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Hyperalgesic response in rats fed sucrose from weaning to adulthood: Role of VMH

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Abstract

The ventromedial nucleus of hypothalamus (VMH) is implicated in food intake, food preference, nociception and its modulation by palatable food. Palatable drink for 5–48 h in adult rat produced hyperalgesia, which is mediated by VMH. The effect of palatable dietary supplement after weaning on the nociceptive response in adult rats has not been reported. Whether or not VMH influences these nociceptive responses is also not known. The present study was therefore undertaken to investigate the effect of VMH lesion on the nociceptive responses in adult rats ingesting (ad libitum) sucrose from weaning. Weanling rats received sucrose solution in addition to drinking water and laboratory pellets (sucrose-fed group), while the control group of rats received laboratory pellets alone. On attaining adulthood, the behavioral responses, namely tail flick latency (TFL), thresholds of tail flick (TF), vocalization during stimulus (SV), and vocalization after discharge (VA) to phasic and formalin pain rating (FP) to tonic noxious stimuli, were noted in pre- and post-VMH lesion states of both groups of rats. In chronic sucrose-fed rats, the TFL was not affected, the thresholds of TF, SV and VA were significantly decreased ($P < .001$) and the FP was increased in comparison to the control group, suggesting a hyperalgesic response to chronic sucrose ingestion. After the VMH lesion, in sucrose-fed rats, the thresholds of TF, SV and VA remained unaltered, while the FP was attenuated and TFL decreased. In control rats, VMH lesion produced a hyperalgesic response to both the phasic and tonic noxious stimuli. The data indicate that chronic sucrose feeding and VMH lesion differentially affect the nociceptive responses to the phasic and tonic noxious stimuli. These results suggest that chronic sucrose feeding from weaning to adulthood produces hyperalgesia to both the tonic and phasic noxious stimuli (except TFL), which is probably mediated by VMH.

Keywords: Chronic sucrose feeding; VMH; Pain threshold; Tail flick latency; Formalin pain

1. Introduction

Ingestion of sucrose for a few minutes immediately relieves the tonic as well as phasic pain (Blass et al., 1987; Mercer and Holder, 1997; Dutta et al., 2001), while for a few hours (5–6 h) to weeks (3 weeks) increases it (Frye et al., 1993; Mukherjee et al., 2000; Roane and Martin, 1990). The former effect is reported in both the neonatal and adult rats and humans, while the latter has only been reported in the adult rats (Frye et al., 1993; Mukherjee et al., 2000; Roane and Martin, 1990). Palatability of sucrose has been implicated in the modulation of nociceptive behavior (Blass and Shah, 1995). Sucrose is innately more palatable to the rat (Scalfani and Clyne, 1987) and can be discriminated as early as postnatal day 9 (Vigorito et al., 1987). Rats select sugar as a dietary supplement without lowering their intake of laboratory pellets to <30–40% of their total calorie intake (Hendley et al., 1987).

The palatability of food has the greatest influence on eating behavior after the ventromedial hypothalamus (VMH) lesion (Carlisle and Stellar, 1969). The VMH is believed to exert its influence on eating as well as nociceptive behavior because its glucose-responsive neurons (Oomura et al., 1967) respond to opioids too (Ono et al., 1980). This property of these neurons enables them to influence food intake (Morley et al., 1983), preference for carbohydrates (Milano et al., 1988) and nociception as well (Sikdar and Oomura, 1985; Mukherjee et al., 2001). Manipulation of the VMH modulates the nociceptive response to

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tonic as well as phasic noxious stimuli (Sikdar and Oomura, 1985; Sinha et al., 1999; Mukherjee et al., 2000). The VMH lesion heightened the nociceptive response and abolished the sucrose fed (for 6–48 h) hyperalgesia in our previous study (Mukherjee et al., 2000). This led us to hypothesize that palatable dietary supplementation during postweaning period determines the nociceptive response in adults, which are mediated by VMH.

Several scientists have examined the effect of sucrose supplementation on the behavior of rats. Neither sucrose supplementation nor high carbohydrate diet in the adult rat for a short duration (2 weeks) has any significant effect on its growth, development and endocrine metabolism (Carlisle and Stellar, 1969; Wardzala et al., 1985; Weick et al., 1983). There is no decrease in their body weight, epididymal fat pad weight, adipose cell size, content of muscle glycogen or triglyceride and levels of plasma glucose and insulin (Wardzala et al., 1985). Nonetheless, there is an elevation in plasma and liver triglycerides, liver glycogen (Wardzala et al., 1985) and impairment of attentional behaviors (Hendley et al., 1987). However, glucose supplementation (parentally) from birth influences the ontogeny of VMH neuronal response to glucose and, on attainment of maturity, their eating behavior and body weight pattern differ from the control rats (Mathur et al., 1983, 1986). The effect of chronic sucrose supplementation during postweaning development on nociceptive responses to various noxious stimuli has not been studied. The present study was therefore directed towards exploring the effect of chronic sucrose supplementation from weaning to adulthood on nociceptive response to phasic and tonic noxious stimuli and the possible role of VMH in the mediation of these responses.

2. Materials and methods

Postnatal day 30 male rat pups were weaned and housed separately in polypropylene cages. The animal facility room temperature was maintained at 26 ± 2 °C with a light–dark cycle of 14:10 h. Each rat received a fresh supply of premeasured food pellets, tap water and 20% sucrose solution ad libitum (sucrose-fed group). The age, weight, sex-matched rats ($n = 13$) were similarly handled except for the availability of the sucrose solution (control group). A separate group of 10 rats served as the controls for the tonic nociceptive behavior since the response could only be studied twice in a rat utilizing one paw at a time, because it interferes with the responses to the other noxious stimuli (unpublished observation).

Intake of food and water was noted daily in both the control and experimental groups of rats, while intake of sucrose was noted in experimental rats alone. The body weights of control and experimental rats were recorded every week. The experiments were conducted when they attained the body weight of 158 ± 6.05 g. On the day of the experiment, both the groups of rats were tested for their responses to phasic (thermal and electrical) and tonic (chemical) noxious stimuli to evaluate the effect of chronic sucrose feeding. The nociceptive responses to each category of noxious stimuli were tested in all the rats albeit on different days in sessions scheduled at 1-week intervals. Ethical guidelines were followed in accordance with the committee for research and ethical issues of the International Association for Study of Pain (Zimmermann, 1983).

To study the role of the VMH in sucrose-fed pain modulation, the above mentioned test schedule was repeated 2 weeks after the VMH lesion. The VMH was lesioned (electrolytically) in both groups of rats. The rats were anaesthetised with ketamine hydrochloride (50 mg/kg ip; KETMIN, Themis Chemicals, Hyderabad, India) and fixed in the stereotaxic apparatus (David Kopf, Tujunga, CA, USA). The electrode was implanted in the VMH utilizing 2.8 mm AP, 0.5 mm L and 9.5 mm V coordinates (Paxinos and Watson,1982). The experimental schedule for testing the phasic and tonic nociceptive responses was repeated 2 weeks after the surgery.

Responses to both phasic and tonic noxious stimuli were recorded: tail flick latency (TFL), threshold for tail flick (TF), threshold for vocalization during stimulus (SV), and threshold for vocalization after discharge (VA) of the phasic pain, and formalin test for tonic pain. TFL was recorded by a TF analgesia monitor (Omnitech, Coulumbus, OH, USA). Each rat was conditioned for 15 min in a plexiglas, well-ventilated restrainer before starting the experiment. After cleaning the tail with alcohol, it was kept over a trough containing a heating coil set at 45 °C. The cut-off latency was set at 30 s. The "on" switch activated both the heat source and the timer. As soon as the rat flicked its tail, the timer was automatically switched off and the latency was noted (Ness and Gebhart, 1986).

The rat was restrained and the threshold of TF was assessed by applying alternating electric current (Grass Stimulator S4-G, Grass Med. Inst., Quincy, MA, USA) (biphasic square wave pulses 40 Hz frequency, 1.5 ms duration and varying current strength for 200 ms) through two-needle electrodes inserted intradermally into the tail. The current strength was increased in steps of 200 μA until the rat flicked its tail. The value was noted. The threshold of electrical stimulus (mA) for eliciting SV was recorded in the same rat by further increasing the current strength gradually until the rat vocalized. The current strength was noted when the vocalization remained restricted to the period of stimulation. The threshold for VA also was recorded in the rat by a further step-wise increase in the current strength until the rat vocalized beyond the period of stimulation (Aimone et al., 1988).

The tonic pain was assessed by the formalin model (Dubuisson and Dennis, 1977). The rat was injected with 5% sterile formalin solution (50 μl) subcutaneously into either of the forepaws and was kept in a specially designed behavioral chamber. The magnitude of pain was rated on a four-point behavioral scale over a 1-h session. The salient

features of the scale are: category 0: when the whole body of the rat was resting or the rat was moving and the body weight was equally borne by all the paws; 1: the injected paw of the rat was partially resting on the floor or it was grooming; 2: the injected paw was kept elevated or tucked into the body; and 3: the injected paw was licked or shaken. The behavioral scores were recorded using a personal computer for 1 h and the time (s) spent in each category (0–3) was recorded for each block of 5 min. The average pain rating for each block was obtained by dividing the sum of T1 + 2(T2) + 3(T3) with the duration (s) of the block (Alreja et al., 1984).

At the end of the experiment, the rats were deeply anaesthetised and injected intracardially with 10% formalin solution to fix the tissues. The rats were sacrificed for the histological verification of the lesion extent and site.

2.1. Data analysis

The data of TFL (s), TF, SV and VA (mA) were analysed using Wilcoxon's rank sum test for the control versus chronic sucrose-fed and control versus VMH lesion group of rats. Paired *t* tests were applied for comparison between pre- and post-VMH lesion in the chronic sucrose-fed rats. For the pain rating, paired *t* tests were used to compare the pre- and postlesion data of control as well as chronic sucrose-fed rats within the groups, while unpaired *t* tests were used to compare the data of control versus VMH lesion and chronic sucrose-fed versus chronic sucrose-fed post-VMH lesion.

3. Results

Bilateral lesion of the VMH was confirmed histologically in brain sections of seven chronic sucrose-fed rats (Fig. 1) and 23 control rats.

3.1. Food and sucrose intake

The mean food intake in the control rats was 10.8 ± 0.8 g in the postweaning week 1, which increased to 16.65 ± 1.1 g in the second week, while in the chronic sucrose-fed rats it was 8.30 ± 0.7 and 16.04 ± 0.09 g, respectively (Table 1). The food intake in the control rats until the period of observation was significantly more compared to chronic sucrose-fed rats (except for the third and the fourth week). However, the total calories (pellets + sucrose) ingested by the chronic sucrose-fed rats were significantly higher during 2–4 and 6–8 weeks (Table 1, Fig. 2). After VMH lesion, the food and sucrose intake significantly increased from the prelesion state in the chronic sucrose-fed rats (Fig. 3).

The body weight did not differ significantly on the day of VMH lesion surgery in both the groups, although the chronic sucrose-fed rats took a longer time (142 days) to attain the body weight of 158.5 ± 6.05 g compared to

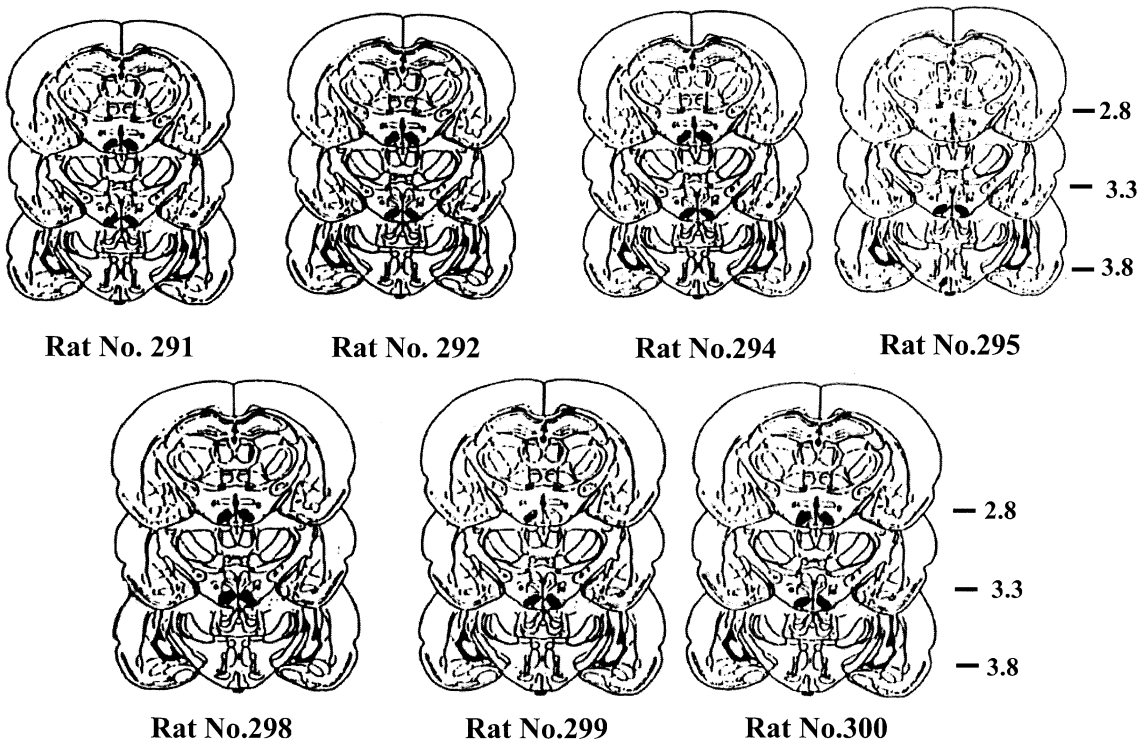

Fig. 1. Figure depicting camera lucida drawings of brain sections corresponding to bregma −2.8, −3.3 and −3.8 showing the extent of lesion in the VMH of seven chronic sucrose-fed rats.

Table 1
Mean daily food intake during a week in control and chronic sucrose-fed rats from weaning to adulthood

Week	Food intake (g mean ± S.D.)		
	Control	Chronic sucrose-fed	*P* value
1	10.80 ± 0.8	8.30 ± 0.7	< .05
2	16.65 ± 1.1	16.04 ± 0.09	NS
3	17.42 ± 0.9	20.09 ± 1.2	< .01
4	17.25 ± 0.3	20.85 ± 0.3	< .01
5	19.67 ± 1.8	16.0 ± 0.1	< .01
6	17.25 ± 0.37	13.51 ± 0.5	< .001
7	20.01 ± 1.7	17.11 ± 0.08	< .05
8	23.42 ± 0.5	17.47 ± 0.48	< .001
9	23.5 ± 0.02	19.38 ± 0.1	< .01
10	26.61 ± 0.2	18.87 ± 0.5	< .001
11	27.2 ± 0.39	18.81 ± 0.1	< .001
12	28.45 ± 0.7	19.68 ± 1.03	< .001
13	28.30 ± 0.2	20.8 ± 0.5	< .001

Table shows the food ingested during a week from weaning to adulthood. The data of both groups was compared using unpaired *t* tests.

controls (121 days). The body weight of sucrose-fed rats increased ($P < .001$) by 4.42 ± 1.90 and 11.57 ± 1.52 g during first and second postlesion week, while in the control rats the body weight increased by 15.10 ± 8.16 and 13.19 ± 8.16 g, respectively.

3.2. TFL

The TFL in the control and the chronic sucrose-fed groups of rats was not statistically different. After the VMH lesion, the TFL decreased significantly ($P < .001$) in both control and sucrose-fed rats (Table 2).

3.3. Threshold of TF

The threshold of TF was significantly ($P < .001$) lower (median 0.1 versus 0.04 mA) in the chronic sucrose-fed group compared to the control group. After the VMH lesion, the threshold value decreased ($P < .001$) in the control group, while it did not decrease in the chronic sucrose-fed group of rats (Table 2).

3.4. Threshold of SV

The threshold of SV was significantly lower ($P < .001$; median 0.07 mA) in the chronic sucrose-fed group compared to the control group (median 0.14 mA). The threshold value after the VMH lesion did not change in the chronic sucrose-fed group, but decreased ($P < .001$) significantly in the control group (Table 2).

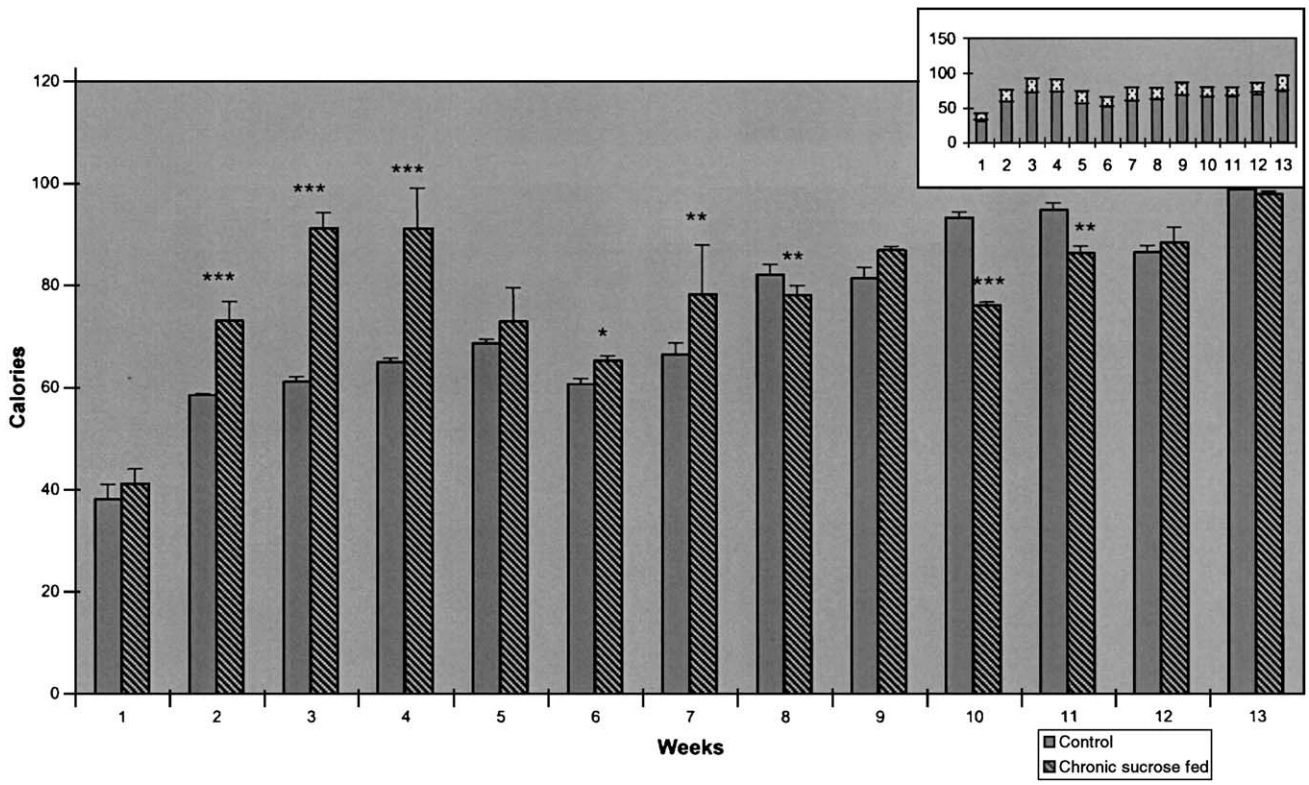

Fig. 2. Shows the total calories ingested per day by the control and chronic sucrose-fed group of rats during the 13-week study. Figure in inset shows the contribution of calories from pellets (filled bars) and sucrose (dotted bars). All values are mean ± S.D. The data were analyzed using paired *t* tests. * $P < .05$, ** $P < .01$, *** $P < .001$.

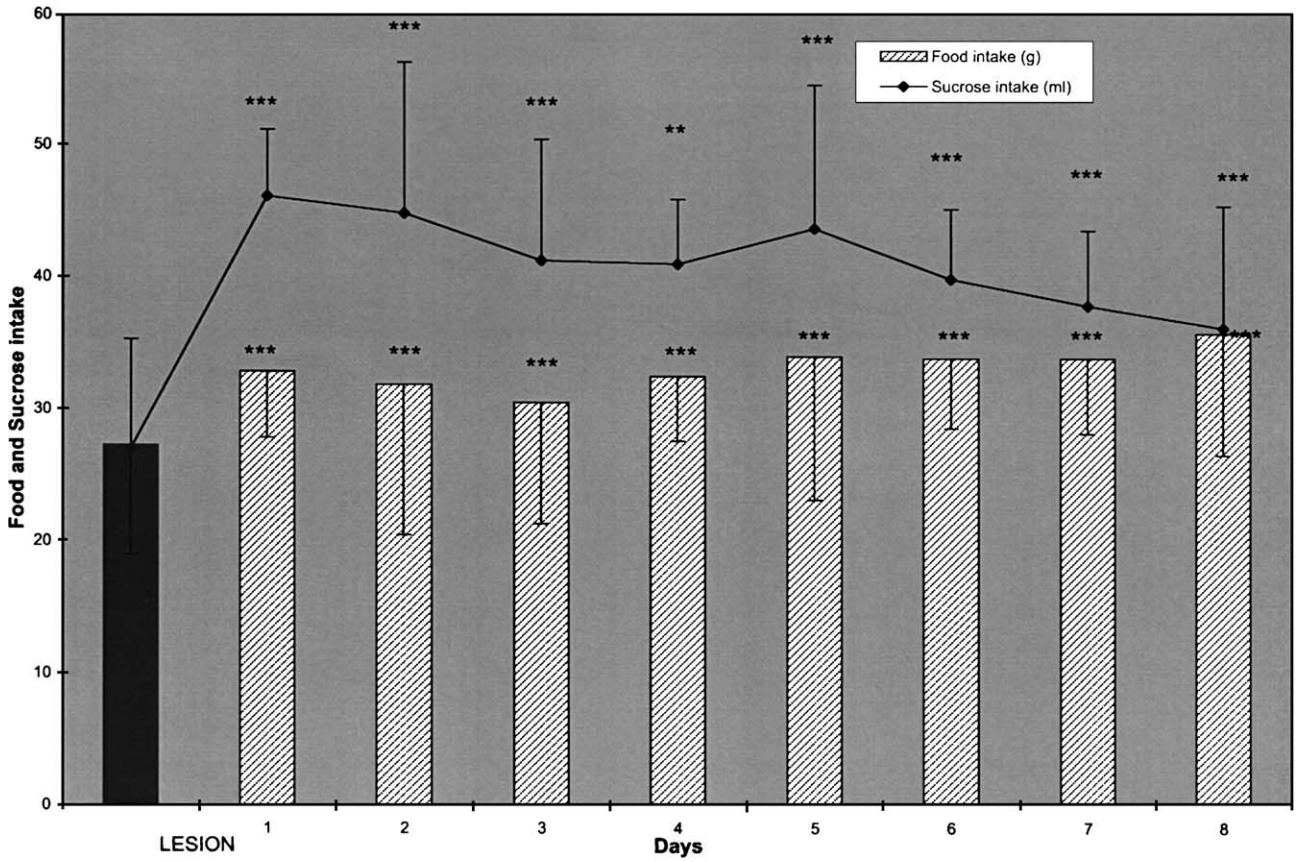

Fig. 3. Shows the effect of VMH lesion on food (bars) and sucrose intake (line graph) in sucrose-fed rats. All values are mean ± S.D. Both food and sucrose intake increased significantly after the lesion (* * $P<.01$, * * * $P<.001$).

3.5. Threshold of VA

The threshold of VA was significantly lower ($P<.01$) in the chronic sucrose-fed group (median 0.09 mA) compared to the control group (median 0.3 mA). However, after the VMH lesion, there was no change in the chronic sucrose-fed group, while it decreased ($P<.001$) in the control group (Table 2).

Table 2
Effect of sucrose feeding from weaning to adulthood on phasic nociceptive responses before and after VMH lesion (median with range)

Tests	Control	VMH lesion	Chronic sucrose-fed	VMH lesion in chronic sucrose-fed
TFL (s)	16.10 (5.93–28.69)	12.40*** (2.36–15.47)	13.37 (7.01–26.62)	$9.71^{\dagger\dagger\dagger}$ (8.10–17.6)
TF (mA)	0.10 (0.04–0.14)	0.05*** (0.02–0.16)	$0.04^{\#\#\#}$ (0.02–0.04)	0.04 (0.04–0.08)
SV (mA)	0.14 (0.04–0.9)	0.08*** (0.06–0.32)	$0.07^{\#\#\#}$ (0.04–0.01)	0.05 (0.4–0.1)
VA (mA)	0.30 (0.06–0.4)	0.10*** (0.02–0.2)	$0.09^{\#\#}$ (0.06–0.2)	0.08 (0.4–0.1)

Effect of chronic sucrose feeding on phasic nociceptive responses. The tail flick latency was not altered after sucrose feeding. The thresholds of TF, SV and VA were significantly reduced in chronic sucrose-fed rats. After VMH lesion in control rats, there was a significant reduction in the TFL and the thresholds indicating hyperalgesia. In chronic sucrose-fed rats, no significant change in the thresholds was observed, while the tail flick latency decreased significantly. The statistical analysis was done by Wilcoxon's rank sum test.

* Indicates comparison between control and lesion.

†Indicates comparison between chronic sucrose-fed and VMH lesion in chronic sucrose-fed groups.

$^{\#}$Indicates comparison between control and chronic sucrose-fed groups.

*** $P<.001$.

††† $P<.001$.

$^{\#\#}$ $P<.01$.

$^{\#\#\#}$ $P<.001$.

3.6. Tonic pain rating

In the chronic sucrose-fed group of rats, the average pain rating during the first block was significantly higher ($P<.001$; 2.51 ± 0.24) than the control group (2.09 ± 0.28), which continued in the second and third blocks (2.5 ± 0.15 and 2.1 ± 0.24, respectively) (Table 3). After the VMH lesion, the pain rating was significantly reduced ($P<.001$; 1.97 ± 0.19) during the first block and it remained decreased ($P<.01$) in the second block. These trends in the tonic pain rating persisted throughout the period of observation although the differences were not statistically significant.

In the control group of rats, the pain ratings during the second and third blocks were significantly higher after the VMH lesion compared to their prelesion value ($P<.001$). This trend in pain rating continued throughout the period of observation.

The average pain rating during the 1-h session was higher in the chronic sucrose-fed group ($P<.001$; 2.06 ± 0.04) compared to the control group (1.75 ± 0.08). However, after the VMH lesion, the pain rating in the control group increased ($P<.001$) to 1.96 ± 0.05, while it decreased ($P<.001$) to 1.77 ± 0.24 in the chronic sucrose-fed group.

Table 3
Effect of sucrose feeding from weaning to adulthood on tonic pain before and after VMH lesion

Block (5 min each)	Control	VMH lesion	Chronic sucrose-fed	VMH lesion in chronic sucrose-fed
I	2.09 ± 0.28	2.16 ± 0.17	$2.51\pm0.24^{\#\#\#}$	$1.97\pm0.19^{\dagger\dagger\dagger}$
II	1.14 ± 0.24	$2.0\pm0.01^{***}$	$2.5\pm0.15^{\#\#\#}$	$1.73\pm0.23^{\dagger\dagger}$
III	1.40 ± 0.07	$1.97\pm0.24^{***}$	$2.1\pm0.24^{\#\#}$	1.63 ± 0.32
IV	1.54 ± 0.22	1.83 ± 0.23	$2.4\pm0.15^{\#}$	$1.74\pm0.7^{\dagger}$
V	1.83 ± 0.22	1.93 ± 0.08	2.1 ± 0.10	2.5 ± 0.34
VI	1.83 ± 0.14	1.83 ± 0.25	1.95 ± 0.09	1.83 ± 0.20
VII	1.85 ± 0.10	$1.97\pm0.02^{*}$	2.3 ± 0.10	1.82 ± 0.35
VIII	1.75 ± 0.22	2.0 ± 0.01	2.0 ± 0.03	1.75 ± 0.33
IX	1.88 ± 0.14	1.96 ± 0.16	1.99 ± 0.01	1.73 ± 0.39
X	1.87 ± 0.13	1.96 ± 0.01	2.0 ± 0.11	1.79 ± 0.35
XI	1.89 ± 0.13	1.89 ± 0.17	1.98 ± 0.05	1.81 ± 0.36
XII	1.78 ± 0.23	2.00 ± 0.01	1.97 ± 0.04	1.80 ± 0.26

The pain rating increased significantly after sucrose feeding from weaning to adulthood, while after VMH lesion there was not much change in their pain rating. VMH lesion in the control group of rats increased the pain rating significantly. The analysis was done by Students t test.

* Indicates comparison between control and VMH lesion group of rats.

# Indicates comparison between control and chronic sucrose-fed rats.

† Indicates comparison between chronic sucrose-fed group of rats before and after VMH lesion.

* $P<.05$.

† $P<.05$.

†† $P<.01$.

\# $P<.05$.

\#\# $P<.01$.

4. Discussion

Chronic sucrose feeding from weaning to adulthood in our rats led to a decrease in the threshold of TF, SV, VA and tonic pain, while the TFL remained unaffected. After the VMH lesion, there was no further attenuation or enhancement in the thresholds of TF, SV and VA, whereas the TFL decreased and the tonic pain rating returned to the control value. The results suggest that chronic sucrose feeding from weaning to adulthood produces a hyperalgesic response to both phasic and tonic pain but is ineffective after VMH lesion.

4.1. Sucrose-fed developing rat model

Our rats had access to a sucrose solution (20%) in addition to the tap water and the laboratory pellets from weaning to adulthood. This schedule was primarily designed to explore the possible influence of a high carbohydrate palatable dietary supplement during the weaning, puberty and postpubertal growth period on the nociceptive responses in adulthood. It is pertinent to study the effect of palatable food during development on nociceptive behavior of the adult because postnatal sensory enrichment influences brain development, and VMH and palatability both influence nociceptive behavior (Mukherjee et al., 2000, 2001; Dutta et al., 2001). Rats innately prefer to ingest sucrose solution over laboratory pellets. Preference for sucrose is apparent as early as postnatal day 9 (Vigorito et al., 1987). After weaning (postnatal day 30), our rats also spontaneously ingested sufficient quantities of sucrose when their diet was supplemented with sucrose. On attaining adulthood, their preference for sucrose was further intensified by the VMH lesion. Lesions of the VMH per se lead to a preference for a high carbohydrate, palatable diet as well as produced hyperalgesia (Turner et al., 1967; Vidal and Jacob, 1980; Mukherjee et al., 2000). Coincidentally, ingestion of sucrose (for 5 h to 3 weeks) also produces hyperalgesia in adult rats (Mukherjee et al., 2000; Frye et al., 1993; D'Anci et al., 1997). This provided us with a rat model wherein a chronic interaction of two vital factors, namely the palatable food and VMH influencing nociceptive responses during development could be studied.

Chronic supplementation of sucrose since weaning may be argued to alter the metabolic-endocrine profile of the rat, thereby influencing the nociceptive response. However, Koyama et al., (1988) reported that 30% sucrose feeding for 6, 8 or 12 weeks in Wistar rats led to higher plasma insulin levels to control glucose load. Moreover, a significant reduction in body weight for both 8- and 12-week-old Wistar rats after sucrose feeding for 2 and 6 weeks has been reported (Koyama et al., 1988). Our sucrose-fed rats also ingested more total calories than the control group, but they took a longer time to gain the comparable body weight. This can be attributed to either the reduced consumption of food pellets vis-a-vis nutrients or to the disturbance in their

metabolic profile or hyperactivity. The former is true because our rats ingested sucrose (20%) at the expense of food pallets, while the latter possibilities cannot be ruled out definitely since we neither evaluated the metabolic-endocrine profile nor the activity patterns of our rats.

4.2. Effect of sucrose feeding—phasic pain

In the present study, sucrose was provided on postnatal day 30 and continued until adulthood. Frye et al. (1993) studied the effect of sucrose feeding for 3 weeks in adult female rats, whereas D'Anci (1999) studied male rats. Both authors reported effects on TFL, but the results are diametrically opposite. Frye et al. (1993) reported hyperalgesia, while D'Anci (1999) reported no effect of sucrose feeding. It appears that the motor response of tail to thermal noxious stimulus is gender-specific (Valenstein et al., 1967). Gender specificity is supported by a smaller proportion of sweet-sensitive pontine neurons in the parabrachial nucleus of males (Di Lorenzo and Monroe, 1989). However, in a recent report by Kanarek et al. (2000), sucrose (32%) feeding for 3 weeks did not affect TFL in both males and females. Our results of TFL further support this contention, although our rats were younger and ingested sucrose for a longer period (13 weeks). In our rats, TFL is specifically spared by sucrose feeding, whereas the thresholds of SV and VA are susceptible (Irwin et al., 1951). Such a differential effect is reported in the literature. Stimulation of the dorsomedial nucleus of the thalamus and septal nuclei do not affect TFL, but affect the threshold of SV and VA, thereby specifically dissociating the TFL mechanism from that of the threshold of SV and VA (Hentall et al., 1991; Mayer and Liebeskind, 1974). The effect on TFL and thresholds for SV, VA were also dissociated in adult male rats fed on sucrose supplemented diet for 48 h (Mukherjee et al., 2000).

These responses varied as a function of time (i.e., the duration of sucrose ingestion). TFL was not affected by sucrose feeding for 6 h, while it decreased by 12 h and continued to remain decreased after 48 h of sucrose feeding (Mukherjee et al., 2000). The thresholds of SV and VA were affected after 6 h of sucrose feeding only, whereas the threshold of TF was not affected even after 48 h. This may indicate a wide dynamic influence of higher centers on the motor neurons and the dissociation of TFL from the threshold of SV and VA mechanisms by sucrose feeding.

It is likely that there is a switch over of the influence exerted by the ingestion of sucrose itself reflecting a temporal variation in the response to the same type of stimulus. The pathway initially activated (within 6 h of sucrose supplementation) may not utilize the nucleus raphe magnus or the periaqueductal gray, but rather the dorsomedial nucleus of the thalamus or septal nuclei or some other area, which is not yet identified. Stimulation of dorsomedial nucleus of the thalamus and septal nuclei do not affect TFL (Irwin et al., 1951). The exact mechanism has not yet been reported, although research in this area is likely to uncover interesting facts about the pain mechanism.

4.3. Effect of sucrose feeding—tonic pain

The nociceptive behavior rating to the tonic noxious stimulus was higher in our chronic sucrose-fed rats indicating hyperalgesia. There are no comparable studies available in the literature. However, in our previous report, sucrose feeding by adult male rats for 6 and 48 h produced no change while it produced analgesia after 12 h (Mukherjee et al., 2000). This analgesic effect is temporary. It may arise as a result of various counteracting responses initiated by hyperalgesia or a tendency towards it, to maintain status quo. It appears that these regulatory mechanisms possibly overshoot leading to analgesia. This was detected in our rats after only 12 h. However, in the present study, such mechanisms are overridden by an uninterrupted access to the sucrose solution for 12–13 weeks. Ingestion of sucrose ad libitum produced hyperalgesia in our rats.

The noxious stimulus of formalin injection itself leads to the secretion of β-endorphin in the hypothalamus (Facchinetti et al., 1992). During formalin pain, significant elevation (until 90 min) of β-endorphin levels in the extracellular fluid collected from the arcuate nucleus has been reported (Zangen et al., 1998). The cell bodies synthesizing β-endorphin predominantly occur in the hypothalamic arcuate nucleus, and their axons and terminals occur in abundance along the wall of the third ventricle (Zakarian and Smyth, 1982). Incidentally, 20 min after ingesting a palatable sweet substance also, an increase in the secretion of β-endorphin in the hypothalamus is detected (Dum et al., 1983). Therefore, we expected chronic sucrose feeding to significantly relieve tonic pain induced by formalin injection. On the contrary, to our surprise, it increased the tonic pain. An increase in pain response to phasic thermal stimuli in sucrose-fed rats is usually explained on the basis of a progressive reduction in β-endorphin secretion by frequent repeated bouts of sucrose ingestion. The palatable feeding produces more opioid secretion than the control feeding in the hypothalamic area, lateral and central amygdaloid nucleus and also other areas in the brain (Jiang and Oomura, 1988; Oomura et al., 1986). The rate of synthesis is probably the limiting factor when the stimulus from sucrose ingestion is more frequent (Schoenbaum et al., 1989). As long as opioids are secreted in sufficient quantities, they regulate the magnitude of pain and an eualgesic state is maintained. This could explain the TFL and threshold of TF results of our 6 or 48 h sucrose-fed rats (Mukherjee et al., 2000). But, it appears that ad libitum availability of a highly palatable sucrose solution in our rats provided a positive reinforcement since rats innately prefer sucrose (Scalfani and Clyne, 1987). Repeated frequent stimuli lead to a decrease in the hypothalamic levels of β-endorphin resulting in hyperalgesia. Moreover, administration of β-endorphin antibodies also produces hyperalgesia in the TF as well as in tonic pain response (Porro et al., 1999).

Chronic sucrose-fed hyperalgesia may not be the primary effect of a gross decrement in β-endorphin levels. There may be a yet unreported alteration in sensitivity leading to the dampening of circadian rhythm, rate of secretion or fluctuation in β-endorphin response to a bout of sucrose ingestion. The possibility of variation in secretion is more because a disturbance in the circadian rhythm of β-endorphin is reported in cluster headache patients (Franceschini et al., 1996). A delayed acrophase during cluster periods is reported compared to the remission period occurs in normal subjects. However, no correlation has been found between the β-endorphin maximum net increase and intensity and/or duration of pain (Franceschini et al., 1996). The mechanism of analgesic action of β-endorphin has been extensively studied and reviewed recently (Narita and Tseng, 1998).

β-endorphin stimulates ε-opioid receptors, which are located in the caudal medulla such as the nucleus raphe obscurus, nucleus raphae pallidus and the adjacent midline reticular formation (Tseng and Collins,1991). Stimulation of ε-opioid receptors facilitates the descending enkephalinergic pathway releasing Met-enkephalin in the spinal cord, which in turn stimulates δ_2-opioid receptors to produce antinociception. This system is different from the other opioid system, although overlap cannot be ruled out.

4.4. Effect of VMH lesion on phasic pain

High concentrations of β-endorphin are found in the arcuate nucleus and the nucleus tractus solitarius (Finley et al., 1981; Mansour et al., 1988). The arcuate nucleus, VMH and nucleus tractus solitarius are richly interconnected. Lesion of the VMH in our sucrose-fed rats led to neither an attenuation nor an accentuation of pain threshold indicating either no influence of lesion on thresholds of pain or the presence of a common process. Mechanical lesion in our rats probably endorses the functional hypoactivity of the VMH neurons consequent to hyposecretion of β-endorphin. The VMH lesion per se produced generalized hyperalgesia in the control rats (Mukherjee et al., 2000; Vidal and Jacob, 1980). On the contrary, in our chronic sucrose-fed rats, VMH lesion did not alter the magnitude of existing hyperalgesic state. This only indicates that the sucrose feeding was ineffective in the VMH lesion group of rats. Alternatively, both the VMH lesion and sucrose feeding are equally effective in sensitizing the nociceptive system either through a common pathway/mechanism thereby supporting the possibility of the VMH as an integral area in the mediation of these effects.

The contention that the effect of sucrose feeding is mediated by the VMH is further supported by the dissociation of effects by the two interventions (e.g. the sucrose feeding and VMH lesion). TFL was not affected by chronic sucrose feeding in our rats but was reduced after the VMH lesion. VMH lesion in control rats produces hyperalgesia in TFL as well as thresholds of pain (TF, SV, VA) and tonic pain (Mukherjee et al., 2000; Vidal and Jacob, 1980). Therefore, the results suggest that a decrement in TFL after the VMH lesion in chronic sucrose-fed rats is clearly suggestive of VMH involvement, albeit the underlying mechanism may be different. The lateral hypothalamic area (LHA) is the extension of the pontine reticular formation into the hypothalamus and receives rich noxious information. The VMH inhibits the neuronal activity of the LHA. Lesion of VMH may, therefore, produce supersensitivity to the noxious stimuli (Jiang and Oomura, 1988; Oomura et al., 1986).

4.5. Effect of lesion on tonic pain

The CNS pattern of activity during tonic pain in the formalin model has recently been described by Porro et al. (1999). The motivational and affective aspects of the late phase of tonic pain are mediated predominantly by the medial thalamus and the fronto-limbic network. Nociceptive processing may involve activation of the anterior cingulate, orbital and prefrontal regions, while the medial prefrontal and lateral visceral area may be involved in vegetative responses to noxious stimuli. The anterior agranular insular cortex, which is connected with the locus coeruleus, ventromedial hypothalamic nucleus, nucleus accumbens and caudate nucleus also is involved in opioid antinociceptive mechanism (Burkey et al., 1996).

It appears that β-endorphins mediate action through the preferred pathway of the VMH, nucleus tractus solitarius and medulla. β-Endorphin act through epsilon opioid receptors, thereby reducing tonic pain and increasing pain thresholds. The nonpreferred pathway of the locus coeruleus possibly produces hyperalgesia. The locus coeruleus pathway is activated as a result of continued stimulation possibly because of a higher threshold or a greater number of interneurons.

The results of our experiments suggest that postweaning chronic sucrose supplementation produces hyperalgesia to tonic as well as phasic noxious stimuli, with the exception of TFL. The effect is probably mediated by the VMH.

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