Enjoyment of Spicy Flavor Enhances Central Salty-Taste Perception and Reduces Salt Intake and Blood Pressure

Qiang Li,* Yuanting Cui,* Rongbing Jin, Hongmei Lang, Hao Yu, Fang Sun, Chengkang He, Tianyi Ma, Yingsha Li, Xunmei Zhou, Daoyan Liu, Hongbo Jia, Xiaowei Chen, Zhiming Zhu

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Abstract—High salt intake is a major risk factor for hypertension and is associated with cardiovascular events. Most countries exhibit a traditionally high salt intake; thus, identification of an optimal strategy for salt reduction at the population level may have a major impact on public health. In this multicenter, random-order, double-blind observational and interventional study, subjects with a high spice preference had a lower salt intake and blood pressure than subjects who disliked spicy food. The enjoyment of spicy flavor enhanced salt sensitivity and reduced salt preference. Salt intake and salt preference were related to the regional metabolic activity in the insula and orbitofrontal cortex (OFC) of participants. Administration of capsaicin—the major pungent component of chili pepper—enhanced the insula and OFC metabolic activity in response to high-salt stimuli, which reversed the salt intensity–dependent differences in the metabolism of the insula and OFC. In animal study, OFC activity was closely associated with salt preference, and salty-taste information processed in the OFC was affected in the presence of capsaicin. Thus, interventions related to this region may alter the salt preference in mice through fiber fluorometry and optogenetic techniques. In conclusion, enjoyment of spicy foods may significantly reduce individual salt preference, daily salt intake, and blood pressure by modifying the neural processing of salty taste in the brain. Application of spicy flavor may be a promising behavioral intervention for reducing high salt intake and blood pressure. (Hypertension. 2017;70:00-00. DOI: 10.1161/HYPERTENSIONAHA.117.09950.)

Key Words: blood pressure ■ brain ■ hypertension ■ orbitofrontal cortex ■ risk factors

High salt intake is a major risk factor for hypertension and is associated with cardiovascular events.1–4 The World Health Organization has proposed salt reduction as the key dietary target for 2025 to reduce mortality from the noncommunicable diseases.4 Most countries in the world have a traditionally high salt intake and consume dietary salt far beyond the 5 g/d recommended by the World Health Organization.5–7 The worldwide prevalence of hypertension has tremendously increased during the previous several decades.8 Thus, effectively restricting salt consumption is crucial for preventing hypertension induced by high salt intake in the population.

Current measures for reducing salt intake include education on healthy lifestyles, a campaign for the use of salt spoons, and the promotion of low-sodium salt with the addition of magnesium and potassium.2 However, traditional cooking habits and changes in the taste of food have dampened the effectiveness of salt reduction at the population level.6,7 High salt intake is associated with altered salt sensitivity and the development of a demand for salted food.9,10 Thus, an alternative strategy for reducing salt intake may be to modify the perception of saltiness.

It is well established that the central gustatory system and the mesolimbic structures are critical for taste signal processing and hedonic responses to foods. Palatability is the consequence of the stimulation of brain reward pathways, which indicates that an individual’s salt preference may be associated with the neural hedonic properties of salted foods.11 The orbitofrontal cortex (OFC), as the secondary taste cortex, has been shown to be specifically associated with hedonic aspects and thus the subjective pleasantness of taste.12 Capsaicin—the major pungent component of chili pepper—has been shown to influence salt sensitivity in humans.13 Epidemiological and experimental studies have indicated an extensive protective role of spicy food or capsaicin against...
It is worth examining whether spicy food consumption may reduce dietary salt intake and whether spicy flavor may affect salty-taste perception. Therefore, we hypothesized that capsaicin administration could reduce salt intake by modifying the neural processing of salty-taste signals. To test this hypothesis, we initially examined the participants’ preferences for salty and spicy flavors, salt intake, and blood pressure in a community-based cross-sectional study. We subsequently validated the effect of capsaicin on the neural perception of saltiness through a randomized, double-blind intervention study. Finally, we validated our hypothesis in rodents using optogenetic approaches.

Methods
Detailed Methods are provided in the online-only Data Supplement. The human study was conducted according to the principles of the Declaration of Helsinki. All protocols and experimental procedures were approved by the institutional ethics committee of the hospital, Institutional Animal Care and Research Advisory Committee, Daping Hospital, Third Military Medical University.

Statistical Analyses
In human study, the baseline characteristics of the participants were compared between the groups using the χ² test for categorical variables and the 2 sample t test for continuous variables. The 2 sample t test was used to evaluate differences in the positron emission tomography images between the groups. Linear regression analysis was performed to assess the relationships among salt intake, salt preference, and changes in regional glucose metabolism. Multivariable adjustment for variables, including age, sex, educational level, work status, fasting blood glucose, body mass index, and waist circumstance, as well as the corresponding 95% confidence intervals (95% CIs), were estimated by covariance analysis with a univariate general linear model. Numeric results are presented as the mean±SD or the mean and 95% CI. The positron emission tomography/computed tomography images were processed using statistical parametric mapping (SPM 8.0), and a critical P=0.005 (uncorrected) with a cluster filter of 5 voxels (1 voxel=8 mm³) was used to identify significant differences. SPM12 maps were superimposed on a standard magnetic resonance imaging brain template. In the animal study, the results are represented as the mean±SEM. Comparisons between the groups were made using a 2-tailed unpaired Student t test or 1-way ANOVA with Tukey multiple comparisons test. The Mann–Whitney nonparametric U test was used to analyze data with an abnormal distribution. Numeric statistical analyses were conducted using SPSS software, version 13.0 (SPSS, Inc), or GraphPad Prism software, version 5.0 (GraphPad Software); a 2-sided P value of <0.05 indicated statistical significance.

Results
Baseline Characteristics of the Study Participants
The baseline characteristics of the participants are presented in Table 1. A preference for higher salt was associated with an older age, heavier physical labor, higher prevalence of hypertension, and lower levels of education. Sensitivity to salt was also changed in the high-salt preference group, along with a less sensitive perception of saltiness (P<0.02) and a higher threshold for declaring a solution to be intolerably salty (P<0.01). Notably, the qualitative analysis indicated a graded effect of salt preference on daily salt intake (P<0.01), with mean values of 11.7±4.8, 13.1±5.3, and 14.3±4.7 g/d in the low-, medium-, and high-salt preference groups, respectively.

The participants with a high salt preference had higher systolic blood pressure (131±15 versus 122±18 mm Hg, respectively; P<0.001) and diastolic blood pressure (82±11 versus 75±11 mm Hg, respectively; P<0.001) than the participants who had a low salt preference. Our findings indicated only minor changes after adjustment for several participant characteristics (Table S2 in the online-only Data Supplement). The participants with a high salt preference had higher salt intake (1.8 g/d; 95% CI, 0.7–2.9 g/d; P<0.01), systolic blood pressure (5.0 mm Hg; 95% CI, 1.7–8.4 mm Hg; P<0.01), and diastolic blood pressure (4.4 mm Hg; 95% CI, 2.0–6.7 mm Hg; P<0.001) than the participants who had a low salt preference.

Spicy Flavor Reduced Salt Intake and Blood Pressure by Modifying Salt Preference and Sensitivity
We subsequently examined whether spicy flavor affected salt intake and blood pressure. A higher salt preference was associated with a lower spice preference, which was represented by the proportion of participants who rated a given capsaicin solution as tolerable (Table 1; P<0.01). Furthermore, a high spice preference was associated with a more sensitive perception of saltiness (Table S1; P=0.001) and a lower threshold for declaring a solution to be intolerably salty (Table S1; P=0.001). These findings indicated that a spice preference may affect salt preference and sensitivity.

Next, we investigated whether spice preference affected salt intake and thus blood pressure. A qualitative analysis indicated that the degree of spice preference affected salt intake and blood pressure (Figure 1A through 1C). The mean salt intake was 13.4±5.1, 10.9±4.5, and 10.3±3.9 g/d in the low, medium, and high-spice preference groups, respectively. Moreover, the participants with a high spice preference had a lower systolic blood pressure (118±15 versus 126±17 mm Hg, respectively; P<0.01) and lower diastolic blood pressure (73±9 versus 78±12 mm Hg, respectively; P<0.01) than the participants who had a low spice preference. These values showed few changes after adjustment for several participant characteristics (Table 2). After adjustment, the participants with a high spice preference maintained a lower salt intake (2.5 g/d; 95% CI, 1.1–3.8 g/d; P<0.001), lower systolic blood pressure (6.6 mm Hg; 95% CI, 2.4–10.9 mm Hg; P<0.01), and lower diastolic blood pressure (4.0 mm Hg; 95% CI, 1.0–7.0 mm Hg; P<0.05) than the participants who had a low spice preference.

Capsaicin Administration Increased Insular and OFC Responses to Salty Taste
High levels of cognitive processing influenced the responses to environmental stimuli, such as salty foods. We subsequently examined how brain metabolism changes in response to a salt stimulus in individuals with high salt consumption. The salt intake and salt preference scores were positively correlated with the regional metabolic activity as determined by positron emission tomography/computed tomography in the insula and OFC under stimulation with 150 or 200 mmol/L NaCl (Figure S1). In humans, responses recorded from the insula have been correlated with the subjective intensity of taste.²⁹
Intensity-dependent changes in metabolic activity were identified in the insula and thalamus using different concentrations of NaCl stimulation (150 and 200 mmol/L; Figure S2). This finding indicated that increased brain metabolic activity is associated with high salt intake and high-salt preference scores in humans.

We subsequently addressed whether changes in salt-induced brain metabolic activity may be modified by capsaicin administration. A human behavioral study showed that capsaicin administration at 0.5 μmol/L did not produce a burning sensation on the tongue but did increase the perception of saltiness. We showed that 0.5-μmol/L capsaicin administration significantly increased activity in the insula and OFC in response to high salt stimulation (Figure 2A and 2B). Notably, the intensity-dependent metabolic activity changes reversed when 0.5 μmol/L capsaicin was added to the solution of 150 mmol/L NaCl (Figure 2C and 2D). Most importantly, the brain regions activated by capsaicin overlapped with the brain regions stimulated by salty taste (Figure 2E). These results indicate that capsaicin can modify the sensation of salt intensity via the activation of brain regions involved in the hedonic experience of salt.

### Peripheral Salty-Taste Signals and Spicy Flavor Evoked Neuronal Population Activities of OFC

We further examined the findings in rodent models. We used a fiber fluorometry method to record the neuronal population activity in terms of calcium wave signals and investigated the potential role of capsaicin in central salty-taste processing in both anesthetized and freely moving mice (Figure 3A and 3B). The calcium waves evoked by different concentrations of NaCl solutions with or without capsaicin exhibited

### Table 1. Characteristics of the Participants in the Cross-Sectional Study*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Low-Salt Preference Group (n=416)</th>
<th>Medium-Salt Preference Group (n=94)</th>
<th>High-Salt Preference Group (n=96)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>39.0±10.5</td>
<td>39.7±9.8</td>
<td>44.0±8.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>202 (48.6)</td>
<td>50 (53.2)</td>
<td>36 (37.5)</td>
<td>0.07</td>
</tr>
<tr>
<td>≥College graduate, n (%)</td>
<td>196 (47.1)</td>
<td>29 (30.9)</td>
<td>11 (11.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heavy physical labor, n (%)†</td>
<td>141 (33.9)</td>
<td>39 (41.5)</td>
<td>50 (52.1)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI, kg/m²‡</td>
<td>23.7±3.6</td>
<td>23.8±3.3</td>
<td>24.4±3.3</td>
<td>0.19</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus, n (%)</td>
<td>11 (2.6)</td>
<td>2 (2.1)</td>
<td>4 (4.2)</td>
<td>0.65</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>76 (18.3)</td>
<td>14 (14.9)</td>
<td>32 (33.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.4±1.3</td>
<td>5.3±1.3</td>
<td>5.4±0.9</td>
<td>0.94</td>
</tr>
<tr>
<td>Salt perception, mmol/L</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>92 (22.1)</td>
<td>20 (21.3)</td>
<td>15 (15.6)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>200 (48.1)</td>
<td>38 (40.4)</td>
<td>36 (37.5)</td>
<td></td>
</tr>
<tr>
<td>&gt;50</td>
<td>124 (29.8)</td>
<td>36 (38.3)</td>
<td>45 (46.9)</td>
<td></td>
</tr>
<tr>
<td>Salty superthreshold, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>500</td>
<td>208 (50.0)</td>
<td>38 (40.4)</td>
<td>28 (29.2)</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td>95 (22.8)</td>
<td>28 (29.8)</td>
<td>33 (34.4)</td>
<td></td>
</tr>
<tr>
<td>&gt;1000</td>
<td>113 (27.2)</td>
<td>28 (29.8)</td>
<td>35 (36.5)</td>
<td></td>
</tr>
<tr>
<td>Spice preference, μmol/L</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>&lt;3</td>
<td>227 (54.6)</td>
<td>61 (64.9)</td>
<td>74 (77.1)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>148 (35.6)</td>
<td>25 (26.6)</td>
<td>18 (18.8)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>41 (9.8)</td>
<td>8 (8.5)</td>
<td>4 (4.1)</td>
<td></td>
</tr>
<tr>
<td>24-h urinary sodium excretion, mmol/24 h</td>
<td>191.4±82.11</td>
<td>221.9±90.47</td>
<td>243.2±80.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Salt intake, g/d§</td>
<td>11.7±4.8</td>
<td>13.1±5.3</td>
<td>14.3±4.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>122±18</td>
<td>124±16</td>
<td>131±15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75±11</td>
<td>77±12</td>
<td>82±11</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BMI indicates body mass index.

*Plus-minus values are means±SD.

†Work status includes light and heavy physical labor. Works like officer, teacher, cashier, cook, driver, doctor, and student are considered light physical labor, whereas works such as farmer, factory worker, wood worker, and construction worker are considered heavy physical labor.

‡The BMI is the weight in kilograms divided by the square of the height in meters.

§Daily salt intake was derived from 24-hour urinary sodium excretion, which has adjusted according to the reference.37,38
distinct waveforms (Figure 3A and 3B), which indicate that salty tastes were encoded by neurons in the OFC. Notably, the time courses of calcium waves are in the range of 2 to 5 seconds, which are highly comparable with the typical activation timing of functional magnetic resonance imaging signals.21 Furthermore, the amplitude of the 200-mmol/L NaCl–evoked waves was significantly higher than the 150-mmol/L NaCl–evoked waves in the anesthetized mice, whereas the amplitude of the 150-mmol/L NaCl–capsaicin-evoked waves was significantly higher than the 150-mmol/L NaCl–evoked waves in both the anesthetized (Figure 3C and 3D) and freely moving (Figure 3E and 3F) mice. Thus, these results suggested that a peripheral salt stimulus could evoke higher OFC activity levels and in a dose-dependent manner, which could be affected by a capsaicin stimulus. Nevertheless, the frequency of the 150-mmol/L NaCl–capsaicin-evoked waves was significantly larger than the 150-mmol/L NaCl–evoked waves in the freely moving mice but not the anesthetized mice (Figure S4A and S4B), which indicates that the OFC neural excitability could be changed by a capsaicin stimulus at normal physiological conditions.

Interventions Related to OFC Altered Salt Preference

Finally, we investigated whether intervening in OFC activity could result in changes in the salty-capsaicin preference through activating or inhibiting neuronal activity by means of optogenetics in mice. The salt preference of mice was determined by calculating the relative ratio of licking actions for different solutions (Methods in the online-only Data Supplement). In 1 example of a naive mouse, the ratio of licks for the 150-mmol/L NaCl solution was significantly higher than the 200-mmol/L NaCl solution (Figure S5A), which indicates that the mouse prefers the former salt level. However, the mice exhibited a significantly lowered preference for the 150-mmol/L NaCl with capsaicin solution than only the 150-mmol/L NaCl solution (Figure S5A), which suggests that the existence of capsaicin may enhance the sensitivity of salt. To confirm the neuronal projection from the primary
taste cortex to OFC, AAV-EGFP (adeno-associated virus–vector expressing enhanced green fluorescent protein; retro) was injected into the OFC of mice, and the retrogradely labeled neurons in the area of insula cortex was identified (Figure 4A). The mice were then injected with AAV-ChR2 (AAV vector expressing channelrhodopsin-2) or AAV-ArchT (archaerhodopsin) for the activation or inhibition of neuronal activities in the OFC. We subsequently analyzed mouse licking behavior in the gustometer with a 30-minute test session during which they underwent optogenetic stimulation or inhibition through an optical fiber (Figure 4B and 4C; Figure SSB). By stimulating the OFC activity, the preference for the 200-mmol/L NaCl solution was further decreased, whereas the preference for the 150-mmol/L NaCl solution significantly increased in the mice (Figure 4D and 4E). In contrast, the mice exhibited a significantly increased preference for the 200-mmol/L NaCl solution and a decreased preference for the 150-mmol/L NaCl solution after OFC activity inhibition (Figure 4D and 4E). These results suggested that the stronger activation of the OFC strengthened the aversion for high-salt solutions and hedonic for low-salt solutions, vice versa. Furthermore, the mice exhibited a decreased preference for the 150-mmol/L NaCl solution with capsaicin during OFC activity stimulation (Figure 4F), which indicates that capsaicin may influence the perception of salty taste in the OFC. To validate the
Figure 3. Neuronal activity in the orbitofrontal cortex of wild-type mice evoked by different salt solution stimuli with capsaicin. 

A, The waveforms of anesthetized mice evoked by deionized water, 150 mmol/L NaCl, 200 mmol/L NaCl, and 150 mmol/L NaCl with 0.5-μmol/L capsaicin solutions, respectively. B, The waveforms of freely moving mice after drinking deionized water, 150 mmol/L NaCl, 200 mmol/L NaCl, and 150 mmol/L NaCl with 0.5-μmol/L capsaicin solutions, respectively. C, The amplitude of calcium signals in anesthetized mice evoked by a concentration gradient of salt solutions with or without capsaicin. **P<0.01 vs without capsaicin. D, The amplitude of calcium signals evoked by each type of solution in anesthetized mice. *P<0.05, ***P<0.001 vs deionized water. (Continued)
effect of optogenetic stimulation on the OFC, we also recorded the spontaneous and optogenetically evoked calcium waves in the OFC of Thy1-ChR2 transgenic mice. Spontaneous and optogenetically evoked calcium waves recorded at a given location had similar waveforms (Figure S5C), which indicates that the optogenetically activated neuronal population is largely the same as the neuronal population involved in the salt preference control pathways.

Discussion

The major findings in this study demonstrate that the enjoyment of spicy taste enhanced the sensitivity to salty taste and lowered the daily salt intake and blood pressure in participants. Furthermore, high salt intake and salt preference were closely correlated with increased brain activity in the insula and OFC of the participants. Capsaicin administration increased activation of the insula and OFC in response to high-salt stimuli, which reversed the salt intensity–dependent differences in activity in the insula and OFC. Similar to humans, salty-taste information processed in the OFC was affected in the presence of capsaicin in mice. Optogenetic stimulation on the OFC strengthened the aversion for high-salt solutions and hedonic for low-salt solutions, which could be reversed by the addition of capsaicin in salt solutions.

The estimation of dietary salt intake using various methodologies has indicated that the mean salt consumption is ≈11.0 to 14.0 g/d (2.5 g salt contains 1 g sodium) in the population,6,22 which far exceeds the dietary salt intake level recommended by the World Health Organization. Substantial effort has been made to reduce salt intake; however, the high consumption of dietary salt in China has hardly changed during the previous several decades.9,23 Similarly, despite numerous initiatives, sodium consumption in the United States has been relatively constant and well above recommended amounts for more than a decade.10 Thus, the identification of an alternative strategy for reducing salt intake is critical for the prevention of salt-induced hypertension.

Chili pepper is perhaps the world’s most widely consumed spice,24–26 and both spicy and hot are reported to be among the most appealing flavors in both China and the United States.27 A human study showed that the administration of capsaicin at a low concentration that does not cause a burning sensation on the tongue may enhance saltiness.13 In this study, we showed that the participants who liked spicy food had a reduction of ≈2.5 g in their daily salt intake, which is noteworthy because the reduction of daily salt intake by 3 g has been shown to produce a protective effect against hypertension and related cardiovascular diseases.28 Furthermore, Lv et al14 also showed that consumption of spicy foods was inversely associated with total and certain cause-specific mortality in a Chinese population. Because chili pepper is the most popular flavor substitute worldwide, the consumption of spicy foods may beneficially reduce dietary salt intake in the general population.

The human preference for salted food is the hedonic response to saltiness and is strongly influenced by its sensory properties.29 The central gustatory system and the mesolimbic structures play critical roles in discerning salt-taste signals as

Figure 3 continued. #P<0.05 vs 150 mmol/L NaCl. E, The amplitude of calcium signals emerged in 300 s after drinking concentration gradient of salt solutions with or without capsaicin in freely moving mice. **P<0.01 vs without capsaicin. F, The amplitude of calcium signals emerged in 300 s after drinking each type of solution in freely moving mice. ***P<0.001 vs deionized water. #P<0.05 vs 150 mmol/L NaCl. Data are presented as the mean±SEM.

Figure 4. Optogenetic intervention on orbitofrontal cortex (OFC) alters the salt preference of wild-type (WT) mice. A, A brain slice of a WT mouse with retrogradely labeled neurons (shown as green spots) in the insula cortex. The scale bar indicates 80 μm. B, Schematic diagram representing the optical fiber stimulation setup, AAV expression, and optical fiber administration on OFC. C, A brain slice of a WT mouse with photosensitive protein (shown as red spots) expressed in the OFC. The black trace represents the optic fiber trace to conduct optogenetic stimulation or inhibition. The scale bar indicates 200 μm. D–F, Ratio of licks for 200 mmol/L NaCl (D), 150 mmol/L NaCl (E), and 150 mmol/L NaCl with 0.5 μmol/L capsaicin (F) solution of ChR2-injected and ArchT-injected mice with or without optogenetic intervention. Data are presented as the mean±SEM. *P<0.05, **P<0.01.
aversive or pleasant. The insula has appeared to be more sensitive to the intensity of taste and is involved in establishing emotionally relevant sensory experiences. The OFC is suggested as a secondary taste cortex that receives inputs from the insula cortex, which has been shown to be particularly associated with hedonic aspects and thus the subjective pleasantness of taste in humans. Moreover, the OFC has been suggested in the control of appetite and food intake. In our human neuroimaging study, we determined that the metabolic activity changes of the OFC in response to salt stimulus were positively correlated with individual daily salt intake and salt preference. Similar findings were obtained in the animal study, with increased OFC activity in response to ascending concentrations of NaCl solutions applied peripherally. Seemingly discrepancy results of salt preference were obtained in the intermingling of OFC activity from our optogenetic experimental studies. Nevertheless, it has also been reported that both pleasant and aversive tastes were presented in the OFC. Furthermore, the OFC has been reported to play a key role in guiding behavior adaptively on the basis of reward value, including receiving rewards and punishments and processing of the values. Thus, it is rationale to assume that the OFC activity represented pleasantness for lower concentrations of salt solution, with an aversion reaction for higher concentrations of salt solution. This finding also explains the dose-dependent increment of neural calcium wave amplitudes recorded at the OFC in response to various concentrations of salt solutions.

In this study, we determined that capsaicin administration increased the metabolic activity in both the insula and the OFC in response to salty-taste stimuli. Notably, capsaicin mediated an increase in brain activity that could reverse the differences in the intensity-dependent activity induced by salty-taste stimuli identified in these brain regions. As reported, a remarkable difference of the OFC from other related brain nuclei was the greater convergence of neurons to taste features, which provided a basis for different behavioral responses to particular combinations of oral sensory stimuli. Taken together with the findings of our optogenetic experimental study on the OFC, it seems that capsaicin may turn the aversion reaction for a salt solution into a lower concentration. Our previous studies have also indicated a protective role of capsaicin in cardiometabolic diseases. Through direct intervention on OFC neuronal activities while monitoring salty-taste preference, we provided additional evidence of the influence of capsaicin on central salty-taste perception (Figure S6).

Perspectives

High salt intake contributes to the pathogenesis of hypertension and its associated cardiovascular events. Healthy lifestyles have been used to reduce salt intake for many years; however, traditional cooking habits and changes in the taste of food have dampened the effectiveness of salt reduction at the population level. This clinical trial combined with an experimental study for the first time shows that salt intake and salt preference were related to the regional metabolic activity in the insula and OFC of individuals. Furthermore, capsaicin administration enhanced insula and OFC metabolism in response to high-salt stimuli; thus, the enjoyment of spicy foods significantly reduced individual salt preference, daily salt intake, and blood pressure by modifying the neural processing of salty taste in the brain (Figure S6). Capsaicin rich in Chili pepper is perhaps the world’s most widely consumed food with spicy flavor. Considering the unsatisfactory effect of the existing salt-reduction strategy, this study provided insights for the enjoyment of spicy flavor as a promising and precise behavioral intervention for reducing high salt intake and blood pressure.

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Disclosures

None.

References


Novelty and Significance

**What Is New?**

- This study is the first investigation to demonstrate the enjoyment of spicy flavor enhances central salty-taste perception.
- Capsaicin administration enhances insula and orbitofrontal cortex metabolism in response to high-salt stimuli and reverses the salt intensity–dependent differences in activity in these brain areas.
- Optogenetic intervention on the orbitofrontal cortex alters salt preference in rodents.

**What Is Relevant?**

- Enjoyment of spicy foods may significantly reduce individual salt preference, daily salt intake, and blood pressure by modifying the neural processing of salty taste in the brain.

- The consumption of foods with spicy flavor promotes a novel lifestyle intervention for reducing high salt intake and blood pressure.

**Summary**

Spicy food supplementation is a promising strategy for salt reduction at the population level, either as a functional dietary factor or in conjunction with conventional lifestyle changes.

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Enjoyment of Spicy Flavor Enhances Central Salty Taste Perception and Reduces Salt Intake and Blood Pressure

Qiang Li, 1,5 Yuanting Cui, 1,5 Rongbing Jin, 2* Hongmei Lang, 1 Hao Yu, 1 Fang Sun, 1 Chengkang He, 1 Tianyi Ma, 1 Yingsha Li, 1 Xunmei Zhou, 1 Daoyan Liu, 1 Hongbo Jia, 3 Xiaowei Chen, 4 Zhiming Zhu, 1*

1Department of Hypertension and Endocrinology, Center for Hypertension and Metabolic Diseases, Daping Hospital, Collaborative Innovation Center for Brain Science, Third Military Medical University, Chongqing Institute of Hypertension, Chongqing 400042, China

2Department of Medical Image, Daping Hospital, Third Military Medical University, Chongqing 400042, China

3Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou 215163, China

4Brain Research Center, Third Military Medical University, Chongqing 400038, China

5These authors contributed equally to this work.

*Correspondence to Zhiming Zhu, Department of Hypertension and Endocrinology, Center for Hypertension and Metabolic Diseases, Daping Hospital, Third Military Medical University, Chongqing Institute of Hypertension, Chongqing 400042, China. E-mail:
zhuzm@yahoo.com. Or to Rongbing Jin, Department of Medical Image, Daping Hospital, Third Military Medical University, Chongqing 400042, China. E-mail: jinrb99@126.com.

Running title: Spicy flavor reduces salt intake

Tel.: +86-23-68767849

Fax: +86-23-68705094
Supplemental Methods

Human Study

SATIETY-1 (Spicy Diet on Salty Taste and Salt Intake)

Study Design

The study design was a multi-center, random-order, double-blind observational study to investigate salty taste and salt intake at the population-level. Six hundred and six individuals were recruited from four cities (Shenyang, Jinan, Chengdu and Chongqing) in China. Written informed consent was provided by all participants. This study aimed to explore the salty taste characterization and salt intake in participants who like or dislike spicy food based on the questionnaires, assessment of spicy preference, salty perception threshold and super-threshold, and blood pressure, as well as the 24-hour urinary sodium excretion/creatinine test.

All of the participants in this study were required to be between 18 and 65 years of age, and to able to comply with all study procedures. The exclusion criteria were as follows: hypogeusia or loss of taste due to neural system disease or oral and digestive disease; capsaicin allergy; poor compliance; recent use of oral diuretics; participation in other pharmacological trials within the past 3 months; acute infection, cancer; serious arrhythmias; drug or alcohol abuse; currently achieve cold, fever, acidosis, dehydration, diarrhea, or vomiting during the course of the study; unwilling or unable to communicate due to the dysnoesia and language disorders; severe neural or psychiatric diseases that would preclude full understanding and cooperation in the study; pregnancy or lactation; and an unwillingness to sign the informed consent.

Diet culture consideration in city selection

There are strike differences in dietary habit in China because of discrepancy in traditional culture and environmental factors, et al. In northern provinces of China, such as Liaoning, Jilin, Heilongjiang, and Shandong, individuals like to salty food but seldom consume spicy food. By contrast, individuals prefer to enjoy spicy food in southern China, such as Sichuan, Chongqing, Guizhou, and Hunan.

Primary Outcome Measure

1. Effects of spicy diet on salt taste

Secondary Outcome Measures

1. Effects of spicy diet on salt intake
2. Effects of spicy diet on obesity parameters
3. Effects of spicy diet on blood pressure

Study Procedures
Taste Testing

Trained assessors administered three taste tests according to a standard protocol: 1) a spicy preference test, in which the participants were instructed to identify the specific solution they liked best with solutions that contained increasing concentrations of capsaicin (1, 3, 5 and 7 μmol/L); 2) a salt perception test, in which the participants were instructed to identify solutions that contained any salt compared with solutions that contained variable but low concentrations of salt (0, 10, 30, 50, and 75 mmol/L); and 3) a salt ‘super-threshold’ test, in which the participants were instructed to indicate which solutions were intolerably salty with solutions that contained variable but high concentrations of salt (500, 750, 1000, and 1500 mmol/L). The participants were blinded to the concentration, and samples were presented in a random order assigned by participant number. The taste testing was performed in a designated room at approximately 22-24 ºC in each study location. All participants fasted for at least 4 hours before the test (no food or beverage with the exception of water). For each taste evaluation, the participants were instructed to rinse their mouth thoroughly with deionized water, sip the stimulus solution, hold it in their mouth for approximately 5 seconds to taste it, and then spit it out. The participants were allowed to taste the solution more than once if they were not certain of the selection. If the selection was difficult, the participant was encouraged to make a selection. The tasting of each sample was followed by a rinse with deionized water. All solutions were provided at room temperature and were poured into plastic cups (180 ml) that contained 10 ml of the sample solution. All participants were instructed to evaluate the hedonic value of the tasting solutions using the VAS. On the scale, “like extremely” was defined as a value of 3, “dislike extremely” was defined as -3, and “neutral” was defined as a value of 0.

The results of the spicy preference test were categorized as low (preference for the solutions with 1 or 3 μmol/L of capsaicin), medium (5 μmol/L), or high (7 μmol/L). Furthermore, self-reported habits for spicy foods were determined, and these results were consistent with the spicy preference test (Table S1).

Diet Questionnaire and Anthropometric Measurements

Between the salty perception test and the salty super-threshold test, a one-hour break was mandatory. During this period, the participants were administered a diet questionnaire and underwent anthropometric measurements. The diet questionnaire included a 19-item general information questionnaire and an 8-item food-preference questionnaire. The food-preference questionnaire included items that elicited participants’ self-reported preferences for salty and spicy foods in their daily life. Anthropometric measurements, including blood pressure, waist circumference, height, and body weight, were performed by trained assessors. Blood pressure measurements were performed using a mercury sphygmomanometer on both arms in the sitting position after 10 minutes of resting. The subject was seated comfortably with the back supported and the upper arm bared without constrictive clothing. Cuffs of the appropriate size were placed around the subject’s arm. The mercury column was slowly deflated at 2 to 3 mm/second, and the first and last audible Korotkoff sounds were taken as systolic and diastolic blood pressure, respectively. Three readings were taken at intervals of two minutes, and the average value was used as the participant’s blood pressure.
Salty Preference Evaluation

Based on a pre-test investigation of common salty foods consumed in China, the salty preference evaluation was performed using the ten most common foods, including fried pork slices with salted pepper, sautéed sliced pork, poached spicy slices of pork or fish, hot pot, salted pickles, bean paste, pickled poth herb mustard, pickled Chinese cabbage, ham, and salted eggs. The participants were instructed to report their frequency of eating these foods in daily, weekly, or monthly periods. For each type of food, the calculated frequency was expressed as follows: 1) daily: 30, 60, and 90 times; 2) weekly: 4, 8, 12, 16, 20, and 24 times; and 3) monthly: 0, 1, 2, and 3 times. Thus, the grouping for salt preference was based on the frequency of the consumption of salty foods, i.e., low salt preference with a frequency of no more than 8 times; medium salt preference with a frequency of no more than 20 times; and high salt preference with a frequency of no less than 24 times. For the participants who reported eating more than one type of salty food, the total frequency was further divided by the number of food types, which yielded the average frequency of salty food intake.

Urinary Sodium Excretion and Laboratory Testing

Measurement of 24-hour urinary sodium excretion was used to estimate the participants’ daily salt intake. To correct for body size and possible collection errors, urinary sodium excretion was normalized to urinary creatinine excretion by calculating the sodium-to-creatinine ratio (mEq/g) from 24-hour urine samples. Participants were given both verbal and written instructions on how to perform the collection. Each participant was provided with a urine collection bottle (50 ml) filled with 5 ml formaldehyde solution (Sigma, USA), a polypropylene graduated cylinder with capacity of 1000 ml, a plastic cask (4000 ml), and a black plastic bag for carrying the bottle. On the first morning of the urine collections, the participants were instructed to discard the first specimen and then to collect all specimens for up to 24 hours, up to and including the first specimen the following day. Urine volume was recorded by the assessors.

SATIETY-2 (Capsaicin on Salty Gustatory Cortices)

Study Design

The experimental design is a randomized, double-blind interventional study using neuroimaging to investigate the changes in salty gustatory cortices following treatment with NaCl solution at different concentrations and with or without capsaicin intervention through brain PET/CT scanning. Sixty volunteers were recruited in Chongqing and underwent PET/CT scanning (Figure S3). As specific requirements for enrollment in the interventional study, participants had to declare that they had no history of taste/smell/neurological disorders, brain tumor, epilepsy, or allergic reaction to 18F-FDG. Participants had limited cigarette consumption (≤3 cigarettes/day).

Interventions

Epidemiologic studies have indicated that salt intake greater than 12 g/day significantly increased the risk of all cardiovascular events. In the current study, the liquid stimulus
of sodium chloride was applied at two concentrations (150 and 200 mmol/L) dissolved in deionized water, with pure deionized water as a control. A previous study in humans has indicated that capsaicin administration at 0.5 μmol/L did not produce a burning sensation but did increase the perception of saltiness. To elucidate the potential salt reduction effect of spicy flavor, 0.5 μmol/L capsaicin was added to the 150 mmol/L sodium chloride stimulus. All participants were instructed to evaluate the hedonic value of salt solutions using the VAS prior to the experiment. On the scale, “very pleasant” was defined as a value of 3, and “very aversive” was defined as -3. The average hedonic value was almost neutral.

**Primary Outcome Measure**

1. Neuroimaging changes in salty gustatory cortices

   After the study was completed, neuroimaging changes in the salty gustatory cortices were determined through direct comparison of images between the groups using the software Statistical Parametric Mapping (SPM 8.0, the Welcome Department of Cognitive Neurology, University College London, UK).

**Sample Size Determination**

The sample size in this study was based on previous human imaging studies on central taste processing. In these studies, the number of participants in each group was approximately ten. Interim analyses were performed on the salt-intensity-dependent changes in the gustatory cortices’ metabolism and the potential effects of capsaicin addition on these changes.

**Randomization and Blinding**

Random numbers were generated using Microsoft Excel, and a randomized block design was implemented to generate five blocks with 6 patients per block to increase the equivalence of the various random groups. All test solutions were prepared by a unique assistant who was not involved in the brain PET/CT scanning or outcome assessment. All blinded test solutions were packaged according to blind codes before the interventions were assigned. Once a participant was eligible for randomization, the investigator assigned the participant a randomized number and supplied the corresponding blinded test solutions with the same randomized number. The participants, investigators, and outcome assessors were unaware of the test solution concentrations.

**Study Procedures**

**PET/CT scan**

**Data Acquisition**

The participants fasted overnight prior to the intravenous injection of a weight-based amount of $^{18}$F-FDG (0.05 mCi/kg). A catheter was placed in an antecubital vein for blood sampling prior to the scan to exclude thrombocytopenia and granulocytopenia. The participants were then recumbent in an isolated, air-conditioned and dimly lighted room with
their eyes blinded for at least 20 mins. Intravenous injection of $^{18}$F-FDG was followed by buccal administration of 10 ml of the test solution for 5 mins. The participants were subsequently recumbent in the same condition as previously described. After approximately 40-min, a combined brain $^{18}$F-FDG PET/CT scan was obtained, with a lead collar covered thyroid. Imaging started with a low-dose CT scan (35 mAs), which was immediately followed by a PET scan for 10 mins. CT images were used for attenuation correction and fusion; no contrast medium was used. Helical CT images were initially acquired using the following parameters: 120 kV and 5-mm-thick sections. The resulting total radiation dose from the low-dose CT scan and the injected radioactive tracer was approximately 3 mSv. Data from fifty-two participants were included in the primary analysis.

**Processing Analysis**

The PET/CT images were processed using Statistical Parametric Mapping (SPM 8.0, the Welcome Department of Cognitive Neurology, University College London, UK). Automated algorithms were implemented to realign each participant’s sequential PET images. A standard whole-brain template provided by the Montreal Neurological Institute (MNI) was used to spatially normalize individual images to the stereotactic space. After realignment and normalization, the images were smoothed spatially with a Gaussian filter of 10 mm in full-width half-maximum. Global normalization and proportional scaling with 0.8 threshold masking were used. To test our hypothesis, a two sample t-test was used to evaluate the differences between the two scans. The resulting T-score maps were subsequently superimposed onto the SPM-MRI template using the standard MNI coordinates to allow visual inspection of the composite images. To determine whether changes in regional glucose metabolism were relevant to salt intake, we performed a multiple regression analysis using PET images with salt intake as a regressor and sex, age, and BMI as covariates. A critical $P\leq 0.005$ (uncorrected) with a cluster filter of 5 voxels (1 voxel = 8 mm$^3$) was used to identify significant differences between two scans.

**Animal Study**

Male C57BL/6J mice (8 weeks) were used for the licking response test and OFC neural signal recordings. At day 0, virus that contained ChR2, ArchT or GCaMP6(s) was injected into the OFC of mice with a glass electrode. After three weeks, an optic fiber (ThorLabs, M89L01) tip was embedded above the OFC encephalic region. The mice recovered for one week and underwent sipper tube training followed by a licking response test in a commercially available gustometer (Davis MS160-Mouse; DiLog Instruments, Tallahassee, FL). Optical fibers were connected via a SMA adaptor to a laser device (FiberOptoMeter v2.0). For the ChR2 activation, the 470 nm switch was turned on to generate blue light pulses. For the ArchT activation, the 565 nm switch was turned on to generate yellow light pulses. The calcium signals after deionized water, 250 mmol/L NaCl, 200 mmol/L NaCl, 150 mmol/L NaCl and 100 mmol/L NaCl with or without 0.5 μmol/L capsaicin solution stimulus on the tongue were recorded and analyzed respectively. For the characterization of virus expression, the brain slices of the mice were cut with a freezing microtome (Leica) and analyzed with a fluorescence microscope (Nikon TE2000-U).
Sipper Tube Training

We used a commercially available gustometer (Davis MS160-Mouse; DiLog Instruments, Tallahassee, FL) to record the licking behavior of each mouse. To encourage the mice to sample from the sipper tube during training and taste testing with NaCl and capsaicin, we removed their water bottles from the home cages 22.5 h before each 30 min test session to ensure they were thirsty when placed in the gustometer. During days 1 and 2, the water-restricted mice were familiarized with the gustometer and trained to lick from sipper tubes (which contained deionized water). A test session began when the mouse took its first lick and lasted 30 min. On day 1, the sipper tube was positioned in the center of the slot with the shutter permanently open. The mouse could drink as much water as possible from the single tube during the session. On day 2, the mice received more limited access to the sipper tubes,— that is, the shutter was opened and a trial that lasted 5 s was initiated once the mouse took its first lick from the sipper tube. At the end of a trial, the shutter was closed for 7.5 s (during which time a different sipper tube that contained water was positioned in the center of the slot) and then reopened, thereby enabling the mouse to initiate another trial of the same duration. In this manner, the mouse could initiate as many trials as possible during a test session.18

Test for Licking Responses

The mice were trained with water as previously described. On the testing day, each mouse was tested with four types of solutions (deionized water, 200 mmol/L NaCl, 150 mmol/L NaCl and 150 mmol/L NaCl with 0.5 μmol/L capsaicin) during a single 30 min test session. Furthermore, to control for concentration order effects across trials, we treated the range of concentrations of a given taste stimulus as a block and programmed our software so that it randomized (without replacement) the sequence of presentation of each stimulus concentration within each block. The mouse was permitted to initiate as many trials (and thus blocks) as possible throughout the 30 min test session. For the data analysis, we standardized the responses to NaCl and NaCl containing capsaicin to the responses to deionized water for each mouse by calculating a tastant/water lick ratio18.

Virus Vectors

pAAV-Syn-ChR2-mCherry, pAOV-CMV-ArchT-tdTomato, pAOV-Syn-GCaMP6(s) and pAAV-Syn-MCS-EGFP-3FLAG (retro) virus vectors were purchased from Obio Technology (Shanghai) Co. Ltd. The titer of the virus was not less than 2×10^{12} v.g./ml.

Virus Injection Surgery

Mice were placed in the induction chamber and anesthetized with 5% isoflurane as the flow rate of oxygen was set at approximately 1.5 liter/min. The depth of anesthesia needed for surgery was indicated by the loss of the righting reflex and pinch withdrawal reflex. The anesthetized mice were placed onto a warming plate (37°C), and the head was fixed in a stereotaxic frame with ear bars. A digital thermometer (Lutron TM-902C) was applied to detect the body temperature. The concentration of isoflurane was switched to 1-1.5% to maintain the mouse at respiration rates of 80-130/min. The eyes of the mice were covered
with ophthalmic ointment for protection. The skull of the mice was exposed by scalp incision. One hundred µL 2% xylocaine solution was subcutaneously injected at the site of brain surgery. Ten minutes after xylocaine injection, the skin and muscles above the brain region were gently removed with fine scissors and a cotton swab. The location of the OFC was 2.6 mm anteriorly, 1.2 mm laterally, and 1.4 mm ventrally from Bregma. After two small burr holes were drilled bilaterally according to the coordinates, 50-60 nl virus were injected into the OFC by air pressure with a glass electrode. The injection glass electrode was left in the brain for 5 minutes to ensure the diffusion of injected virus. The incision on the scalp was carefully sutured with 6-0 suture. The mice were intraperitoneally injected with $2 \times 10^5$ U/ml penicillin to protect against infection.

**Optic Fiber Placement**

The methods of anesthesia and preparation of the skull were in accordance with that of the virus injection surgery. After the soft tissue cover on the skull was removed, hydrogen peroxide was wiped on the surface of the skull until no soft tissue remained. The scalp was adhered to the side of the skull by tissue adhesive (3M Vetbond, America). The location of the OFC coordinates was the same as the virus injection surgery. A 0.5 mm×0.5 mm hollow was rubbed by the cranial drill until the skull of the target region became gauzy. A syringe needle was used to wipe off the residual skull slice and the nether dura. The fiber tip cannula was connected with an optogenetics patch cable through the mating sleeve (Thorlabs). The assembled fiber was inserted into the OFC encephalic region by the clamp holder of the stereotaxic apparatus. The surface of the skull was dried, followed by the addition of industrial strength dental cement between the base of the implantable fiber and the skull. The optogenetics patch cable was extracted out with the mating sleeve sheathing on the cannula. The mice were maintained on the warming plate until awake for the recovery of body temperature.

**Blue and Yellow Light Stimulation**

Optical fibers (ThorLabs, M89L01) were connected via a SMA adaptor to a laser device (FiberOptoMeter v2.0). For the ChR2 activation, the 470 nm switch was turned on to generate blue light pulses. For the ArchT activation, the 565 nm switch was turned on to generate yellow light pulses. For the licking response experiments, the mice were administered 1s light stimulation at a frequency of 0.25 Hz, and the stimulation was sustained for the entire 30 min test session. For the calcium signal evoking experiments, the duration of one single pulse of blue light was 20 ms.

**Staining with Fluorescent Ca$^{2+}$ Indicator and Optical Fiber Recordings**

C57BL/6J wild-type mice were anesthetized with 5% isoflurane and fixed on the stereotaxic apparatus. A small craniotomy was performed above the OFC area. The injection solution that contained OGB-1 (Invitrogen) was prepared according to previous studies. We filled 1 µl of the dye-containing solution into a patch pipette and inserted 1.5 mm for the OFC staining. Approximately 30 min after dye application, the fiber tip was inserted into the stained region with a micromanipulator to the depth, thereby providing the maximal
fluorescence intensity. The insertion was halted a minimum of 100 μm above the staining depth to avoid lesion of the stained area. The calcium signals after deionized water, 250 mmol/L NaCl, 200 mmol/L NaCl, 150 mmol/L NaCl and 100 mmol/L NaCl with or without 0.5 μmol/L capsaicin solution stimulus on the tongue were recorded and analyzed, respectively. For the recordings of the awake mice, the fiber was fixed by dental cement immediately after the AAV-GCaMP6s virus injection. After 21 days, the mice were transferred to the gustometer for sipper tube training followed by brief-access to each type of solution for 60 s. The calcium signals that emerged after the shutter closed in each test session (10 min) were recorded and analyzed.

**Histology and Fluorescence Imaging**

For the characterization of ChR2 and ArchT expression, animals were transcardially perfused with 4% PFA 10 days post-injection, and the brains were post-fixed for 24 hours. We cut 50-mm-thick sections with a freezing microtome (Leica) and stored them in PBS. The brain slices were analyzed with a fluorescence microscope (Nikon TE2000-U).

**References**


### Supplemental Tables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Low spice preference group (n=362)</th>
<th>Medium spice preference group (n=191)</th>
<th>High spice preference group (n=53)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Degree of liking-disliking spicy foods</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>— no. (%)</td>
<td></td>
<td></td>
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<tr>
<td>Dislike</td>
<td>91 (25.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<tr>
<td>Mild like</td>
<td>158 (43.7)</td>
<td>17 (8.9)</td>
<td>4 (7.5)</td>
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<td>Like</td>
<td>104 (28.7)</td>
<td>151 (79.1)</td>
<td>7 (13.2)</td>
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<tr>
<td>Strong like</td>
<td>9 (2.5)</td>
<td>23 (12.0)</td>
<td>42 (79.3)</td>
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<td>Intensity of spice preference — no. (%)</td>
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<td></td>
<td></td>
<td>&lt;0.001</td>
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<td>No spicy</td>
<td>90 (24.9)</td>
<td>0 (0.0)</td>
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<td>23 (12.0)</td>
<td>5 (9.4)</td>
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<td>Moderate spicy</td>
<td>82 (22.7)</td>
<td>157 (82.2)</td>
<td>8 (15.1)</td>
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<tr>
<td>Strong spicy</td>
<td>11 (3.0)</td>
<td>11 (5.8)</td>
<td>40 (75.5)</td>
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<tr>
<td>Frequency of consuming spicy foods</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>— no. (%)</td>
<td></td>
<td></td>
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<tr>
<td>1 time/wk or less</td>
<td>201 (55.5)</td>
<td>15 (7.9)</td>
<td>0 (0.0)</td>
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<tr>
<td></td>
<td>2-6 times/wk</td>
<td>≥7 times/wk</td>
<td>Salty perception — (mmol/L)</td>
<td>Salty super-threshold — (mmol/L)</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------</td>
<td>-------------</td>
<td>-----------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>103 (28.5)</td>
<td>48 (25.1)</td>
<td>4 (7.5)</td>
<td></td>
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<td></td>
<td>58 (16.0)</td>
<td>128 (67.0)</td>
<td>49 (92.5)</td>
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<td>Salty perception</td>
<td>10</td>
<td>60 (16.6)</td>
<td>50 (26.2)</td>
<td>17 (32.1)</td>
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<tr>
<td>perception</td>
<td>30</td>
<td>159 (43.9)</td>
<td>89 (46.6)</td>
<td>26 (49.0)</td>
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<td>&gt;50</td>
<td>143 (39.5)</td>
<td>52 (27.2)</td>
<td>10 (18.9)</td>
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<tr>
<td>Salty super-threshold</td>
<td>500</td>
<td>138 (38.1)</td>
<td>108 (56.5)</td>
<td>28 (52.8)</td>
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<tr>
<td>threshold</td>
<td>750</td>
<td>106 (29.3)</td>
<td>41 (21.5)</td>
<td>9 (17.0)</td>
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<tr>
<td>threshold</td>
<td>&gt;1500</td>
<td>118 (32.6)</td>
<td>42 (22.0)</td>
<td>16 (30.2)</td>
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Table S2. Effects of salty preference on salt intake and blood pressure *

<table>
<thead>
<tr>
<th>Variables</th>
<th>Salty preference group</th>
<th>Original measurement</th>
<th>Unadjusted</th>
<th>Model 1†</th>
<th>Model 2‡</th>
<th>Model 3§</th>
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<tr>
<td>Salt intake —</td>
<td>Low</td>
<td>11.7</td>
<td>0 (reference)</td>
<td>0 (reference)</td>
<td>0 (reference)</td>
<td></td>
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<tr>
<td>g/day (95% CI)</td>
<td>Medium</td>
<td>13.1</td>
<td>1.3 (-0.1, 2.7)</td>
<td>0.9 (-0.1, 2.0)</td>
<td>0.9 (-0.1, 2.0)</td>
<td></td>
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<tr>
<td></td>
<td>High</td>
<td>14.3</td>
<td>2.6 (1.3, 3.9)***</td>
<td>1.8 (0.7, 2.9)**</td>
<td>1.8 (0.7, 2.9)**</td>
<td></td>
</tr>
<tr>
<td>Systolic blood —</td>
<td>Low</td>
<td>122</td>
<td>0 (reference)</td>
<td>0 (reference)</td>
<td>0 (reference)</td>
<td>0 (reference)</td>
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<tr>
<td>pressure —</td>
<td>Medium</td>
<td>124</td>
<td>1.7 (-2.7, 6.1)</td>
<td>-0.2 (-3.5, 3.2)</td>
<td>0.1 (-3.2, 3.4)</td>
<td>-0.1 (-3.4, 3.2)</td>
</tr>
<tr>
<td>mmHg (95% CI)</td>
<td>High</td>
<td>131</td>
<td>9.2 (5.1, 13.4)***##</td>
<td>5.5 (2.0, 8.9)***#</td>
<td>5.4 (2.1, 8.8) ***#</td>
<td>5.0 (1.7, 8.4)***#</td>
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<tr>
<td>Diastolic blood —</td>
<td>Low</td>
<td>75</td>
<td>0 (reference)</td>
<td>0 (reference)</td>
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<td>pressure —</td>
<td>Medium</td>
<td>77</td>
<td>2.1 (-1.2, 5.3)</td>
<td>1.0 (-1.4, 3.3)</td>
<td>1.1 (-1.2, 3.4)</td>
<td>1.0 (-1.4, 3.3)</td>
</tr>
<tr>
<td>mmHg (95% CI)</td>
<td>High</td>
<td>82</td>
<td>6.6 (3.8, 9.5)***#</td>
<td>4.6 (2.2, 7.0)***#</td>
<td>4.6 (2.2, 6.9)***#</td>
<td>4.4 (2.0, 6.7)***#</td>
</tr>
</tbody>
</table>

*CI denotes confidence interval.
†Model 1 includes adjustment for age, gender, educational level, work status.
‡Model 2 adjusts for Model 1 parameters as well as BMI and diabetes.

§Model 3 adjusts for Model 2 parameters as well as daily salt intake.

** P<0.01, *** P<0.001, medium or high salty preference groups vs. low salty preference group. # P<0.05, ## P<0.01, high salty preference groups vs. medium salty preference group.
Figure S1. Brain regions with positive correlations between salt intake/preference and glucose metabolism in response to two different concentrations of high salt stimuli. (A) The images represent multiple regression analysis with salt intake as a regressor and sex, age, and BMI as covariates (n=8 for 150 mmol/L NaCl group and n=10 for 200 mmol/L NaCl group). The highlighted regions indicate areas that exhibit positive correlations with individual salt intake levels. (B and C) Linear regression analysis was performed between normalized glucose metabolism in the insula/OFC and salt preference scores. The peak coordinates of each VOI are shown in parentheses. The significance threshold was set at \( P < 0.005 \), and SPM\( \{t\} \) maps were superimposed on a standard magnetic resonance imaging brain template.
Figure S2

Figure S2. Dose-dependent increases in glucose metabolism in the insula, OFC, and thalamus. (A) The images represent two sample t tests for the 200 mmol/L NaCl group (n=8) vs. the 150 mmol/L NaCl group (n=8). The highlighted regions indicate areas that exhibit different glucose metabolism between groups. (B-E) VOIs (2-mm radius) centered at the peak voxel of clusters within the image space were established to calculate the relative metabolism in the two groups. The peak coordinates of each VOI are provided in parentheses. The significance threshold was set at $P < 0.005$, and SPM(t) maps were superimposed on a standard magnetic resonance imaging brain template. *** $P<0.001$, the 200 mmol/L NaCl group vs. the 150 mmol/L NaCl group.
Figure S3. Flow chart of participant randomization in neuroimaging study. Participants were grouped according to specific interventions: Capsaicin: 0.5 μmol/L capsaicin dissolved in deionized water; 150_NaCl+Cap: 150 mmol/L sodium chloride solution with 0.5 μmol/L capsaicin added; 200_NaCl: 200 mmol/L sodium chloride solution; Control: deionized water; and 150_NaCl: 150 mmol/L sodium chloride solution. Notably, to elucidate the potential salt-reduction effect of spicy flavor, only sodium chloride at 150 mmol/L was combined with 0.5 μmol/L capsaicin, and this condition was compared with the higher concentration of sodium chloride (200 mmol/L).
Figure S4. Frequency of neuronal activity in the orbitofrontal cortex of WT mice evoked by different salt solution stimuli with capsaicin. The frequency of calcium signals evoked by deionized water, 150 mmol/L NaCl, 200 mmol/L NaCl and 150 mmol/L NaCl with 0.5 μmol/L capsaicin solutions in anesthetized mice, respectively. (B) The frequency of calcium signals in freely moving mice after drinking deionized water, 150 mmol/L NaCl, 200 mmol/L NaCl and 150 mmol/L NaCl with 0.5 μmol/L capsaicin solutions, respectively. *** $P < 0.001$ vs deionized water. # $P < 0.05$, ## $P < 0.01$ vs 150 mmol/L NaCl.
Figure S5. Optogenetic intervention on OFC alters the salt preference of WT mice. (A) The ratio of licks for 200 mmol/L, 150 mmol/L NaCl and 150 mmol/L NaCl with 0.5 μmol/L capsaicin solution during the brief-access taste testing. ** P <
0.01 vs 200 mmol/L NaCl. # $P < 0.05$ vs 150 mmol/L NaCl. (B) Experimental flow chart. Optical fiber placement was performed 21 days after AAV injection, followed by sipper tube training and a brief-access test in a gustometer after recovery for one week. (C) Spontaneous and optogenetically evoked calcium waves of ChR2-expressing OFC neurons of Thy1-ChR2 transgenic mice.
Figure S6. Schematic representation of capsaicin administration enhancing central salty perception and thus affecting daily salt intake and blood pressure in humans.