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Effects of Saccharin Intake on Hippocampal and Cortical Plasticity in Juvenile and Adolescent Rats

Jong-Sil Park[1], Sang Bae Yoo[2], Jin Young Kim[2], Sung Joong Lee[1], Seog-Bae Oh[1], Joong-Soo Kim[1], Jong-Ho Lee[2], Kyungpyo Park[1], Jeong Won Jahng[2,†], and Se-Young Choi[1,*]

Departments of [1]Physiology and [2]Oral and Maxillofacial Surgery, Dental Research Institute, Seoul National University School of Dentistry, Seoul 110-749, Korea

The sensory system is developed and optimized by experiences given in the early phase of life in association with other regions of the nervous system. To date, many studies have revealed that deprivation of specific sensory experiences can modify the structure and function of the central nervous system; however, the effects of sensory overload remains unclear. Here we studied the effect of overloading the taste sense in the early period of life on the synaptic plasticity of rat hippocampus and somatosensory cortex. We prepared male and female Sprague Dawley rats with *ad libitum* access to a 0.1% saccharin solution for 2 hrs per day for three weeks after weaning on postnatal day 22. Saccharin consumption was slightly increased in males compared with females; however, saccharin intake did not affect chow intake or weight gain either in male or in female rats. We examined the effect of saccharin-intake on long term potentiation (LTP) formation in hippocampal Schaffer collateral pathway and somatosensory cortex layer IV - II/III pathways in the 6-week old saccharin-fed rats. There was no significant difference in LTP formation in the hippocampus between the control group and saccharin-treated group in both male and female rats. Also in the somatosensory cortex, we did not see a significant difference in LTP among the groups. Therefore, we conclude that saccharin-intake during 3∼6 weeks may not affect the development of physiological function of the cortical and hippocampal synapses in rats.

Key Words: Hippocampus, Experience dependency, Sensory overloading, Somatosensory cortex, Synaptic plasticity, Taste

INTRODUCTION

Sensation is necessary for animals to extract information from the external environment and is achieved by the activation of sensory receptor cells, afferent neurons and sensory cortex in the central nervous system [1,2]. The efficacy and sensitivity of the sensory system is thus influenced by the development and optimization of sensory neuronal circuits [3,4]. The development of the sensory system depends on the sensory experience, especially during the early phase of an animal's life and actively optimizes itself according to the environmental exposure [5-7].

Neuronal circuits in the sensory system are closely connected with other nerve systems for the efficient handling of sensory information [8]. For example, taste sensory information principally projects into the gustatory cortex but also targets to other brain areas such as other cerebral cortices, the hippocampus, and amygdala for the correct storage and recall of taste memory and to develop appropriate innate and instinctive responses such as preference and aversion. It therefore follows that deprivation or overloading of a certain sensory experience may affect the function of those receiving brain regions [9]. It is well-known that visual deprivation promotes the somatosensory or auditory system in animal models as well as in humans [10]. Especially, the abnormal sensory stimulation in the early life (e. g. juvenile and adolescent periods) can disturb the normal structure and function of brain circuits [11,12].

The effect of sensory deprivation on neuronal circuits has been investigated intensively, however the effect of sensory overload has received less attention. This may be due to the lack of animal models. Visual and somatosensory systems are the most intensively studied senses in terms of sensory deprivation. On the other hand, sensory overloading is technically difficult since it requires a well-controlled exposure (the time and intensity) of 'excessive' sensory stimulation and animals are always exposed to basal visual/auditory stimulation in normal state. Thus there is a need for the development of an animal model for sensory

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*Corresponding to: Se-Young Choi, Department of Physiology, Seoul National University School of Dentistry, 28, Yeongeon-dong, Jongno-gu, Seoul 110-749, Korea. (Tel) 82-2-740-8650, (Fax) 82-762-5107, (E-mail) sychoi@snu.ac.kr
†Co-corresponding author: Jeong Won Jahng, (Tel) 82-2-2072-0739, (Fax) 82-2-766-4948, (E-mail) jwjahng@snu.ac.kr

ABBREVIATIONS: ACSF, artificial cerebrospinal fluid; LTP, long term potentiation; fEPSP, extracellularly recorded excitatory post-synaptic potential; HPA, hypothalamus-pituitary-adrenal axis.

overloading with an appropriate choice of sensory experience.

For this purpose, we chose 'taste' sense as a relatively simple to apply excessive sensory experience. We developed an animal model with overloaded taste experience using saccharin, which is a well known artificial sweetener with strong sweet taste even at very low concentrations. After exposure to saccharin in the early period, we attempted to identify functional changes in brain circuits including hippocampus and somatosensory cortex by monitoring the formation of long term potentiation (LTP), an archetypal form of the synaptic plasticity.

METHODS

Animals

Sprague-Dawley rats were purchased (Samtako Bio, Osan, Korea) and cared for a specific-pathogen-free barrier area with constant control of temperature (22±1°C), humidity (55%), and a 12/12 hr light/dark cycle (lights-on at 07:00 h). Standard laboratory food (Purina Rodent Chow, Purina Co., Seoul, Korea) and membrane filtered purified water were available *ad libitum*. Animals were cared according to the Guideline for Animal Experiments, 2000, edited by the Korean Academy of Medical Sciences, which is consistent with the NIH Guidelines for the Care and Use of Laboratory Animals, revised 1996. All animal experiments were approved by the Committee for the Care and Use of Laboratory Animals at Seoul National University.

Taste-overloading animal model

Nulliparous females and proven breeder males were used for breeding in the laboratory of the animal facility, and the pups were reared in a controlled manner to minimize and standardize unwanted environmental stimulation from *in utero* life. Twelve hours after confirming delivery, pups were culled to five males and five females per litter, fostered, and then left undisturbed until weaning on postnatal day 22. On weaning day, pups were caged by sex in groups of 2 or 3 and then the experimental groups received 2 h of *ad libitum* access to a 0.1% saccharin (Sigma Co., St Louis, MO, USA) solution daily between 09:00 h and 11:00 h with free access to chow and water. The concentration of saccharin solution used in this study (0.1%) has been proven as a preferred taste to rodents [13]. Pups in the control groups remained with free access to chow and water omitting the saccharin access. The amounts of saccharin solution consumed during each drink session were recorded daily. Body weight gain and 24 hr food intake were measured twice per week, and the pups were sacrificed at 6 weeks of age to collect the brain slices.

Slice preparation

Coronal slices (400 μm) from the somatosensory cortex and transverse slices (400 μm) from the hippocampus were prepared as described previously [14]. After decapitation, brains were removed rapidly and placed in cold, oxygenated (95% O_2 and 5% CO_2) low-Ca^{2+} / high-Mg^{2+} dissection buffer composed of 5 mM KCl, 1.23 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM dextrose and 212.7 mM sucrose. Slices were transferred to a holding chamber in an incubator containing oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF) composed of 124 mM NaCl, 5 mM KCl, 1.23 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 26 mM $NaHCO_3$ and 10 nM dextrose at 28~30°C for at least 1 hr before recording.

Electrophysiology

Slices were transferred to a recording chamber at 28~30°C, which was perfused with ACSF saturated with 95% O_2 and 5% CO_2 at a flow rate of 2 ml/min. Synaptic responses were recorded every 15 sec in layer II/III of somatosensory cortex or in CA1 of hippocampus and evoked with 0.2 ms current pulses with the stimulus intensity of approximately 40~60% of the maximum response. The stimulations were delivered with a bipolar stimulating electrode (200 μm diameter; FHC, Bowdoinham, ME, USA) placed approximately in the layers IV or Schaffer collateral fiber. Microelectrodes filled with ACSF (1~2 MΩ) were used for extracellular recordings. Synaptic responses were quantified as the initial slope of the extracellularly recorded excitatory postsynaptic potential (fEPSP) in CA1, or as the amplitude of the maximum negative field potential in layer III. Changes in the amplitude of the field potential correlate with changes in the initial slope of EPSPs recorded in layer III neurons [15]. LTP was induced by theta-burst stimulation, which consisted four trains of ten bursts (each with four pulses at 100 Hz). Only data from slices with stable recordings (<5% change over the baseline period) were included in the analysis. All data are presented as mean± SEM normalized to the preconditioning baseline.

Data analysis

Data were analyzed by one-way analysis of variance (ANOVA) and preplanned comparisons with the groups performed by *post hoc* Fisher's Protected Least Significant Difference (PLSD) test, using StatView software (Abacus, Berkeley, CA, USA). Cumulative saccharin intake data were further analyzed by repeated measures ANOVA. Significance was set at $p<0.05$, and all values were presented as means±SEM.

RESULTS

Saccharin intake, food intake and body weight gain

Male and female pups received 0.1% saccharin solution *ad libitum* for 2 hr daily for 3 weeks after weaning, and chow and water were freely available during this period. Daily saccharin intake tended to be increased in males compared to females but was without statistical significance (Fig. 1A). The cumulative intake of saccharin solution gradually increased in males compared to females; however, repeated measures analysis of variance revealed no gender effect on the cumulative saccharin intake (Fig. 1B). Total saccharin intake during 3 weeks of period was 129.17±36.18 g in male and 107.47±31.61 g in female pups. Two hours of daily saccharin access did not appear to affect the consumption of standard chow and weight gain both in male and female pups. That is, weight gain of the saccharin groups (Chow/Sac) did not differ from the control groups (Chow) in males and females, respectively (Fig. 1C and 1E). Daily saccharin access slightly increased the chow intake of male pups during the experimental period; however, the

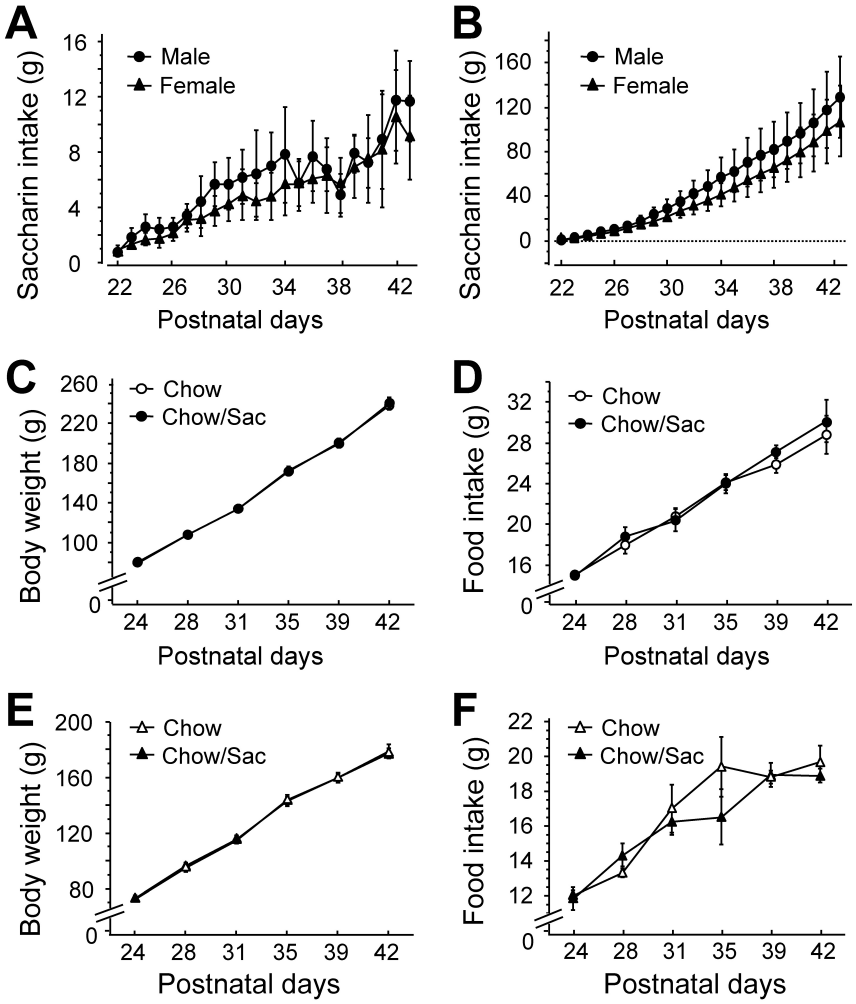

Fig. 1. Daily saccharin intake and body weight of male and female rats. (A) Amount of saccharin solution consumed during daily drinking sessions. Male (circle) and female (triangle) Sprague-Dawley pups had *ad libitum* access to 0.1% saccharin solution for 2 hr daily for 3 weeks following weaning on postnatal day 22. (B) Total saccharin consumption during each drinking session. (C, D) Body weight gain and food intake of male rat by saccharin intake. Food intake and body weight gain were monitored with (filled circle) or without (open circle) the exposure to 0.1% saccharin for 2 hr daily for 3 weeks during the indicated experimental period. (E, F) Body weight gain and food intake of female rat by saccharin intake. Food intake and body weight gain were monitored with (filled triangle) or without (open triangle) the exposure to 0.1% saccharin for 2 hr daily for 3 weeks during the indicated experimental period. All results are presented as mean±SEM from more than five individual rats.

intake differences between Chow/Sac and Chow only males on each measured day were not statistically significant (Fig. 1D). Although the female Chow/Sac group showed a transient decrease in chow intake on postnatal day 35 compared with Chow only control females, no overall difference during the whole experimental period was observed (Fig. 1F).

Effect of saccharin-intake on LTP formation in male rat hippocampus

We monitored LTP formation at hippocampus Schaffer collateral-CA1 synapses of Chow/Sac and Chow only 6-week old male rats using fEPSP recordings. Theta burst stimulation in Chow/Sac male rats produced LTP with 122.25± 9.82% (of baseline responses during the 50～60 min after stimulation) potentiated synaptic response (n=4 slices from 3 animals), whereas theta burst stimulation in Chow only male rats showed LTP with 121.13±8.59% increased responses (n=5 slices from 3 animals; Fig. 2A, B). Hippocampal LTP formation did not show a significant difference in Chow/Sac male rats compared with that in Chow only male rats (Fig. 2C).

Effect of saccharin-intake on LTP formation in female rat hippocampus

We also monitored LTP formation at hippocampal Schaffer collateral-CA1 synapses of Chow/Sac and Chow control 6-week old female rats. Theta burst stimulation in Chow/Sac female rat produced LTP response 125.98±10.17% of control (n=5 slices from 3 animals) and Chow control female rat shows LTP with 127.23±7.39% increased responses (n=6 slices from 5 animals; Fig. 3A, B). In summary, hippocampal LTP formation did not show a significant difference female or male saccharin fed rats compared to control, suggesting that the hippocampal synaptic plasticity was unchanged by juvenile saccharin exposure (Fig. 3C).

Effect of saccharin-intake on LTP formation in male rat somatosensory cortex

To monitor the effect of the taste sensory overloading on synaptic plasticity in other areas of cerebral cortex, we targeted the layer IV- II/III pathway of the somatosensory cortex and compared LTP formation in Chow/Sac and Chow control 6-week old male rats. Theta burst stimulation in

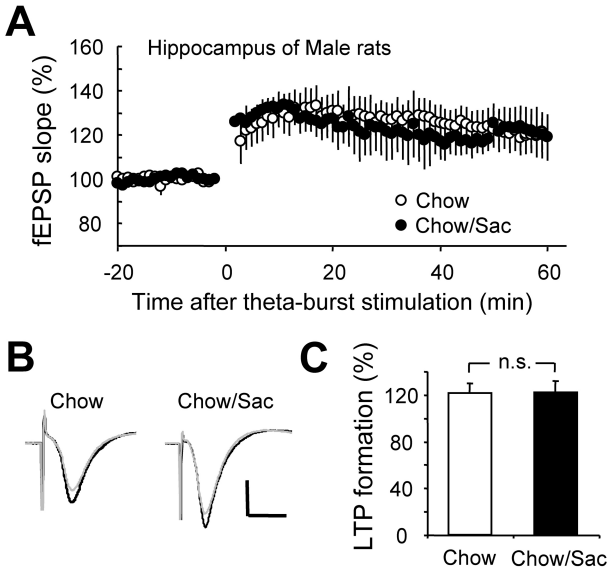

Fig. 2. Effect of saccharin intake on LTP induction in hippocampal Schaffer collateral pathway in male rat. (A) Hippocampal slices were prepared from 6∼7 weeks old male Sprague-Dawley rat with (filled circle) and without (open circle) *ad libitum* access to 0.1% saccharin solution for 2 hr daily for 3 weeks, and recorded fEPSP in Schaffer collateral-CA1 synapses. Average changes in the fEPSP slope induced by theta burst stimulation are depicted. (B) Typical field potential traces from experiments performed in saccharine-treated group (right) and chow only group (left) are shown. The superimposed traces are averages of four consecutive responses recorded 1 min before (black traces) and 1 hr after (gray traces) theta burst stimulation. (C) The magnitude of LTP formation of baseline responses is shown depicted. All results are presented as mean±SEM from more than four independent trials. n.s., not significant statistical difference ($p > 0.05$).

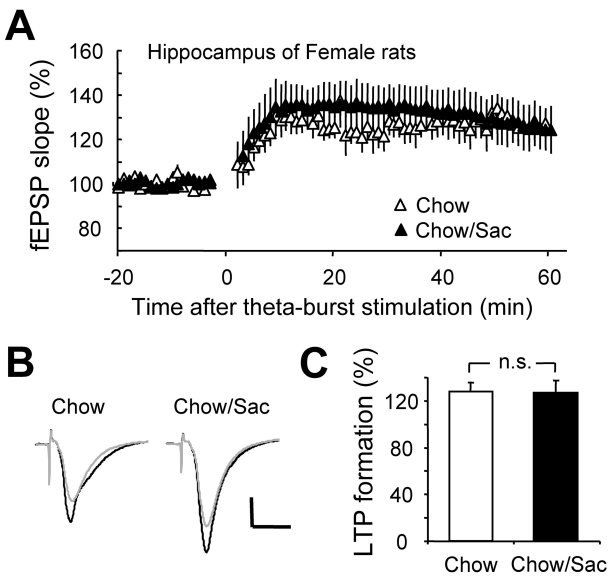

Fig. 3. Effect of saccharin intake on LTP induction in hippocampal Schaffer collateral pathway in female rat. (A) Hippocampal slices were prepared from 6∼7 weeks old female rat with (filled circle) and without (open circle) 0.1% saccharin intake, and recorded fEPSP in Schaffer collateral-CA1 synapses. Average changes in the fEPSP slope induced by theta burst stimulation are depicted. (B) Typical field potential traces from experiments performed in saccharine-treated group (right) and chow only group (left) are shown. The superimposed traces are averages of four consecutive responses recorded 1 min before (black traces) and 1 hr after (gray traces) theta burst stimulation. (C) The magnitude of LTP formation of baseline responses is shown depicted. All results are presented as mean±SEM from more than five independent trials. n.s., not significant statistical difference ($p > 0.05$).

Chow/Sac male rats produced LTP with 108.91±4.67% potentiated response (n=7 slices from 5 animals), Chow control male rat formed LTP with 108.46±12.49% increased responses (n=3 slices from 3 animals; Fig. 4A, B). LTP formation in the somatosensory cortex did not show a significant difference in Chow/Sac male rats compared with that in Chow only male rats (Fig. 4C).

Effect of saccharin-intake on LTP formation in female rat somatosensory cortex

Finally we tried to monitor the difference in LTP formation at layer IV- II/III pathway of somatosensory cortex between Chow/Sac and Chow control 6-week old female rats. Theta burst stimulation in Chow/Sac female rat produced LTP with 117.32±14.50% increased responses (n=3 slices from 3 animals) and Chow control female rat shows LTP with 118.19±10.91% increased responses (n=5 slices from 4 animals; Fig. 5A, B). Like in male rats, LTP formation in the somatosensory cortex also did not show a substantial difference in the female model, suggesting that synaptic plasticity in somatosensory cortex is not grossly affected by juvenile saccharin treatment (Fig. 5C).

DISCUSSION

Recently, sensory overloading and its effect on neuronal systems have gathered interest from many neuroscientist and physiologists with the accumulation of several lines of evidence. For example, visual sensory overloading in juvenile and adolescent animals increases the chance of photic seizure [16]. Auditory cortical reorganizations were monitored from rats exposed with environmental noise [17], and cats exposed with dense tone pip ensemble (4∼20 kHz) [18]. However, the effect of sensory overloading on the structure and function of neuronal circuits and its related physiological mechanism have not yet been investigated.

We attempted to create an animal model of excessive taste sensory experience in the early period of life. Taste is one of the chemical senses handled by taste receptor cells in the tongue and signaled by afferent fibers including facial and glossopharyngeal nerve, via the solitary tract nucleus, to the gustatory cortex [19]. In spite of the caveat that the physiological and anatomical map and sensory coding in rat gustatory cortex are not yet fully understood, taste sense was a strong candidate for a sensory overloading model because it is possible to control the duration and/or intensity of sensory stimulation. For instance, taste sensory stimulation can be self-administered or administered by the experimenter. We also chose the preferable stimulation with sweet taste. With taste sense it is

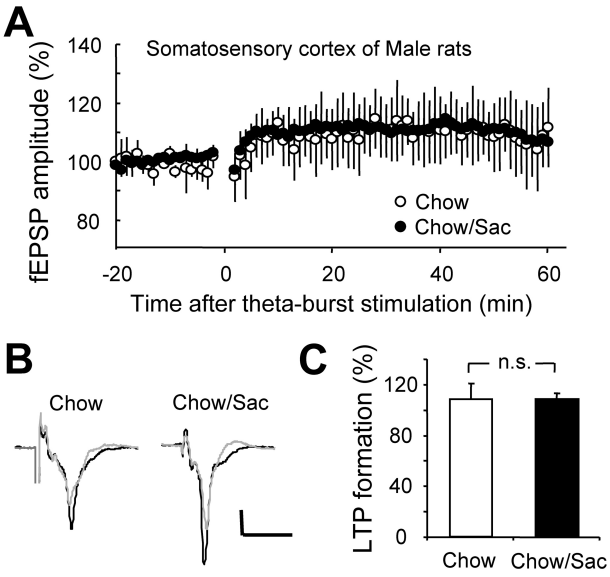

Fig. 4. Effect of saccharin intake on LTP induction in somatosensory cortex layer IV- II/III pathway in male rat. (A) Somatosensory cortical slices were prepared from 6∼7 weeks old male rat with (filled circle) and without (open circle) 0.1% saccharin intake, and recorded fEPSP in layer IV- II/III synapses. Average changes in the fEPSP amplitude induced by theta burst stimulation are depicted. (B) Typical field potential traces from experiments performed in saccharine-treated group (right) and chow only group (left) are shown. The superimposed traces are averages of four consecutive responses recorded 1 min before (black traces) and 1 hr after (gray traces) theta burst stimulation. (C) The magnitude of LTP formation of baseline responses is shown depicted. All results are presented as mean±SEM from more than three independent trials. n.s., not significant statistical difference ($p > 0.05$).

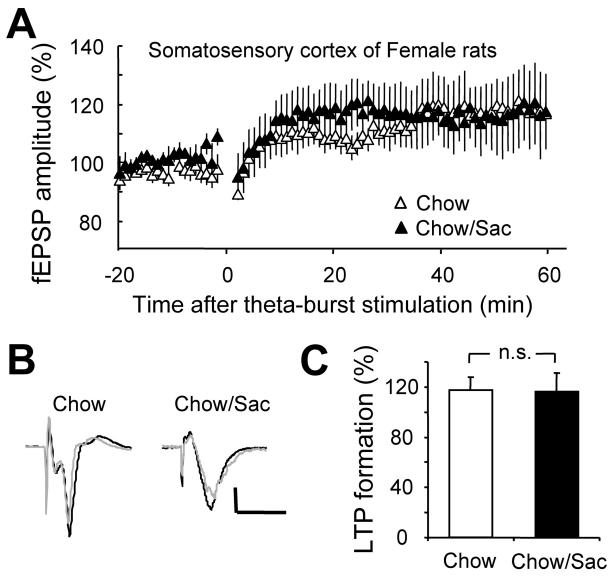

Fig. 5. Effect of saccharin intake on LTP induction in somatosensory cortex layer IV- II/III pathway in female rat. (A) Somatosensory cortical slices were prepared from 6∼7 weeks old female rat with (filled circle) and without (open circle) 0.1% saccharin intake, and recorded fEPSP in layer IV- II/III synapses. Average changes in the fEPSP amplitude induced by theta burst stimulation are depicted. (B) Typical field potential traces from experiments performed in saccharine-treated group (right) and chow only group (left) are shown. The superimposed traces are averages of four consecutive responses recorded 1 min before (black traces) and 1 hr after (gray traces) theta burst stimulation. (C) The magnitude of LTP formation of baseline responses is shown depicted. All results are presented as mean±SEM from more than three independent trials. n.s., not significant statistical difference ($p > 0.05$).

also possible to select continuous administration, thereby producing tolerance and dependence, or intermittent administration to maximize sensitization. Most useful of all, we can choose to apply the taste stimulation only during a certain period such as juvenility or adolescence, which is very useful to find experience-dependent changes in an animal model.

Saccharin is a typical stimulant for rat with preferred sweet taste [20-22]. It is reported that the restricted daily application of 0.1% saccharin diminished the stress-induced corticosterone release without changes in amount of food intake and body weight [23]. It is noteworthy that the hypothalamus-pituitary-adrenal (HPA) axis ('stress axis') can be controlled by the hippocampus; it seemed likely, therefore, that there may be a link between sweet taste sensory overloading and hippocampal function. We monitored the effect of saccharin intake on the hippocampal Schaffer collateral synaptic plasticity. In this trial, we maximized the sensory intensity not only by the concentration of saccharin but also by way of restriction of saccharin self-administration to 2 hr per day, because continuous administration of palatable food did not change the stress-induced corticosterone release in rat [24]. We have previously reported that not only the hippocampal function but also HPA axis activity responding to reward stimuli exhibit gender differences [25], therefore we also separately monitored the difference in LTP formation between genders. Gender-specific effects of early life manipulations on brain monoamine levels [26], the HPA axis activity and neuro-behaviors [27,28] of rats have also been reported.

It is reported that the deprivation of sensory stimulation not only changes the cortical circuitry for the targeted sense but also produces compensatory changes in other sensory cortical circuitry [29]. Referred to as 'cross-modality', it was found that dark rearing affects the circuitry not only of visual cortex but also of somatosensory cortex in rat [29], and blindness lead to improved perceptual skills with modified occipital cortical circuitry in humans [30,31]. We therefore studied whether saccharin intake could mediate changes in synaptic plasticity in somatosensory cortex.

Gustatory signals are thought to be handled by the hippocampus for taste memory storage, the recall process, and are closely related to its emotional state. However, our results show that hippocampal LTP formation was intact without significant difference between control and saccharin intake rats and without any difference between male and female. These results suggest that taste sensory overloading does not affect the basic learning and memory process in the Schaffer collateral pathway. Previously Goel et al. reported that a visual sensory deprivation model showed intact synaptic plasticity in hippocampus [29], suggesting that hippocampal circuit is not detectably affected by changes in environment or experience. We also observed similar results on the effect of saccharin intake on the somatosensory cortex, which showed no significant difference between the two groups and genders. The lack of synaptic

plasticity changes in our results may have at least two explanations. One is that sensory overloading (compared to sensory deprivation) does not express detectable cross-modality or the technique used was not sensitive enough to detect such changes. The other is that cross-modality triggered by gustatory sensory overloading is relatively limited because gustatory cortex is not as widely spread and lacks the precise mapping of the visual and somatosensory cortices.

Even though our experimental procedure with saccharine intake was based on previous reports, there still remains the possibility that the concentration of saccharin and/or the duration/period of treatment were not sufficient to evoke detectable changes in synaptic plasticity. Confirmation of the saccharin-intake effect using more varied conditions would be a valuable study. Gustatory information has a strong connection to the emotion and reward centers, therefore further investigation into the changes in other brain regions, including amygdala, nucleus accumbens, and ventral tegmental area, may answer these unsolved questions and help in our understanding of the structural and functional changes in neuronal circuitry by sensory overloading.

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