means no significant. R^2 is Pearson's correlation coefficient.

min: $F(3, 243) = 0.220$, $p = 0.8827$; 1–2 min: $F(3, 243) = 7.022$, $p = 0.000151$; 2–3 min: $F(3, 243) = 5.446$, $p = 0.00122$; 3–4 min: $F(3, 243) = 5.603$, $p = 0.000990$; 4–5 min: $F(3, 243) = 5.000$, $p = 0.00220$; 5–6 min: $F(3, 243) = 4.708$, $p = 0.00326$; 6–7 min: $F(3, 243) = 6.636$, $p = 0.000252$; 7–8 min: $F(3, 243) = 5.895$, $p = 0.000672$; 8–9 min: $F(3, 243) = 4.536$, $p = 0.00409$; 9–10 min: $F(3, 243) = 2.769$, $p = 0.0423$; Bonferroni post hoc test: 0–1 min: $p = 0.572$; 1–2 min: $p = 4.32 \times 10^{-32}$; 2–3 min: $p = 8.86 \times 10^{-26}$; 3–4 min: $p = 3.39 \times 10^{-16}$; 4–5 min: $p = 3.81 \times 10^{-23}$; 5–6 min: $p = 7.88 \times 10^{-19}$; 6–7 min: $p = 1.69 \times 10^{-32}$; 7–8 min: $p = 3.50 \times 10^{-29}$; 8–9 min: $p = 1.84 \times 10^{-22}$; 9–10 min: $p = 2.57 \times 10^{-16}$; Fig. 3B). Many papers have suggested that a higher immobility time during the first 6 min, except the first 1 min, reflects despair behavior, which is used for the assessment of antidepressants. Therefore, we investigated whether the innate fear induced by TMT exposure affected the immobility time during TST 1 h after the TMT. Multiple comparisons among four groups were performed by one-way ANOVA, which was followed by Bonferroni post hoc test and indicated that the TMT exposure increased the immobility time in water-intake mice compared to MO exposure ($F(3, 46) = 9.857$, $p = 4.54 \times 10^{-5}$; Bonferroni post hoc test: H_2O–TMT mice: $p = 0.0031$ vs. H_2O–MO mice, Fig. 3C). However, NaCl intake suppressed the increase in the immobility time after the TMT exposure (NaCl–TMT mice: $p = 0.344$ vs. H_2O–TMT mice, Fig. 3C), indicating that the NaCl intake prevented the TMT-induced despair behavior. In our previous work, we demonstrated that the learned despair behavior could be observed in the last 5 min of 10-min TST in subsequent of the first 5 min [41,43]. Therefore, we investigated whether the NaCl intake affected the induction of learned despair behavior. Two-way repeated measures ANOVA revealed that NaCl intake and TMT exposure affected the immobility time during TST $(3, 93) = 10.33$, $p = 3.03 \times 10^{-5}$, Fig. 3D). Bonferroni comparison test indicated that the immobility time during the last 5 min in every group was increased compared to that in the first 5 min of TST (H_2O–MO mice in the first 5 min: $p = 1.42 \times 10^{-32}$ vs. H_2O–MO mice in the last 5 min; H_2O–TMT mice in the first 5 min: $p = 1.49 \times 10^{-13}$ vs. H_2O-TMT mice in the last 5 min; NaCl–MO mice in the first 5 min: $p = 1.91 \times 10^{-13}$ vs. NaCl–MO mice in the last 5 min; NaCl–TMT mice in the first 5 min: $p = 1.28 \times 10^{-10}$ vs. NaCl–TMT mice in the last 5 min; Fig. 3D). These results indicated that the NaCl intake enhanced the active coping behavior and induced fear-based despair-like behavior, while the learned despair-like behavior was not affected by NaCl intake. There was no correlation between the immobility time during the first or last 5 min and the freezing time during the first 5 min in each group when the mice were exposed to MO (Pearson's R: H_2O–MO in TST (0–5 min): $R^2 = 0.034$; NaCl–MO in TST (0–5 min): $R^2 = 0.2898$; H_2O–MO in TST (5–10 min): $R^2 = 0.034$; NaCl–MO in TST (5–10 min): $R^2 = 0.2898$; Fig. 3E,F). However, NaCl intake showed a correlation between the immobility time during the first or last 5 min and the freezing time during the first 5 min in each group when the mice were exposed to the TMT (Pearson's R: H_2O–TMT in TST (0–5 min): $R^2 = 0.0027$; NaCl–TMT in TST (0–5 min): $R^2 = 0.3438$; H_2O–TMT in TST (5–10 min): $R^2 = 0.063$; NaCl–TMT in TST (5–10 min): $R^2 = 0.6394$; Spearman's rank correlation: H_2O–TMT in TST (0–5 min): $p = 0.4277$; NaCl–TMT in TST (0–5 min): $p = 0.0426$; H_2O–TMT in TST (5–10 min): $p = 0.4431$; NaCl–TMT in TST (5–10 min): $p = 0.0045$; Fig. 3G,H). This indicates that mice with lower freezing levels exhibited shorter immobility times. Fisher's r-to-z transformation revealed that during the second 5 min of TMT exposure, Pearson's correlation coefficient of 2% NaCl intake was significantly different from that of water-intake mice ($z = 2.967$, $p = 0.00301$, Fig. 3H). These results suggest that the sodium-intake mice exhibited lower freezing levels during inescapable innate fear of TMT exposure and shorter immobility times during the first 5 min of exposure to TST.

3.4 Effect of 2% NaCl Intake on the Correlation between Immobility Time during TST and Central Preference during TMT Exposure

Because the freezing time during the first 5 min of exposure to TMT was correlated with the central preference, such as distance traveled, the number of entries, and the time spent in the central zone (Fig. 2I–K), we investigated whether the immobility time during TST was correlated with the preference of the central zone during the first 5 min of exposure to TMT to induce innate fear. The immobility time during both the first and last 5 min of TST in all groups was not correlated with any preference for the central zone during MO exposure (distance traveled: H_2O–MO in TST (0–5 min): $R^2 = 0.0018$, $p = 0.855$; NaCl–MO in TST (0–5 min): $R^2 = 0.0216$, $p = 0.519$, Fig. 4A; H_2O–MO in TST (5–10 min): $R^2 = 0.00718$, $p = 0.855$; NaCl–TMT in TST (5–10 min): $R^2 = 0.0568$, $p = 0.259$, Fig. 4B; number of entries: H_2O–MO in TST (0–5 min): $R^2 = 0.0015$, $p = 0.894$; NaCl–MO in TST (0–5 min): $R^2 = 0.1158$, $p = 0.250$, Fig. 4E; H_2O–MO in TST (5–10 min): $R^2 = 0.00263$, $p = 0.947$; NaCl-TMT in TST (5–10 min): $R^2 = 0.021$, $p = 0.535$, Fig. 4F; time spent: H_2O–MO in TST (0–5 min): $R^2 = 0.0003$, $p = 0.603$; NaCl–MO in TST (0–5 min): $R^2 = 0.0023$, $p = 0.709$, Fig. 4I; H_2O–MO in TST (5–10 min): $R^2 = 0.0040$, $p = 0.777$; NaCl–TMT in TST (5–10 min): $R^2 = 0.0359$, $p = 0.433$, Fig. 4J). However, although the immobility time during TST in both the first and last 5 min in water-intake mice were not correlated with any central preference during first 5 min of TMT exposure, NaCl intake showed a correlation between the immobility time during TST in both the first and last 5 min and central preference during first 5 min of TMT exposure, excepting the time spent in the central zone and the immobility

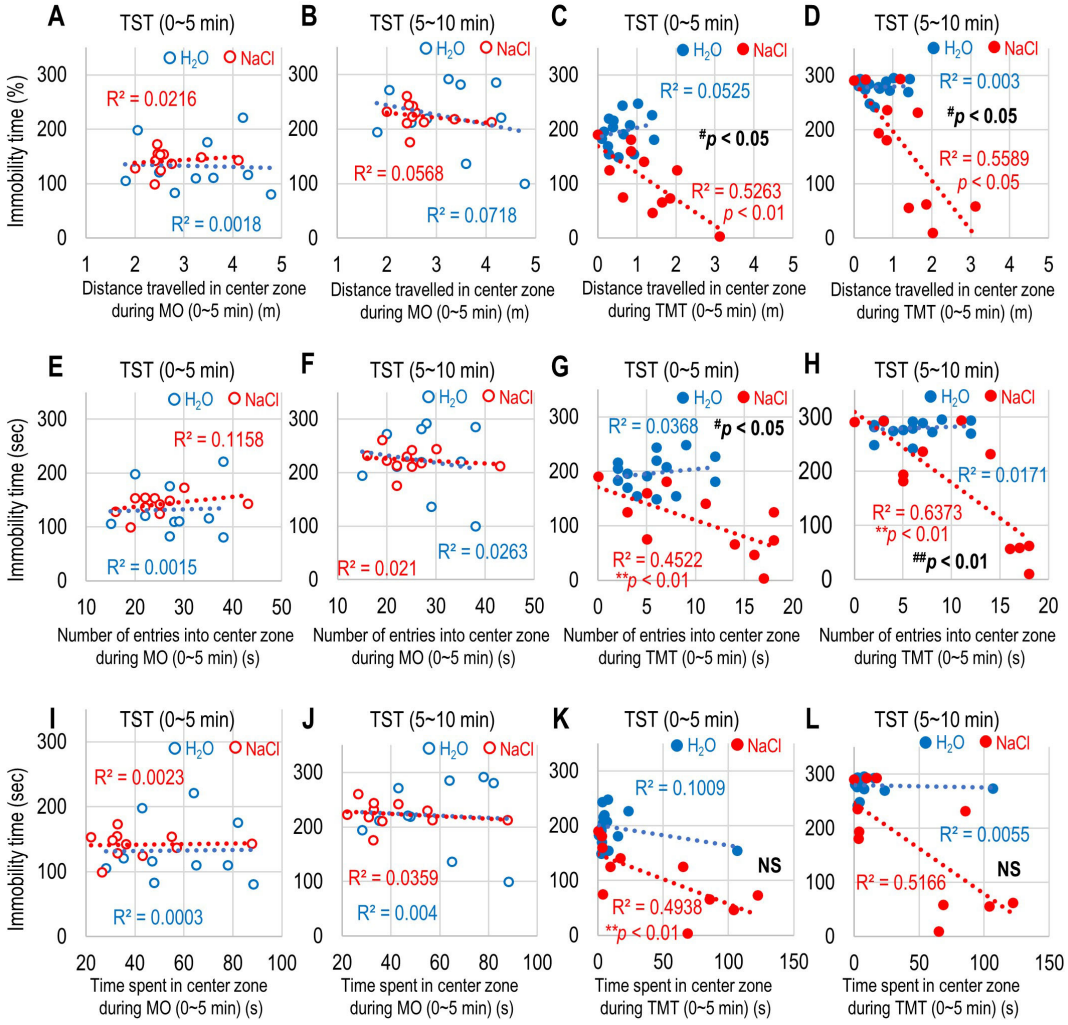

Fig. 4. Correlation between immobility time and preference of the central zone during mineral oil (MO) and 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) exposure. (A–D) Correlation between the distance traveled in the central zone during the first 5 min of MO and the immobility time during the first 5 min of tail suspension test (TST) (A), during the first 5 min of MO and the immobility time during the second 5 min of TST (B), during the first 5 min of TMT and the immobility time during the first 5 min of TST (C), and during the first 5 min of TMT and the immobility time during the second 5 min of TST (D). (E–H) Correlation between the number of entries in the central zone during the first 5 min of MO and the immobility time during the first 5 min of TST (E), during the first 5 min of MO and the immobility time during the second 5 min of TST (F), during the first 5 min of TMT and the immobility time during the first 5 min of TST (G), and during the first 5 min of TMT and the immobility time during the second 5 min of TST (H). (I–L) Correlation between the time spent in the central zone during the first 5 min of MO and the immobility time during the first 5 min of TST (I), during the first 5 min of MO and the immobility time during the second 5 min of TST (J), during the first 5 min of TMT and the immobility time during the first 5 min of TST (K), and during the first 5 min of TMT and the immobility time during the second 5 min of TST (L) for H_2O–MO (n = 9), H_2O–TMT (n = 15), NaCl–MO (n = 11), and NaCl–TMT mice (n = 11). Statistical significance (**$p < 0.01$, *$p < 0.05$) are noted. NS means not significant. R^2 is Pearson's correlation coefficient.

time during last 5 min of TST in sodium-intake mice (distance traveled: H_2O–TMT in TST (0–5 min): $R^2 = 0.0525$, $p = 0.334$; NaCl–TMT in TST (0–5 min): $R^2 = 0.526$, $p = 0.0165$, Fig. 4C; H_2O–TMT in TST (5–10 min): $R^2 = 0.003$, $p = 0.781$; NaCl–TMT in TST (5–10 min): $R^2 = 0.5589$, $p = 0.0233$, Fig. 4D; number of entries: H_2O–TMT in TST (0–5 min): $R^2 = 0.0368$, $p = 0.504$; NaCl–TMT in TST (0–5 min): $R^2 = 0.452$, $p = 0.0251$, Fig. 4G; H_2O–TMT in TST (5–10 min): $R^2 = 0.0171$, $p = 0.725$; NaCl–TMT in TST (5–10 min): $R^2 = 0.637$, $p = 0.0139$, Fig. 4H; time spent: H_2O–TMT in TST (0–5 min): $R^2 = 0.1009$, $p = 0.713$; NaCl–TMT in TST (0–5 min): $R^2 = 0.494$, $p = 0.000807$, Fig. 4K; H_2O–TMT in TST (5–10 min): $R^2 = 0.0055$, $p = 0.8894$; NaCl–TMT in TST (5–10 min): R^2

= 0.517, p = 0.0956; Fig. 4L). Fisher's r-to-z transformation revealed that during the first 5 min of TMT exposure and the immobility time, Pearson's correlation coefficient of sodium-intake mice was significantly different from that of water-intake mice (distance traveled: TST (0–5 min): z = 2.5248, p = 0.0116, Fig. 4C; TST (5–10 min): z =2.2393, p = 0.0251, Fig. 4D; number of entries: TST (0–5 min): z = 2.21184, p = 0.0270, Fig. 4G; time spent: TST (0–5 min): z = 2.6847, p = 0.00726, Fig. 4H). These results indicate that the NaCl intake caused mice to adapt to the fear and aversive environment, and the preference of the central zone during TMT exposure may be a coping behavior to prevent fear stress–induced despair during TST.

3.5 Effect of 2% NaCl Intake on Mouse Anxiety Levels in the EPM and the Correlation between Anxiety Level and Immobility Time during TST

The sodium-intake mice exhibited a central preference in the test box during the TMT exposure. It has been suggested that decreased levels of anxiety increase exploratory behavior in the open field box, while increased anxiety levels result in less locomotion and staying close to the walls of the open field box for longer [49,50], although this is controversial because higher preference of the central zone does not correlate with anxiety levels in mice. Therefore, we examined whether NaCl intake affected the anxiety level using the EPM [45,51] and whether the NaCl intake produced a correlation between anxiety level and immobility time during TST. The distance traveled in the open arm in sodium-intake mice was similar to that of water-intake mice (NaCl-intake mice; $t(17)$ = 1.4026, p = 0.1787 vs. H_2O-intake mice; Fig. 5C). However, both the number of entries and the time spent in the open arm were significantly increased in the sodium-intake mice compared to the water-intake mice (number of entries: NaCl-intake mice: $t(17)$ = 4.4462, p = 0.000354 vs. H_2O-intake mice, Fig. 5D; time spent: NaCl-intake mice: $t(17)$ = 1.8758, p = 0.0380, Fig. 5E). In addition, the sodium-intake mice spent more time in the central zone than the water-intake mice, although both groups had a similar number of entries in the central zone (number of entries: NaCl-intake mice: $t(17)$ = 1.6436, p = 0.1186 vs. H_2O-intake mice, Fig. 5F; time spent: NaCl-intake mice: $t(17)$ = 2.1429, p = 0.0453; Fig. 5G). Two-way repeated measures ANOVA revealed that the time course of the immobility time during TST in sodium-intake mice was similar to that of water-intake mice ($F(1, 197)$ = 0.8694, p = 0.3622, Fig. 5H). The immobility time during the first 5 min, except the first 1 min (1–6 min), in sodium-intake mice was also similar to that of water-intake mice (NaCl-intake mice: $t(17)$ = 0.4795, p = 0.6377, Fig. 5I). In addition, learned despair during the second 5 min compared to the first 5 min in sodium-intake mice was at a similar level to water-intake mice ($F(1, 41)$ = 0.6168, p = 0.4419, Fig. 5J). In the next experiment, we investigated whether the distance traveled, number of entries, and the time spent in the open arm were correlated with the immobility time during the first and second 5 min of TST. However, Spearman's signed rank test revealed that no behavior was correlated with the immobility time during the first and second 5 min of TST (distance traveled and first 5 min of TST: H_2O-intake mice: p = 0.6362, NaCl-intake mice: p = 0.9787, Fig. 5K; number of entries and first 5 min of TST: H_2O-intake mice: p = 0.7514, NaCl-intake mice: p = 0.8944, Fig. 5L; time spent and first 5 min of TST: H_2O-intake mice: p = 0.8028, NaCl-intake mice: p = 0.1509, Fig. 5M; distance traveled and second 5 min of TST: H_2O-intake mice: p = 0.5526, NaCl-intake mice: p = 0.0872, Fig. 5N; number of entries and second 5 min of TST: H_2O-intake mice: p = 0.9867, NaCl-intake mice: p = 0.7495, Fig. 5O; time spent and second 5 min of TST: H_2O-intake mice: p = 0.5334, NaCl-intake mice: p = 0.7495, Fig. 5P). These results indicate that the anxiety level in sodium-intake mice was not correlated with their immobility time during TST. Therefore, central preference in sodium-intake mice might not reflect their anxiety level.

4. Discussion

The role of salt intake in psychiatric disease remains controversial. Because patients with cardiovascular disease often have mental illnesses such as major depressive symptom [52,53], high salt intake has been suggested to be a risk factor for psychiatric diseases [6,8]. In contrast, it has been reported that low daily salt intake is a significant risk factor for mental distress [10] and that higher intake may protect people from depression [11,54]. This suggests that having a restricted or low salt intake to prevent the aggravation of cardiovascular diseases may cause depressive symptoms [54]. High dietary salt intake is thought to promote neuroinflammation, which can cause depression-like behaviors in rodents [8]. We previously reported that an increase in proinflammatory cytokines was associated with despair behaviors [44,55]. Therefore, if high salt intake increases the level of cytokines in the brain, this could induce depression-like behavior. Chronic salt intake has been shown to increase active coping behavior during inescapable stress by FST [8,20]. In the present study, we observed that immobility time during the TST shortened in mice with 5 consecutive days of 2% NaCl intake compared to those with water intake. TST is similar to FST because mice generally struggle to escape when subjected to tail suspension as an inescapable stress. However, if they have learned they are in an inescapable situation, most mice stop struggling and exhibit despair-like behavior [56]. Immobility time has been widely used to evaluate whether antidepressant drugs affect despair-like behavior [31]. In the present study, mice with a 2% NaCl intake exhibited shorter immobility time in the first 5 min period, except for the first 1 min (1–6 min), indicating their despair behavior was decreased by the 5 days of sodium intake. However, their immobility time during the second 5 min period of the TST was longer than that of

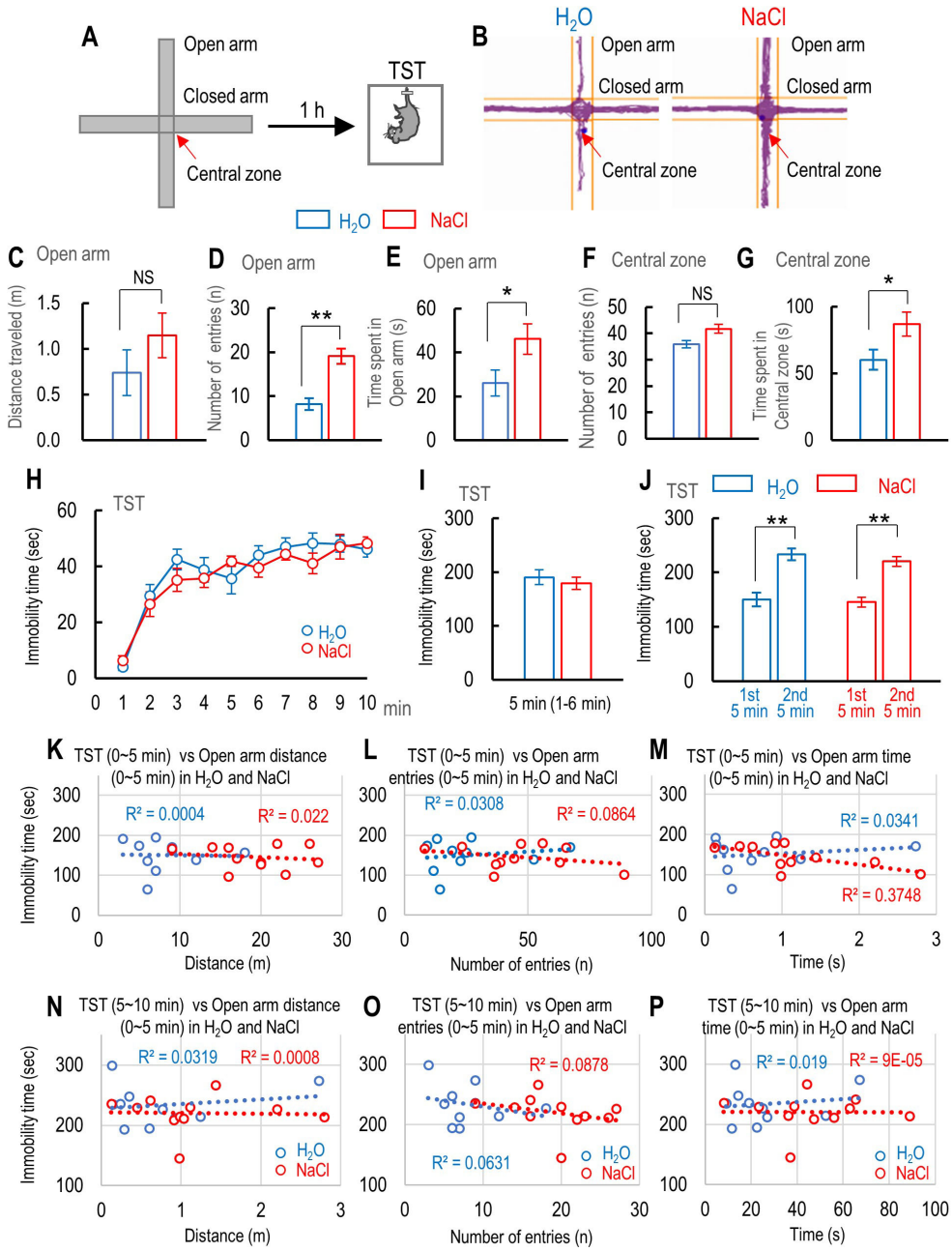

Fig. 5. The effect of 2% NaCl intake on mouse anxiety levels while using the elevated plus maze (EPM) and the correlation between immobility time during tail suspension test (TST) and the anxiety level. (A) Schematic drawing shows the EPM and the schedule of TST. (B) Traces of water- and sodium-intake mice moving on the EPM. (C–E) Comparison between water- and sodium-intake mice in terms of distance traveled (C), number of entries (D), and time spent (E) in the open arm of the EPM. (F, G) Comparison between the number of entries (F) and time spent (G) in the central zone of the EPM for water-intake mice (n = 10) and sodium-intake mice (n = 11). (H) The time course of immobility time during 10 min of TST for water-intake mice (n = 10) and sodium-intake mice (n = 11). (I) Immobility time during the first 6 min, except the first 1 min, of TST for water-intake mice (n = 10) and sodium-intake mice (n = 11). (J) Summary of cumulative immobility time during the first (0–5 min) and last 5 min (5–10 min) 1 h after exposure to inescapable innate fear for water-intake mice (n = 10) and sodium-intake mice (n = 11). (K–M) Correlation between immobility time during the first 5 min of TST and the distance traveled (K), number of entries (L), and time spent in the open arm of the EPM (M) for water-intake mice (n = 10) and sodium-intake mice (n = 11). (N–P) Correlation between immobility time during the second 5 min of TST and the distance traveled (N), number of entries (O), and time spent in the open arm of the EPM (P) for water-intake mice (n = 10) and sodium-intake mice (n = 11). Statistically significant differences between two Pearson's correlation coefficients by Fisher's r-to-z transformation($^{\#\#}p < 0.01$, $^{\#}p < 0.05$) are indicated. NS means not significant. R^2 is Pearson's correlation coefficient.

the first 5 min. We have previously suggested that immobility time during the second 5 min period of the TST was longer due to the induction of learned despair [41,43]. The present results indicate that despair behavior was reduced in mice with a 2% NaCl intake for 5 consecutive days, but that learned despair was not affected. Therefore, active coping against the aversive situation during the TST can reduce despair-like behavior, and a high salt intake can have a similar effect to an antidepressant.

The role of high salt intake in the development of psychiatric diseases is still controversial. High salt intake is a risk factor for multifaceted cardiovascular diseases [57] such as hypertension [1] and renal disease [2]. Mental diseases are common in patients with coronary heart disease and may be associated with a substantial increase in cardiovascular morbidity and mortality [58]. It has been suggested that excessive salt intake causes stress-related psychiatric disorders by increasing one's sensitivity to psychological stress through the activation of stress-sensitive vasopressin neurons and neuronal inflammation in the PVN [8,20]. Therefore, researchers have raised the possibility that active coping behavior in high salt–intake mice might result in their hyper-responsivity to acute stress [6,8,20]. In contrast, acute hypernatremia inhibits neuronal inputs to CRH neurons in the PVN and blunts stress-induced activation of the HPA axis [59]. Moreover, chronic high salt intake for 5 days suppresses the expression of CRH in the PVN during the recovery period after restrained stress [18]. Secretion of CRH from the PVN drives HPA axis activity via adrenocorticotropic hormone (ACTH) and enhances the secretion of cortisol by the adrenal gland cortex. It has been suggested that both CRH secretion and increased levels of cortisol contribute to the signs and symptoms of depression [60]. Therefore, it is conceivable that high salt intake suppresses the secretion of CRH from the PVN during inescapable stress and may prevent the development of depressive-like symptoms in mice. The predator odor of TMT also increases the level of CRH and of corticosterone in mice [61,62].

In the present study, it is possible the observed increase in immobility time during the TST 1 h after exposure to TMT was because the corticosterone was increased by TMT, thereby activating the glucocorticoid receptor and inducing dysregulation of the HAP axis [63,64]. The main findings of the present study are that central preference during TMT exposure was negatively correlated with the immobility time during TST. Furthermore, the freezing time during the first 5 min of TMT exposure was correlated with immobility time during the TST. These results indicate that central preference during inescapable innate fear stress in mice with a 2% NaCl intake for 5 consecutive days induced active coping behavior. Additionally, the shorter immobility time during the TST indicates that the active coping behavior was improved by 2% NaCl intake for 5 days, whereas despair-like behavior during the TST was suppressed by 2% NaCl intake. Active coping is a qualitative factor of resilience and is defined as the ability to cope mentally or emotionally with a crisis and quickly return to pre-crisis status [65]. Active coping strategies and resilience are associated with the maintenance of psychological adjustment [66]. The preference by mice to enter the central zone during their exposure to inescapable innate fear suggests their resilience was increased by intake of 2% NaCl for 5 days, thus allowing active coping behavior against fear and aversive situations. The correlation coefficients between immobility time during the TST and both the distance traveled and the number of entries in the central zone during TMT exposure were significantly different between mice with 2% NaCl intake for 5 days and control mice. Therefore, we suggest that 2% NaCl intake for 5 days gave mice the resilience to adapt to fear and to inescapable stress by enhancing their active coping behaviors.

It has been demonstrated that low dietary sodium causes anxiogenic effects in rats. In the present study, we found that 2% NaCl intake for 5 days increased the distance traveled, the number of entries, and the time spent in the open arm of the EPM. This indicates that high sodium intake reduced the anxiety level compared to control mice. Therefore, 2% NaCl intake has an anxiolytic effect. However, behaviors that indicated a lower anxiety level, such as the distance traveled, the number of entries, and the time spent in the open arm, were not correlated with the immobility time during the TST. It has been suggested that lower levels of anxiety lead to increased exploratory behavior and that increased anxiety induces less locomotion and a preference to stay close to the walls of the field [49]. However, inconsistent results were observed in mice with a lower anxiety level during an open field test [49]. Therefore, taken together, the correlation between central preference and immobility time was not due to a lower anxiety level, but might be due to increased resilience for inducing active coping behavior during inescapable innate fear stress. Because excessive salt intake is a risk factor for cardiovascular disease, the American Heart Association (AHA) recommends a daily intake of less than 3.75 g for the general population [67], with moderate range for dietary sodium (3–5 g/day). These intake levels are associated with the lowest risk of cardiovascular disease and mortality [68]. In the present study, we used C57BL6 mice to investigate the effect of 2% NaCl intake. It has been demonstrated that a 2% NaCl intake for 7 consecutive days did not change serum osmolality compared to water-intake mice [20]. However, another group reported that 2% NaCl intake for 5 consecutive days increased the blood sodium concentration. Therefore, the effect of a short-term high salt diet on mental health and cardiovascular disease requires further investigation. In particular, we need clarify whether the active coping behavior in an aversive environment correlate with the blood sodium concentration. Furthermore, we should investigate what the lowest level of blood sodium concentration can lead to ac-

tive coping behavior in the future.

5. Conclusions

A NaCl intake of 2% for 5 days resulted in a negative correlation between central preference during inescapable innate fear stress and immobility time during a TST-induced aversive situation. The results of EPM indicate that a 2% NaCl intake decreases mouse anxiety levels. However, none of the behaviors related to anxiety in the EPM was correlated with immobility time during the TST. This indicates that the central preference of sodium-intake mice increases their resilience to innate fear stress and leads to active coping behavior that prevents depression-like behavior. Although excessive salt intake poses a risk for the development of cardiovascular disease, an appropriate amount of intake is important for acquiring resilience and for the induction of active coping to deal with daily stress, thus preventing the development of mood disorders.

Abbreviations

AHA, American Heart Association; ANOVA, Analysis of variance; CRH, Corticotropin-releasing hormone; EPM, Elevated plus maze; FST, Forced swimming test; HPA, Hypothalamic–pituitary–adrenal; MO, Mineral oil; NS, no significant; PTSD, Posttraumatic stress disorder; PVN, Paraventricular nucleus of hypothalamus; SEM, Standard error of the mean; TMT, 2,5-dihydro-2,4,5-trimethylthiazoline; TST, Tail suspension test.

Author Contributions

KS and TMat conceived and designed the experiments. RH and TMur designed the behavioral study and performed the experiments. RH, TMur, RK and KS performed the experiments and analyzed the data. TMat and KS wrote the first draft of the manuscript. RH, TMur, RK, and TMat reviewed and critiqued the manuscript. RH, TMur, RK, TMat, and KS analyzed and interpreted the data. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was conducted according to the guidelines of the Declaration of Helsinki and was ap-proved by the Institutional Animal Care and Use Committee of Ohu University, which complies with the criteria mandated by the Japanese Law for the Humane (No. 2018-29, 2019-39, and 2020-17). This study was carried out in compliance with the ARRIVE guidelines.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Osborn JW, Fink GD, Sved AF, Toney GM, Raizada MK. Circulating angiotensin II and dietary salt: converging signals for neurogenic hypertension. Current Hypertension Reports. 2007; 9: 228–235.

[2] Lin C. Stroke and diets – a review. Tzu Chi Medical Journal. 2021; 33: 238–342.

[3] Arcand J, Wong MMY, Santos JA, Leung AA, Trieu K, Thout SR, *et al*. More evidence that salt increases blood pressure and risk of kidney disease from the Science of Salt: a regularly updated systematic review of salt and health outcomes (April-July 2016). The Journal of Clinical Hypertension. 2017; 19: 813–823.

[4] Toney GM, Stocker SD. Hyperosmotic activation of CNS sympathetic drive: implications for cardiovascular disease. The Journal of Physiology. 2010; 588: 3375–3384.

[5] Kato S, Tsukamoto T, Mizoshita T, Tanaka H, Kumagai T, Ota H, *et al*. High salt diets dose-dependently promote gastric chemical carcinogenesis inHelicobacter pylori-infected Mongolian gerbils associated with a shift in mucin production from glandular to surface mucous cells. International Journal of Cancer. 2006; 119: 1558–1566.

[6] Gilman TL, George CM, Andrade MA, Mitchell NC, Toney GM, Daws LC. High Salt Intake Lowers Behavioral Inhibition. Frontiers in Behavioral Neuroscience. 2019; 13: 271.

[7] Hare DL, Toukhsati SR, Johansson P, Jaarsma T. Depression and cardiovascular disease: a clinical review. European Heart Journal. 2014; 35: 1365–1372.

[8] Gilman TL, Mitchell NC, Daws LC, Toney GM. Neuroinflammation Contributes to High Salt Intake-Augmented Neuronal Activation and Active Coping Responses to Acute Stress. International Journal of Neuropsychopharmacology. 2019; 22: 137–142.

[9] Angelakis I, Gooding P, Tarrier N, Panagioti M. Suicidality in obsessive compulsive disorder (OCD): a systematic review and meta-analysis. Clinical Psychology Review. 2015; 39: 1–15.

[10] Shimizu Y, Kadota K, Koyamatsu J, Yamanashi H, Nagayoshi M, Noda M, *et al*. Salt intake and mental distress among rural community-dwelling Japanese men. Journal of Physiological Anthropology. 2015; 34: 26.

[11] Goldstein P, Leshem M. Dietary sodium, added salt, and serum sodium associations with growth and depression in the U.S. general population. Appetite. 2014; 79: 83–90.

[12] LeMoult J, Ordaz SJ, Kircanski K, Singh MK, Gotlib IH. Predicting first onset of depression in young girls: Interaction of diurnal cortisol and negative life events. Journal of Abnormal Psychology. 2015; 124: 850–859.

[13] Adam EK, Sutton JM, Doane LD, Mineka S. Incorporating hypothalamic–pituitary–adrenal axis measures into preventive

interventions for adolescent depression: are we there yet? Development and Psychopathology. 2008; 20: 975–1001.
[14] Scherf-Clavel M, Wurst C, Nitschke F, Stonawski S, Burschka C, Friess L, *et al*. Extent of cortisol suppression at baseline predicts improvement in HPA axis function during antidepressant treatment. Psychoneuroendocrinology. 2020; 114: 104590.
[15] Young EA, Altemus M, Lopez JF, Kocsis JH, Schatzberg AF, deBattista C, *et al*. HPA axis activation in major depression and response to fluoxetine: a pilot study. Psychoneuroendocrinology. 2004; 29: 1198–1204.
[16] Menke A. Is the HPA Axis as Target for Depression Outdated, or Is There a New Hope? Frontier in Psychiatry. 2019; 10: 101.
[17] Cowen PJ. Not fade away: the HPA axis and depression. Psychological Medicine. 2010; 40: 1–4.
[18] Krause EG, Pati D, Frazier CJ. Chronic salt-loading reduces basal excitatory input to CRH neurons in the paraventricular nucleus and accelerates recovery from restraint stress in male mice. Physiology & Behavior. 2017; 176: 189–194.
[19] Smith JA, Wang L, Hiller H, Taylor CT, de Kloet AD, Krause EG. Acute hypernatremia promotes anxiolysis and attenuates stress-induced activation of the hypothalamic–pituitary–adrenal axis in male mice. Physiology & Behavior. 2014; 136: 91–96.
[20] Mitchell NC, Gilman TL, Daws LC, Toney GM. High salt intake enhances swim stress-induced PVN vasopressin cell activation and active stress coping. Psychoneuroendocrinology. 2018; 93: 29–38.
[21] Cabib S, Campus P, Colelli V. Learning to cope with stress: psychobiological mechanisms of stress resilience. Reviews in the Neurosciences. 2012; 23: 659–672.
[22] Lu A, Steiner MA, Whittle N, Vogl AM, Walser SM, Ableitner M, *et al*. Conditional mouse mutants highlight mechanisms of corticotropin-releasing hormone effects on stress-coping behavior. Molecular Psychiatry. 2008; 13: 1028–1042.
[23] Kendler KS, Karkowski LM, Prescott CA. Causal Relationship between Stressful Life Events and the Onset of Major Depression. American Journal of Psychiatry. 1999; 156: 837–841.
[24] Javidi H, Yadollahie M. Post-traumatic Stress Disorder. Journal of Occupational and Environmental Medicine. 2012; 3: 2–9.
[25] Jaiswal M, Seki K, Yoshida S. Molecular mechanism of noradrenaline during the stress-induced major depressive disorder. Neural Regeneration Research. 2018; 13: 1159–1169.
[26] Wood SK, Bhatnagar S. Resilience to the effects of social stress: Evidence from clinical and preclinical studies on the role of coping strategies. Neurobiology of Stress. 2015; 1: 164–173.
[27] Kautz M, Charney DS, Murrough JW. Neuropeptide Y, resilience, and PTSD therapeutics. Neuroscience Letters. 2017; 649: 164–169.
[28] Horn SR, Feder A. Understanding Resilience and Preventing and Treating PTSD. Harvard Review of Psychiatry. 2018; 26: 158–174.
[29] Consoli D, Fedotova J, Micale V, Sapronov NS, Drago F. Stressors affect the response of male and female rats to clomipramine in a model of behavioral despair (forced swim test). European Journal of Pharmacology. 2005; 520: 100–107.
[30] Wang Q, Liu Y, Zhang J, Wang W. Corticotropin-Releasing Factor Receptors in the Locus Coeruleus Modulate the Enhancement of Active Coping Behaviors Induced by Chronic Predator Odor Inoculation in Mice. Frontiers in Psychology. 2019; 10: 3028.
[31] Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. Neuroscience & Biobehavioral Reviews. 2005; 29: 571–625.
[32] El Yacoubi M, Bouali S, Popa D, Naudon L, Leroux-Nicollet I, Hamon M, *et al*. Behavioral, neurochemical, and electrophysiological characterization of a genetic mouse model of depression. Proceedings of the National Academy of Sciences. 2003; 100: 6227–6232.
[33] Rubinow DR, Schmidt PJ. Sex differences and the neurobiology of affective disorders. Neuropsychopharmacology. 2019; 44: 111–128.
[34] Magon N, Kalra S. The orgasmic history of oxytocin: Love, lust, and labor. Indian Journal of Endocrinology and Metabolism. 2011; 15: 156.
[35] Weissman MM. Sex Differences and the Epidemiology of Depression. Archives of General Psychiatry. 1977; 34: 98–111.
[36] Weissman M. Sex differences in rates of depression: cross-national perspectives. Journal of Affective Disorders. 1993; 29: 77–84.
[37] Neumann ID, Landgraf R. Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends in Neurosciences. 2012; 35: 649–659.
[38] Curtis KS. Estrogen and the central control of body fluid balance. Physiology & Behavior. 2009; 97: 180–192.
[39] Endres T, Fendt M. Aversion- vs fear-inducing properties of 2,4,5-trimethyl-3-thiazoline, a component of fox odor, in comparison with those of butyric acid. The Journal of Experimental Biology. 2009; 212: 2324–2327.
[40] Blanchard RJ, Blanchard DC. Crouching as an index of fear. Journal of Comparative and Physiological Psychology. 1969; 67: 370–375.
[41] Fukuwada N, Kanno M, Yoshida S, Seki K. Gαq protein signaling in the bed nucleus of the stria terminalis regulate the lipopolysaccharide-induced despair-like behavior in mice. AIMS Neuroscience. 2020; 7: 438–458.
[42] Mul JD, Zheng J, Goodyear LJ. Validity Assessment of 5 Day Repeated Forced-Swim Stress to Model Human Depression in Young-Adult C57BL/6J and BALB/cJ Mice. Eneuro. 2016; 3: ENEURO.0201–ENEU16.2016.
[43] Fujita S, Yoshida S, Matsuki T, Jaiswal MK, Seki K. The α1-adrenergic receptors in the amygdala regulate the induction of learned despair through protein kinase C-beta signaling. Behavioural Pharmacology. 2021; 32: 73–85.
[44] Sekio M, Seki K. Lipopolysaccharide-induced depressive-like behavior is associated with α_1-adrenoceptor dependent down-regulation of the membrane GluR1 subunit in the mouse medial prefrontal cortex and ventral tegmental area. The International Journal of Neuropsychopharmacology. 2014; 18: pyu005.
[45] Carobrez AP, Bertoglio LJ. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neuroscience & Biobehavioral Reviews. 2005; 29: 1193–1205.
[46] Shoji H, Kunugi H, Miyakawa T. Acute and chronic effects of oral administration of a medium-chain fatty acid, capric acid, on locomotor activity and anxiety-like and depression-related behaviors in adult male C57BL/6J mice. Neuropsychopharmacology Reports. 2022; 42: 59–69.
[47] Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplantation. 2013; 48: 452–458.
[48] Kimura K, Minami R, Yamahama Y, Hariyama T, Hosoda N. Framework with cytoskeletal actin filaments forming insect footpad hairs inspires biomimetic adhesive device design. Communications Biology. 2020; 3: 272.
[49] Ennaceur A. Tests of unconditioned anxiety — Pitfalls and disappointments. Physiology & Behavior. 2014; 135: 55–71.
[50] Sturman O, Germain P, Bohacek J. Exploratory rearing: a context- and stress-sensitive behavior recorded in the open-field test. Stress. 2018; 21: 443–452.
[51] Kulesskaya N, Voikar V. Assessment of mouse anxiety-like behavior in the light–dark box and open-field arena: Role of equipment and procedure. Physiology & Behavior. 2014; 133: 30–38.

[52] Ogunmoroti O, Osibogun O, Spatz ES, Okunrintemi V, Mathews L, Ndumele CE, *et al*. A systematic review of the bidirectional relationship between depressive symptoms and cardiovascular health. Preventive Medicine. 2022; 154: 106891.
[53] Sreenivasan J, Kaul R, Khan MS, Malik A, Usman MS, Michos ED. Mental health disorders and readmissions following acute myocardial infarction in the United States. Scientific Reports. 2022; 12: 3327.
[54] Leshem M. Salt need needs investigation. British Journal of Nutrition. 2020; 123: 1312–1320.
[55] Kurosawa N, Shimizu K, Seki K. The development of depression-like behavior is consolidated by IL-6-induced activation of locus coeruleus neurons and IL-1β-induced elevated leptin levels in mice. Psychopharmacology. 2016; 233: 1725–1737.
[56] Can A, Dao DT, Terrillion CE, Piantadosi SC, Bhat S, Gould TD. The tail suspension test. Journal of Visualized Experiments. 2012; 2021: e3769.
[57] Baldo MP, Rodrigues SL, Mill JG. High salt intake as a multifaceted cardiovascular disease: new support from cellular and molecular evidence. Heart Failure Reviews. 2015; 20: 461–474.
[58] De Hert M, Detraux J, Vancampfort D. The intriguing relationship between coronary heart disease and mental disorders. Dialogues in Clinical Neuroscience. 2018; 20: 31–40.
[59] Krause EG, de Kloet AD, Flak JN, Smeltzer MD, Solomon MB, Evanson NK, *et al*. Hydration State Controls Stress Responsiveness and Social Behavior. Journal of Neuroscience. 2011; 31: 5470–5476.
[60] Bao A-, Meynen G, Swaab DF. The stress system in depression and neurodegeneration: Focus on the human hypothalamus. Brain Research Reviews. 2008; 57: 531–553.
[61] Morrow BA, Elsworth JD, Roth RH. Fear-like biochemical and behavioral responses in rats to the predator odor, TMT, are dependent on the exposure environment. Synapse. 2002; 46: 11–18.
[62] Day HEW, Masini CV, Campeau S. The pattern of brain c-fos mRNA induced by a component of fox odor, 2,5-dihydro-2,4,5-Trimethylthiazoline (TMT), in rats, suggests both systemic and processive stress characteristics. Brain Research. 2004; 1025: 139–151.
[63] Holanda VAD, Oliveira MC, Da Silva Junior ED, Calo' G, Ruzza C, Gavioli EC. Blockade of nociceptin/orphanin FQ signaling facilitates an active copying strategy due to acute and repeated stressful stimuli in mice. Neurobiology of Stress. 2020; 13: 100255.
[64] Pavlides C, Watanabe Y, Magariños AM, McEwen BS. Opposing roles of type i and type II adrenal steroid receptors in hippocampal long-term potentiation. Neuroscience. 1995; 68: 387–394.
[65] de Terte I, Stephens C. Psychological Resilience of Workers in High-Risk Occupations. Stress and Health. 2014; 30: 353–355.
[66] Smith MM, Saklofske DH, Keefer KV, Tremblay PF. Coping Strategies and Psychological Outcomes: the Moderating Effects of Personal Resiliency. The Journal of Psychology. 2016; 150: 318–332.
[67] Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, *et al*. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Journal of the American College of Cardiology. 2019; 74: 1376–1414.
[68] Mente A, O'Donnell M, Yusuf S. Sodium Intake and Health: What Should We Recommend Based on the Current Evidence? Nutrients. 2021; 13: 3232.