



# The functional link between tail-pinch-induced food intake and emotionality and its possible role in stress coping in rats

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## Abstract

Tail pinch facilitates eating in rats. We investigated an unidentified link between tail-pinch-induced eating behavior and individual emotionality in male Sprague–Dawley rats. Anxiety-like behavior was assessed on the elevated plus maze (EPM) and in the open field test (OFT). Tail-pinch-induced eating was observed as follows: After a 30-min habituation period, the tail pinch was applied for 5 min, followed by a 30-min recovery period. During the habituation and recovery periods, rats were allowed to access food ad libitum. During the recovery period, 14 of 24 rats ate more food than during the habituation period. Thus, we named them “high responders” and the others as “low responders”. The food intake was significantly greater, while the times spent in the open arms in the EPM and in the center area in the OFT were significantly shorter in high responders than in low responders. This result suggests that the rats consuming more food after mild stress have higher anxiety.

**Keywords** Tail pinch · Hyperphagia · Anxiety · Stress-induced eating

## Abbreviations

5-HT	Serotonin
RHA/Verh	Roman-high avoidance
RLA/Verh	Roman-low avoidance
TPF	Tail pinch with food
TP	Tail pinch without food
CF	Control with food
EPM	Elevated plus maze test
OFT	Open field test
TPF-high	High responders to tail pinch stress
TPF-low	Low responders to tail-pinch stress

## Introduction

In humans, it is known that emotional stress can affect eating behavior, and that this emotion-induced change of eating behavior depends on the variability across both individuals and emotions [1]. For example, a previous survey revealed that an almost equal number of participants reported eating more and eating less in response to stress [2], and emotional eaters, identified by questionnaire [3], were more likely to report overeating than were non-emotional eaters [4]. Laboratory study also reported that emotional eaters consumed more sweet, high-fat foods in response to emotional stress compared to non-emotional eaters [5]. Stress-induced eating may be one factor that contributes to the development of obesity [6]. The convergence of these lines of evidence has impelled researchers to investigate the mechanism of stress-induced eating.

Tail-pinch-induced food intake is known as one animal model of stress-induced eating. Tail pinch was first observed to induce eating behavior in rats in 1975 [7]. Many studies since then have been trying to elucidate the possible mechanisms underlying this response and have suggested that dopamine, serotonin (5-HT), and other endogenous molecules such as opiates, neuropeptide Y, and corticotropin-releasing factor are involved in this response [7–15]. Although such evidence has accumulated, the relationship

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between stress-induced eating and individual psychological characteristics has not been well examined.

Several studies investigated eating behavior and neuroendocrine responses to tail-pinch stress using psychogenetically selected lines of rats that differ in emotion- and coping-related strategies: The Roman high-avoidance (RHA/Verh) rats represent a “proactive coper” and Roman low-avoidance (RLA/Verh) rats represent a “reactive coper”. Martin [16] reported that RLA/Verh rats exhibited significantly shorter latencies to bite and eat solid food during tail pinch than the RHA/Verh rats. In addition, Giorgi et al. [17] reported that RHA/Verh rats displayed more robust active coping activity (clamp gnawing) and less fear-related behavior (freezing, self-grooming) than RLA/Verh rats, and had higher dopamine output in the medial prefrontal cortex. However, it is not known whether such emotional influences extend to tail-pinch-induced food intake in non-selected lines of rats.

Not only does emotion regulate eating, but eating may also regulate emotions; i.e., eating may provide an outlet for emotional tension. It is thought that tail-pinch-induced eating has a function as a relief from tail-pinch stress, but there is only slight evidence that eating after tail pinch has a function of helping the rat to cope or extinguish the behavioral and physiological reaction. Several behavioral responses induced by tail-pinch are accompanied by monoaminergic release; those have a role for stress relief [17–19]. This prompted us to hypothesize that the food intake after mild stress may have a certain function against stress status and alter subsequent behavior. So far, however, there is no integrated analysis that has determined the functions of the eating response to tail pinch considering the individual emotionality and selectivity of eating.

Elucidating the involvement of emotionality on stress-induced behavior and the effect of stress-induced eating behavior on other behaviors is important for understanding the physiological meaning of stress-induced eating and to prevent overeating induced by stress, especially for emotional eaters. Thus, we designed the present study to elucidate (1) the functional link between the amount of food intake immediately after the tail pinch and the anxiety-like behavior and locomotor activity, and (2) the effect of food intake immediately after the tail pinch on subsequent spontaneous activity such as locomotor activity, grooming, and food intake. We analyzed the data among groups based on the self-selectivity of stress-induced eating behavior.

## Methods

### Animals

Forty-eight 6-week-old male Sprague–Dawley rats (Kyudo, Tosu, Japan) weighing 167–200 g were individually housed

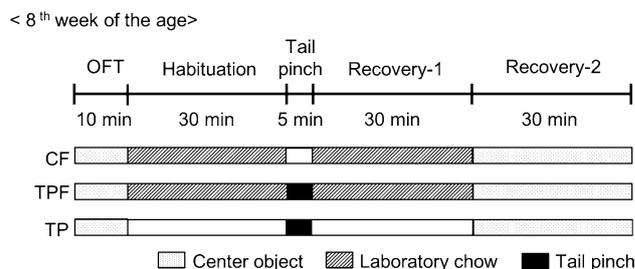
and fed ad libitum. Housing conditions were thermostatically controlled at 22–24 °C and maintained on a 12-h dark/light schedule (lights on at 8:00–20:00). Daily food intake was measured every day at 10:00–10:30. Rats were assigned to the following three groups at random: tail pinch with food (TPF,  $n = 24$ ), tail pinch without food (TP,  $n = 12$ ), and control with food (CF,  $n = 12$ ).

### Outline and experimental conditions

At 7 weeks of age, anxiety-like behavior was assessed on an elevated plus maze (EPM). At 8 weeks of age, the open field test (OFT) was performed in an experimental field. Subsequently, the habituation period of the tail-pinch procedure was started in the same field, followed by tail-pinch and recovery periods (Fig. 1). All sessions were automatically recorded with a computer-based videotracking system (Ethovision v1.96, Noldus Info. Tech., Netherlands). All tests were conducted between 11:00 and 16:00 h. Brightness conditions of each test were about 46 lx for the EPM at 50 cm height from the floor and 33 lx for the OFT and tail-pinch procedure at the floor level. Each apparatus was cleaned after the test session of each rat to prevent olfactory cues from affecting the behavior of subsequently tested rats. The experiments were performed under the control of the Ethics Committee of Animal Care and Experimentation in accordance with the Guiding Principles for Animal Care Experimentation, Kyushu Institute of Technology, Japan, and with the Japanese Law for Animal Welfare and Care.

### Elevated plus maze test (EPM)

The EPM was performed according to the way that has been established in our laboratory [20]. The elevated plus maze consisted of two open arms (50 × 10 cm) and



**Fig. 1** Time course of the experiment at 8 weeks of age. Immediately after the open field test (OFT), the center object was removed and habituation period was started. After 30-min habituation, tail pinch was applied for the group of tail pinch with food (TPF) and the group of tail pinch without food (TP). During habituation and recovery-1, food was available only for the group of control with food (CF) and TPF. At the end of recovery-1, the food was removed and the center object was put again on the center of the field, and then recovery-2 was started

two closed arms (50 × 10 cm), which had 39-cm-high walls. The arms extended from a square center platform (10 × 10 cm) and were arranged so that those of the same type were opposite to each other. The apparatus was elevated to 50 cm above the floor. Each rat was placed on the center platform and allowed to move freely for 5 min. We recorded the time spent in the open and closed arms and the number of entries into each arm as an index of the rats' inherent anxiety activity.

### Open field test (OFT)

The open field apparatus was a square field (49 × 49 × 49 cm), in which an object (φ7 × 4 cm) was placed at the center. Each rat was placed along one side in the apparatus at the beginning of the test and was allowed to move freely for 10 min. We recorded the total distance moved as an index of locomotor activity, time spent outside of the center area (21 × 21 × 21 cm) as an index of anxiety behavior, and duration spent grooming as an index of stress-related behavior.

### Tail pinch procedure

Tail pinch procedure was performed in the same field of the OFT continuously. After the OFT, the object at the center was removed from the field and laboratory chow (six pieces of solid chow, approximately 20 g) was put in the field, and rats were allowed to habituate to the environment for 30 min (habituation period). At the end of habituation period, the chow was removed from the field. Then a binder clip (15 mm wide, and its inner section was isosceles triangle with 8-mm height and 7-mm base) was placed 4 cm from the tip of the tail for 5 min for TPF and TP, but not for CF. The rats were not restrained during tail pinch and some of them were chasing their tail or gnawing the clip. After taking off the clip, the chow was put in the field again, and the tail-pinch-induced food ingestion was measured for 30 min (recovery-1). Then chow was removed from the field and the center object was put again in the field, and consecutive behaviors were measured for 30 min (recovery-2).

We measured the amount of food ingested during the habituation and recovery-1 periods. In the recovery-1 period, four of 24 rats in the TPF showed no feeding response to tail pinch, and six rats consumed smaller amounts of food in recovery-1 than in the habituation period. We categorized these ten rats as low responders to tail-pinch stress (TPF-low), and the rest as high responders (TPF-high).

After the end of experiment, the rats were put in their home cage. Then we assessed overnight food intake in their home cage for approximately 20 h.

### Statistical analysis

To evaluate the effect of tail pinch on food intake in the experimental field, the main effects of time (habituation and recovery periods) and group (CF, TPF-high, and TPF-low), and their interaction were examined by two-way repeated ANOVA. When a significant *F* value was observed, this was examined further by comparisons between groups at each time point.

One-way ANOVA was used for comparisons of body weight on the experimental day, the amount of food intake on the day before the experimental day, the percentage of time spent in the open arms and the ratio of entries into the open arms to the total number of entries to all arms in the EPM, and for the locomotor activity, the time spent in the center area, and the time spent grooming during the OFT among the CF, TPF, and TP groups. These parameters were also compared by paired *t* test between TPF-high and TPF-low. Correlation coefficients between the amount of food intake during the recovery period and behavioral parameters on the EPM and during the OFT were also evaluated in the TPF.

One-way ANOVA was also used for comparisons of the locomotor activity, time spent in the center area, and time spent grooming during the habituation, recovery-1, and recovery-2 among groups (CF, TP, TPF-high, and TPF-low). The amount of overnight food ingestion was compared using one-way ANOVA among these groups. When a significant *F* value was observed, this was examined further by Tukey–Kramer HSD.

The data are expressed as mean ± SEM. Statistical significance was accepted at *p* < 0.05. The statistical analyses were performed with JMP Pro (ver.13.1.0, SAS Institute, Cary, NC, USA) at Chiba Prefectural University of Health Sciences.

## Results

### Precondition of the tail pinch procedure

As shown in Table 1, there was no significant difference in the rats' weight on the experimental day, the amount of food intake on the day before the experimental day, the percentage of time spent in the open arms and the percentage of entries in the open arms on the EPM, and the total distance moved, the time spent in the center area, and the time spent grooming in the OFT among the CF, TPF, and TP.

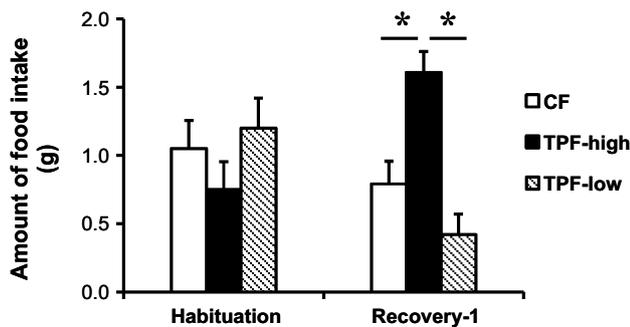
### Effect of tail pinch on food intake

There was no significant difference in the amounts of food ingested during the habituation period among CF, TPF-high,

**Table 1** General parameters

	CF, <i>n</i> = 12	TPF, <i>n</i> = 24	TP, <i>n</i> = 12
Weight, g	309 ± 6	315 ± 4	314 ± 6
Food intake before experimental day, g	26.4 ± 0.9	26.4 ± 0.5	26.8 ± 1.1
Percentage of time spent in open arms on the EPM, %	16 ± 3	21 ± 4	14 ± 3
Percentage of entries into open arms on the EPM, %	43 ± 5	42 ± 3	36 ± 6
Total distance moved in the OFT, m	42.7 ± 2.6	47.7 ± 3.1	46.7 ± 4.9
Time spent in the center area in the OFT, s	99 ± 13	95 ± 11	95 ± 11
Time spent grooming in the OFT, s	48 ± 10	64 ± 7	53 ± 13

Data are means ± SEMs. There was no significant difference among control with food (CF), tail pinch with food (TPF), and tail pinch without food (TP) groups. *EPM* elevated plus maze test, *OFT* open field test ( $p > 0.05$ )

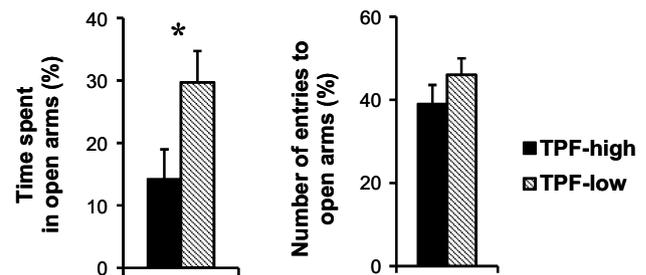


**Fig. 2** The amount of food ingested during 30 min of habituation and recovery-1 periods in CF and high responders and low responders in tail pinch with food (TPF-high and TPF-low, respectively). Data are means ± SEMs. \*A significant difference was observed between groups ( $p < 0.05$ )

and TPF-low ( $1.1 \pm 0.2$ ,  $0.8 \pm 0.2$ , and  $1.2 \pm 0.2$  g, respectively). During the recovery-1 period, on the other hand, the amount food ingested was significantly greater in TPF-high than in CF and TPF-low ( $1.6 \pm 0.2$ ,  $0.8 \pm 0.2$ , and  $0.4 \pm 0.2$  g, respectively, Fig. 2).

### Tail-pinch-induced food intake and emotionality

In the TPF including TPF-high and TPF-low, significant negative correlation was observed between the amount of food intake during recovery-1 and the time spent in the center area in the OFT ( $r = -0.53$ ,  $p < 0.05$ ). Comparing the TPF-high and TPF-low, the body weight on the experimental day ( $315 \pm 6$  and  $315 \pm 4$  g) and the amount of the food intake on the day before the experimental day ( $26.4 \pm 0.7$  and  $26.4 \pm 0.8$  g) were not significantly different between the groups. The ratio of time spent in the open arms to the time spent in either kind of arms in the EPM was significantly shorter in the TPF-high than the TPF-low ( $14 \pm 5$  and  $30 \pm 5\%$ , respectively, Fig. 3, left). On the other hand, the ratio of the number of entries into the open arms to the total number of entries into either kind of arms in the



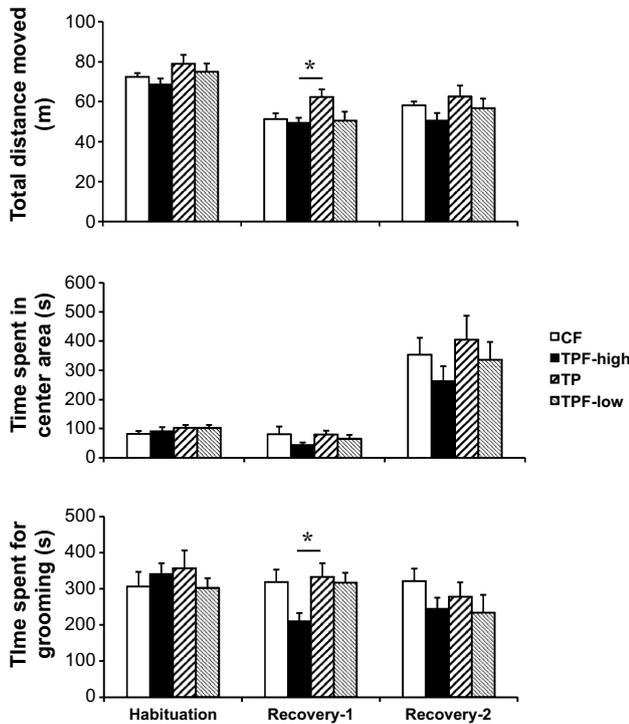
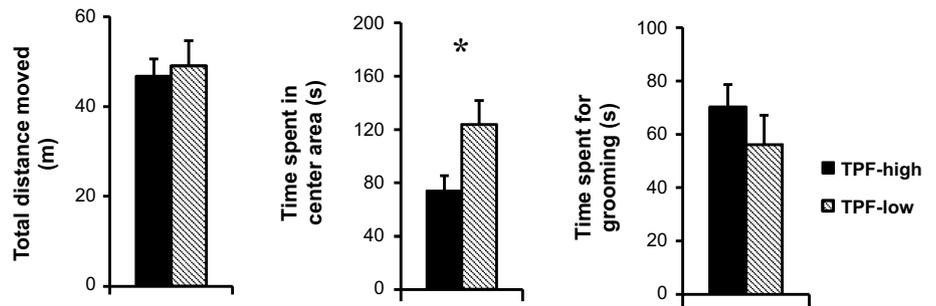
**Fig. 3** Ratio of time spent in open arms to the time spent in open and closed arms during 5 min of elevated plus maze test (EPM) in TPF-high and TPF-low (left). The ratio of the number of entries into open arms to the number of entries into open and closed arms during EPM (right). Data are means ± SEMs. \*A significant difference was observed between groups ( $*p < 0.05$ )

EPM did not differ significantly between the groups ( $39 \pm 5$  and  $46 \pm 4\%$  for TPF-high and TPF-low, respectively, Fig. 3, right). Although the total distance moved in the OFT did not differ significantly between the groups ( $46.7 \pm 3.8$  and  $49.0 \pm 5.6$  m for TPF-high and TPF-low, respectively, Fig. 4, left), a significant difference was observed in the time spent in the center area in the OFT; the shorter duration in the TPF-high ( $74 \pm 11$  s) implied a higher anxiety than in the TPF-low ( $124 \pm 18$  s, Fig. 4, middle). There was no significant difference in the time spent grooming between the groups ( $70 \pm 8$  and  $56 \pm 11$  s for TPF-high and TPF-low, respectively, Fig. 4, right). These results indicated that the individuals who consumed more food after the stress could show higher anxiety behavior and that neither body size, feeding condition, nor general activity could affect the feeding response immediately after the tail-pinch stress.

### Effect of food intake immediately after the tail pinch on subsequent behaviors

During the habituation period, there was no significant difference in the total distance moved in the open field among the groups (Fig. 5, top). During the recovery-1 period, the

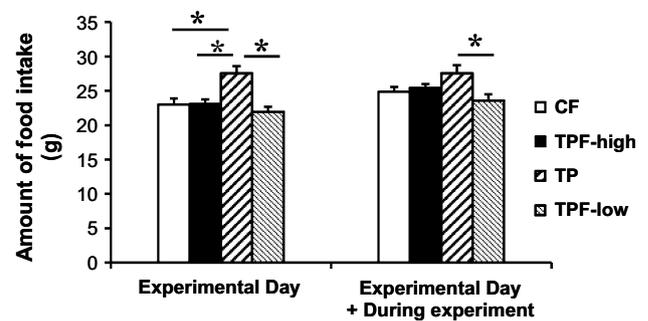
**Fig. 4** Total distance moved (*left*), the time spent in center area (*middle*), and the time spent grooming (*right*) in TPF-high and TPF-low during 10 min of the open field test (OFT). Data are means  $\pm$  SEMs. \*A significant difference was observed between groups ( $p < 0.05$ )



**Fig. 5** Total distance moved (*upper panel*), the time spent in the center area (*middle panel*), and the time spent grooming (*lower panel*) during habituation, recovery-1, and recovery-2 in the CF, TPF-high, TP, and TPF-low. Data are means  $\pm$  SEMs. \*A significant difference was observed between groups ( $p < 0.05$ )

total distance moved in the open field was significantly greater for TP than for TPF-high. During the recovery-2 period, there was no significant difference among the groups. During habituation, recovery-1, and recovery-2, there was no significant difference among the groups in the time spent in the center area of the open field (Fig. 5, middle). The time spent grooming did not differ among the groups during the habituation and recovery-2 periods, while it was shorter in the TPF-high than TP during the recovery-1 period (Fig. 5, bottom).

The amount of overnight food ingestion after the experiment in the TP group was significantly greater than that in CF, TPF-high, and TPF-low (Fig. 6). Even when the amount



**Fig. 6** Amount of food consumed in the post experiment overnight period and the sum of the amount of food consumed during the experiment and the subsequent overnight period by CF, TPF-high, TP, and TPF-low. Data are means  $\pm$  SEMs. \*A significant difference was observed between groups ( $p < 0.05$ )

of food ingested during the experiment was combined with the amount of food ingested after the experiment in CF, TPF-high, and TPF-low, the significant difference between TP and TPF-low was still observed (Fig. 6).

## Discussion

The main finding in the present study is that rats which expressed a certain degree of eating behavior as a response to tail pinch showed higher anxiety behavior during EPM and OFT. This result suggests that individuals with higher susceptibility to stress-induced food intake may have higher anxiety.

### Tail-pinch-induced feeding behavior

In the present study, four of 24 rats did not show eating behavior after the tail pinch. In previous studies [7, 8, 14], 90–100% of rats have shown tail-pinch-induced behaviors, i.e., eating, gnawing, and licking. The lower rate in the present study could be due to methodological differences. For example, in these previous studies, rats were allowed to access the food during 20 s of tail pinch [7, 8]. On the other hand, in the present study, chow was removed at the start

of the tail pinch and put in again after the end of the tail pinch. The methods of the tail pinch, e.g., pinching materials, duration, and application point of the tail, varied from study to study. Thus, it is difficult to conclude the reason why there were non-responders in the present study. Nevertheless, we successfully identified a difference in emotionality between high responders and low responders using our tail-pinch method. We characterized the high responders as rats that ate more food during the 0–30 min after the tail pinch (recovery-1) than during the 30-min of habituation period, and low-responders as rats that ate no or less food during the recovery-1 than during the habituation period. We first compared the emotionality between the two groups of rats expressing different stress responses.

### **Involvement of emotionality in tail-pinch-induced eating**

In the present study, there was no significant difference in the amount of food intake during the habituation period between TPF-high and TPF-low. On the other hand, during recovery-1, the amount of food intake was significantly greater in TPF-high than TPF-low. The body weight on the experimental day and the amount of food intake before the experimental day did not differ between the groups, while the percentage of time spent in the open arms was significantly higher in the TPF-low than the TPF-high in the EPM test. In addition, the time spent in the center area was longer in the TPF-low than in the TPF-high in the OFT. Moreover, in the TPF, including TPF-high and TPF-low, a significant negative correlation was observed between the amount of food intake during recovery-1 and the time spent in the center area in the OFT. These results indicated that anxiety could be involved in the tail-pinch-induced eating behavior. The group that displayed stress-induced eating behavior could have emotionality with higher anxiety.

This result is partly consistent with the results from previous pharmacological studies. Hawkins et al. [11, 12] reported that activation of the 5-HT<sub>2</sub> receptor produced an anxiolytic effect, and decreased the amount of food intake in response to tail pinch in rats. Our results suggest that lower anxiety is one psycho-behavioral characteristic which has a functional link to food intake in response to tail pinch.

### **The possible role of eating for stress coping**

Our second interest was the possible role of the tail-pinch-induced food intake. We compared the behaviors after the tail-pinch stress among the four groups. During the recovery-1 period, in which animals were allowed to eat food only in CF, TPF-high, and TPF-low but not in TP, the distance moved in the open field was longer in the TP than in the TPF-high. There is a possibility that the time spent eating

food in TPF-high could affect their activity in this period. Nevertheless, there was no significant difference in the total distance moved among the CF, TPF-high, and TPF-low, even though the amount of food intake of TPF-high was twice that of CF and three times that of TPF-low. Considering this, the difference between TP and TPF-high cannot be attributed to the eating duration alone, but the tail-pinch stress could increase the rats' activity when food is not available after the stress. On the other hand, the time spent grooming was shorter in the TPF-high than in the TP, but it did not differ among TP, CF, and TPF-low. Thus, the shorter time spent grooming in the TPF-high could be due to the longer time spent eating, rather than the effect of tail pinch per se. The lack of difference among groups during recovery-2 indicated that the effect of tail pinch had only temporary effects on general activities and anxiety behaviors, and that eating behavior after the tail pinch did not affect the subsequent behaviors assessed in the present study. Thus, we could not show a significant role of eating as stress coping behavior. Further studies are needed to elucidate this.

The overnight food ingestion after the experiment in the TP was significantly greater than that in the CF, TPF-high, and TPF-low. Even when the amount of food ingested during the experiment was combined with the amount of food ingested after the experiment in the CF, TPF-high, and TPF-low, the significant difference between TP and TPF-low was still observed. These results indicate that in the TP, the food intake after the experiment compensated for the food intake immediately after the stress. On the other hand, in the TPF-low, the smaller amount of food intake was not compensated for by the food intake during the subsequent overnight period. Nevertheless, there was no significant difference between CF and the other three groups. This result suggested that the tail pinch adopted in the present study has only temporary and trivial effects on short-term food intake.

## **Conclusions**

In conclusion, the present study indicates that individuals susceptible to stress-induced food intake could have emotionality of higher anxiety. In addition, eating behavior after the tail pinch has a limited effect on subsequent behaviors assessed in the present study, even in the subgroup analysis based on self-selectivity of eating. Further studies are needed to clarify the possible role of stress-induced eating as a stress-coping behavior.

**Author contribution** NA designed and performed the experiment, analyzed the data, interpreted the results, and drafted the manuscript. KN, AM, and SA helped to perform the experiment, to interpret the results, and to draft the manuscript.

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### Compliance with ethical standards

**Ethical approval** All procedures performed in this study were accordance with national and institutional guidelines for the care and use of animals.

**Conflict of interest** The authors declare that they have no conflicts of interest.

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