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1 Novel flavours paired with glutamate condition increased intake in older adults in the

2 absence of changes in liking

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12

13 Abstract

- 14 Previous research on the repeat exposure to a novel flavour combined with monosodium
- 15 glutamate (MSG) has shown an increase in liking and consumption for the particular
- 16 flavour. The aim of the current work was to investigate whether this could also be
- 17 observed in the case of older people, since they are most affected by undernutrition in the
- 18 developed world and ways to increase consumption of food are of significant importance
- 19 for this particular age group. For this study, 40 older adults (age 65-88) repeatedly
- 20 consumed potato soup with two novel flavours (lemongrass and cumin) which were either
- 21 with or without a high level of MSG (5%w/w). A randomized single blind within-subject
- design was implemented, where each participant was exposed to both soup flavours three
- times over 6 days, with one of the soup flavours containing MSG. After three repeat
- 24 exposures, consumption increased significantly for the soups where the flavours had
- contained MSG during the repeated exposure (mean weight consumed increased from 123
- to 164 g, p=0.017), implying that glutamate conditioned for increased wanting and
- consumption, despite the fact that the liking for the soup had not increased.
- 28

29 Highlights

- Glutamate can condition increased food consumption in older adults
 - Older adults increased consumption of novel flavoured soups paired with glutamate
 - Older adults did not increase liking of novel flavoured soups paired with glutamate
- 32 33

31

34 Keywords

35 Conditioned liking, conditioned intake, wanting, consumption, glutamate

36 Introduction

37 While repeated exposure alone can produce increased liking for food/beverage flavours (Methven, Langreney, & Prescott, 2012), it is thought that most flavour or food preferences 38 39 are formed through associative learning in which the flavour is paired with a liked taste such as sweetness (flavour-flavour learning, FFL; (Yeomans, Mobini, Elliman, Walker, & 40 Stevenson, 2006) or with ingested nutrients (flavour-consequence learning, FCL) including 41 42 carbohydrates (Yeomans, Leitch, Gould, & Mobini, 2008) and fat (Kern, McPhee, Fisher, 43 Johnson, & Birch, 1993). Nutrient pairing has been shown to lead to produces reliably strong increases in liking for a paired flavour in some studies (Brunstrom & Mitchell, 2007; 44 45 Kern, et al., 1993; Yeomans, Leitch, et al., 2008) but not all (Remy, Issanchou, Chabanet, & Nicklaus, 2013). Typically the effect is only successful under conditions in which the 46 nutrient is valued. Hence, learning is strongly dependent on hunger state, an observation 47 which emphasizes the key role of the metabolic consequences of the nutrients in 48 49 facilitating the increased flavour liking (Mobini, Chambers, & Yeomans, 2007). It is however 50 noted that where the taste is generally associated with nutritive value, for example 51 sweetness and sugar, FFL can still result from low-nutritive value (low calorie) sweetness 52 pairings (Privitera, Mulcahey, & Orlowski, 2012).

53

FCL has also been demonstrated with glutamate in the form of its sodium salt, 54 monosodium glutamate (Prescott, 2004; Yeomans, Gould, Mobini, & Prescott, 2008). The 55 metabolic underpinnings of this process are less clear than they are for the similar effects 56 produced by sugar or fat, both sources of energy. However, glutamate is an amino acid and 57 58 the human body requires sources of amino acids and protein, so the fact that glutamate increases savoury taste and food pleasantness may increase motivation to consume foods 59 60 as a response to ensure adequate protein intake (Murphy, 1987). There are also data suggesting an important metabolic role for glutamate as an intestinal energy source 61 (Reeds, Burrin, Stoll, & Jahoor, 2000). 62

Motivation to consume sufficient protein is the basis of the "protein leverage hypothesis" whereby it is proposed that humans have an appetite for protein which within low protein diets can lead to excessive energy intake. In a recent study by Gosby, et al. (2011), lowering protein in the diet (from 15% to 10%) led to higher total energy intake which was

67 predominantly consumed through savoury-flavoured foods. This supports the theory that68 savoury taste signals for protein.

69

70 Repeatedly pairing a novel soup flavour with ingested glutamate was found to produce a 71 substantially stronger preference than either simple repeated exposure to the soup flavour 72 or to the soup flavour paired with glutamate but ingested in only nominal amounts, a 73 condition equivalent to FFL (Prescott, 2004). In a subsequent study (Yeomans, Gould, et al., 74 2008), conditioned flavour liking from ingested glutamate added to soups was also 75 observed. In addition, this later study also examined the impact of glutamate-conditioned 76 flavours on post-conditioning intake and hunger. Relative to a control condition in which 77 the soup did not contain added MSG, pairing with MSG produced a significant increase in 78 ad-libitum soup intake following conditioning. As well, tasting the soup whose flavour had 79 been paired with MSG increased ratings of hunger, again an effect not evident in the control condition. 80

81

82 In contrast to measures of liking, hunger might be considered a motivational consequence of ingested MSG in such conditioning paradigms. Distinctions between affective (liking) and 83 84 motivational (wanting) aspects of consumption have been highlighted by Berridge (Berridge, 1996, 2009), initially as an explanatory principle underlying drug addictions. The 85 86 two psychological processes of liking and wanting operate at explicit (deliberative) and 87 implicit (automatic) levels (Finlayson & Dalton, 2012) and there have been a number of 88 studies that focused on developing methodologies in order to understand the extent to which this distinction applies to food consumption (Finlayson, King, & Blundell, 2006, 2007) 89 90 and the importance of the distinction in providing explanations for food-related issues such as obesity and binge-eating (Finlayson, Arlotti, Dalton, King, & Blundell, 2011; Finlayson, 91 92 King, & Blundell, 2008; Griffioen-Roose, Finlayson, Mars, Blundell, & de Graaf, 2010; 93 Temple, et al., 2009).

94

However, whereas obesity is of concern to the main stream western populations, the
incidence of under-nutrition is widespread among older people (Age Concern, 2006) and

factors that may increase intake or hunger are consequently of practical interest. One of 97 the physiological factors that results in reduced food intake is the natural decline of taste 98 99 and olfaction due to ageing (Kenway, et al., 2004; Methven, Allen, Withers, & Gosney, 100 2012; Schiffman & Warwick, 1993), or due to diseases and medication (Nolan, 2001; Schiffman, 2007; Schiffman, et al., 2000; Winkler, Garg, Mekayarajjananonth, Bakaeen, & 101 102 Khan, 1999), which can lead to a reduced appreciation and interest in food. Moreover, 103 perhaps as a consequence of such sensory deficits, it is not at all clear that either FFL or FCL operate as effectively in elderly populations as they do in the younger age groups that have 104 105 been studied to date.

106

Here we report the results of a study in which the pairing of MSG with a novel soup flavour
produced conditioned increases in soup consumption in the absence of conditioned liking.
In this particular study, we investigated how repeated exposure to MSG could influence
both liking for paired soup flavour as well as willingness to consume the soup in older
healthy adults.

112

113 Materials and Methods

114 Design

This single blind study used a within subjects design to measure the effect of MSG-115 116 enhanced soup on liking and consumption by older adults, following repeat exposure. The 117 participants were asked to attend a pre-training conditioning session (session 1, S1) and a 118 post-training conditioning session (session 8, S8) at a central location (the university). At the pre-training conditioning session they were required to take home 6 cans of soup for 119 120 their consumption over the week. Two different flavours of soup, cumin and lemongrass, were used account for mere exposure effects of increased liking to the flavour regardless of 121 122 the addition of MSG. Each flavour was consumed on alternate days throughout the week (Table 1). The order in which the flavours were consumed was balanced across 123 participants. For each group one soup contained added MSG, the other did not. In pre-124 125 and post- training conditioning sessions, the soups tasted and consumed did not contain 126 added MSG.

TABLE 1 ABOUT HERE

128

127

129 Participants

Forty volunteers (10 men, 30 women; 65-88 years old, mean age: 73.7±5.5 years) were 130 131 recruited from a database of healthy older volunteers whom had previously stated an interest in taking part in research studies. Each participant received a participant 132 133 information sheet and on agreeing to take part in the study they completed a consent form. A screening questionnaire determined their eligibility to take part in the study; 134 135 exclusion criteria were the existence of relevant food allergies or intolerances, taking medications known to affect appetite or taste and age under 65 years. The volunteers self-136 reported height and weight, from which their BMI was calculated. The majority of 137 participants were in the healthy weight BMI category, the mean BMI was 23.7 kgm⁻², the 138 median was 23.1 kgm⁻², and the overall range was 18.6 to 31.9 kgm⁻². No volunteers were 139 reportedly underweight (<18.5 kgm⁻²), eight were in the overweight category (25 to 29.9) 140 kgm^{-2}) and two in the obese category (30 to 39.9 kgm^{2}). 141

142

Participants were allocated randomly to one of two groups (MSG⁺-and MSG⁻Group 1 or 143 144 Group 2) which determined which of the soups to be taken home contained added MSG and which did not. Each group was then further segmented in terms of which flavour soup 145 they would consume on the pre- and post- conditioning training sessions (the one exposed 146 with MSG or the one exposed without MSG). The two groups did not differ in terms of 147 gender (both groups had 5 men and 15 women) or differ significantly in terms of age 148 (MSG⁺: 72.8 ±4.9 years; MSG⁻: 74.6 ±6.1 years, p=0.298). One participant failed to attend 149 150 post-trainingconditioning, so her results were excluded from the study. The project was 151 given approval to proceed by the University of Reading Research Ethics Committee (study 152 number 11/41).

153

154 Samples

155 A potato soup was prepared as a base and two different concentrated flavour

preparations, lemongrass and cumin (Treatt, Bury St Edmunds, UK) were added to create 156 the two different soup flavours. The soups were then processed in a canning retort at 157 121°C in either 400 ml or 120 ml cans, the larger size for the consumption tests and the 158 159 smaller sizes for the repeat exposure tastings. The particular flavours chosen were considered novel for this age group and were used in order to avoid any effects of 160 161 familiarity on liking and consumption of the soups. All soups were prepared in the pilot 162 plant in University of Reading, following good manufacturing practices (GMP) and Hazard Analysis Critical Control Points (HACCP). To half of the soup samples provided during the 163 164 training conditioning sessions (see below) 5% MSG w/w was added.

165

166 Procedure

Pre-training-conditioning session (session 1): Volunteers tasted both of the two flavours of 167 soup and rated their liking for them on a standard 9-point hedonic category scale (1 = 168 dislike extremely and 9 = like extremely). They were also asked to rank their 169 170 preferencestate which of the two samples they preferred. Soups were presented in a 171 balanced order and participants were asked to rinse their palate with water between 172 samples. Additional questions were asked on familiarity of each soup flavour as well as questions on appetite and mood. These were rated using unstructured line scales (results 173 174 scaled 0-100) with anchors at both ends (Table 1). They were then presented one soup for consumption. Each volunteer was asked to eat as much soup as they wanted; they were 175 provided with both a bowl of soup (250 ml) alongside an additional thermos flask of soup 176 (total amount of soup was 400ml). Consumption weight was calculated. Then participants 177 178 were asked further questions on appetite (Table $\frac{1}{2}$ Q7-8).

179

Training-Conditioning sessions (Sessions 2 – 7): The volunteers took home six cans of soup
(120 ml), three of each flavour, to consume on alternate days over the 6 days with one
flavour containing added MSG. Instructions were given to each volunteer on how to reheat
the soup and they were asked to consume one can of soup (120ml) at their normal lunch
time each day, recording the time they ate and any other comments on a chart provided.

Post-training-conditioning (Session 8): During the post-trainingconditioning session, held at 186 187 the central location (University), the volunteers repeated the liking and preference test and 188 answered the same questions on familiarity, appetite and mood as in the pre-189 trainingconditioning, and in the same order (session 1). They were also presented with the 190 same soup for consumption as they had been in session 1. Moreover, they were asked to 191 eat the same breakfast at the same time on both the pre- and post- conditioning training 192 days and not to consume anything apart from water between breakfast and lunchthe midday session. Both pre- and post- conditioningtraining sessions were commenced at 193 194 midday with participants seated in isolated sensory booths. 195 196 TABLE **1-2** ABOUT HERE 197 198 Statistical analysis Analysis of the data was conducted with XLSTAT (Addinsoft, version 2011.4.01). 199 Correlation tests were done using Pearson correlation matrix. Comparison of measures 200 201 between flavours and sessions were carried out using t tests. The effect of soup flavour, 202 exposure to MSG and gender on the difference in consumption, liking and familiarity pre-203 and post training conditioning were carried out using analysis of variance. Difftest 204 (Statbasics, version 2.00) was used to determine the significance of the preference results. 205 For all tests, significance was established at p<0.05. 206 207 Results 208 Liking 209 Liking ratings for the soups were low with mean liking ratings across both flavours being 5.7 and 5.4 out of 9 at sessions 1 and 8 respectively. The liking of the soups was significantly 210 211 affected by the flavour (F(1,148)=3.90, p=0.05) with the lemongrass scoring lower than the 212 cumin flavour in both S1 (mean rating 5.3 compared to 6.1) and S8 (5.3 compared to 5.6). 213 However, liking was not significantly affected by the session (pre- to post conditioningtraining) (F(1,148)=0.49, p=0.48), the exposure group (F(1,148)=1.05, p=0.31) 214

or gender (F(1,148)=0.21, p=0.65). There were no significant interactions between any of
the treatment effects.

217

218	The difference in liking between the pre- and post <u>conditioning</u> training was not
219	significantly affected by the soup flavour (F(1,73) =0.95, p=0.33), whether that flavour had
220	been previously paired with MSG (F(1,73) =0.01, p=0.93), or gender (F(1,73) =0.60, p=0.44),
221	and there was no significant interaction between flavour and exposure group
222	(F(1,73)=0.90, p=0.35) (Figure 1). There was a significant correlation between familiarity
223	and liking, across all soup flavours and sessions (r=0.25, p=0.002).
224	
225	The scores for "would you choose this soup" were below the mid-point on a 100 point
226	scale (mean values of 37.8 and 35.6 across both soup flavours at S1 and S8, respectively).
227	There were no significant effects of flavour (F(1,147)=2.59, p=0.11), session (F(1,147=0.23,
228	p=0.63), exposure group (F(1,147=1.14, p=0.29) or gender (F(1,147=0.09, p=0.76) on the
229	readiness to choose the soup, and no significant interactions between these factors.
230	
231	FIGURE 1 ABOUT HERE
232	Preference
233	In neither S1 nor S8, was t <u>T</u> here was <u>no significant</u> any difference in the number of subjects
234	who preferred <u>the cumin one soup flavour<u>ed soup</u> over another the lemongrass flavoured</u>
235	soup in either S1 or S8 (n=22 cumin, n=17 lemongrass; at both sessions).
236	

237 Familiarity

238 Familiarity was rated low for both soups in S1 (mean values out of 100: cumin 30.3,

lemongrass 20.6), confirming initial judgments of flavour novelty. Significant effects on

240 changes in familiarity between the pre- and post- exposure sessions were seen as a

241 function of flavour (F(1,71)=3.89, p=0.025) and gender (F(1,71)=3.89, p=0.05), but not

exposure group (F(1,71)=0.15, p=0.70). Familiarity increased significantly from S1 to S8 for

the lemongrass flavour (t (76)= 3.44, p=0.001) but not for the cumin flavour (t (74) = 1.17,

244	p=0.25). The apparent difference in change in familiarity as a function of exposure with
245	MSG shown in Figure 2 was not significant (interaction between flavour and exposure
246	group: F(1,71)=1.27, p=0.26), whereas the familiarity of the lemongrass flavour increased
247	between the sessions whether exposed with MSG or not.
248	
249	FIGURE 2 ABOUT HERE
250	
251	Appetite and Mood
252	The participants pre-lunch hunger, desire to eat and mood ratings did not change
253	significantly between sessions (Figure 3) (t(74)=0.93, p=0.35; t(74)=0.63, p=0.53;
254	t(74)=0.05, p=0.96 respectively) . The mean hunger and desire to eat ratings were mid-
255	scale implying the participants were moderately hungry at the time of the tests, and the
256	mean mood rating was high implying the participants were in a pleasant mood.
257	
258	FIGURE 3 ABOUT HERE
259	
260	Although post consumption the overall mean satiety ratings were not higher in S8
261	compared to S1 (t(76)=1.29, p=0.2), the mean values were lower than the mid-point of the
262	scale (S1: 43.2; S8: 35.2), implying that the participants had not eaten to fullness. However,
263	when asked if they could eat any more of the soup the values were also low on the scale,
264	with no significant differences between S1 and S8 (21.5 compared to 30.2; t(75)=1.45,
265	p=0.15).
266	
267	There were no significant effects of gender (F(1,70)=0.02, p=0.89), soup flavour
268	(F(1,70)=0.09, p=0.77), exposure group (whether the soup flavour had been exposed to
269	MSG during the <u>conditioning</u> training period) (F(1,70)=0.04, p=0.85), nor session (pre- or
270	post <u>conditioning</u> training) (F(1,70)=1.63, p=0.21) on post-consumption satiety ratings and
271	no significant interactions of any of the treatment effects. Similarly, there were no
272	significant effects of gender (F(1,69)=0.03, p=0.87), soup flavour (F(1,69)=0.01, p=0.92),

exposure group (F(1,69)=2.08, p=0.15), nor session (F(1,69)=2.11, p=0.15) on the "could
you eat more" rating and no significant interactions of any of the treatment effects.

275

276 Compliance and Comments

277 During the 6 day exposure period the participants were asked to note whether they ate the

soups and to provide free-text comments. Of the 39 participants, 32 (82%) ate all of the

soup provided on all days, 1 person forgot to eat the soup on one day, 5 participants ate

280 only a quarter or half of the portion each day and one participant did not provide

compliance information. Twenty-six participants (67%) commented that the soups were

salty and of these comments 16 (41%) were specific to the soups containing MSG.

283

284 Consumption

Following the liking ratings, and prior to the consumption test, participants were asked to
rate "how much could you eat of this soup now?". These ratings were below the mid-point
on a 100 point scale (means: 40.5 and 38.1, across both soup flavours at S1 and S8). There
were no significant effects of flavour (F(1,146)=2.29, p=0.13), session (F(1,146=0.31,
p=0.58), exposure group (F(1,146=1.72, p=0.19) or gender (F(1,146=0.48, p=0.49) on the
expected consumption of the soup, and no significant interactions between these factors.

291

However, pairing with MSG over the conditioning sessions did lead to a significant effect on intake at the post-conditioning session. There was an increase in consumption of the soups between S1 and S8, but only in the condition in which where the soup had been previously paired with MSG (F(1,34) =6.34, p=0.017) (Figure 4). The mean consumption of soups which had been paired with MSG increased from 123 g at the pre-conditioning session to 164 g at the post conditioning session (standard error 24), whereas the consumption of soups not paired with MSG remained virtually constant (130 g and 121 g respectively).

There was a positive correlation between soup consumption across both sessions with liking (r=0.61, p<0.0001) and familiarity (r=0.29, p=0.009). However, there was no 302 correlation between consumption and rated hunger (r=0.13, p=0.24). There were no 303 changes in consumption associated with soup flavour (F(1,34) =0.49, p=0.49) or gender 304 (F(1,34) =0.69, p=0.41).

305

306

FIGURE 4 ABOUT HERE

307

308 Discussion

The major finding from this study is that repeat exposure to novel-flavoured soups with 309 310 MSG increased consumption by older people after three at-home exposures. This finding is 311 consistent with previous research that MSG can condition both appetite and consumption 312 (Yeomans, Gould, et al., 2008). However, unexpectedly, the current data failed to show an increase in conditioned liking for the soup flavours. The failure to show an increase in 313 314 hedonic ratings was consistent with the relatively low scores both pre- and postconditioning for the question "would you choose this soup". The lack of increased liking 315 316 following conditioning, contrary to expectations, is unlikely to be a function of the soup flavours themselves, novel though they were. Previous research has used highly novel, 317 indeed somewhat unpalatable, flavours while still showing increased liking over repeat 318 319 exposures, even without consumption (Prescott, 2004).

320

321 A more likely cause is the soups' salt content, an important determinant of the flavour acceptability of foods. In particular, appropriate salt levels are dependent on the context of 322 323 the particular food (Sullivan & Birch, 1990). The average sodium level in UK soups is 224 mg/100ml (data taken from eight UK supermarket canned soups), which is comparable to 324 the soup with no MSG used in this study (255 mg/100ml). However, we subsequently 325 326 determined that the sodium level of the soup with 5% MSG was 983 mg per 100ml, so the 327 120 ml samples that were consumed provided 1180 mg sodium. We suggest, therefore, 328 that the soups are highly likely to have been unpalatably salty. Comments received from 329 many of the participants to the effect that some of the soups were too salty support this 330 interpretation. Of course, the MSG- soups also failed to show changes in liking, but this 331 may have reflected an entirely different mechanism, for example, insufficient exposure to

333

334 Thus, although conditioned increases in consumption are most often accompanied by 335 increases in liking (Yeomans, Gould, et al., 2008) these two processes were affected 336 differently by the added MSG in this study. Effectively, this implies that FFL, the high Na sodium paired with the soup flavours, was acting to offset any conditioned liking that might 337 338 have arisen as a result of pairing MSG with the flavour. In contrast, the ingested MSG paired with the flavour facilitated FNL, which provided the basis for conditioned increases 339 340 in post-conditioning intake. In effect, we report here a serendipitous finding of conditioned 341 intake in the absence of conditioned liking. As to whether this dissociation is a function of 342 other aspects of the design apart from the high Na-sodium content of the soups is not 343 clear. Multiple exposures can in fact increase liking for a flavour that is initially unpalatable due to low Na-sodium content (Methven, Langreney, et al., 2012), so the possibility exists 344 345 that further exposure in this case may also have led to increased liking.

346

One interpretation of this dissociation is that it reflects separate processes inherent in the 347 liking/wanting distinction. There are substantial difficulties in disentangling liking and 348 349 wanting as reasons for intake, as both factors are likely to coincide most often (Havermans, 350 2011). Moreover, the learning processes behind each are likely to coincide. Thus, the 351 results from Yeomans, Gould, et al. (2008) showed that pairing a flavour with an ingested 352 nutrient such as glutamate under conditions of hunger produced both increased liking for the flavour and increased consumption of it. But conceptually, these two processes can be 353 distinguished. As an illustration, a simple case in which they are dissociated might be where 354 we would be willing to eat a food that was otherwise highly unpalatable if we were 355 sufficiently hungry. 356

357

Of course, food intake can occur for a variety of reasons not associated with motivational states, including such cases as mindless eating (Braude & Stevenson, 2014). However, in the present study the increased intake was specifically associated with the addition of MSG to the soup. Moreover, other recent studies have provided evidence of liking/wanting

362 dissociation. Repeat exposure has been shown to lead to increased consumption of snack foods without subsequent increase of liking in the case of obese and non-obese women 363 364 (Temple, et al., 2009). In the study conducted by Finlayson, King and Blundell (2007) they 365 attempted to develop a methodology in order to dissociate wanting from liking and they 366 also found that more differences in liking were observed when the participants were 367 hungry than when satiated. These studies, plus the data from the present study, strongly indicate that, since motivation and liking can be dissociated, at least potentially, it is crucial 368 369 to measure both phenomena.

370

Lastly, this research provides evidence that repeat exposure to novel foods with glutamate 371 372 could result in increased appetite and consumption in older people. This is important for 373 two reasons. Firstly, in these populations, under-nutrition is of particular concern, so strategies in increase food consumption are needed. Secondly, almost all studies on FFL 374 and FNL have been conducted on young adults or children, and so there has been a need to 375 376 establish that the same processes of learning occur in elderly populations, who may differ not only in their perceptual abilities but also particularly in their responses to novel foods 377 378 (Meiselman, King, & Gillette, 2010). A previous study of a frail elderly population (Essed, 379 van Staveren, Kok, & de Graaf, 2007) over a period of 16 weeks found no effect on rated 380 pleasantness and measured consumption when 0.3% MSG was added to the animal protein 381 part of a cooked meal. It may be that higher levels of MSG are required to trigger a change 382 in consumption, perhaps due to post-ingestive effects. The concentration of MSG used in the present study was substantially higher than in previous studies, therefore, it remains to 383 be determined whether lower levels (0.5 to 1%) are sufficient to trigger post-ingestive 384 385 effects and increase food consumption.

386

In addition, while the volunteers participating in the present study were all older people,
they were all healthy and none were reportedly underweight. It would be of benefit to
further study frailer older adults at risk of under nutrition. For those in elderly care homes
or elderly care wards in hospital, malnutrition is estimated to be 60% in those over 65 (Age
Concern, 2006). Since the glutamate rather than the sodium was responsible for the
increase wanting, the use of other sources of glutamate apart from MSG may be preferable

393 as a supplement to foods in such situations. The results may be generalizable to the use of 394 natural food sources of glutamate and 5' ribonucleotides (Dermiki, et al., 2013; Dermiki, et 395 al., 2014). Thus, our studies have shown that a combinations of natural ingredients can 396 lead to higher umami taste in savoury meals than can be achieved by the addition of MSG alone (Dermiki, et al., 2013). Future work should focus on the longevity of this increase in 397 appetite and consumption after MSG has been removed from the food. If the increase is 398 completely due to FCL then constant addition of MSG may be required in food, since there 399 400 is some evidence that such learning can extinguish (O'Sullivan, Alexander, Ferriday, & Brunstrom, 2010). However if FFL is important, especially in novel foods, then only a small 401 402 number of exposures to MSG may be required, since such learning is thought to be 403 permanent (Baeyens, Crombez, Bergh, & Eelen, 1988).

404

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- 514

- **Table 1:** Design of the experiment (-MSG indicates no MSG added to soup, +MSG indicates
- 516 5% (w/w) MSG added to soup)

GROUP	SESSION 1	SESSIONS 2-7	SESSION 8
	Pre-conditioning session	Conditioning sessions	Post-conditioning session
Group 1	Liking and preference for	This group consumed the	Liking and preference for
	Lemongrass AND Cumin	following soups on	Lemongrass AND Cumin
	Group 1 <i>a</i> :	alternate days:	Group 1a:
	consumption Lemongrass		consumption Lemongrass
	Group 1b: consumption	Lemongrass +MSG,	Group 1b: consumption
	Cumin	Cumin –MSG	Cumin
Group 2	Liking and preference for	This group consumed the	Liking and preference for
	Lemongrass AND Cumin	following soups on	Lemongrass AND Cumin
	Group 2a: consumption	alternate days:	Group 2a: consumption
	Lemongrass		Lemongrass
	Group 2b: consumption	Lemongrass -MSG,	Group 2b: consumption
	Cumin	Cumin +MSG	Cumin

- **Table <u>12</u>**: Questions and anchors used in session 1 and 8 using unstructured 100mm
- 520 line scales.

Question	Anchors
Would you choose to have this particular soup?	No, not at all – Yes, very much
How much could you eat now of this soup?	None at all – A lot
How familiar are you with the flavour of this soup?	Not at all – Very familiar
How hungry are you now?	Not at all – Extremely
How strong is your desire to eat?	Very weak – Very strong
How good is your mood?	Unpleasant – Pleasant
Do you feel satiated (are you full)?	Not at all – Extremely
Could you eat more of this particular soup?	Not at all – Yes, definitely

522

523

524 FIGURE CAPTIONS

- 525 Figure 1: Mean (+/- standard error) change in liking of soup flavours from session 1 to
- 526 session 8 where participants had previously been exposed to the flavour with or without
- 527 MSG (MSG+, MSG- respectively)

528

- 529 Figure 2: Mean (+/- standard error) change in familiarity ratings between session 1 and 8
- 530 for cumin and lemongrass in those previously exposed to the flavour with or without MSG

531 (MSG+, MSG- respectively)

532

- 533 Figure 3: Mean (+/- standard error) ratings of hunger, desire to eat and mood, all
- 534 pre- consumption, at sessions 1 and 8 (Ratings from Questions 4, 5 and 6 in Table <u>12</u>)

- 536 Figure 4: Mean (+/- standard error) consumption of soup in session 1 and session 8 where
- 537 participants were either exposed to the flavour with MSG (MSG+) or exposed to the flavour
- 538 without MSG (MSG-).