Capsaicin-sensitive nerves and energy homeostasis

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CHAPTER 2

DEAFFERENTATION AFFECTS SHORT-TERM BUT NOT LONG-TERM CONTROL OF FOOD INTAKE

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ABSTRACT

Rats were treated neonatally with capsaicin (CAP) to investigate the involvement of vagal afferents in food intake control and body weight regulation. In the first set of experiments, rats were offered increasing concentrations of sucrose (10%-15%-20%-40%) in short-term feeding tests of 1 h. At the end, 10% was offered again to see whether CAP rats modified their intake after repeated exposure to different concentrations of sucrose solution. Results demonstrated that CAP animals overconsume persistently compared to vehicle (VEH) controls. This overconsumption is most pronounced and variable at 10% trials. Hypertonic 40% sucrose-solution resulted in a small but significant drop in intake in CAP rats. Overall, if the concentration of sucrose solution is more then 10%, sucrose ingestion of CAP and VEH rats does not depend on the concentration of sucrose solution and remains relatively constant during all trials. In another experiment, rats were exposed to a high-fat condensed milk suspension (CMS) for 5 days. CAP rats initially overconsumed from this CMS compared to VEH. This was accompanied by a decreased intake in chow. However, over the 5 day period CAP animals adjusted their CMS and chow intake to control levels. During both experiments there were no differences in body weight gain between CAP and VEH. Together, these results suggest that capsaicin-sensitive vagal C-fibers are involved in the control of volume ingestion and short-term food intake control but are not required for long-term control of energy intake.

1 INTRODUCTION

Ingested nutrients are complex stimuli, with multiple mechanical and chemical properties that can elicit a range of neural and humoral feedback signals for termination of intake (26). There are strong indications that sensory neurons innervating the gastrointestinal tract are very important in the process of satiation and short-term satiety signaling. The extrinsic and intrinsic innervations of the upper gastrointestinal tract and the extrinsic projections to the brain are part of a negative feed back loop in the process of meal satiation and meal termination. The vagus nerve is a very important

mediator in this neural gut-brain axis and has been extensively studied by different kinds of vagotomies (1, 20, 27, 30, 34, 36) or by using the neurotoxin capsaicin (4, 22, 31-33, 37). The vagus nerve is composed of mainly afferent fibers (70 to 90%) but it contains efferent fibers as well (2, 21). Vagotomy destroys both afferent and efferent fibers, which complicates the interpretation of the observed effects on food intake. Capsaicin in large doses damages small unmyelinated primary sensory cells and fibers (12, 24) and is often used as a tool to study the involvement of vagal primary afferent fibers in the control of food intake.

Ablation of neural afferents with capsaicin attenuates the suppression of feeding behavior (18, 22, 31) and hindbrain fos expression (10, 19, 25) normally seen after intraperitoneal (IP) CCK administration. Also, hindbrain extracellular responses to near-celiac artery infusion of CCK were abolished in capsaicin treated rats (22). Moreover, capsaicin treated rats (CAP) show distinct overconsumption compared to their vehicle controls (VEH) depending on the diet (5, 6, 14) or novelty of the food (5, 15). Kelly and colleagues (14) found that animals treated with capsaicin at an adult age showed concentration-depended volume intake of sucrose solution after an initial overconsumption of the novel diet. They suggest that capsaicin treated animals regulate more on calories, while controls regulate on volume intake. These deficits may have consequences for the long-term regulation of body weight. Therefore, our aim was to investigate whether systemic neonatal capsaicin treatment leads to permanent disturbances in food intake control and body weight regulation. To dissociate effects on the short-term control of food intake from the long-term control, two experiments were performed at adult age. In one experiment, animals were offered different concentrations of sucrose in short-term feeding tests over 8 weeks. In the second experiment, possible effects on the long-term control of food intake were studied by exposing the animals to a high-fat condensed milk suspension (CMS) for 5 days. In both experiments, body weight was measured daily.

2 METHODS

2.1 Animals and housing

30 Male wistar rats, bred in our own laboratory were used and housed in climate controlled rooms (22 °C ± 2) under a 12h:12h light-dark cycle (lights on at 8:00 am). These rats were previously treated with capsaicin or vehicle capsaicin at the age of 2 days (see below). Food and water were ad libitum available, unless mentioned otherwise. Capsaicin treated pups and vehicle treated pups grew up separately -to avoid selective mother care- in litters of 5-9 pups, in the proportion 5-7 male, 2 female (non-treated). After weaning at the age of 23 days, rats were individually housed in clear plexiglas cages (25x25x30 cm).

2.2 Capsaicin treatment

Capsaicin (8-methyl-N-vallinyl 6 nonenamide, Sigma Chemical, The Netherlands) was given neonatal when pups were 2 days old (n=16) by a subcutaneous injection (50 mg/kg). This was done under 100% O_2 conditions to avoid hypoxia. Capsaicin was dissolved in a vehicle consisting of 10% ethanol (10%) and 5% cremophore (pig-40-hydrogenated castor oil, gift of Novo Nordisk, Denmark) -0.9% sodium chloride solution (90%). As a control, vehicle solution was injected s.c. (n=14). Each animal was given the same volume of 50 µl based on an average weight of the pups of 8 grams.

After treatment, body weight was measured weekly. One capsaicin animal died during the nursing period and one capsaicin-treated animal died during the experimental period for unclear reasons. At the age of 15 weeks efficacy of the capsaicin treatment was assessed by testing the corneal chemosensory response (23, 24). This test involved application of one drop of 0.1% capsaicin solution into the left eye. One vehicle-treated animal was tested to see if the solution was effective to trigger a behavioral response. The vehicle-treated animal immediately exhibited a strong reaction (> 10 wipes) of the affected eye, while capsaicin-treated animals did not exhibit any response to the application of 0.1% capsaicin solution. Absence of the corneal chemosensory response indicated that the small unmyelinated afferent fibers

of the trigeminal nerve, and presumably all other small unmyelinated primary afferents, were successfully destroyed by capsaicin.

2.3 Experimental procedure

2.3.1 Experiment 1: short-term sucrose intake test

At the age of 16 weeks, animals were trained for 2 subsequent days for the experimental procedure to avoid neophobia. 1 hour before the lights turned on, animals were food deprived. At 10:30 am, the training started. During the training period, animals were allowed to drink from a 10% sucrose solution for only 10 seconds to avoid metabolic consequences of the ingested sucrose [24]. One animal did not drink sucrose and was therefore excluded from the experiment. At the age of 17 weeks, the experiment started. The experimental procedure follows the experiment described in Kelly et al. (14). Sucrose was offered in ml scaled cylinders to the animals on different days for 60 minutes in the order of 10%, 15%, 20%, 40% and 10%. Each percentage of sucrose was offered three times with at least 4 days between the subsequent trials. In total, each animal was offered 15 times a sucrose solution of a certain concentration. During these short-term sucrose tests, water was ad libitum available. One capsaicin animal died during the experiment. Therefore, in total seven capsaicin-treated animals and eight vehicle-treated animals where used in the experiment.

2.3.2 Experiment 2:Long-term food intake

In the second set of experiments, another group of rats (capsaicin, n=7; vehicle, n= 6) were offered a suspension of a high-fat diet consisting of condensed milk and corn oil (3:1). This condensed milk suspension (CMS) was offered in drinking bottles continuously for 5 days. Rats were not trained before the start of the experiment. During the experiment, animals still had free access for their chow and water. CMS and chow intake as well as body weight were measured daily.

2.4 Statistical analysis

Data from the sucrose intake were analyzed with an ANOVA repeated measurements with time and food intake as within subject factors and group as between subject factor. Posthoc pairwise comparisons (Bonferroni) were done based on estimated marginal means. Two-tailed student's t-test was used for unpaired observations. Differences were considered significant if $p < 0.05$.

3 RESULTS

3.1 Experiment 1

3.1.1 Short-term sucrose intake

Rats were offered increasing concentrations of sucrose-water in short-term feeding tests of 1 hour. Each concentration was offered 3 times, with at least 4 days between the consecutive trials. The initial 10% concentration was offered again in the last trial set to see whether repeated experience with different concentrations of sucrose solution resulted in altered sucrose intake in CAP rats. Figure 1 presents the sucrose and caloric intake of CAP and VEH animals. The average volume intake per trial set (3 trials) shows that there is a significant overconsumption in neonatally treated CAP animals independent of the concentration; ANOVA repeated measurements ($F_{1,13} = 63.17$, $p < 0.001$, figure 1A). There is a significant main effect of sucrose concentration ($F_{4, 52} = 36.69$, $p < 0.001$) and more detailed analysis reveals that in CAP treated animals, the 40% causes a significant drop in sucrose intake ($p < 0.05$); during the last trial set of 10% animals consumed significantly more compared to all other sucrose trials ($p < 0.05$). In VEH animals, the first trial set of 10% sucrose ingestion is significantly lower than the 20% trials and during the last trial set of 10% animals consume significantly more compared to the 40% trials ($p < 0.05$). There is no significant sucrose concentration × group interaction ($F_{4, 52} = 4.92$, NS). When calories are considered (figure 1B), there is also a main effect of sucrose concentration ($F_{4, 52} = 293.71$, $p < 0.001$), and a significant sucrose concentration × group interaction ($F_{4, 52} = 4.34$, $p < 0.01$). Post hoc analysis reveals that higher sucrose concentration leads to significant higher calorie intake during the short-term feeding tests in CAP ($p < 0.001$) and VEH rats ($p < 0.001$). The increased sucrose intake after capsaicin treatment is

reflected in a significant higher calorie intake in CAP rats ($F_{1, 13} = 1561.38$, $p < 0.001$).

Figure 1C shows the variations in sucrose intake within one trial set of a certain sucrose concentration. The increased sucrose-intake in CAP is most pronounced at both trial sets (first and last trials) of the 10% trials. There is a main effect of time ($F_{14, 128} = 10.98$, $p < 0.001$), and time × group interaction ($F_{14,182} = 2.12$, $p < 0.01$). Post hoc comparisons reveal significant changes in volume intake during the 10% and 40% trial sets in CAP animals. VEH rats only show significant changes in consumption during the first 10% trial set. In CAP animals, there is a significant increase each trial in the first 10% trial set ($p < 0.01$); the last 10% sucrose exposures resulted in a significant higher intake in the second trial of the 10% concentration set compared to the first and last trial ($p < 0.05$). 40% sucrose intake caused a significant drop in the second trial of this trial set ($p < 0.001$). In VEH controls, the first trial experience resulted in a significant lower 10% sucrose intake compared to the other 2 trials of 10% ($p < 0.05$); the last 10% trials did not differ significantly from each other. Also, there are no significant differences between the 40% trials in VEH.

Figure 2 shows a typical curve of the average rate of sucrose ingestion in CAP and VEH animals. The third trial of the first trial set of 10% and the second trial of the last trial set are shown. At these points, the overconsumption in CAP is most pronounced. No significant differences in rate consumption are observed between CAP and VEH animals in the first 7-10 minutes of the feeding test. Significant differences between CAP and VEH after this time point are more likely due to the delayed satiety response after capsaicin treatment.

3.1.2 Body weight

Figure 3 presents the growth curve of CAP and VEH animals during the sucrose intake experiment. Body weight of CAP animals and their VEH controls did not differ significantly (397.9 g ± 9.0 406.5 g ± 9.5 resp.) at the beginning of the experiment. There is a significant increase in body weight over time ($F_{14,196} = 192.44$, $p < 0.001$). Yet, there is no significant time × group

interaction ($F_{14,196}$ = 1.07, NS). Also, body weight growth during the experiment did not differ between CAP and VEH rats ($F_{14,196}$ = 0.67, NS).

3.2 Experiment 2: CMS intake, chow intake and body weight changes

Figure 4A shows the intake of CMS in CAP and VEH rats during 5 days. ANOVA repeated measurements analysis reveals a significant effect of time ($F_{4,40}$ = 4.10, $p < 0.01$) and significant effect of treatment ($F_{1,10}$ = 117.19, $p < 0.001$). There is no significant time × treatment interaction ($F_{4,40}$ = 0.83, NS). Post hoc analysis reveals a significant 45% higher intake of the CMS in CAP rats on the first day when compared to their VEH controls ($p < 0.05$). However, the CMS in CAP drops significantly on day 3 to control levels ($p < 0.01$).

ANOVA repeated measurements reveals a main effect of time on chow intake ($F_{4,40}$ = 4.52, $p < 0.01$) and a significant main effect of treatment ($F_{1,10}$ = 103.00, $p < 0.001$); but does not show significant time × group interaction ($F_{4,40}$ = 0.83, NS). Post hoc analysis with Bonferroni-test does not result in significant differences at a certain time point (figure 4B). When the caloric intake of CAP and VEH rats is considered on the first day to CMS exposure, results indicate that CAP rats consume significantly more of the CMS and less of chow compared to VEH controls on day 1 ($p < 0.05$; figure 5).

A main effect of time on body weight is seen after analysis of the data ($F_{4,40}$ = 19.90, $p < 0.001$, figure 4C). However, here as well, no significant time × group interaction is observed ($F_{4,40}$ = 0.54, NS). There is a significant effect of group on body weight ($F_{1,10}$ = 22.96, $p < 0.001$). Pair wise comparison with the Bonferroni test does not reveal significant body weight changes between CAP and VEH rats on a specific time point.

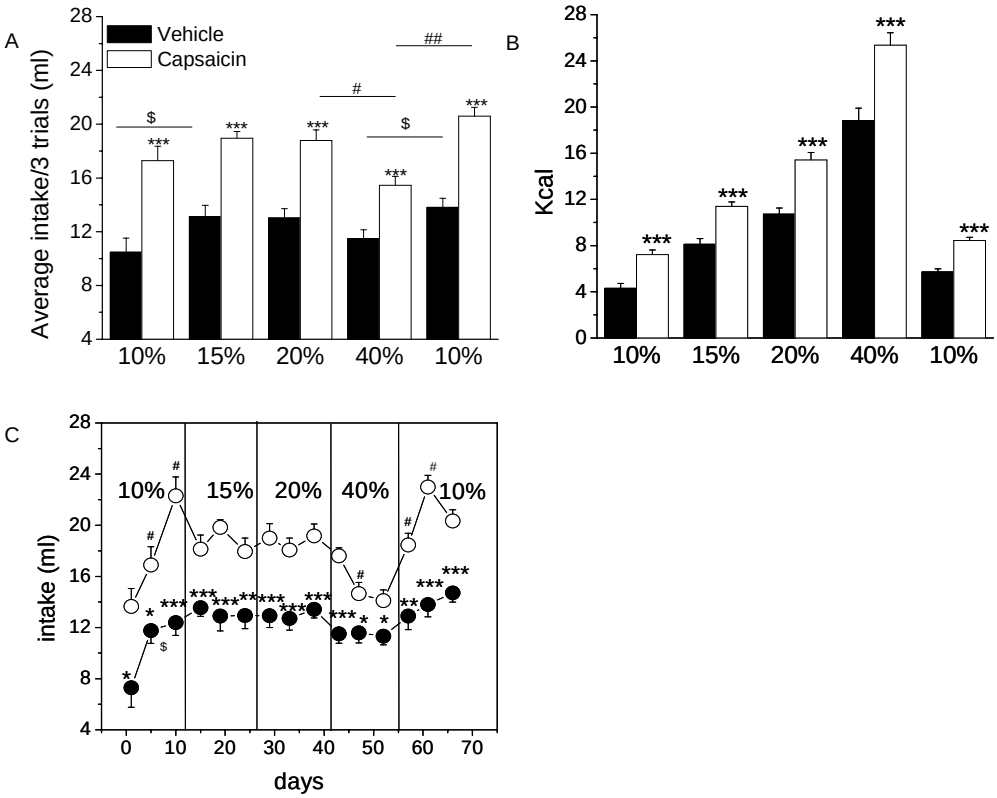

Figure 1

*A) Average sucrose intake per trial set in CAP and VEH animals. B) Average caloric intake per trial set in CAP and VEH animals. C) Sucrose intake per trial in CAP and VEH animals. *, **, *** significantly different from VEH controls, $p < 0.05$, $p < 0.01$, $p < 0.001$ resp.; #, ## significant differences within CAP animals, $p < 0.05$ and $p < 0.01$ resp.; $, significant differences within VEH animals, $p < 0.05$.*

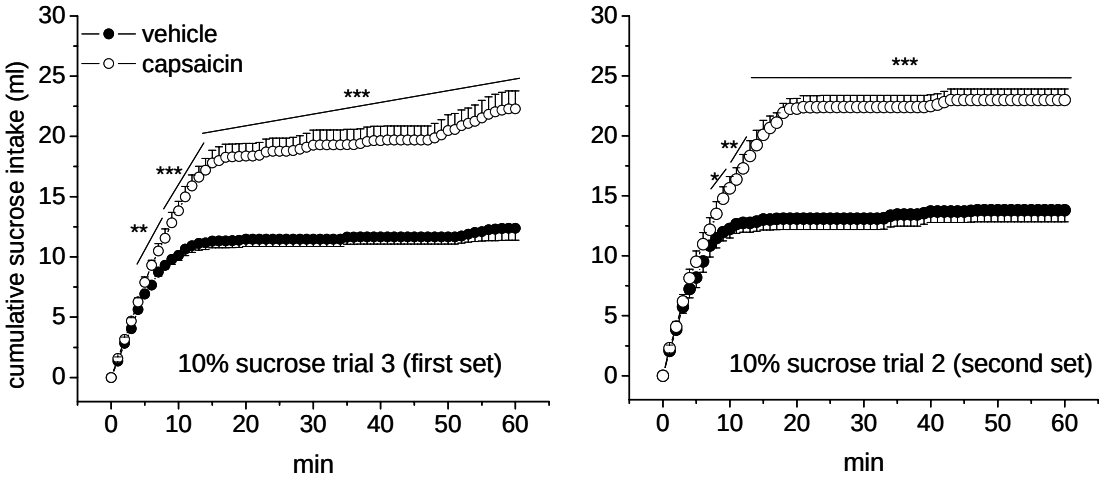

Figure 2
*Ingestion of sucrose in CAP and VEH animals during the 3thd trial of the first trial set of 10% and the second trial of the last trial set of 10%. measurements of consumption are plotted every 5 minutes. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.*

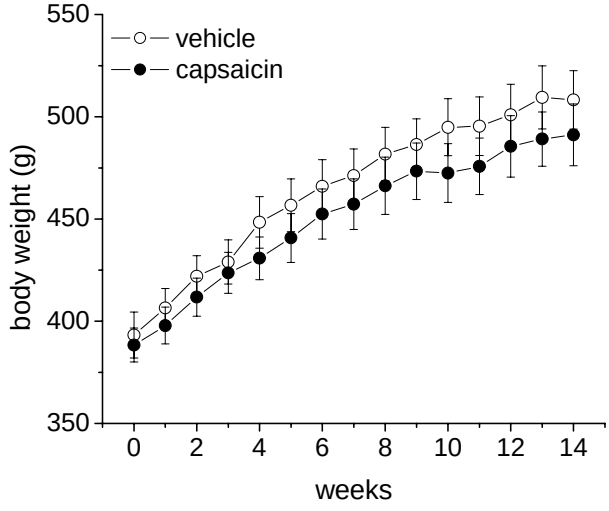

Figure 3
Body weight increase of CAP and VEH animals during the exposure to the different sucrose trials.

4 DISCUSSION

4.1 Short-term sucrose intake

In the present study, CAP rats showed a persistent overconsumption of sucrose solution compared to VEH controls. This overconsumption was independent of the sucrose concentration, but was most pronounced in the 10% sucrose trials. The large variation in the CAP animals between subsequent trials of the 10% solution suggests that at this concentration vagal afferents play an important role in short-term satiation. This result is in agreement with findings of Kelly and colleagues (14) as well as Curtis and Stricker (6) who also showed that animals with chemical ablation of their primary afferents at adult age overconsume from a 10% sucrose solution. However, in contrast to our finding, Kelly and colleagues (14) found that adult capsaicin-treated animals learned to control their intake to vehicle control levels when sucrose concentrations of 15% or higher were offered. Thus, it appears that neonatal or adult capsaicin treatment has not the same effects on short-term food intake.

One of the first questions that arise is whether differences in neophobia between CAP and VEH animals can explain the present results. However, animals were trained before the experimental protocol started and the overconsumption in CAP rats was persistent throughout the experiment. These arguments indicate that it is unlikely that a difference in neophobia between CAP and VEH is the key to explain the overconsumption in CAP animals. Moreover, Davis and Smith (7) have shown that the rate of ingestion of liquid diets is an indicator for the acceptance of the compound. In this experiment, no significant differences were observed in the initial rate of sucrose intake between CAP animals and their controls. Therefore, the increased sucrose intake in CAP rats is likely to be explained by a delayed satiety mechanism and not to changes in rate of sucrose ingestion or differences in acceptance of the sucrose solution.

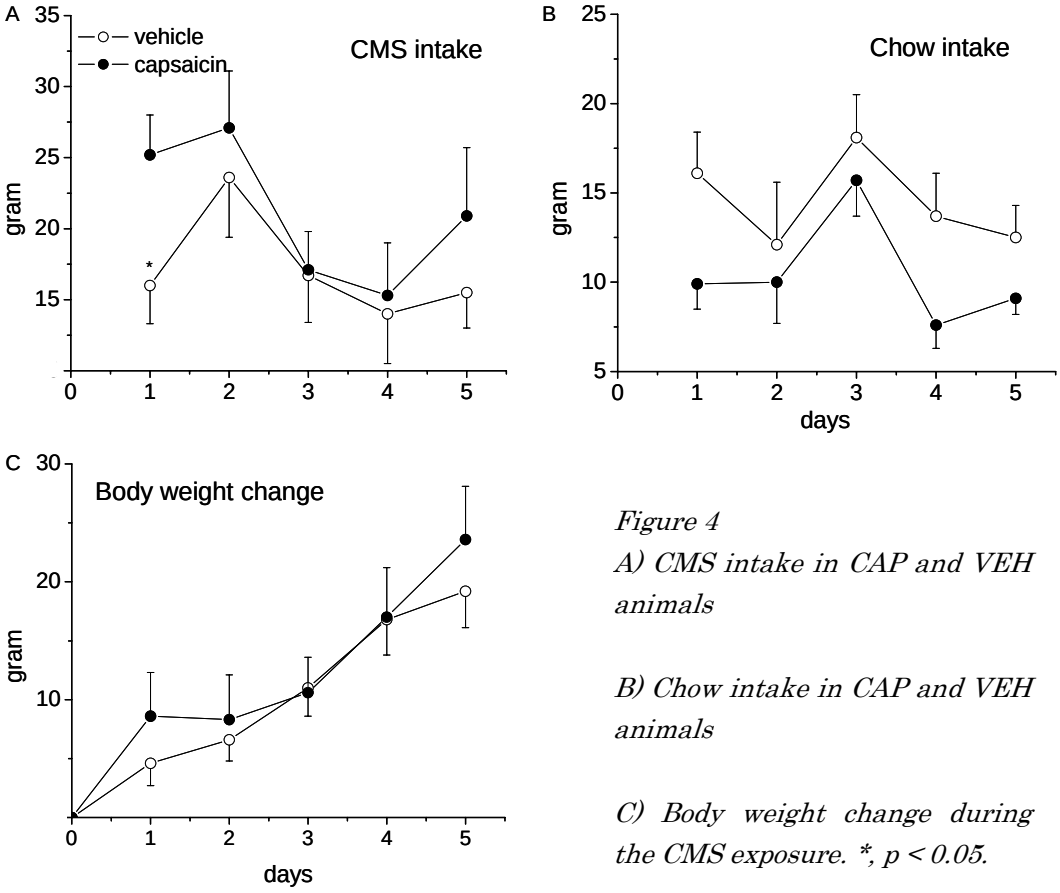

Figure 4
A) CMS intake in CAP and VEH animals

B) Chow intake in CAP and VEH animals

*C) Body weight change during the CMS exposure. *, $p < 0.05$.*

Another factor that should be addressed is that differences in taste sensitivity could play a role for the observed differences between capsaicin-treated rats and their controls. Kido et al. (16) reported that taste papillae in the tongue and palate are richly innervated by VR1-immunoreactive nerve fibers. However, rats tested 15 hours after desensitization by capsaicin

injection did not show differences in behavioral responses to salty and sour stimuli compared to control animals. Yet, the CAP animals appeared to find bitter solutions slightly less aversive than their controls (28).

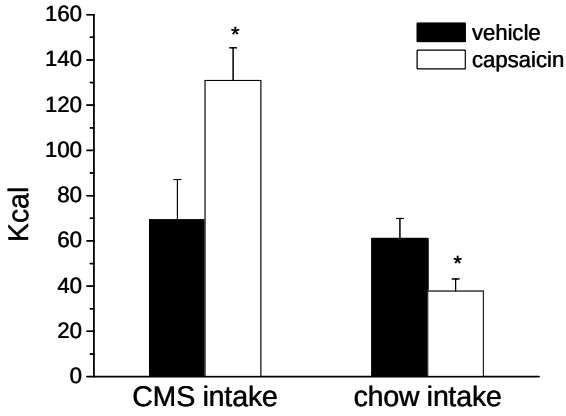

Figure 5
*Caloric intake of CMS and chow on the first day of exposure to the CMS. *, $p < 0.05$.*

Also, Karrer and Bartoshuk (13) report a decrease in taste sensitivity after capsaicin desensitization in humans for high concentrations of citric acid or quinine. Although sucrose was not tested in this set up, it might be possible that the sucrose taste is less intense after systemic capsaicin treatment. This might account partly for the observed results in this study. However, this explanation is not very likely since differences between CAP and VEH rats are independent of sucrose concentration.

Feedback signals like mechanical stimuli such as stomach distension are important contributors in signaling satiation and meal termination. It is interesting that in this experimental set up, VEH animals show a constant volume intake in the trial sets of 15% through 40% sucrose. This indicates that in this set up, at higher sucrose concentrations, VEH animals regulate on volume and not on calories. It appears that CAP animals can regulate on volume as well, since there are no significant differences within the trial sets of 15% and 20%. The same volume is consumed at each of the 3 trials, indicating that volume is still sensed to some degree by the gastrointestinal tract in CAP animals. Indeed it has been shown in multiple ways that there are still capsaicin-resistant fibers which signal gastric distension. Ritter et al. reported that hindbrain extracellular responses to near-celiac artery infusion of CCK were abolished in capsaicin-treated rats, while responses to gastric distension were not diminished (22). Also, reduction of food intake in response to gastric loading is not attenuated in capsaicin treated rats (23). Moreover, gastric balloon distension still induces c-Fos expression in the dorsal vagal complex in capsaicin-treated rats (3). Finally, Drew et al. (8) showed that in somata of cultured neonatal rat dorsal root ganglia neurons different types of sensory neurons have distinct mechanosensitive phenotypes. They found that capsaicin-sensitive neurons showed higher thresholds for the induction of an inward current and lower peak currents than other mechanosensitive neurons. This would be in agreement with the current results, where CAP animals show a regulated, but higher satiety level compared to controls. It might be possible that the low-threshold population is mostly sensitive to capsaicin and that these high-threshold mechanoreceptors are stimulated when a greater amount of sucrose solution is consumed. Thus, the present results suggest that volume distension is still an important satiation factor in CAP rats, even though it may be that low- versus high- threshold receptors are differentially affected after capsaicin treatment.

Finally, osmotic concentration of the consumed compound is another important factor in the process of satiation for food as suggested by Weller et al. (35). The present results may suggest that capsaicin treated animals are still able to sense osmotic concentration of ingested food. The amount of sucrose consumption drops in CAP when 40% solution is offered in the second trial;

40% is hypertonic and the drop in intake suggests that osmoreceptors are still functional after capsaicin treatment. These results are in agreement with findings of Kelly et al. (14) who also found that 40% sucrose intake drops significantly in CAP and VEH compared to lower sucrose concentrations.

In conclusion, the persistent, but regulated overconsumption of sucrose at the higher concentrations and the variable overconsumption in the 10% sucrose trials of CAP rats suggest that vagal deafferentation disrupts a specific feedback signal, presumably from intestinal glucoreceptors, leaving other feedback signals from mechanoreceptors and osmoreceptors partly intact. In intact animals these intestinal and gastric feedback signals form a coordinated satiety signal.

4.2 Long-term intake of CMS

We found an initially higher intake of sweetened CMS on the first day in neonatally treated CAP rats. This difference disappeared on the second day due to an increased intake in VEH animals. On the third day, both VEH and CAP rats drop their intake and remain constant for the other remaining 2 days. Since our animals were not trained to this new diet, reduced neophobia in CAP animals could explain the present results. Especially since VEH animals show a tendency to increase their condensed milk intake the second day of exposure. Similar experiments were performed by investigators of Berthoud's lab (3). In this study, they did short-term feeding tests with high-fat diets in CAP animals that were treated at adult age. In this experimental set up, animals were trained to the new diet to reduce neophobia. However, they also showed that adult capsaicin-treated animals initially overconsume of an unfamiliar high-fat diet. After multiple exposures, intake in CAP rats equaled that of their VEH controls. In the present experiment, the overconsumption of the sweetened CMS in CAP animals is reflected in a decrease in caloric intake of chow as well as a tendency to a higher body weight increase on the first day of exposure to CMS compared to VEH rats.

All together, these results point to the possibility that repeated experience of the sweetened CMS results in an adjustment of the initial overconsumption so that intake on the 3thd day is similar to control levels. This compensation is also reflected in body weight gain. After an initial

tendency to a higher body weight increase in CAP animals this difference disappeared on the second day of the condensed milk exposure and body weight gain is exactly the same on the 3thd day in CAP and VEH rats. It may be that long-term signals related to fat do play a role in this compensation. Since we did not observe significant differences in body weight gain between CAP and VEH animals, it might be that CAP animals are more sensitive to signals related to long-term regulation of energy homeostasis as leptin (submitted). This could be a compensatory pathway since they miss a substantial part of neurons which control short-term food intake. The fact that CAP and VEH rats do not differ significantly in absolute body weight supports this thought.

Thus, these results suggest that CAP animals after initial overconsumption are capable to adjust their intake to control levels after a metabolic experience of a novel diet. These results do support that CAP animals are capable to adjust their energy intake over a longer term period even though they miss a substantial part of their feedback signaling involved in the process of meal satiation.

4.3 Mechanisms of satiety

We showed that CAP animals treated neonatally show a transient overconsumption of different concentrations of sucrose offered in short-term feeding tests. Moreover, they do show an initial overconsumption of a sweetened CMS offered for longer period. However, they seem to learn to reduce their intake to control levels over time. In line with our results, previous research of Chavez et al. (4), Kelly et al. (15) showed that adult capsaicin-treated animals overconsume of an unfamiliar high-fat diet, but not of fat-free cakes or familiar chow. A suggestion made by Kelly et al. [24] is that the overconsumption is dependent on how fast the novel food can generate satiety signals in the gastro-intestinal tract on locations which are not damaged by capsaicin-sensitive nerves. Sucrose is faster metabolized than fat and could therefore generate faster a satiety signal compared to fat. The fact that overconsumption is more pronounced when sweetened CMS is offered - which is a high-fat food- or when low concentrations of sucrose solution (10%) are offered supports this idea. If the time frame wherein the novel food is

capable to produce satiety signals would be the only factor, one may expect that a decrease in sweetened condensed milk intake in CAP would already occur the second day. However, it is not until the 3thd day before CAP significantly reduced their condensed milk intake. Another important difference between our experiment and the results of Kelly et al. (14) is that systemic capsaicin treatment at the 2nd day after birth resulted in a persistent overconsumption during all sucrose concentrations. Indeed, it might be that the initial overconsumption reflects the loss of negative feed-back signals from fat or low concentrations of sucrose. However, in our studies it appears that even when higher concentrations of sucrose are consumed, this is not sufficient to bring the satiety levels to control levels after a first metabolic exposure. Therefore, other factors must be involved as well.

First, an explanation could be that other populations of afferent neurons which might play a role in the process of satiation are affected in CAP rats. Anatomical studies have shown that systemic capsaicin treatment leads to degeneration of a distinct population of visceral afferents. Mainly the unmyelinated C-afferents and small myelinated δ-fibers are affected, but the effects are not limited to those of dorsal root and nodose-vagal origin (3, 11, 24).

Also, neonatally denervated animals may develop compensatory mechanisms to cope with the loss of primary afferents. These factors could (partly) explain the observed differences between adult and neonatal treated CAP animals. Second, it might be that adult treatment or neonatal treatment with capsaicin does not destroy exactly the same populations of vagal afferents. It is described that the distribution of the capsaicin-sensitive VR1 receptor is slightly different in neonatal and adult animals. Dvorakova and Kummer (9) found that VR1 is not neuron-specific but is transiently expressed on cardiomyocytes during ontogeny thereby pointing to a developmental role of this cation channel. Although they did not investigate structures which are critical in the control of food intake, one might expect this phenomenon in these structures as well. Consequently, neonatal treatment and adult treatment does not necessarily lead to deletion of the exact same neuronal population.

Finally, other factors as taste and reward sensation could differ between CAP and VEH rats. In general, it is considered that meal size is

determined by the balance between negative feedback signals from the gastrointestinal tract and reinforcing signals from gustatory and olfactory sensations (29). The experiments of Lucas and Sclafani (17) indicate that unlike the satiating effect of intestinal carbohydrate and fat, the reinforcing actions of these nutrients are not mediated by capsaicin-sensitive visceral afferents. It might be that a first exposure and during short-term food intake tests, the balance between those 2 factors is towards the reinforcing feedback in CAP rats, since they miss a substantial part of the negative feedback signals involved in meal termination. However, when food is offered for a longer period -as is in the second experiment where CMS is offered next to their regular chow- the integration of metabolic experience as well as physiological changes over the longer-term could learn the animal to control their food intake.

4.4 Body weight regulation

The present results demonstrate that CAP animals are able to regulate their energy homeostasis on the long-term. In the short-term food intake experiment as well as in the long-term food intake study, body weight increase did not differ significantly between CAP animals VEH animals. This indicates that CAP animals do compensate for their loss in feedback signals. One explanation could be that the increased intake during a meal produces longer lasting satiation and therefore also longer intermeal intervals and consequently fewer meals. Another explanation could be by a sensitization of long-term signals as leptin, or by increased sensitivity of humoral and/or capsaicin-insensitive neural short-term signals as metabolic factors and gastric distension. We found indeed indications that CAP rats are more sensitive to the anorexigenic action of leptin (unpublished data).

4.5 Conclusion

These results show that capsaicin-sensitive afferents play an important role in the control of meal termination in short-term sucrose intake tests. CAP animals show a persistent overconsumption compared to VEH and this is independent of the sucrose concentration. The current results suggest that in CAP animals volume is an important factor in the control of sucrose intake at sucrose concentrations of 15% and higher. Furthermore, the variable

overconsumption in 10% sucrose intake suggests that metabolic factors might play a role as well in short-term sucrose intake. However, when a novel diet is presented over a longer-term period, then after an initial higher intake CAP animals are still able to adjust their intake to control levels. This indicates that although short-term signaling is modified in CAP, they still are capable to control their energy intake on the long-term. Also, body weight increase did not differ between CAP animals and VEH controls. This suggests that through the integration of metabolic experience on the short-term with long-term signals about energy status neonatally treated capsaicin animals are still capable to maintain their metabolic homeostasis.

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