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RESEARCH ARTICLE

# REVISED Sweet taste does not modulate pain perception in adult humans [version 2; peer review: 3 approved]

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## Abstract

**Background**: Sugar is routinely used to comfort neonates undergoing painful procedures, and animal studies have shown that sucrose increases the time to withdrawal from painful stimuli. However, there are no published studies examining the effects of sweet substances on heat pain thresholds and percept in adult humans.

**Methods**: Healthy adult volunteers (n=27, aged 18-48 years) were recruited to a controlled, double-blind, randomised, cross-over study to characterise the effect of tasting solutions of equivalent sweetness (10% sucrose and 0.016% sucralose) on warm detection and heat pain thresholds and the percept ratings of painfully hot stimuli. The effect of anticipation of a sweet taste on heat pain threshold was also assessed.

**Results**: Tasting either sucrose or sucralose had no significant effect on the percept of an individually titrated hot stimulus (54.5±4.2 and 54.9±3.2 vs 53.2±3.5 for water, 0-100 visual analogue scale), on the warm detection or heat pain threshold (43.3±0.8, 43.2±0.8 vs 43.0±0.8°C). Anticipation of a sweet substance similarly did not affect heat pain thresholds.

**Conclusions**: Sucrose and sucralose solutions had no analgesic effect when assessed using heat detection thresholds and percept ratings of painfully hot stimuli despite being perceived as sweeter and more pleasant than water. These findings are in contrast to results reported from previous animal studies in which thermal analgesia from sweet solutions is robust. Given the ubiquitous availability of sugar rich drinks in the modern environment, the lack of observable effect may be due to an insufficient hedonic value of the test solutions when compared to the experience of a laboratory rodent. Alternatively, sweet tastes may have a specific effect on pain tolerance rather than the threshold and acute percept measures assayed in this study.

## Keywords
Pain, Sucrose, Sweetness, Endogenous Analgesia, Hedonia

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**REVISED** **Amendments from Version 1**

Clarifications of methods and extended discussion around motivation and hedonic value of sweet substances and around limitations of the experiment with regard to pain tolerance versus pain threshold and our inability with the small sample to make reliable inferences about the lack of sex differences in the analgesic effect.

**Any further responses from the reviewers can be found at the end of the article**

## Introduction

Sugar is in routine clinical use in the neonatal intensive care setting to comfort neonates undergoing painful procedures[1,2]. A number of studies have demonstrated that sucrose has an analgesic effect in immature rodents, particularly in neonatal rats, acting to increase the thermal sensory threshold[3,4]. A recent study has demonstrated that active consumption of sucrose also produces an analgesic effect in adult rats[5], slowing withdrawal from a painful thermal stimulus. This rapid, robust and transient effect was observed when the rats actively consumed the sucrose and also after training when it was provided passively. The analgesic effect was not prevented by opioid, noradrenaline or dopamine antagonists but was blocked by cannabinoid CB1 receptor antagonists. The analgesic effect was seen with artificial sweeteners and also seen when rats anticipated a sweet solution but were provided with water.

A limited number of studies have examined the effect of sweet tastes on pain thresholds in human adults and children[6,7]. These studies suggest that sucrose can alter both the perception and tolerance of pain, although there are some suggestions that this is true only in males[8] or in individuals with a preference for sugary tastes[6]. Studies in adult humans have largely used tolerance of cold pain as an assessment: a suprathreshold test which is both unpleasant and known to act as a generalised stressor[9], making these studies less comparable to the rodent studies which have typically assessed pain thresholds using escapable heat stimuli. Furthermore, factors behind the anticipation of sweet and rewarding substances have not been studied in the context of pain sensation.

Therefore, we aimed to develop a simple assay to assess the analgesic effect of sweet taste in adult humans. We examined the effects of sweet taste on the perception and tolerance of painful thermal stimuli in adults. We used both calorific (sucrose) and zero-calorie (sucralose) sweetened solutions alongside a neutral control liquid (water) and assessed their effects on the detection of thermal stimuli, threshold for painful heat stimuli and the pain felt on delivery of a calibrated hot stimulus (i.e. to assess stimulus and percept locking). The effect of anticipation of sweet taste on thermal detection and tolerance was also investigated. In contrast to previous results in the adult rat, our findings did not demonstrate an equivalent analgesic effect of sweet taste in adult humans. We discuss our results with reference to the existing literature on the phenomenon of sweet taste or sucrose analgesia.

## Methods

The study was approved by University of Bristol Faculty of Biomedical Science Research Ethics Committee (reference 60062).

### Population

A power calculation was undertaken for the main outcome measure of the effect of sweet taste on pain intensity in response to a standardised thermal stimulus established at baseline for each subject as being ~60/100mm on a VAS scale. We expect to see a 15% reduction in that pain score based on previous studies which would be biologically and clinically meaningful. The variance of the pain scores was expected to be 12mm (SD, based on data from Brooks *et al.,* 2017[10]). With alpha of 0.05 and beta of 0.9 this gives a sample size of 18 subjects for repeated measures testing (G*power).

Healthy volunteer subjects were recruited between December 2017 and March 2018 for entry to the study using poster and email advertisements (see *Extended data*)[11] at the University of Bristol. A total of 27 participants (22F:5M) aged 18–48 years took part in the assessments (see *Underlying data* for demographics)[11]. After participants had expressed an interest in the study, they were sent a participant information leaflet by email (see *Extended data*)[11]. Participation in the study was precluded by the presence of acute or chronic pain, a neurological disease, a diagnosed medical or psychiatric condition, diabetes or impaired glucose tolerance, use of recreational drugs, use of analgesic medications within the preceding 48 hours, or pregnancy. One participant was excluded due to their disclosing a diagnosis of depression and anxiety (see participant flow diagram in *Extended data*)[11].

### Enrolment

Those who wished to proceed then attended a single session which incorporated completion of an inclusion questionnaire (see *Extended data*)[11], provision of informed written consent for study entry and the use of any data for research (see consent form in *Extended data*)[11] and data collection. The session took place in a purpose-built consultation room within the Clinical Research and Imaging Centre at the University of Bristol, at a date and time agreed with participants. Sessions were conducted by ERM (who is a female medical doctor) and no other staff members were present during the experiments. Sessions lasted between 1 and 1.5 hours each. Subjects were told that the objective of the study was to examine how the sensation of pain interacts with sweet flavours – they were not explicitly told the underlying study hypothesis regarding sweet-taste induced analgesia.

### Calibration

Participants were asked to rate their current level of thirst on a visual analogue scale (VAS) consisting of a 100mm line labelled with "Not thirsty at all" at one end to "Extremely thirsty" at the other[12]. Participants marking the scale at a level greater than 30mm were encouraged to drink water to quench their thirst, and then repeated the rating. This was repeated up to three times until the value was below 30mm. Four participants who

continued to record a thirst score >30mm despite free access to water were later excluded from analysis due to concerns that thirst may confound the results. Sensitivity analysis with inclusion of these participants did not demonstrate any difference in analgesic effects.

Participants were seated comfortably at a table in a temperature-controlled room with their non-dominant arm resting supinated on a soft support on the table-top. A contact thermode (30×30mm, Medoc TSA-II, Israel) was positioned on the volar forearm (C6 dermatome) and secured in place by a Velcro strap. All instructions to participants were standardised and read from a script (provided as *Extended data*)[11]. For quantitative sensory testing (QST), the wording of the instructions was standardised in accordance with the DFNS (German Research Network on Neuropathic Pain) protocol[13]. Warm detection threshold (WDT) was assessed by applying a temperature ramp at 1°C.s$^{-1}$ from a baseline of 32°C (chosen as the average temperature of the immediately exposed forearm:[14]) and participants were instructed to press a button when they first felt the thermode becoming warm. Heat pain threshold (HPT) was assessed by applying an identical temperature ramp with the instruction to press the button when the warm sensation changed to painful. After an initial familiarisation trial assessment of WDT and HPT, baseline measurements were taken by applying two temperature ramps in short succession separated by 6s at 32°C[15] with the participants instructed to indicate their WDT on ramp 1 and HPT on ramp 2. This was repeated three times at 2 min intervals and mean values calculated.

For the fixed stimulus rating (FSR) experiment, the thermode temperature was incremented using a pseudo-random sequence to identify a stimulus level that produced a pain rating of 60mm on a 100mm pain VAS using the method of limits[16]. Thermode temperature increased at a rate of 4°C/s and was held at the fixed temperature for 5s. The pain rating was provided by making a mark on a 100mm line anchored with the words "Not painful at all" at the left-hand end and "Extremely painful" at the right-hand end. A new blank scale was presented for the application of the next stimulus. Four participants who did not achieve a rating >50mm even at the maximum temperature of 48°C were excluded from analysis of the FSR component.

## Taste test
Following this initial calibration QST, participants blindly rated the sweetness and pleasantness of the test solutions. Three test solutions used were selected to enable a comparison between calorific and non-calorific sweet substances. The index solution of 10% sucrose was chosen as a concentration equivalent to that commonly contained in sweet beverages[17] and sucralose was used as a sweet-tasting, non-calorific control at a concentration (0.016%) selected to be equivalently sweet to 10% sucrose[18]. Weaker solutions of 5% sucrose and 0.008% sucralose were also prepared to assess comparative pleasantness. Sucrose was purchased as caster sugar (Silver Spoon) from a supermarket and food-grade sucralose (Bulk Powders) was purchased online. Both were diluted using potable tap water

and the same tap water was used as a neutral control liquid. Fresh solutions were made up on each testing day. For each test a 10–15ml sample of liquid (comfortable volume determined individually) was held in the mouth for 10s, after which the participant spat out the solution and rinsed their mouth with water.

Each participant rated the solutions by making marks on two 100mm VAS lines, one for sweetness from "Not sweet at all" to "Extremely sweet", and the other reflecting pleasantness marked "Neutral" to "Extremely pleasant" on the right. Participants were instructed to leave the second scale blank and instead mark a box provided if they found the solution at all unpleasant ('Dislike'). This assessment was repeated at the end of the test session after all the pain assessments in order to ensure that the reward value of the solutions had not changed significantly.

## Test protocol
On each test day, the experimenter made up the solutions as above and numbered them, making a record of the number allocated to each solution. The flasks were then given to an independent researcher in the university department who re-labelled the flasks (such that the original label was not visible) and documented the re-allocated numbers in a file to which the experimenter had no access until all data collection sessions were complete. Prior to the beginning of the data collection period, a random number generator was used to generate 30 random sequences of the appropriate numbers with which solutions were labelled, and each participant was allocated to a sequence in order of date and time of attendance.

To allow assessment of the influence of sweet taste on thermal thresholds participants were given a plastic cup containing a solution (10% sucrose, 0.016% sucralose or water), which they conveyed to their mouth using their dominant hand. Solutions were presented randomly in blocks of three, with both participant and observer blinded to the identity of the solutions, as described above. Warm detection and heat pain threshold were assessed while participants held 10–15ml of the solution in their mouth. The two-ramp thermal protocol was used to measure WDT followed by HDT. After completion, participants expelled the solution and rinsed their mouth as desired. Each solution was presented three times giving a total of nine trials.

A similar protocol was used to assess the effect of the test solutions on the FSR. The solutions were again presented blind in block randomised order as described above. The calibrated heat stimulus was delivered at the individually pre-determined temperature for 5s, after which the participant marked the 100mm pain VAS.

To assess the effects of anticipation of a sweet solution on WDT and HPT, participants were told that the heat ramp experiment would be repeated first with no solution, then three times with the same sweet solution. The two-ramp protocol was delivered as described above, first in the absence of solution, then repeated with samples of 10% sucrose, water, and 10%

sucrose, in a fixed order. One participant who was unable to comply with instructions was excluded from analysis of the anticipation experiment.

### Data analysis
Data is presented as mean ± standard error of the mean (SEM). Statistical tests were performed in Prism 7.0 (GraphPad). The taste preferences and effects of solution on thermal percept were assessed with repeated measures ANOVA with Bonferroni post-hoc test. Linear regression analysis was used to assess the influence of reward on the change in pain ratings.

## Results
### Participant demographics
A total of 27 participants (22F:5M) aged 18–48 years took part in the assessments. See flow chart (*Extended data*)[11] for details regarding number of participants included in each analysis.

### Sucrose and sucralose are equally sweet and rewarding
Sucrose (10%) and sucralose (0.016%) were perceived as equally sweet both at the start and the end of the experiment (Figure 1A) and both were sweeter than water. Likewise, both sucrose and sucralose were perceived to be equally pleasant on both initial and subsequent assessments and both were more pleasant than water (Figure 1B). Several participants rated solutions as unpleasant (three for sucrose, three for sucralose and two for water) and so did not provide pleasantness VAS ratings (marked 'Dislike').

A previous study in humans demonstrated an analgesic effect of sweet solutions only in those who preferred high concentration sweet solutions[6]. To assess whether there was a population of participants who had a preference for less sweet solutions, participants also rated the sweetness and pleasantness of weaker solutions of 5% sucrose and 0.008% sucralose. 5% sucrose was rated to be significantly less sweet than 10% sucrose but the difference in sweetness rating between 0.016% and 0.008% sucralose did not reach significance. Both the sweet solutions were rated as significantly sweeter than water. Analysis of the pleasantness ratings of the solutions demonstrated that 10% sucrose was rated as significantly more pleasant than water, although not significantly more pleasant than 5% sucrose. The same was true of high and low concentrations of sucralose. There was no clear separation into populations who preferred low to high concentrations of sweet solution. Given these results, only the higher concentrations of sucrose (10%) and sucralose (0.016%) were used in all subsequent tests. Tap water was used as a neutral, non-rewarding control solution.

### Sweet taste has no effect on heat pain perception
The effects of the test solutions on the perceived painfulness (assessed by VAS score) of the calibrated stimulus (FSR) was assessed while the participant held test solution in their mouth. There was no significant difference in the FSR in the presence of 10% sucrose, 0.016% sucralose or water control (Figure 2A). This suggests that sweet taste did not affect the perceived painfulness of a calibrated thermal stimulus.
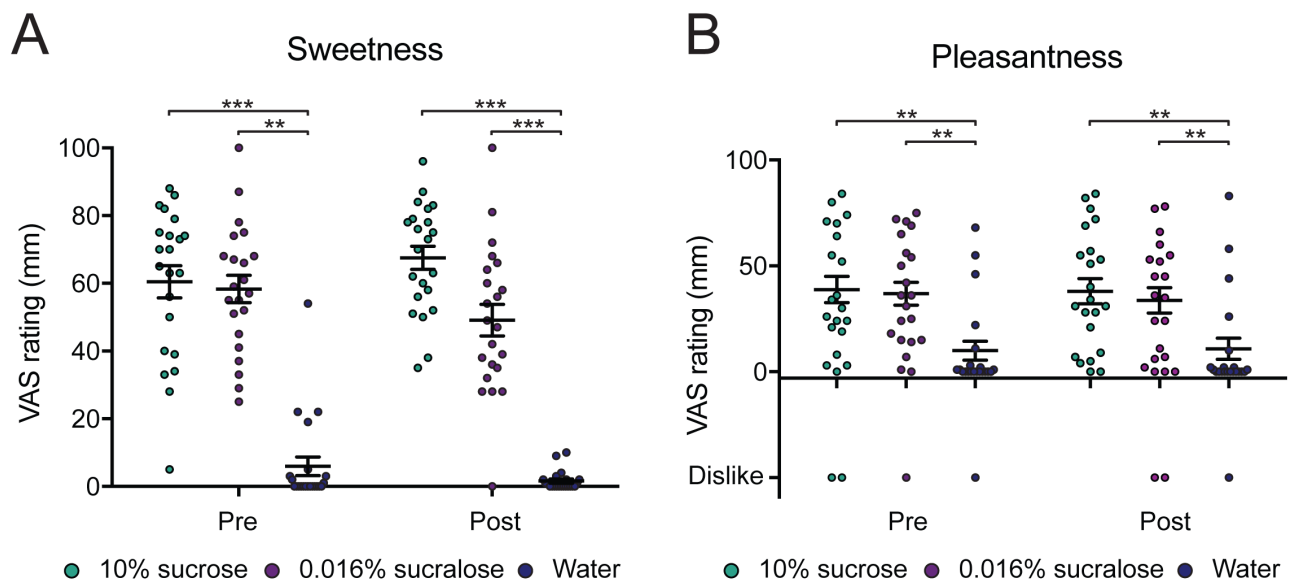


**Figure 1. The sweetness and pleasantness of the solutions before and after the experiment. A**. Sucrose and sucralose were found to be equally sweet on both initial (sucrose 60.5±4.7mm, sucralose 58.3±4.0mm) and final assessments (sucrose 67.5±3.5mm, sucralose 49.1±4.6mm), and both were significantly sweeter than water. **B**. Sucrose and sucralose were perceived to be equally pleasant on both initial (sucrose 38.9±6.2mm, sucralose 37.0±5.4mm) and final assessments (sucrose 38.1±5.9mm, sucralose 35.1±5.8mm). Water was significantly less pleasant than sucrose or sucralose at both assessments. Individuals rating the solutions as unpleasant ('Dislike') were excluded from the analysis. There was no significant change in the sweetness or pleasantness ratings of sucrose, sucralose or water between the beginning and end of the experiment (pre vs post). (*** - p<0.001; ** - p<0.01, RM-ANOVA with Bonferroni post hoc tests, n=22). VAS, visual analogue scale.
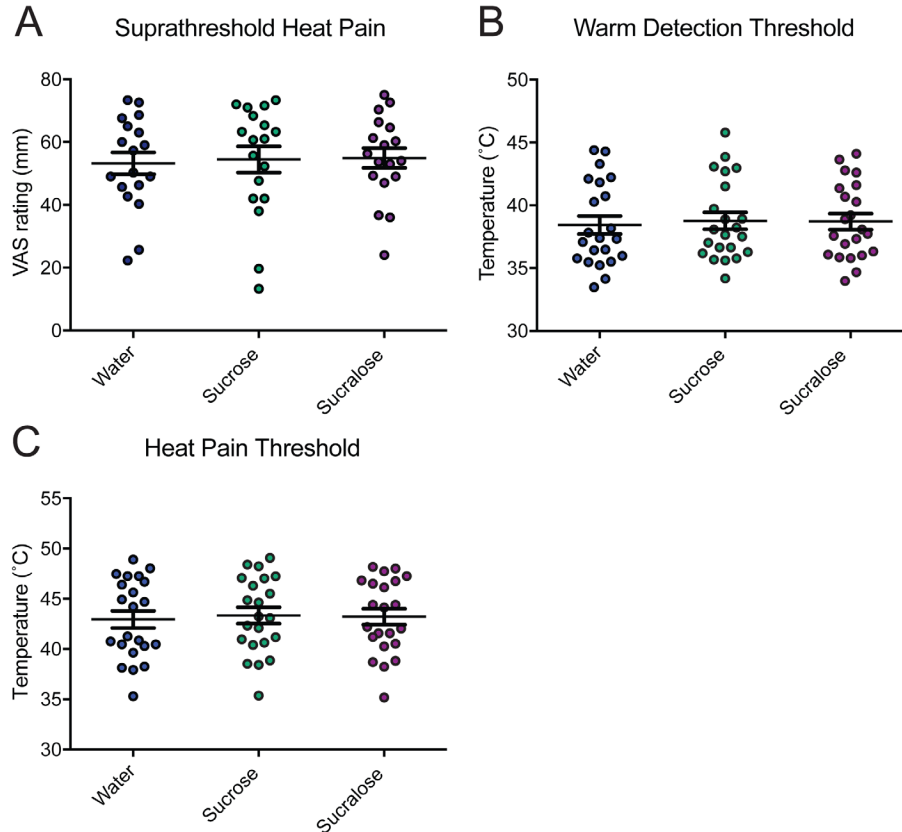
**Figure 2. Effect of sweet taste on fixed stimulus rating, warm detection and heat pain thresholds. A**: The mean visual analogue scale (VAS) pain rating when presented with a heat stimulus which had previously produced a rating of 60mm in the absence of solution. There were no differences between the VAS in the presence of 10% sucrose, 0.016% sucralose or water (p>0.05, n=18). **B**: No difference was observed in the warm detection threshold in the presence of 10% sucrose, 0.016% sucralose or water (p>0.05, n=22). **C**: There was no significant difference in the mean heat pain threshold recorded in the presence of 10% sucrose (43.3±0.8°C), 0.016% sucralose (43.2±0.8°C) or water control (43.0±0.8°C).

### Sweet taste has no effect on warm detection threshold or heat pain threshold

The effects of sweet taste on the WDT and HDT were assessed consecutively while participants held test solution in their mouth. There was no significant difference between WDT or HPT measured in the presence of 10% sucrose, 0.016% sucralose or water control (Figure 2B, 2C).

### Anticipation of a sweet taste does not affect thermal sensitivity

Animal studies have demonstrated that rats trained to anticipate sucrose show an increase in thermal withdrawal latency when they are presented with water instead of sucrose[5], suggesting that the anticipation of sucrose alone is sufficient to affect thermal sensitivity. In order to investigate this in human subjects, we told participants that they would receive the same sweet solution on three occasions. They were then presented first with sucrose, then with water, and subsequently with sucrose again. The protocol was designed such that an effect of sucrose anticipation on thermal sensitivity would be revealed by the presence of an increased heat pain threshold on presentation of water.

At the start of this experimental phase, warm detection and heat pain thresholds were re-assessed in the absence of any solution and subsequent measurements in the presence of solutions were compared to these baselines. There was no significant difference between the HPT at baseline and on the first presentation of sucrose. There was no significant change in heat pain threshold on presentation of water when sucrose was anticipated, and subsequent presentation of sucrose did not lead to a significant change in thresholds. These results demonstrate that anticipation of sucrose did not have an analgesic effect, regardless of whether or not sucrose is received (Figure 3).

### Inter-individual perception of sweetness or pleasantness does not influence pain

The pleasantness VAS ratings of the sweet solutions in comparison to water showed considerable variation across individuals (from -31 to +74mm for 10% sucrose and -31 to +71mm for 0.016% sucralose, n=19). Regression analysis was used to assess whether there was any correlation between the relative pleasantness of solutions and their analgesic effect (Figure 4). There was no significant correlation between the relative pleasantness rating of 10% sucrose and the difference in
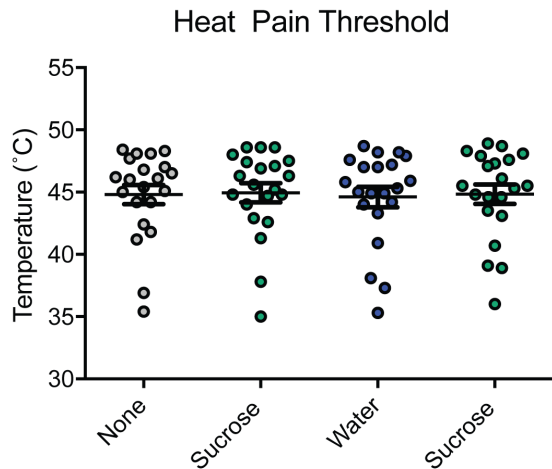
## Heat Pain Threshold



**Figure 3. Anticipation of sucrose does not alter heat pain thresholds.** The mean heat pain thresholds recorded at baseline (44.8±0.8°C) and on presentation of 10% sucrose (45.0±0.8°C), water (44.6±0.8°C) and 10% sucrose (44.8±0.8°C) in sequence (p>0.05, n=21).

heat pain threshold or the fixed stimulus rating ($r^2$=0.036 and 0.0002, respectively) (Figure 4A and 4B). There was no significant correlation between the relative pleasantness rating of 0.016% sucralose and the change in FSR ($r^2$=0.05) (Figure 4C and 4D). There was an unexpected and weak negative relationship between relative pleasantness rating of 0.016% sucralose and difference in heat pain threshold, though the slope of the regression line failed to reach statistical significance ($r^2$=0.18, p=0.06, Figure 4C). Overall these results demonstrate that there is no positive association between the pleasantness of any sweet solution and its effect on pain perception.

## Discussion

It is commonly observed that humans who are in a negative emotional state seek solace in the form of sweet foods and drinks[19]. Recent evidence further suggest a relationship between pain suffering and emotional eating that is driven by anxiety sensitivity[20,21] and chronic pain has been associated with an increased prevalence of eating disorders in young people[22]. Studies in rats show that, similarly to humans, highly palatable
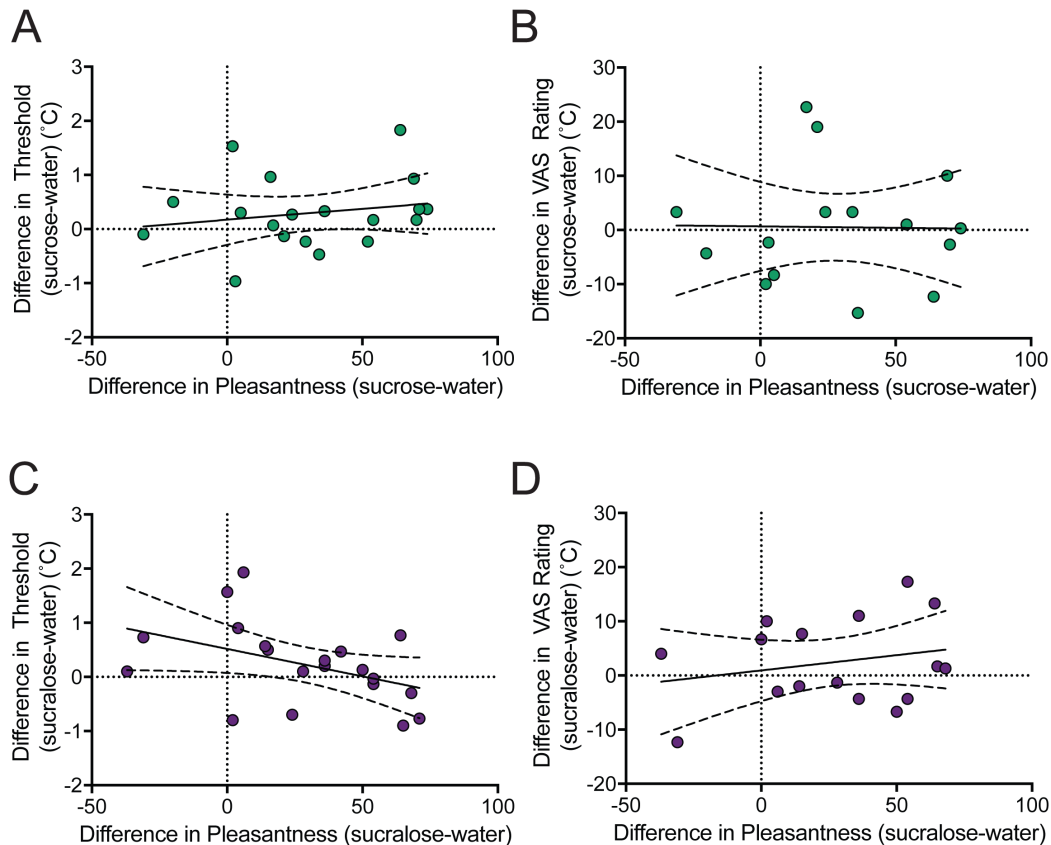


**Figure 4. Relationship between pleasantness of test solutions and heat pain. A**: The difference in heat pain thresholds measured in the presence of 10% sucrose and water (control) plotted against the difference in pleasantness for each individual (linear regression r2=0.036, ns, n=19). **B**: The difference in fixed stimulus rating reported in the presence of 10% sucrose and water plotted against the difference in pleasantness for each individual (linear regression, r2=0.0002, ns, n=15). **C**: The difference in heat pain thresholds measured in the presence of 0.016% sucralose and water control plotted against the difference in pleasantness for each individual (linear regression, r2=0.18, ns, n=20). **D**: The difference in fixed stimulus rating reported in the presence of 10% sucrose and water control plotted against the difference in pleasantness for each individual (linear regression, r2=0.05, ns, n=16). Graphs show regression lines with 95% confidence intervals (dotted). VAS, visual analogue scale.

foods are sought in response to stress[23], or anxiety[24], and suggest that limited intake of sucrose may in fact dampen the physiological responses to stress[23,25]. We have previously, shown in adult rats that a sucrose solution is sufficient to elicit short term thermal analgesia during consumption[5]. We therefore posed the question of whether a similar neurophysiological phenomenon to sweet taste may occur in adult humans.

Contrary to our initial hypothesis, we found no convincing effect of sweet taste on thermal pain perception in our study. The possible reasons for the absence of an effect, which is in contrast to previous studies in both humans and rodents, are discussed below.

## Hedonic value

A possible mechanism by which sweet flavours affect pain sensation is via the reward/hedonic pathway. While the interaction between chronic pain and affect has been extensively described and discussed[26,27], there are also reports of modulation of subjective perception of acute pain and even of nociceptive withdrawal reflexes by pleasant stimuli including images and odours[28,29]. These effects have been attributed to activation of limbic structures which exert modulatory effects on nociceptive signals at a spinal level[30]. A previous study investigating the effects of sweet flavours on pain perception suggested that the reward or hedonic value of the sweet substance was important, as an analgesic effect was only seen in individuals who had a preference for the sweeter solutions[6].

Studies in adult rats have demonstrated that both chocolate and sucrose had an analgesic effect in the naïve state, but not after induction of nausea or in the context of a conditioned aversion to sucrose[31]. Furthermore, it has been shown that a solution which does not normally have a positive hedonic value or analgesic effect, in this case sodium chloride, does have an analgesic effect when the solution becomes desirable in the context of sodium depletion[32]. These studies suggest that it is the reward value, rather than the specific taste or the pharmacological properties of the solution, which confers the analgesic effect.

In our study, the majority of participants reported the test solutions to be pleasant, and there was no correlation between an individual's pleasantness rating and pain perception. In comparison to the homologous rodent experiment[5], the hedonic value of a sugar solution may be considerably less to modern humans who live in a sugar rich environment than to a rat raised on a monotonous chow diet. Thus, it is possible that holding a simple sugar/sweetener solution in the mouth, although rated as pleasant, did not have sufficient reward value to produce an analgesic effect in adult humans[33]. Use of highly palatable sweet food or prior mild water deprivation may instead be required to observe a significant analgesic effect[34].

## Modality of pain stimulus

Most previous studies demonstrating sucrose-induced analgesia in humans have used the cold pressor test[6,8,35,36]. Analgesic effects on pressure pain have also been observed in adult humans during consumption of sweet and highly palatable

food or drink[34]. Our study shows that the analgesic phenomenon does not – at least in the context presented to participants in our study - appear to extend to modulation of thermal sensitivity and heat pain thresholds or percept.

Different models of pain and hyperalgesia are sensitive to modulation by specific classes of exogenous analgesics (e.g. opioids, NMDA receptor antagonists[37,38]). The modality of pain sensation affected by sucrose or hedonic consumption is therefore likely to depend on the mechanism of endogenous analgesia. While sweet taste analgesia in neonatal animals is thought to depend on endogenous opioid signalling[39–41], analgesia from hedonic drinking in adult animals is thought to require endocannabinoid signalling[5]. Data from human newborns are equivocal but suggest a non-opioid mechanism[42,43]. Thus, engagement of differing neurotransmitter systems and endogenous analgesic pathways by the various reported sucrose analgesia paradigms may variably affect different nociceptive modalities.

## Motivation and affect

Unlike measurements of pain thresholds (i.e. nociception), cold pain tolerance as measured by the cold pressure test reflects a broader psychological response to pain and is confounded by stress[9,44]. A study examining cold perception in young adults while holding a 24% sucrose solution in their mouth observed an analgesic effect on cold pain tolerance but not sensory thresholds[36]. Similarly, changes to pressure pain tolerance but not pressure threshold were also observed in adults[34]. Sweet taste in adults may therefore preferentially modify the motivational or affective aspects of the pain experience, as revealed in assays of pain tolerance, rather than the threshold and acute pain percept (nociceptive) measures tested in the current study. Further investigation of the phenomenon using assays for thermal pain tolerance[45] would therefore be of interest.

In concordance with previous human studies[6,8,35,36], participants in the current study were asked to hold the sweet solutions in their mouths during the test stimulus. The lack of a motivational component in our tasting assay, as well as the cognitive demand on participants to hold the solutions in their mouths without drinking, could also help explain some of the discrepancy with the previous study in adult rats in which the animals were required to actively seek and consume the sweet solution[5].

A concentration of 24% sucrose is typically recommended prior to heel lancing and venepunctures in neonates[2], and this concentration was reported as effective in the cold pressor task of pain tolerance in adult humans[36]. It is therefore possible that a thermal analgesic effect could have been seen had we used a higher concentrations (>24%) of sucrose in our participants. In our study, we chose a sucrose concentration of 10% (0.29M) as that most commonly used in sweetened beverages[46], and within the range of concentrations associated with the highest liking (0.21M-0.3M sucrose)[47]. It is noteworthy that two participants in the current study rated 10% sucrose as 'unpleasant'. 0.25M to 0.5M sucrose (8.6% to 17.2%) is considered a flexion point in the 'liking' rating, such that concentrations of sucrose above this are characterised by a decrease in liking by 'sweet dislikers',

which make up around 20% of adults aged 18–34[47]. Therefore, increasing the sweetness of the taste solution would require stratification of the volunteer group by sweet preference to eliminate the confound of aversion in such sweet 'dislikers'[47].

### Influence of age

Some animal studies have reported that analgesic effects of passive sucrose are seen only in neonatal rats[39], whereas studies in adult rats have demonstrated a clear analgesic effect with active consumption[5,31,48], suggesting an age-dependence on the context in which sucrose analgesia is apparent. Similarly, in humans an effect was demonstrated only in children and not in adults[6]. In children, the analgesic effect of sweet taste was restricted to cold thresholds but not tolerance to cold[49]. It is therefore possible that purely anti-nociceptive effects of sucrose are only present in neonatal and immature humans, which may account for the lack of effect in this adult population.

### Power and Sex

A limitation of our study is the relatively low number of participants. Like previous studies in adults[8,36] our experiments were powered to detect differences in thermal thresholds of 15% or more (a decrease of >9mm on a VAS score). While it is possible that smaller but still clinically relevant differences (<15%) between groups may be detectable if more participants were included in the study. However, the effect size detected in our investigation (of an increase in pain VAS of ~1mm with sucrose/sucralose) does not provide any evidence of a clinically meaningful effect.

Sex differences in the phenomenon have also been described in humans; for example, the analgesic effect of sucrose in the cold pressor test was observed in adult males but not females[6,8,35]. On the other hand, sweet taste analgesia in an assay of pressure pain tolerance in adults revealed differences in only female participants, a finding attributed to a greater preference for sweets in women than men[34]. In rats, a significant effect of sucrose on thermal sensitivities was seen in both sexes[5]. Although our study included both male and female participants and an exploratory sensitivity analysis of the two populations independently did not alter our findings, the low proportion of male participants (5 versus 22 female) means that our study is not sufficiently powered to detect a sex-specific differences.

### Conclusion

In conclusion, our study has demonstrated no clear effect of sweet-tasting solutions (either calorific or non-calorific) on the perception of warm temperature or heat pain in adult humans. This finding differs from some previously reported studies in humans and observations made in rodents. Potential explanations for the discrepancy include that the experimental solutions used lacked sufficient hedonic value for the study participants. Differences in the modality of sensory testing and motivational component of the taste assay may also contribute to variability in observations of the sucrose analgesia phenomenon. We hypothesise that alternative substances such as commercial sweet drinks or chocolate, which have a stronger association with pleasure than our clear, unbranded sugary liquid, may have a more substantial have a more substantial hedonic value and therefore greater potential analgesic effect. Further work is therefore required to investigate the effects of sucrose and sweet taste on human sensory physiology.

### Data availability

#### Underlying data

Data.Bris: Mooney 2020 WellcomeOpenRes. https://doi.org/10.5523/bris.11ruhu8y19oxe2ihn9qb11igk3[11]

This project contains the following underlying data:

- Fig1A_Sweetness.csv

- Fig1B_Pleasantness.csv

- Fig2A_Suprathreshold.csv

- Fig2B_WDT.csv

- Fig2C_HPT.csv

- Fig3_HPT.csv

- Fig4.csv

- sucrose_data_mastersheet.xlsx

#### Extended data

Data.Bris: Mooney 2020 WellcomeOpenRes. https://doi.org/10.5523/bris.11ruhu8y19oxe2ihn9qb11igk3[11]

This project contains the following extended data:
- Sucrose study documents.pdf (participant information leaflet, inclusion questionnaire, consent form, email and poster advertisements, participant instructions script)

- Participants.pdf (participant flow chart)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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**PubMed Abstract** | **Publisher Full Text**
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# Open Peer Review

## Current Peer Review Status: ✓ ✓ ✓

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**Version 2**

Reviewer Report 11 August 2020

https://doi.org/10.21956/wellcomeopenres.17677.r39837

✓ **Steve Davidson** [iD]

Department of Anesthesiology and Pain Research Center, University of Cincinnati, College of Medicine, Cincinnati, OH, USA

The revisions and sidebar discussions are appropriate.

*Competing Interests:* No competing interests were disclosed.

*Reviewer Expertise:* Neurobiology of pain

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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**Version 1**

Reviewer Report 14 July 2020

https://doi.org/10.21956/wellcomeopenres.17239.r39255

✓ **Naomi J Meesters** [iD]

Department of Pediatrics, Division of Neonatology, Erasmus MC-Sophia Children's Hospital, Rotterdam, The Netherlands

This study focuses on the effect of sweet taste on heat pain threshold in adults. The study method is clearly and extensively described. Many of the factors that could have influenced the pain

threshold are taken into account.

I mainly focused on a comparison with the existing evidence regarding this subject in neonates. I have some concerns/questions regarding the methods that were used to study the effect of pain threshold in adults:

1. Even though a power calculation was performed, I wonder if the expected 15% reduction on the VAS scale is realistic. In contrast to analgesics, there are no (or only minor) negative consequences related to the administration of sweet tasting solutions. Therefore a smaller reduction in pain score could also be clinically relevant.

2. It was already mentioned in the introduction that a difference between males and females was described. Therefore I do not understand why it was chosen to include 22 female and 5 males. In the discussion it was described that and exploratory sensitivity analysis did not show any difference finding, but I wonder if this is reliable considering the low number of males that were included.

3. For neonates, a much higher concentration of sucrose is studied most often and used in clinical care (24%). Why was a concentration of 10% used in this study? It is explained that it was used because of it's equivalence to sweet beverages, but at this point it is unclear if the solution did not reduce pain threshold because it was not sweet enough or if it can not reduce pain threshold in general.

4. Another difference with neonatal studies is the way the sweet solution was administered. The underlying mechanisms of the pain reducing effect of sucrose in neonates are not fully understood and possibly multifactorial. While in neonates the sucrose will be ingested, in this study the participants were instructed to hold the liquid in the mouth.

5. In neonates often a waiting period (mostly two minutes) is used between the administration of sucrose and the painful procedure. In this study, the measurement of the pain threshold was performed during the experience of tasting the sweet solutions. It is not known if it might take a certain period before the sweet tasting has an effect on the pain threshold.

**Is the work clearly and accurately presented and does it cite the current literature?**
Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

*Competing Interests:* No competing interests were disclosed.

*Reviewer Expertise:* Neonatal pain.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Author Response 28 Jul 2020

**Alexander Davies**, University of Oxford, Level 6 West Wing, John Radcliffe Hospital, Oxford, UK

**Reviewer 3**

**Reviewer:***This study focuses on the effect of sweet taste on heat pain threshold in adults. The study method is clearly and extensively described. Many of the factors that could have influenced the pain threshold are taken into account.*

*I mainly focused on a comparison with the existing evidence regarding this subject in neonates. I have some concerns/questions regarding the methods that were used to study the effect of pain threshold in adults:*

   *Even though a power calculation was performed, I wonder if the expected 15% reduction on the VAS scale is realistic. In contrast to analgesics, there are no (or only minor) negative consequences related to the administration of sweet tasting solutions. Therefore a smaller reduction in pain score could also be clinically relevant.*

   **Authors:**  We agree with the reviewer that a smaller reduction in pain score could still be clinically relevant and have added the following statement to the discussion:

"A limitation of our study is the relatively low number of participants. Like previous studies in adults (Lewkowski et al., 2003; Kakeda & Ishikawa, 2011) our experiments were powered to detect differences in thermal thresholds of 15% or more (a decrease of >9mm on a VAS score). While it is possible that smaller but still clinically relevant differences (<15%) between groups may be detectable if more participants were included in the study.  However, the effect size detected in our investigation (of an increase in pain VAS of ~1mm with sucrose/sucralose) does not provide any evidence of a clinically meaningful effect."

 **Reviewer:**  *It was already mentioned in the introduction that a difference between males and females was described. Therefore I do not understand why it was chosen to include 22 female and 5 males. In the discussion it was described that and exploratory sensitivity analysis did not show any difference finding, but I wonder if this is reliable considering the low number of males that were included.*

**Authors:**    An equal ratio of sexes, although desired, was not prospectively specified. Participation was on a voluntary basis and owing to limitations on the length of the study

recruitment was stopped having reached 27 individuals. We agree that balanced sexes would be preferable to identify sex-specificity, and in this regard the study is underpowered. We have added the following sentence to the discussion:

"...the low proportion of male participants (5 versus 22 female) mean our study is not sufficiently powered to detect a sex-specific differences."

**Reviewer:** *For neonates, a much higher concentration of sucrose is studied most often and used in clinical care (24%). Why was a concentration of 10% used in this study? It is explained that it was used because of it's equivalence to sweet beverages, but at this point it is unclear if the solution did not reduce pain threshold because it was not sweet enough or if it can not reduce pain threshold in general.*

**Authors:** We agree with the reviewer that 10% sucrose may not be sweet enough to increase the pain threshold. However, we also caution the use of very high sucrose concentrations owing to the possibility of sweetness aversion in a subset of volunteers. We have therefore added the following to the discussion:

A concentration of 24% sucrose is typically recommended prior to heel lancing and venepunctures in neonates (Stevens et al., 2004), and this concentration was reported as effective in the cold pressor task of pain tolerance in adult humans (Lewkowski et al., 2003). It is therefore possible that a thermal analgesic effect could have been seen had we used a higher concentrations (>24%) of sucrose in our participants. In our study, we chose a sucrose concentration of 10% (0.29M) as that most commonly used in sweetened beverages (Ventura et al., 2011), and within the range of concentrations associated with the highest liking (0.21M-0.3M sucrose (Iatridi et al., 2019). It is noteworthy that two participants in the current study rated 10% sucrose as 'unpleasant'. 0.25M to 0.5M sucrose (8.6% to 17.2%) is considered a flexion point in the 'liking' rating, such that concentrations of sucrose above this are characterised by a decrease in liking by 'sweet dislikers', which make up around 20% of adults aged 18-34 (Iatridi et al., 2019). Therefore, increasing the sweetness of the taste solution would require stratification of the volunteer group by sweet preference to eliminate the confound of aversion in such sweet 'dislikers' (Iatridi et al., 2019).

**Reviewer:** *Another difference with neonatal studies is the way the sweet solution was administered. The underlying mechanisms of the pain reducing effect of sucrose in neonates are not fully understood and possibly multifactorial. While in neonates the sucrose will be ingested, in this study the participants were instructed to hold the liquid in the mouth.*

**Authors:** We agree that the neonatal phenomenon is likely to be multifactorial. Therefore, in this study we explicitly set out to examine the requirement for sweet taste without the possible confound ingestion. Our results point to sweet taste alone being insufficient for sucrose's potential analgesic effect. We also acknowledge discrepancies between this study and previous animal experiments in which sucrose analgesia is observed:

"The lack of a motivational component in our tasting assay, as well as the cognitive demand on participants to hold the solutions in their mouths without drinking, could also help explain some of the discrepancy with the previous study in adult rats in which the

animals were required to actively seek and consume the sweet solution".

**Reviewer:** *In neonates often a waiting period (mostly two minutes) is used between the administration of sucrose and the painful procedure. In this study, the measurement of the pain threshold was performed during the experience of tasting the sweet solutions. It is not known if it might take a certain period before the sweet tasting has an effect on the pain threshold.*

**Authors:** The rationale for the short duration between pairs of stimuli was to directly compare with the rat experiments in which analgesia was observed within seconds of the onset of sucrose consumption. Equally, the analgesic phenomenon in rats appeared to extinguish fairly quickly after cessation of consumption (Davies et al., 2019). We therefore think it unlikely for a delayed analgesic effect to appear beyond the tasting period.

The short testing duration also reduced the burden on participants holding the test solutions in their mouths for long periods of time. However, we agree that longer duration tasting and/or sucrose concentration may be required to observe a measurable effect.

We thank the reviewer for their time and their careful and considered assessment our work.

**References for reviewer 3**

Davies AJ, Kim D, Park J, Lee JY, Vang H, Pickering AE & Oh SB. (2019). Hedonic drinking engages a supraspinal inhibition of thermal nociception in adult rats. Pain 160, 1059-1069.

Iatridi V, Hayes JE & Yeomans MR. (2019). Quantifying Sweet Taste Liker Phenotypes: Time for Some Consistency in the Classification Criteria. Nutrients 11.

Kakeda T & Ishikawa T. (2011). Gender differences in pain modulation by a sweet stimulus in adults: A randomized study. Nursing & Health Sciences 13, 34-40.

Lewkowski MD, Ditto B, Roussos M & Young SN. (2003). Sweet taste and blood pressure-related analgesia. Pain 106, 181-186.

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Ventura EE, Davis JN & Goran MI. (2011). Sugar content of popular sweetened beverages based on objective laboratory analysis: focus on fructose content. Obesity (Silver Spring) 19, 868-874.

***Competing Interests:*** No competing interests were disclosed.

Reviewer Report 14 July 2020

https://doi.org/10.21956/wellcomeopenres.17239.r39254

✔️  **Steve Davidson** 🆔

Department of Anesthesiology and Pain Research Center, University of Cincinnati, College of Medicine, Cincinnati, OH, USA

This is a clearly executed and written study to examine whether sweet taste alters the perception of threshold warm and hot stimuli. The conclusion, supported by the data, is that sweet taste does not alter thermal heat perception in adult humans. The study was carefully designed, although limited in scope.

A few issues reduce the overall impact of the study which should be addressed.

1. The study was designed to identify differences from the mean pain score of 15% in the treatment group. This is a highly ambitious change in threshold perception given that many of the best analgesics do not perform this well. Therefore, the study may not have been adequately powered to find smaller (more likely) differences, i.e., the limited number of subjects tested may have prevented finding a smaller, but true difference.

2. The number of male subjects to female subjects (about a 1:4 ratio) prevents meaningful inference of the interpretation of sex differences in this study, a point of interest which would have been worth the effort. Gender and sex are not synonyms.

3. There are a few speculative statements in the study without adequate citation or literature support including:
   a) that humans seek sugary foods during pain
   b) living in a sugar rich environment dampened the potential analgesic effects of this study.
   c) commercial drinks and chocolate are not valid for comparison because of many additional odors and flavors compared to sugar water. The goal of this study is not to test whether reward alters pain thresholds (otherwise cocaine would be just as appropriate) - it is specifically to test sweet taste.

4. In the animal studies, and perhaps some of the previous human studies, subjects may have been allowed to actually consume the sweet food/drink, however in this study subjects had to hold it in their mouths during testing and then spit it out. This may have produced the unwanted consequence of high cognitive demand to resist the desire of consumption, potentially confounding the interpretation.

5. Pain tolerance is discussed, although not tested in this study. There is no discussion of pain tolerance in animal studies, to parallel the discussion on threshold.

6. The timing between heating stimulus rounds may have been too short. What is the rationale for choosing this protocol?

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

*Competing Interests:* No competing interests were disclosed.

*Reviewer Expertise:* Neurobiology of pain

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

> Author Response 28 Jul 2020
> **Alexander Davies**, University of Oxford, Level 6 West Wing, John Radcliffe Hospital, Oxford, UK
>
> **Reviewer 2**
> *This is a clearly executed and written study to examine whether sweet taste alters the perception of threshold warm and hot stimuli. The conclusion, supported by the data, is that sweet taste does not alter thermal heat perception in adult humans. The study was carefully designed, although limited in scope.*
>
> *A few issues reduce the overall impact of the study which should be addressed.*
>
> *1.* **Reviewer:** *The study was designed to identify differences from the mean pain score of 15% in the treatment group. This is a highly ambitious change in threshold perception given that many of the best analgesics do not perform this well. Therefore, the study may not have been adequately powered to find smaller (more likely) differences, i.e., the limited number of subjects tested may have prevented finding a smaller, but true difference.*
>
> **Authors:** Our power calculation was based on differences observed in previous similar studies. Lewkowski et al identified a change in cold tolerance of more than 18% when participants held sweet solutions in their mouths (Lewkowski *et al.*, 2003). Kakeda & Ishikawa observed a 19% increase in pain threshold time in the cold pressor test in men

with sucrose water held in their mouths relative to distilled water (Kakeda & Ishikawa, 2011). We agree that further participants would be required to detect smaller differences. As addressed below, increasing the number of males specifically would address the question of sex-specificity in the proposed analgesic effect seen in other studies. We have added the following sentences to the discussion:

"A limitation of our study is the relatively low number of participants. Like previous studies in adults (Lewkowski et al., 2003; Kakeda & Ishikawa, 2011) our experiments were powered to detect differences in thermal thresholds of 15% or more (a decrease of >9mm on a VAS score). While it is possible that smaller but still clinically relevant differences (<15%) between groups may be detectable if more participants were included in the study.  However, the effect size detected in our investigation (of an increase in pain VAS of ~1mm with sucrose/sucralose) does not provide any evidence of a clinically meaningful effect."

*2.* **Reviewer:** *The number of male subjects to female subjects (about a 1:4 ratio) prevents meaningful inference of the interpretation of sex differences in this study, a point of interest which would have been worth the effort. Gender and sex are not synonyms.*

**Authors:** We agree that sex is the correct term to be use and this has been updated in the revised manuscript. An equal ratio of sexes, although desired, was not prospectively specified. Participation was on a voluntary basis and, owing to limitations on the length of the study recruitment period, was stopped having reached 27 individuals. We agree that balanced sexes would be preferable to identify sex-specificity, and in this regard the study is underpowered. We have added the following sentence to the discussion:

"...the low proportion of male participants (5 versus 22 female) mean our study is not sufficiently powered to detect a sex-specific differences."

*3.* **Reviewer:** *There are a few speculative statements in the study without adequate citation or literature support including:*
*a) that humans seek sugary foods during pain*

**Authors:** Evidence in humans suggests positive associations between sweet, palatable food-seeking/consumption and depression (Westover & Marangell, 2002; Knuppel *et al.*, 2017), anxiety (Penaforte *et al.*, 2019) and chronic stress (Tryon *et al.*, 2013), all of which are frequently co-morbid with chronic pain (Attal *et al.*, 2011; Woda *et al.*, 2016; Sieberg *et al.*, 2018).

Anecdotal evidence suggests chronic pain sufferers seek solace in the form of hedonic consumption (Janke & Kozak, 2012), which may be driven indirectly through anxiety-triggered sensitivity for emotional eating (Janke *et al.*, 2016; Kauffman *et al.*, 2019). Chronic pain has also been associated with an increased prevalence of eating disorders in young people, and may include over-eating as well as under-eating conditions (Sim *et al.*, 2017). Obesity, for example, is often co-morbid with chronic pain (Okifuji & Hare, 2015). Chronic pain patients also show increased gustatory sensitivity to sweet substances (Small & Apkarian, 2006), suggesting changes to brain circuity that may make them more susceptible

to sweet foods.

Negative emotions and stress may pre-dispose for the consumption of sweet and palatable foods with the aim of relieving the aversive state (Gibson, 2006). Studies in rats also show that highly palatable foods are sought in response to a stressful (Ulrich-Lai *et al.*, 2011), or anxiolytic state (Ghitza *et al.*, 2006), and suggest that limited intake of sucrose may in fact dampen the physiological responses to stress (Ulrich-Lai *et al.*, 2010; Ulrich-Lai *et al.*, 2011).

We agree that in its simplicity the statement 'humans seek sugary foods during pain' could be misleading and that the evidence for a link between chronic pain and sugary foods is more nuanced. We have therefore removed this statement from the abstract and introduction, and moved to the discussion where we have made the following changes to better reflect on the speculative nature of the association:

"It is commonly observed that humans who are in a negative emotional state seek solace in the form of sweet foods and drinks (Gibson, 2006). Recent evidence further suggest a relationship between pain suffering and emotional eating that is driven by anxiety sensitivity (Janke et al., 2016; Kauffman et al., 2019) and chronic pain has been associated with an increased prevalence of eating disorders in young people (Sim et al., 2017). Studies in rats show that, similarly to humans, highly palatable foods are sought in response to stress (Ulrich-Lai et al., 2011), or anxiety (Ghitza et al., 2006), and suggest that limited intake of sucrose may in fact dampen the physiological responses to stress (Ulrich-Lai et al., 2010; Ulrich-Lai et al., 2011)."

*b)* **Reviewer:** *living in a sugar rich environment dampened the potential analgesic effects of this study.*

**Authors:** This statement on access to sweet foods (comparing lab rodents to humans) is not in doubt. However, we agree that we have no evidence that this played a role in damping the human analgesic response to sweet taste – this is speculative rather than evidence based but we prefer to keep it in the discussion as an interesting hypothesis. The main point however, is that sucrose solution alone is relatively less rewarding than the complex nutrition and flavour of sweet foods typically consumed by humans. We have therefore removed this sentence and cite recent evidence that foods high in fat and carbohydrate are more rewarding, calorie for calorie, than foods high only in fat or carbohydrate (DiFeliceantonio *et al.*, 2018).

*c)* **Reviewer:** *commercial drinks and chocolate are not valid for comparison because of many additional odors and flavors compared to sugar water. The goal of this study is not to test whether reward alters pain thresholds (otherwise cocaine would be just as appropriate) - it is specifically to test sweet taste.*

**Authors:** We have of course taken a reductive approach to the question of whether sucrose, so often a component of highly palatable foods, is sufficient to elicit thermal analgesia in adult humans as it appears to do in neonates and in rats. However, we feel it is important to place the study into context with real life, in which sugar is purposely consumed with additional flavours and odours, likely due to the greater reward obtained from consumption

of these foods as outlined above. It will be interesting therefore for future studies to investigate whether more nutritionally complex palatable food stuffs are sufficiently rewarding to elicit thermal analgesia using a similar protocol. We have modified the final paragraph to reflect this potential for future work:

"We hypothesise that alternative substances such as commercial sweet drinks or chocolate, which have a stronger association with pleasure than our clear, unbranded sugary liquid, may **have a more substantial hedonic value and therefore greater potential** analgesic effect."

*4.* **Reviewer:** *In the animal studies, and perhaps some of the previous human studies, subjects may have been allowed to actually consume the sweet food/drink, however in this study subjects had to hold it in their mouths during testing and then spit it out. This may have produced the unwanted consequence of high cognitive demand to resist the desire of consumption, potentially confounding the interpretation.*

**Authors:** This is an interesting point and would again emphasise the cognitive nature of sucrose reward mediating any potentially analgesic effect rather than taste perception alone. We have added a sentence to the discussion:

"The lack of a motivational component in our tasting assay, **as well as the cognitive demand on participants to hold the solutions in their mouths without drinking,** could also help explain some of the discrepancy with the previous study in adult rats in which the animals were required to actively seek and consume the sweet solution".

*5.* **Reviewer:** *Pain tolerance is discussed, although not tested in this study. There is no discussion of pain tolerance in animal studies, to parallel the discussion on threshold.*

**Authors:** The valency afforded to pain tolerance renders it susceptible to cognitive factors (Cimpean & David, 2019). Therefore, we hypothesise that thermal pain tolerance, rather than merely pain intensity (threshold), may be preferentially affected by sweet consumption, as has been shown for cold pain tolerance in humans (Lewkowski *et al.*, 2003).

Owing to the subjective nature of pain and suffering, one must be cautious in attempting to ascribe the phenomenon of pain tolerance to animals. Indeed, a change in thermal withdrawal latencies in rats could indicate either a change in nociceptive threshold or an increased tolerance of a suprathreshold stimuli. Without verbal confirmation of the pain threshold we are not able to distinguish these effects. Furthermore, eliciting pain in animals without the ability to escape the painful stimulus would be unethical. We have therefore restricted our discussion to human studies, and instead add the following proposal for further work:

"Further investigation of the phenomenon using assays for thermal pain tolerance (Lue et al., 2018) would therefore be of interest."

*6.* **Reviewer:** *The timing between heating stimulus rounds may have been too short. What is the rationale for choosing this protocol?*

**Authors:** The rationale for the short duration between pairs of stimuli was to directly compare with the rat experiments in which analgesia was observed within seconds of the onset of sucrose consumption. The short testing duration also reduced the burden on participants to hold the test solutions in their mouths for long periods of time. Intervals of 4-6 s between thermal stimuli has previously been validated as producing reproducible thermal thresholds in normal subjects (Wasner & Brock, 2008), therefore we do not expect this to confound our results. We have added this reference to the methods section.

We thank the reviewer for taking the time to thoughtfully assess our work and are grateful for their feedback, which has helped to greatly improve the paper.

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***Competing Interests:*** No competing interests were disclosed.

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Reviewer Report 09 June 2020

✅ **Richard Hulse**

School of Science and Technology, Nottingham Trent University, Nottingham, UK

This study performed by Mooney and colleagues investigates the impact taste, focussing upon sweet taste, has upon pain perception. A large body of literature presents that by influencing taste pathways such as via sucrose and sweet taste can influence pain. A number of differing experimental approaches including human and animal studies have elucidated an analgesic capacity for sugar consumption. This is widely acknowledged in the clinical setting such as sugar administration to infants whom undergo painful clinical procedures. This study targets an adult human setting to evaluate how sweet taste may influence the perception of heat induced sensory experiences targeting both perception and tolerance of heat induced pain. However, how sweet taste can influence differing modalities and the fundamental neural axis' associated with sensory

perception is underrepresented in the article, especially in the context of heat pain in adults. Could the authors provide an expansion of the included literature to provide a broader introduction to the study, providing scope for further discussion?

Interestingly the article initially presents itself with the context that the human population widely seek sugar in times of 'pain or discomfort seek solace in the form of sweet foods and drinks'. This statement highlights a phenomenon that is of significant importance. It is widely appreciated that pain is a fundamental physiological protective mechanism, as well as being a significant burden to the clinical setting inclusive of the patient, carer and healthcare systems. Additionally, during these times modern dietary composition and mental health are under the spotlight. Can this initial statement become expanded with additional literature to support how the authors see this study fitting into a wider research landscape. For example, is there data available to highlight increased sugar dietary consumption in pain patients or possible association with pain treatments or other consumed drugs? Consideration of the utilisation and benefit of using sweet taste would also be appreciated in the introduction to allow a greater audience to appreciate this work.

An aspect of the study is to elucidate whether there is a disconnect between heat pain perception and tolerance. The justification of this; both as anatomical and neuronal function was tentatively outlined in the introduction, an aspect of this was provided in the discussion as hedonic/reward via the limbic system. Could a detailed expansion of this be provided as currently 'modulatory' actions is used as a term and putative mechanisms of actions would be appreciated by the reader?

The proposed methodology and general experimental design were appropriate, with overall structure well considered.

Comments
- In reference to the dose of sugar given; in the discussion it was highlighted that young adults in ref 26 were given a higher concentration of sucrose to induce analgesia. Could an aspect of discussion include reasoning around why a 10% dosing was utilised for this study, and how this differing dose may have implications in the data presented in this study. Also what dose is given in the clinic?

- 32°C used as a baseline why was this chosen? Room temp is usually 20-25. Does this negate detection thresholds?
- 6 seconds between heating ramps? Is this sufficient time to provide recovery both dissipation of heat from the skin and also neuronal activity? For example skin temperature takes time to return to normal temperature post stimulation with varying temperature ramps whether hot or cold (McMullan 2004[1], Hulse 2012[2]). Additionally, how the sensory afferents respond to these 'rapid fire' temperature spikes i.e. induction of nociceptor sensitisation/desensitisation.
- Why was second dose of less concentrated sugar data not presented? Could this be included?

- Figure 3 warm detection threshold test performed and mentioned but not presented. Could this be incorporated?

- A sentence in discussion could possibly be revised as it suggests that this article identifies an antinociceptive affect, which it did not. Recommendation to delete *italic* text.

'It is therefore possible that purely antinociceptive effects of sucrose, *as measured by thermal sensitivity in our study,* are only present in neonatal and immature humans, which may account for the lack of effect in this adult population.'

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**Is the work clearly and accurately presented and does it cite the current literature?**
Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

*Competing Interests:* No competing interests were disclosed.

*Reviewer Expertise:* Neurobiology, pain.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Author Response 30 Jun 2020
**Alexander Davies**, University of Oxford, Level 6 West Wing, John Radcliffe Hospital, Oxford, UK

**Responses to Reviewer**

The authors would like to thank the Reviewer for their time and careful assessment of our manuscript. We provide answers to each of the points raised by the Reviewer in the text

below.

***Reviewer:*** *This study performed by Mooney and colleagues investigates the impact taste, focussing upon sweet taste, has upon pain perception. A large body of literature presents that by influencing taste pathways such as via sucrose and sweet taste can influence pain. A number of differing experimental approaches including human and animal studies have elucidated an analgesic capacity for sugar consumption. This is widely acknowledged in the clinical setting such as sugar administration to infants whom undergo painful clinical procedures. This study targets an adult human setting to evaluate how sweet taste may influence the perception of heat induced sensory experiences targeting both perception and tolerance of heat induced pain. However, how sweet taste can influence differing modalities and the fundamental neural axis' associated with sensory perception is underrepresented in the article, especially in the context of heat pain in adults. Could the authors provide an expansion of the included literature to provide a broader introduction to the study, providing scope for further discussion?*

**Author response:** A reduction in nocifensive responses to both thermal and mechanical stimuli by intraoral sucrose (7.5%) has been demonstrated in neonatal rats (Anseloni *et al.*, 2002). The endogenous analgesia pathway in neonates may involve a descending inhibitory mechanism (Anseloni *et al.*, 2005), potentially suppressing both sensory modalities at the spinal level. In this study we set out specifically to test the effect of sweet taste on thermal sensory thresholds in adults. This had been reported in animal studies but there were no data using a thermal assay in humans. Additionally, thermal stimulation (using a heat ramp device) represents a convenient method to apply a well-controlled stimulus of defined duration, therefore standardising the amount of time participants must hold sweet solutions in their mouth. The thermal stimulation paradigm allows a focus on C-fibre and Adelta-fibre pathways, which ascend the spinothalamic tract. Intersection of this pathway with brainstem centres such as the parabrachial nucleus and periaqueductal grey (Bernard *et al.*, 1995) offers a mechanism for the supraspinal modulation of nociception that we have previously proposed in the equivalent adult rat model of sucrose analgesia (Davies *et al.*, 2019). Whether mechanosensory information ascending via the dorsal-column pathways may additionally be intercepted by central modulatory mechanisms associated with reward is yet to be established.

***Reviewer:*** *Interestingly the article initially presents itself with the context that the human population widely seek sugar in times of 'pain or discomfort seek solace in the form of sweet foods and drinks'. This statement highlights a phenomenon that is of significant importance. It is widely appreciated that pain is a fundamental physiological protective mechanism, as well as being a significant burden to the clinical setting inclusive of the patient, carer and healthcare systems. Additionally, during these times modern dietary composition and mental health are under the spotlight. Can this initial statement become expanded with additional literature to support how the authors see this study fitting into a wider research landscape? For example, is there data available to highlight increased sugar dietary consumption in pain patients or possible association with pain treatments or other consumed drugs? Consideration of the utilisation and benefit of using sweet taste would also be appreciated in the introduction to allow a greater audience to appreciate this work.*

**Author response:** Chronic pain has been associated with an increased prevalence of eating

disorders in young people, and may include over-eating as well as under-eating conditions (Sim *et al.*, 2017). Obesity, for example, is often co-morbid with chronic pain; each condition negatively impacts the other, though the relationship is complex involving genetic and environmental factors (Okifuji & Hare, 2015). Anecdotal evidence suggests chronic pain sufferers may seek solace in the form of hedonic consumption (Amy Janke & Kozak, 2012), though again it is unclear whether this is related to an analgesic effect *per se*. We emphasise that the negative results of the current study do not allow us to speculate on the use or otherwise of sweet taste to engage such cognitive mechanisms at this time. However, we agree that this is an interesting area of study, and one that should benefit from further research into the neurobiology of the reward-pain axis and how the system may become imbalanced in injury or disease.

*Reviewer: An aspect of the study is to elucidate whether there is a disconnect between heat pain perception and tolerance. The justification of this; both as anatomical and neuronal function was tentatively outlined in the introduction, an aspect of this was provided in the discussion as hedonic/reward via the limbic system. Could a detailed expansion of this be provided as currently 'modulatory' actions is used as a term and putative mechanisms of actions would be appreciated by the reader?*

**Author response:** Nociception, which encodes pain intensity, is discriminate, whereas pain tolerance has valency and can be affected by cognitive factors (Cimpean & David, 2019). Placing our results into context with the literature, we hypothesise that pain tolerance, and not merely pain intensity (threshold), may be preferentially affected by sweet consumption, as has been shown for cold pain tolerance (Lewkowski *et al.*, 2003). The wide distribution of pain processing throughout the brain and it's separation into cognitive and emotional contexts provides numerous pathways for modulation of the pain experience (Tracey & Mantyh, 2007). We do not yet understand the neural circuits of sweet taste that appears able to suppress the valency of pain, but pathways related to reward, or the expectation of reward, as engaged by hedonic consumption (including sweet tasting food/drink) are likely to be involved. Further investigation of the potential central mechanisms of thermal sensory modulation by sucrose or other sweet tastes in humans awaits the further validation of assays for thermal pain tolerance (Lue *et al.*, 2018) that are ethically sound and sufficiently engage the relevant cognitive pathways.

The proposed methodology and general experimental design were appropriate, with overall structure well considered.

**Comments**
*1. In reference to the dose of sugar given; in the discussion it was highlighted that young adults in ref 26 were given a higher concentration of sucrose to induce analgesia. Could an aspect of discussion include reasoning around why a 10% dosing was utilised for this study, and how this differing dose may have implications in the data presented in this study. Also what dose is given in the clinic?*

**Author response:** We chose a concentration of 10% sucrose as this is the most commonly used concentration of sugar (typically sucrose) in sweetened beverages, including fruit juices (Ventura *et al.*, 2011). Concentrations of sucrose employed in studies of procedural

analgesia in human neonates range from 7.5% to 50%, with 24% sucrose recommended prior to heel lancing and venepunctures (Stevens *et al.*, 2004). It is therefore possible that an analgesic effect could have been seen at higher concentrations of sucrose in our participants, as observed in the cold pressor task of pain tolerance (Lewkowski *et al.*, 2003). It is noteworthy that two participants rated 10% sucrose as 'unpleasant'. Between 0.25M and 0.5M sucrose (8.6% and 17.2%, respectively) is considered a flexion point in the 'liking' rating, such that concentrations of sucrose above this are characterised by a decrease in liking by 'sweet dislikers', which make up around 20% of adults aged 18-34 (Iatridi *et al.*, 2019). Indeed 10% sucrose (0.29M) is within the range of concentrations associated with the highest liking (0.21M-0.3M) (Iatridi *et al.*, 2019). Increasing the sweetness of the solution would therefore likely require stratification of the volunteer group by sweet preference to eliminate the confound of aversion in sweet 'dislikers'. We have added a brief discussion of these limitations in the revised manuscript.

*2. 32°C used as a baseline why was this chosen? Room temp is usually 20-25. Does this negate detection thresholds?*

**Author response:** Although the ambient temperature of the environment was indeed between 20-25°C, a Peltier device set to this temperature would be perceived as 'cold'; this is because the average skin temperature of the immediately exposed forearm is approximately 33°C (Barcroft & Edholm, 1946). Therefore 32°C was chosen to provide an imperceptible baseline for the temperature ramps, which we do not expect to interfere with the accurate detection of warm and thermal pain threshold. This baseline temperature is also standard for measuring warm detection and thermal pain thresholds in quantitative sensory testing protocols (Rolke *et al.*, 2006)**.** This has been clarified in the methods sections of the revised manuscript.

*3. 6 seconds between heating ramps? Is this sufficient time to provide recovery both dissipation of heat from the skin and also neuronal activity? For example skin temperature takes time to return to normal temperature post stimulation with varying temperature ramps whether hot or cold (McMullan 2004, Hulse 2012). Additionally, how the sensory afferents respond to these 'rapid fire' temperature spikes i.e. induction of nociceptor sensitisation/desensitisation.*

**Author response:** Heat ramps were performed in pairs with short (6 s) intervals: Warm detection thresholds were measured on the first ramp, and heat pain thresholds were measured on the second ramp. However, to clarify, a period of recovery of 2 min was then allowed before a repeat of the paired stimuli. We acknowledge that repeated short interval thermal stimulation could lead to sensitization, however we believe this was avoided by the longer interval between trials, thus allowing the skin temperature to return to normal. We do not expect a single warm ramp (to 39°C) to significantly affect subsequent thermal nociception (i.e. detection of painful heat); indeed the heat pain threshold measurements (average 43°C) were well within the range of healthy individuals (Rolke *et al.*, 2006)**.** We clarify these methodological details in the revised manuscript.

*4. Why was second dose of less concentrated sugar data not presented? Could this be included?*

**Author response:** As we describe in the paper, lower concentrations of sucrose (5%) and

sucralose (0.008%) were tested for sweetness and pleasantness in a pilot study. As we found no difference in the ratings for either concentration, we continued the sensory testing with only the higher concentrations (10% sucrose and 0.016% sucralose equivalent), due to reasons outlined above.

*5. Figure 3 warm detection threshold test performed and mentioned but not presented. Could this be incorporated?*

**Author response:** For experiments on the effect of anticipation of sucrose only the heat pain thresholds were recorded. This has been clarified in the revised manuscript.

*6. A sentence in discussion could possibly be revised as it suggests that this article identifies an anti-nociceptive affect, which it did not. Recommendation to delete* italic *text: 'It is therefore possible that purely anti-nociceptive effects of sucrose,* as measured by thermal sensitivity in our study*, are only present in neonatal and immature humans, which may account for, the lack of effect in this adult population'.*

**Author response:** We thank the reviewer for highlighting this grammatical error. This sentence has now been removed.

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***Competing Interests:*** The authors declare no competing interests.