



HHS Public Access

Author manuscript

Food Qual Prefer. Author manuscript; available in PMC 2018 January 01.

Published in final edited form as:

Food Qual Prefer. 2017 January ; 55: 26–34. doi:10.1016/j.foodqual.2016.08.003.

Perceptual and Affective Responses to Sampled Capsaicin Differ by Reported Intake

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Abstract

The present study was conducted to a) generate suprathreshold dose-response functions for multiple qualities evoked by capsaicin across a wide range of concentrations, and b) revisit how intensity ratings and liking may differ as a function of self reported intake. Individuals rated eight samples of capsaicin for perceived burn and bitterness, as well as disliking/liking. Measures of reported preference for chili peppers, chili intake frequency, prior experience and personality measures were also assessed. Here, we confirm prior findings showing that burn in the laboratory differs with reported chili intake, with infrequent consumers reporting more burn. We extend these findings by exploring how capsaicin perception varies by reported liking, and measures of variety seeking. We also address the question of whether differences in burn ratings may potentially be an artifact of differential scale usage across groups due to prior experience, and not chronic desensitization, as is typically assumed. By using generalized scaling methods and recalled sensations, we conclude the differences observed here and elsewhere are not likely due to differences in how participants use rating scales.

Keywords

psychophysics; generalized scaling; chemesthesis; capsaicin; memory; chili pepper intake; personality

Introduction

The chili pepper (*Capsicum Solanaceae*) is widely used as an ingredient in many cuisines around the world (Lembeck, 1986), with consumption frequencies that may exceed once per day. Surprisingly, the etiology of chili pepper preference is still not well understood, despite

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Conflict of interest

AAN has no conflicts to declare. JEH has received speaking or consulting fees from corporate clients in the food industry. Additionally, the Sensory Evaluation Center at Penn State routinely conducts taste tests for industrial clients to facilitate experiential learning for undergraduate and graduate students. None of these organizations have had any role in study conception, design or interpretation, or the decision to publish these data.

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several decades of study. Different motives and reasons have been proposed to explain the widespread popularity of chili peppers. Some researchers have speculated their wide use may be due to the biological or pharmacological properties of capsaicin (i.e. anti-bacterial properties, or gustatory sweating) (Abdel-Salam, 2015; Lee, 1954). Other factors that have been identified include culture (Abdel-Salam, 2015), personality traits (Byrnes and Hayes, 2013, 2015, 2016; Rozin, 1980) and gender (or masochism) (Bègue, Bricout, Boudesseul, Shankland et al., 2015; Byrnes and Hayes, 2015; Rozin, 1980; also see Abdel-Salam, 2015). While the relative weight of these reasons as drivers of consumption remains unclear, it is well understood that chilies elicit a burning sensation. This burn, in the mouth and elsewhere on the body, is primarily due to capsaicin (PubChem CID: 1548943) and dihydrocapsaicin (PubChem CID: 107982), the two main capsaicinoids found in chili peppers. These compounds are potent agonists of the heat pain receptor TRPV1.

The term chemesthesis was originally coined to describe touch and pain sensations that are initiated by chemical stimuli (Green, 2016). Examples of oral chemesthesis include tingling, buzzing, cooling, and warming. These sensations are clearly distinct from classical taste sensations (i.e. sweet, sour, salty, bitter, and umami) (Green, 1996). In regard to oral sensation, capsaicin is one of, if not the most, systematically investigated chemesthetic stimulus (e.g., (Green, 1991; Green and Hayes, 2003, 2004; Lawless, Rozin, and Shenker, 1985; Prescott and Stevenson, 1995)).

Despite decades of research investigating the oral burn evoked by capsaicin, response to capsaicin across a wide range of concentrations has not been evaluated in a large group of untrained participants using modern scaling psychophysical methods. Within the psychophysical literature, varied concentrations of capsaicin have been used in many previous studies; unfortunately, it is not possible to extract a single suprathreshold dose response function from these reports due to different delivery systems (liquid solution, cotton swab, filter paper, etc.), type of exposure (sip and spit, sip and swallow, regional application, etc.) and characteristics of the task given to study participants (different scales, or different descriptors such as ‘overall sensation’, ‘irritation’, ‘pepper heat’, ‘burn’, etc.). To identify appropriate doses for use in subsequent experiments in our laboratory, we desired such a function.

One conventional method for estimating perceived heat from chilies is the Scoville Test, which generates an estimate of perceived intensity in units known as Scoville Heat Units (SHU) (Scoville, 1912). However, due to methodological problems with the classical Scoville Test (see (Gillette, Appel, and Lego, 1984; Govindarajan, Shanthi, and Dhanaraj, 1977; Todd, Bensinger, and Biftu, 1977)), efforts have been made to improve the method of estimating the burn produced by chili peppers and capsaicinoids. Because there is a simple ordered relationship between perceived burn and capsaicinoid concentration, instrumental methods using high performance liquid chromatography-mass spectrometry (HPLC-MS) and gas chromatograph-mass spectrometry (GC-MS) to determine the capsaicinoid content in chili peppers and chili pepper containing foods have been developed (e.g. (Gillette et al., 1984; Othman, Ahmed, Habila, and Ghafar, 2011; Peña-Alvarez, Ramírez-Maya, and Alvarado-Suárez, 2009; Todd et al., 1977; Welch, Regalado, Welch, Eckert et al., 2014)). These instrumental methods, which have been validated with human sensory data, are often

used as a standard method to estimate heat from various foods or ingredients. One example is work by Gillette and colleagues (Gillette et al., 1984), who used a trained panel (n=10) with fixed references for 'slight', 'moderate', and 'approaching strong' stimuli to estimate a dose response function for n-vanillyl-n-nonamide, a synthetic capsaicin analog, as well as extracts of ground peppers (chilies); however capsaicin itself was not included in their report. Their report later inspired two standard methods from the American Society for Testing Materials (e.g., ASTM E1083-00 and E1396-90), but again, these methods were based on trained panels using fixed intensity references. Additionally, many prior studies in this area have focused solely on burn, irritation or bite; however, capsaicin is known to elicit bitterness in addition to burning in some individuals (e.g. Green and Hayes, 2003; Nolden, McGeary, and Hayes, 2016). Accordingly, we chose to address this specific gap in the literature by obtaining intensity estimates for multiple qualities across a wide range of capsaicin concentrations, similar to recent work conducted on ethanol (Nolden and Hayes, 2015).

Greater liking or frequency of chili pepper consumption has been associated with reductions in the reported burn of sampled capsaicin (Cowart, 1987; Lawless et al., 1985; Prescott and Stevenson, 1995; Stevenson and Yeomans, 1993). Based on these data, it was widely assumed that regular consumption of chili pepper results in chronic capsaicin desensitization, based on observations that desensitization can occur with exposure in the laboratory and can last over days (reviewed in Hayes, 2016). However, Stevenson and Prescott put forth an alternative explanation that remains untested; namely, observed differences between intake groups may be due to prior experience that influences scale usage rather than true desensitization (Stevenson and Prescott, 1994). This hypothesis suggests that individuals who frequently consume chili peppers have a larger frame of reference outside of the laboratory regarding chili burn compared to those who do not eat chili peppers regularly; thus, when given the same stimuli in the laboratory, frequent consumers use the scale differently, and rate the stimuli as less intense. It remains untested whether differences in capsaicin responses (i.e., perceived burn) across chili pepper intake groups are a result of desensitization due to repeated dietary exposure or merely due to prior context that alters use of the rating scale.

The primary aims of the present study were to a) generate a dose-response curve for capsaicin over a wide concentration range using untrained participants without fixed references, and b) reevaluate associations between perceived burn, bitterness and liking of sample capsaicin and chili pepper consumption groups, and investigate whether this relationship is due to diet-induced desensitization or possible context effects. As secondary aims, we also explored the relationship between sampled capsaicin and a trait-based measure of food adventurousness, operationalized via the VARSEEK scale. Here, individuals evaluated eight samples of capsaicin for their bitterness and burning intensity, along with liking/disliking. They also answered questions regarding chili pepper preferences, intake frequency, prior experience, and personality. This study confirms prior work, and extends current knowledge regarding capsaicin perception.

Materials and Methods

Participants

Adults were recruited from The Pennsylvania State University and surrounding community to participate in two 30 minute visits that were scheduled one week apart at the Sensory Evaluation Center at Penn State. Interested individuals completed a brief online questionnaire to see if they met the following study criteria: not pregnant nor breast feeding, non-smoker, no tongue, cheek or lip piercing, no difficulty swallowing or history of choking, no known taste or smell defect, not taking prescription pain medication, no hyperactive thyroid and no history of chronic pain. Individuals meeting these criteria answered additional questions regarding their liking and intake of foods containing chili peppers. Recruitment was stratified by gender and by liking and intake of chili peppers. These groups included no/low, medium, and high liking, and intake of chili peppers. Participants' self-reported liking of spicy foods, and frequency of intake for a variety of foods containing chili pepper were used to bin participants into groups. At the end of the study, 82 participants (34 men) had completed both sessions, with an average age of 32 (\pm 0.9) years. A majority of participants reported Caucasian ancestry (n=72), with low representation from Asian (n=7) and Black (n=2) individuals; one individual chose not to disclose ancestry. Procedures were IRB approved, informed consent was obtained, and participants were compensated for their time with a small cash payment.

Stimuli and sampling procedure

Sampled stimuli included 0.11, 0.275, 0.55, 1.1, 2.75, 5.5, 11 and 22 ppm natural capsaicin from Sigma-Aldrich (Sigma #360376). This natural product actually contains a mix of capsaicin and dihydrocapsaicin (~65%/~35%, respectively, with small variations from lot to lot), but due to their very similar potency, and Sigma's nominal branding as capsaicin, it will be referred to simply as capsaicin for the remainder of the document. In each visit, participants sampled 4 different concentrations of capsaicin, with each participant rating all 8 concentrations across the two visits. Sample sets were counterbalanced across participants, and presented in increasing and alternating order, with two possible orders (0.11, 0.55, 2.75, and 11; and 0.275, 1.1, 5.5 and 22 ppm). This order was chosen both to limit simple carry-over (by presenting lower concentrations first, as is commonly done in threshold testing), and to reduce the potential for sensitization. Prior data (Green, 1991) indicates greater sensitization occurs following higher concentrations (30 ppm) relative to lower concentrations (3 ppm), so the presentation order used here should minimize sensitization, as the highest stimulus is presented last. Further considerations regarding sensitization are discussed in more detail below.

All stimuli were made from a single stock solution where capsaicin was dissolved in 95% USP grade ethanol. This stock was diluted with reverse osmosis (RO) water to reach the final concentrations, and supplemented with ethanol to standardize all stimuli to equal ethanol concentration of 0.1% (v/v). All stimuli were presented at room temperature in 10mL aliquots in plastic medicine cups labeled with a random three-digit blinding code.

Prior to tasting any stimuli, participants rinsed with room temperature RO water. Participants were instructed to place the entire stimuli in their mouth, swish for 10 seconds, spit it out, and wait 10 seconds before making their ratings. There was a minimum 2.5-minute break between each stimulus, where participants were asked to rinse with water until they no longer perceived any sensation in their mouth; this break was enforced via software. Repeated exposure to capsaicin within a single session can result in sensitization or desensitization depending on the interstimulus interval (ISI) that is used: ISIs of 1.5 minutes or less result in sensitization while ISIs of ~3.5 to 5.5 minutes result in desensitization (Green, 1989, 1991; Green and Rentmeister-Bryant, 1998). Here, an initial ISI of 2.5 minutes was used, as prior work with whole mouth stimuli suggests this interval does not result in sensitization or desensitization (Nasrawi and Pangborn 1990). After the 2.5 minute break, participants rated the intensity of any sensation they were still experiencing (i.e., any residual burn). If ratings were greater than 1.4 ('barely detectable'), then participants waited another minute before continuing on to the next stimuli. Participants were not made aware of the minimum rating required to skip over the additional minute. Therefore, it is very unlikely participants clicked 'no sensation' to avoid the additional wait time and finish sooner. This step was included as a secondary means to limit any carryover effects that might inflate subsequent ratings. However, it should be noted that this choice is also a potential limitation, as changing the ISI might alter the relative occurrence of sensitization versus desensitization. In summary, we cannot know with certainty whether participants might have experienced sensitization or desensitization for subsequent capsaicin stimuli within a session; however, we believe the design tradeoffs made here represent a reasonable attempt to efficiently manage competing concerns about sensitization, desensitization and carry-over effects.

Psychophysical Scaling and Practice/warm-up for scales

Participants rated the burning and bitterness of capsaicin and other non-sample related items on a horizontal general labeled magnitude scale (gLMS). On the left side, the scale is labeled at 0 with "NS" (no sensation) and on the right labeled 'the strongest imaginable sensation of any kind' at 100. Labels were placed at 1.4, 6, 17, 35 and 51 ('BD'; barely detectable, 'weak', 'moderate', 'strong' and 'very strong'; respectively). Participants were instructed to not let whether or not they liked or disliked each stimuli influence his or her ratings for intensity. They indicated their affective responses on a generalized bipolar hedonic scale (e.g. Byrnes and Hayes, 2013) for sampled capsaicin and other non-sampled items (see below). This scale ranges from 'strongest disliking of any kind' at -100 on the left to 'the strongest liking of any kind' at 100 on the right, with 'neutral' at 0 at the center point.

Prior to rating any samples, participants were given written instructions on the use of the gLMS (Snyder, Prescott, and Bartoshuk, 2006) and rated 15 remembered or imagined sensations (e.g. Hayes, Allen, and Bennett, 2013). Similarly, participants received instructions for the use of the hedonic scale, followed by a practice session where participants rated 8 items. These items, both for the gLMS and generalized hedonic scale orientation included food and non-food items in order to emphasize that the scales are to be used in context to all sensations.

Measures of liking and intake of foods containing chili peppers

Affective responses were also collected for 38 items that were not tasted in the laboratory. These items included 15 spicy foods, 14 non-spicy foods and 9 non-food related items. Participants also rated several remembered intensities on a gLMS, including the intensity for the spiciest meal or food they could remember experiencing, and the remembered burn from commercially available hot, medium and mild salsa. They reported intake frequency for a variety of foods, which included questions related to chili peppers and chili pepper containing foods as well as non-spicy foods that were included as controls. Specific wording was as follows: 'How often do you consume ... [hot sauce; chili peppers; habanero peppers; red pepper flakes; spice mix containing chilies; fried foods; sweet snacks (candy, chocolate, baked goods); salty snacks (pretzels, potato chips, popcorn); ice cream or frozen yogurt]?' Participants selected either: never, less than once/month, 1–3 times/month, 1–2/week, 3–4/week, 5–6/week, once/day or 2 or more times/day. Stated preference of spicy food was measured by asking participants to report their preferred heat/spice level when ordering food at a restaurant by selecting either: 'I avoid eating spicy foods', 'mild', 'medium', 'spicy' and 'very spicy'. Participants' motives for either consuming or avoiding consuming chili peppers were also obtained (Supplemental Figure 1). Lastly, participants were asked to rate 'How much do you like the burn of chili pepper in your food' and 'How much do you like the taste of chili pepper in your food' on a 7-point hedonic scale, ranging from dislike extremely, to like extremely.

Personality measures

To measure participants desire to seek a variety of foods, they endorsed a subset of statements from the Variety Seeking Tendency Scale (VARSEEK). Participants indicated how much they agreed, or disagreed with each statement (completely disagree, disagree, neither disagree nor agree, agree, or completely agree), and these were coded with values from 0 to 4. Answers were summed to give an overall score of variety seeking, to estimate participants' willingness to try new or unusual foods. A total of 6 statements (of 8) were provided, so potential scores here ranged from 0 to 24, allowing us to differentiate between non-adventurous and very adventurous consumers.

Statistical analysis

Prior to subsequent analyses, the self reported food intake was annualized to express estimated consumption frequency on a yearly basis (e.g. less than once per month = 6, one to three times per month = 24, one to two times per week = 62.4, etc.). Sex was coded as 0 for women and 1 for men.

All analyses were conducted using SAS 9.2 (Cary, NC). Analysis of variance (ANOVA) was conducted on annualized intake frequency data to determine if intake significantly differed by sex. A Cochran-Mantel-Haenszel (CMH) test was conducted to determine the relationship between reported preference of spice/heat level (mild, medium, spicy) and sex. Pearson correlation coefficients were conducted via *proc corr* and regression was conducted via *proc reg*. Repeated measures ANOVA were conducted via *proc mixed* to determine associations between groups (e.g. intake and spice preference groups) with bitterness, burning and liking/disliking ratings of sampled capsaicin. Group differences at each concentration were tested

using the ‘slice’ option in SAS. Comparison of slopes from regression models was evaluated between non-normalized and normalized data using *proc glm*.

Results

Variability in liking and intake of spicy foods among participants

Participants were asked a series of questions regarding intake frequency of chili peppers and foods containing chili pepper, in addition to reporting their stated preference of spiciness/hotness level in their food, and how much they like the burn and taste of chili peppers. Intake frequencies were annualized for chili peppers, sweet snacks, salty snacks, fried foods, and ice cream/frozen yogurt; these are summarized in Table 1. There was no evidence of a significant difference between men and women for intake frequency for any of the food items (p 's>0.05), and the VARSEEK scores (Table 1) did not significantly differ between men and women ($t=1.17$; $p=0.6$). However, preferred spice level (no heat/avoid, mild, medium, spicy, very spicy) significantly differed by gender (CMH $\chi^2=12.6$; $p=0.01$), with men reporting preferring a higher spice level than women.

Participants were also asked to answer questions regarding why they do, or alternatively, why they do not consume spicy foods (Supplemental Table 1). Overall, participants reported they ate spicy foods because they ‘liked the way it tasted’ ($n=49$), followed by ‘liking the burn’ ($n=11$), for it’s ‘health benefits’ ($n=3$) and because ‘it is what their family eats’ ($n=5$). Conversely, of participants reporting not liking spicy foods, reasons given were that they: ‘are too hot’ ($n=23$), ‘don’t feel well when they eat them’ ($n=6$), ‘don’t like the taste’ ($n=3$), or other reasons ($n=2$).

In the questionnaire, participants rated how much they liked the burn of chili peppers in their food, followed by how much they liked the taste of chili peppers in their food. Reported liking of both burn and taste were then used in separate regression models to predict annualized intake frequency of chili peppers. Chili pepper intake was estimated by taking the sum of annualized intake of hot sauce, habanero peppers, chili peppers, red pepper flakes, and spice mix containing chilies. This summed measure of chili pepper intake was significantly correlated with both the reported liking of burn ($r=0.37$; $p=0.0005$) and ‘taste’ ($r=0.37$; $p=0.0005$) of chili peppers, consistent with prior work (Byrnes and Hayes 2015, 2016). A stepwise regression with both variables (liking of taste and burn) resulted in ‘taste’ ceasing to be a significant predictor of intake when burn was included in the model, suggesting these are largely redundant measures. Reported liking of the burn of chili peppers explained 14.3% of the variability in annual intake frequency of chili peppers.

Psychophysical response to sampled capsaicin

Participants rated both burn and bitterness for all concentrations of capsaicin. As expected, intensity ratings for each increased with increasing concentrations. As expected, burn response increased at a faster rate than bitterness (Figure 1, top). As shown in the top of Figure 1, separate linear models were fit to model the mean perceived intensity ratings after \log_{10} transforming the capsaicin concentrations: Burn = $23+21.5$ [log ppm] ($R^2=0.99$) and

Bitter = $7.9 + 3.35 [\log \text{ ppm}]$ ($R^2=0.91$); both models had slopes that were significantly different from zero.

As anticipated, hedonic ratings for sampled capsaicin decreased as concentrations increased, as shown in the bottom panel of Figure 1. At the lowest concentration given, 0.11 ppm, capsaicin was rated just above neutral, suggesting some participants found the sample pleasant. In fact, a sizeable fraction of participants rated at least some of the capsaicin concentrations between 'neutral' and 'strongest liking of any kind'. Frequency counts for participants rating above neutral were 27, 29, 42, 39, 24, 16, 15 and 14, for 0.11, 0.275, 0.55, 1.1, 2.75, 5.5, 11 and 22 ppm capsaicin, respectively. While none of these counts exceed the significance threshold for a binomial preference test (51 of 82, at $\alpha = 0.05$), this highlights that some participants liked the burn, despite the overall downward trend seen in Figure 1.

Notably, the intensity ratings of burn and bitterness did not show a large change in intensity between 11 and 22 ppm, yet mean hedonic ratings for 11 and 22 ppm continued to drop from -29.4 ± 4.6 to -38.6 ± 4.7 . To determine if bitterness and burning responses were associated with liking ratings, we explored correlations between each sensation across the different concentrations of capsaicin. Bitterness ratings were significantly associated with liking at the lowest capsaicin concentration ($r = -0.34$; $p=0.0016$), but bitterness was not correlated with liking for any other concentrations ($p's > 0.4$). Burning response was significantly correlated with liking for 2.75, 5.5, and 11 ppm ($r = -0.32, -0.36$ and -0.35 ; $p's < 0.003$, respectively). No significant correlations between burn response and liking were observed for any other concentrations ($p's > 0.07$).

Self-reported intake of chili peppers is associated with capsaicin perception

Based on the summed measure of chili pepper intake (using the various annualized intake measures, as described above), participants were segmented into low and high consumers of chili peppers using on a median split of 103.2 times per year. The mean burn ratings of sampled capsaicin in these two groups are shown in Figure 2 (top). Separate repeated measures ANOVAs were used to determine whether intake group (high versus low) associated with the perceived burn, bitterness and disliking/liking of the sampled capsaicin.

For burn, concentration [$F(7,560)=178.0$; $p<0.0001$], intake group [$F(1,80)=9.2$; $p=0.003$] and the interaction (concentration by intake group) [$F(7,560)=3.5$; $p=0.001$] were significant. Significant differences between intake groups were observed for the 1.1, 5.5, 11, and 22 ppm capsaicin samples (Figure 2). For bitterness, there was a significant main effect of concentration [$F(7,560)=6.35$; $p<0.0001$], but bitterness did not differ by intake group [$F(1,80)=1.68$ $p=0.2$], and there was no evidence of an interaction [$F(7,560)=1.19$; $p=0.3$].

Capsaicin concentration was significantly associated with hedonic ratings of sampled capsaicin [$F(7,560)=24.0$; $p<0.0001$], as shown in Figure 2 (bottom). Also, the main effect of intake group [$F(1,80)=4.9$; $p=0.03$] and the intake group by concentration interaction [$F(7,560)=2.9$; $p=0.005$], were significant for the disliking/liking ratings. Decomposition of significant effects via the slice option in SAS revealed significant differences at 2.75, 5.5, 11, and 22 ppm capsaicin, as shown in Figure 2 (bottom).

Self-reported preference for spice level is associated with capsaicin perception

Participants were asked to state their preference for spice/heat in their food using one of five categories: 'no heat/avoid', 'mild', 'medium', 'spicy', 'very spicy'. Given endorsements of 4, 23, 27, 20, and 8, respectively, we combined the bottom two categories, and top two categories, resulting in 3 groups of roughly equal size for further analysis (n's of 27, 27, and 28, respectively). Figure 3 summarizes the mean burn ratings of sampled capsaicin segmented by preferred spice level. As above, separate repeated measures ANOVA models were created to test effects of capsaicin concentrations and preferred spice level on burn, bitterness and liking/disliking ratings. In all three models, concentration was significantly associated with liking ($p < 0.0001$), as would be expected. Preferred spice/heat level was significantly associated with burn ratings [$F(2,79)=3.8$; $p=0.02$], with the top group (spicy and very spicy) reporting the lowest burn (Figure 4). There was no evidence of an interaction between preference group by concentration [$F(14,553)=1.2$; $p=0.2$], suggesting that the effect of group was uniform over the entire concentration range. Given the significant main effect, we compared differences at each concentration, and significant differences were observed at 1.1, 5.5, 11 and 22 ppm, with participants in the spicy/very spicy group ($n=28$) reporting lower burn compared to the other two groups. Notably, the no heat/avoid/mild group ($n=27$) and the medium group ($n=27$) did not differ from each other. We did not observe any relationship between preferred spicy/heat level and rated bitterness from sampled capsaicin [$F(14,553)$; $p=0.9$].

As would be expected, stated preference for spice/heat was associated with hedonic ratings of sampled capsaicin [$F(2,79)=11.3$; $p < 0.0001$], and there was evidence of a preference level by concentration interaction [$F(14,553)=2.2$; $p=0.007$], indicating the magnitude of the differences in liking between the groups changed in size across concentration. This can be seen in the bottom of Figure 3, where the gap between groups increases as concentration increases, and significant differences were observed at the higher capsaicin concentrations.

Variability in variety seeking measures (VARSEEK) is associated with capsaicin perception, liking and intake

Participants indicated their agreement/disagreement with 6 statements from the VARSEEK (see methods). These endorsements were used to generate a score indicating a participant's willingness to try new foods and overall adventurousness in their diet. From these scores, participants were placed into either a non-adventurous or adventurous group using a median split of the overall sum (median=17). Once again, repeated measures ANOVA models were used across all sampled capsaicin concentrations to determine if group (non-adventurous versus adventurous) associated with burning, bitterness and liking/disliking ratings of sampled capsaicin. Unlike the intake group data above, we did not see any effect of food adventurousness (i.e., personality) on burn [$F(1,80)=0.75$; $p=0.3$]; nor, was there an association between food adventurousness and bitterness [$F(1,80)=0.4$; $p=0.5$]. In contrast, hedonic ratings of sampled capsaicin associated with food adventurousness group [$F(1,80)=3.7$; $p=0.05$], and the group by concentration interaction was significant [$F(7,560)=2.7$; $p=0.007$]. Further analysis revealed the group differences were larger at higher concentrations, as shown in Figure 4.

We also tested food adventurousness/variety seeking against reported intake of chili peppers and state preference for spice levels and reported intake of chili peppers. There was a trend of reported frequency of combined chili pepper intake to be associated with individual VARSEEK scores [$F(1,80)=3.2$; $p=0.07$]. Similarly, reported spice preference was significantly associated with variety seeking group ($X^2(2,n=82)=9.9$; $p=0.007$), with greater frequency of adventurous consumers stating a preference for spicy or very spicy heat level in their food.

Investigating context effects: exploring associations between capsaicin perception and chili pepper intake with prior experiences

Previously, Stevenson and Prescott speculated that participants who consume chili peppers frequently potentially have a broader context of burn intensity compared to individuals whom choose to avoid chili peppers. In an attempt to determine whether or not participant's prior experience influenced his or her ratings, we also asked participants to rate the remembered intensity for 'commercially available... [mild], [medium] and [hot] salsa', as well as the 'spiciest meal they have ever eaten' on a gLMS. Mean ratings of remembered intensity of the three salsas and spiciest ever are shown in Figure 5, segmented by the same intake groups as used in Figure 2. In repeated measures ANOVA on ratings of remembered intensity of mild, medium and hot salsa, the main effect of intake group was significant [$F(1,80)=8.6$; $p=0.004$], with the low intake group reporting more burn (Figure 5). Also, the salsa type by intake group interaction was significant [$F(2,160)=3.1$; $p=0.04$]. In individual pairwise comparisons for each salsa level, significant differences were observed for mild and hot salsa ($p=0.02$ and 0.0003 , respectively), with medium salsa showing a similar trend ($p=0.054$). Figure 5 also shows the group means for 'spiciest meal ever experienced'; these data were analyzed separately via an independent sample t-test, and in sharp contrast to the salsa data, there was no evidence of an effect of group ($t=1.3$; $p=0.3$).

To further test if prior experience results in a contextual effect that alters scale usage and thus ratings of burn in a laboratory setting, ratings of spiciest ever were used to normalize the burn ratings of sampled capsaicin. These normalized ratings were calculated by dividing the rating of spiciest ever by the mean rating of spiciest ever across all participants (mean=66.33). This generated a normalization factor for each participant. Each individual's rating for each capsaicin sample was then multiplied by this factor; thus, their ratings for capsaicin sampled in the laboratory were now expressed relative to the spiciest meal they had ever experienced.

Linear regression (via *proc glm*) was used to test if the slopes for the functions predicting non-normalized and normalized burn ratings of sampled capsaicin were significantly different between intake groups. A significant difference in slopes would suggest prior experience influences ratings of burn collected in a laboratory setting on the gLMS, suggesting greater intake frequency gives individuals a broader context (i.e., a stronger internal referent) against which to compare stimuli given in the laboratory. The burn from sampled capsaicin differed by group even after normalization ($F(1,1280)=43.0$; $p<0.0001$), with the low intake group reporting greater burn, which is wholly consistent with the non-normalized data reported above. However, within the normalized data, the burn by group

interaction no longer reached the criteria for significance [$F(7,1280)=1.8$; $p=0.08$]. Nonetheless, this analysis suggests group differences persist even when ratings are adjusted for prior experience.

Discussion

Prior literature exploring capsaicin response lacked details on responses across a broad range of concentrations. Methods used in prior studies have also been inconsistent regarding the specific qualities they measures and used different methods for presenting stimuli, making it difficult to extrapolate across studies. Also, many previous reports focused exclusively on ‘overall intensity’ or ‘irritation’; however, capsaicin can also elicit bitterness in some individuals (Green and Hayes, 2003, 2004). Finally, prior studies have used a wide assortment of scaling methods, making it difficult to make comparisons and draw generalized conclusions about the psychophysical function for capsaicin. Therefore, our first aim here was to generate such quality specific functions for capsaicin over a wide range of concentrations.

Here, dose response functions were generated for both bitter and burn ratings. A priori, we expected to use a power function to do so, given our use of a gLMS to collect these data. However, when a log-log plot was created, it was clearly apparent that a straight line was not a good fit for modeling burn as a function of capsaicin concentration. Thus, a logarithmic function was used instead of a power function, as a straight line fit the data very well in a semi-log plot (see Figure 1). The concentration required for a specific desired level of burn can be readily calculated by rearranging the equation from Figure 1 as follows:

$$[\log_{10} \text{ppm}] = \frac{2(\text{Burn} - 23)}{43}$$

Thus, to achieve an average burn of moderate (**17** on a gLMS), the logged concentration of capsaicin required would be:

$$\frac{2(17 - 23)}{43} = -0.279 \log_{10} \text{ppm}$$

Taking the antilog of -0.279 gives a final concentration of 0.53 ppm in water needed to have a moderate burn. This approach can be used for any desired intensity.

In terms of perceived quality, capsaicin is predominately perceived as burning, and this increases as a function of concentration. At lower concentrations (<0.275 ppm), bitter and burn intensities are similar (~weak), however; these sensations quickly diverge, with bitterness increasing at a much lower rate, compared to burn. Due to these differences in slope for bitterness and burn response functions, we conclude that participants are able to distinguish between bitterness and burn perceptions, despite a lack of prior training with exemplars for these qualities. We also believe that the bitterness ratings are not merely a matter of ‘hedonic dumping’ – wherein the participant rate a stimulus as bitter merely because it is aversive – as we also gave individuals the opportunity to rate their affective

responses to the same stimuli. Also, we observed that bitterness intensity was not significantly associated with liking/disliking ratings, with the exception of the lowest capsaicin concentration (0.11 ppm). Conversely, burn was significantly associated with hedonic ratings for three concentrations (2.75, 5.5 and 11 ppm). This suggests that burn, rather than bitterness, is the primary driver of liking for sampled capsaicin.

A variety of personality measures like sensation seeking and sensitivity to reward have been previously associated with preference and intake of chili peppers (e.g. (Byrnes and Hayes, 2013, 2015, 2016; Rozin, 1980)). To extend these findings, we included 6 items from the VARSEEK questionnaire to estimate willingness to try new foods, seek a varied diet and food-adventurousness. We found individuals with a greater willingness to try new foods were more likely to state a preference for higher degree of spiciness in their food, and these individuals also reported less disliking for sampled capsaicin. While this specific effect for VARSEEK has not been reported previously, it reinforces a substantial and growing body of evidence that multiple personality traits influence liking and intake of chili containing foods.

Present data confirm previous findings of an association between ratings of sampled capsaicin and frequency of chili pepper intake, and we show here that such effects occur across a very wide range of concentrations (0.11 to 22 ppm). Here, greater intake was associated with an increased liking (i.e., Byrnes and Hayes, 2013 2015) and decreased burn perception of capsaicin (i.e., Lawless et al., 1985) compared to those consuming chili pepper less frequently. We expand on prior findings by showing intake also associates with remembered intensity of commercially available salsas (mild, medium and hot), suggesting effects of intake not only associate with the burn from sampled capsaicin within the laboratory, but also with chili pepper containing products consumed outside of the laboratory. Critical to this conclusion is the prior observation that judgments of intensity made from memory are similar to those made from sampled stimuli, for both tastants and chemesthetic stimuli (see Stevenson and Prescott, 1997).

Here, stated preference for spice level in food also associated with burn and liking/disliking of sampled capsaicin. Notably, for intensity, the 'avoid/mild' and 'medium' preference groups were more similar to each other, while differing from the 'spicy/very spicy' group. This implies that higher levels of exposure are required to cause in diet-induced capsaicin desensitization, whereas the amount of exposure experienced by the medium spice level group was not efficient to reduce burn relative to the 'avoid/mild' group. Accordingly, the minimal amount of exposure required to induce desensitization warrants further exploration in future work.

To explicitly test Stevenson and Prescott's (1994) hypothesis of prior experience/scale usage, we used two approaches. First, we compared ratings for the spiciest meal ever consumed, and the means did not differ between the groups, suggesting that the groups did not differ in their exposure to very intense stimuli outside the laboratory. However, this interpretation relies on two assumptions that bear further discussion. A gLMS was used here to collect intensity ratings; this scale was developed to make ratings generalized to all possible sensations, and not just burn or oral sensations. While all scales may suffer from context effects, prior work shows the Labeled Magnitude Scale (LMS) anchored to 'oral

sensation' exhibits smaller context effects than category scales or the LMS when it is anchored to 'taste' (Lawless, Horne, and Speirs, 2000). Although the robustness of the gLMS to context effects has never been formally tested, it does not seem unreasonable to assume that the generalization process should help minimize such effects, especially given the improvements seen for the non-generalized LMS when the top anchor is switched from 'taste' to 'oral sensations'. Also, our conclusion assumes individuals can accurately recall intensities from memory: prior work suggests this is the case (see Stevenson and Prescott, 1997). For additional confirmation, we tested the Stevenson and Prescott (1994) hypothesis using a second method: by normalizing burn ratings for sampled capsaicin obtained in the laboratory against that individual's remembered rating of the spiciest meal ever consumed. When ratings in the laboratory were expressed as fraction of the most intense meal that person had ever consumed, those who eat capsaicin-containing foods more frequently had lower burn ratings for sampled capsaicin. Taken together, these two analyses suggest that prior experience does not associate with chili pepper intake or perceived burn ratings of sampled capsaicin. In turn, this implies that context effects potentially induced by differences in past experiences with chili peppers and spicy foods do not affect laboratory ratings, as speculated previously (Stevenson and Prescott, 1994).

Limitations

Here, 8 capsaicin concentrations were presented across 2 days in ascending alternating order to determine suprathreshold psychophysical functions for multiple qualities evoked by capsaicin. A minimum wait of 2.5 minutes between stimuli was used, and participants were encouraged to wait longer if any sensation(s) remained. While logistically efficient, this approach has some potential limitations that should be acknowledged. This design was intended to limit i) simple carryover, and ii) sensitization, which has been observed when a constant concentration is presented repeatedly within a test session. Critically, each of these factors may inflate ratings of subsequent stimuli. Previously, Green (1991) observed more sensitization following higher concentrations compared to lower concentrations (30 ppm versus 3ppm), so use of an ascending series should help mitigate potential sensitization. However, capsaicin may also cause desensitization when the ISI is longer (Green, 1991). Thus, allowing too much time between stimuli may instead depress subsequent ratings if the break between stimuli is too long. Nasrawi and Pangborn (1990) used an ISI of 2.5 min for a series of five whole mouth solutions, finding that, on average, this did not result in sensitization or desensitization. Here, we used the same ISI with whole mouth stimuli, with one additional modification: we required participants to rate the residual burn at the end of the ISI near zero before they could proceed to the next sample. This was done to minimize carryover that might inflate subsequent ratings. However, this choice may have increased the potential for desensitization, due to a longer ISI, which would in turn serve to depress ratings. Given the potential for carryover, sensitization and desensitization, there is no way to perfectly optimize the ISI. An alternative would be test a single stimulus on 8 different days, but this choice would increase participant dropout, be logistically difficult, and likely cause learning/practice effects. Accordingly, while acknowledging that we are unable to make definitive conclusions regarding the extent of potential sensitization or desensitization in our participants, we also believe the approach used here reflects a reasonable compromise

that balances competing concerns. Even if one believes our design may have systematically biased the reported psychophysical functions upward or downward (which we do not believe to be the case, for the reasons given above), any such deviation would only alter the generalizability of the reported functions, and not influence the conclusions regarding users and non-users, or personality. As with any dose-response study, the functions may differ in other populations or with other methods, and should be treated more as a guideline and not as a lawful rule.

Conclusions

In summary, this study expands our current knowledge of perceptual and affective responses to capsaicin. As expected, reported intake of chili peppers was associated with perceived burn of sampled capsaicin, consistent with previous findings (e.g. Lawless et al., 1985; Prescott and Stevenson, 1995). Specifically, individuals who reported consuming chili peppers more frequently reported lower burn ratings from sampled stimuli compared to individuals who reported consuming chili pepper less frequently. Two theories have been proposed previously to explain the observed difference in burn ratings between intake groups. The predominant explanation given in the literature is capsaicin desensitization induced by dietary exposure. However, an alternative explanation has been raised: specifically, differences in prior experience between intake groups may influence how individuals use the scale in the laboratory (Stevenson and Prescott, 1994). That is, the observed differences may not be perceptual differences per se, but differential judgment processes in transforming the percept into a response on a scale. By investigating the recalled intensity spiciest meal ever experienced, we have shown – using two different analyses – that prior experience does not appear to explain differences in burn ratings of sampled capsaicin between the two intake groups. This suggests that past and present findings are indeed due to capsaicin desensitization as a result of regular chili pepper intake, and not merely a scaling artifact. More pragmatically, present data also provide quality-specific response functions that can be used to guide selection of appropriate capsaicin concentrations in future psychophysical studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was supported in part by the National Institutes of Health via an institutional Clinical and Translational Sciences TL1 Predoctoral Fellowship from the National Center for Advancing Translational Sciences [TR000125], and a Ruth L. Kirschstein National Research Service Award (NRSA) F31 Predoctoral Fellowship from the National Institute of Deafness and Communication Disorders [F31DC01465] to AAN. Additional support was provided by United States Department of Agriculture Hatch Project [PEN04565] funds, and discretionary funds from the Pennsylvania State University.

The authors would like to thank Cordelia Running, Ryan Elias, Shane McDonald, and David Bolliet for thoughtful discussion of this work, and Gabrielle Lenart for helping to collect these data. We also thank the staff of the Sensory Evaluation Center at Penn State for their assistance in executing the study, as well as our participants for their time and participation.

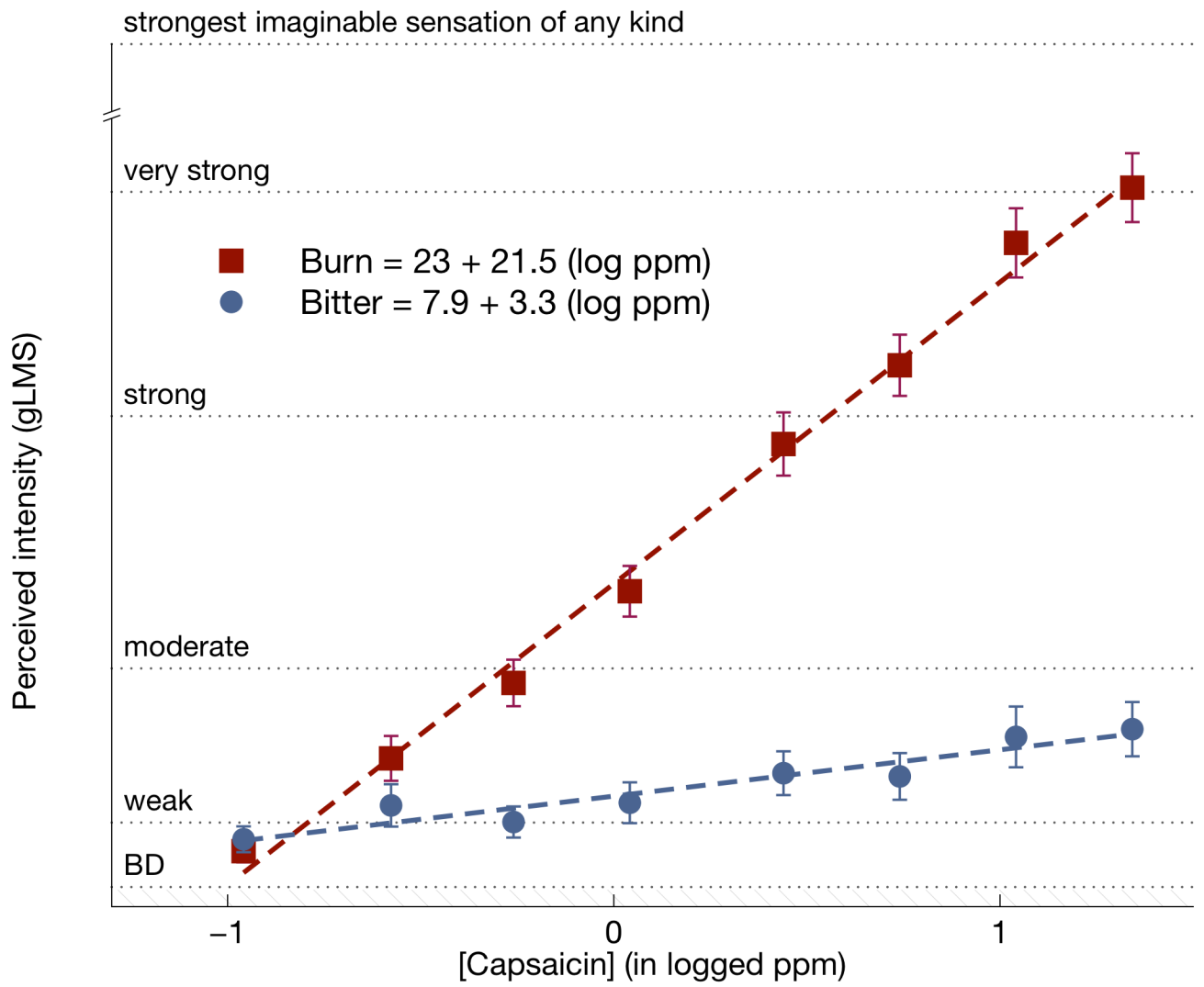
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Highlights

- Burn from sampled capsaicin and recalled sensations were obtained
- As expected, burn and liking differed by self reported chili intake
- When controlling for prior experience, differences persisted across groups
- Burn and liking also differed by variety seeking and preferred heat level
- A suprathreshold dose response function for capsaicin is provided



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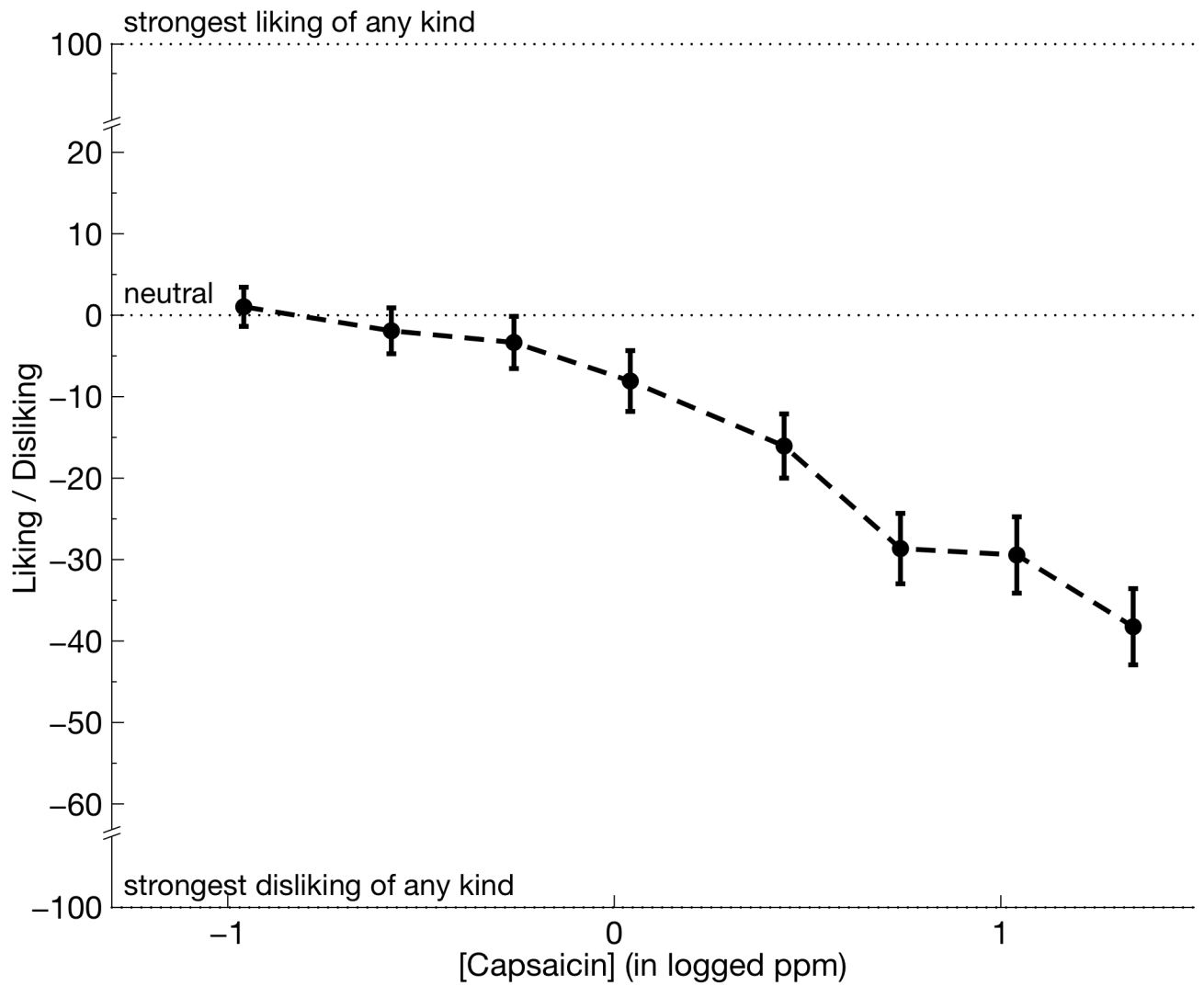
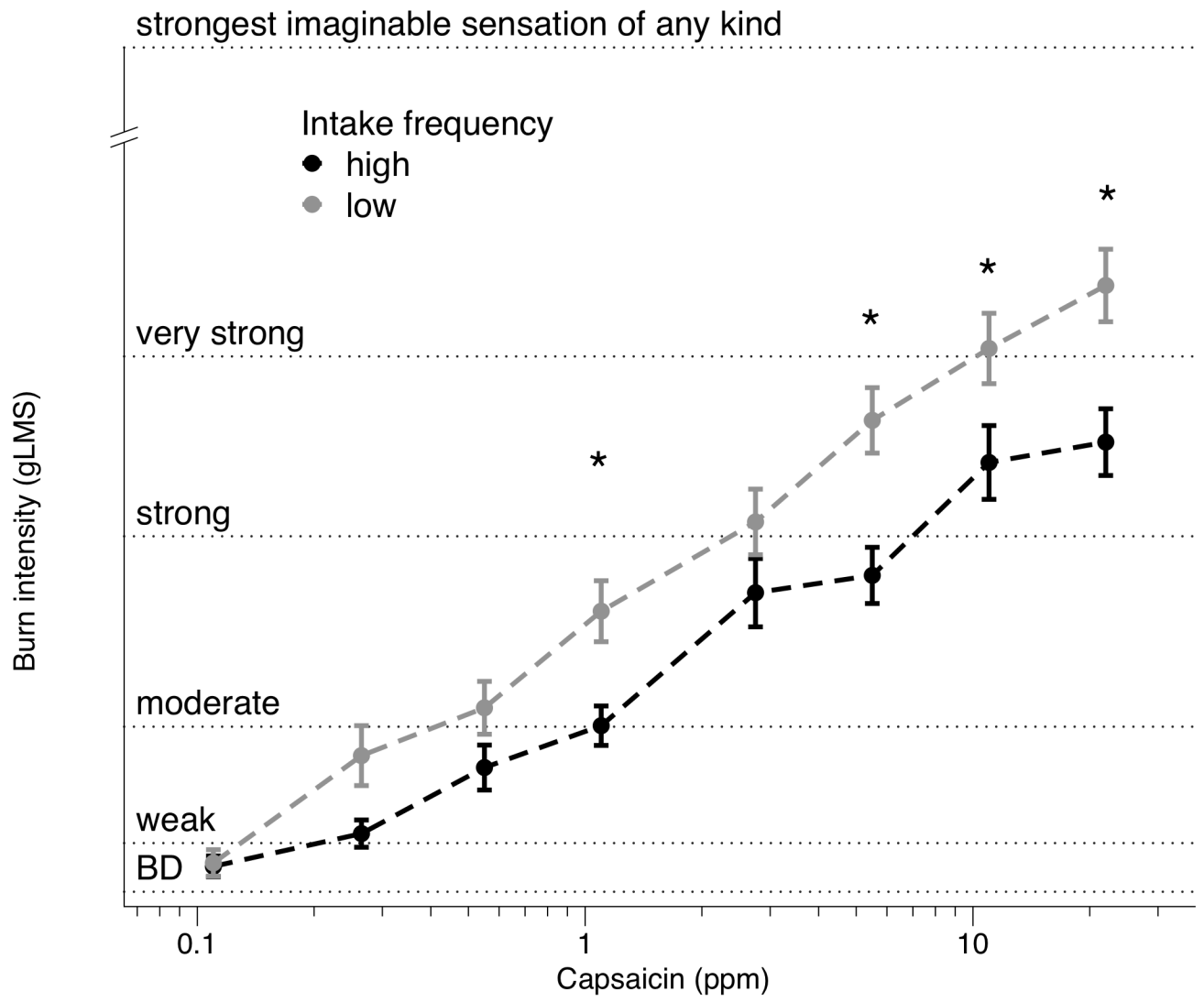


Figure 1.

(A) Group means and standard errors for burn and bitter ratings are shown for 8 different concentrations of capsaicin (0.11, 0.275, 0.55, 1.1, 2.75, 5.5, 11 and 22 ppm); data were collected over 2 days, with participants receiving 4 stimuli per day. The x and y axes are plotted as linear scales, but the capsaicin concentrations (in ppm) were \log_{10} transformed prior to plotting to facilitate fitting a simple linear equation, resulting in a semi-log plot. (B) Same as above, but showing liking/disliking ratings are shown for the same capsaicin samples; error bars indicate standard errors.



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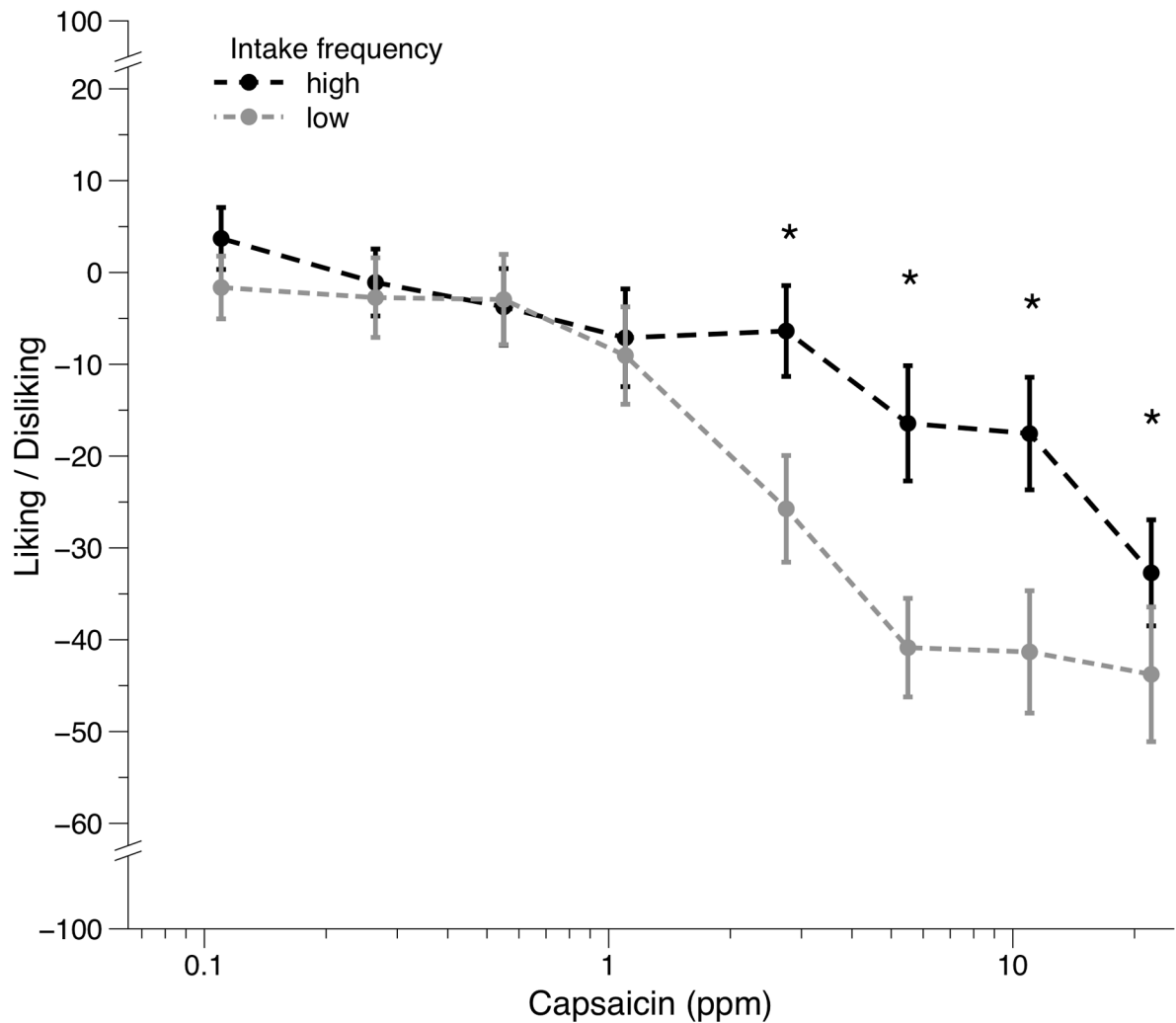
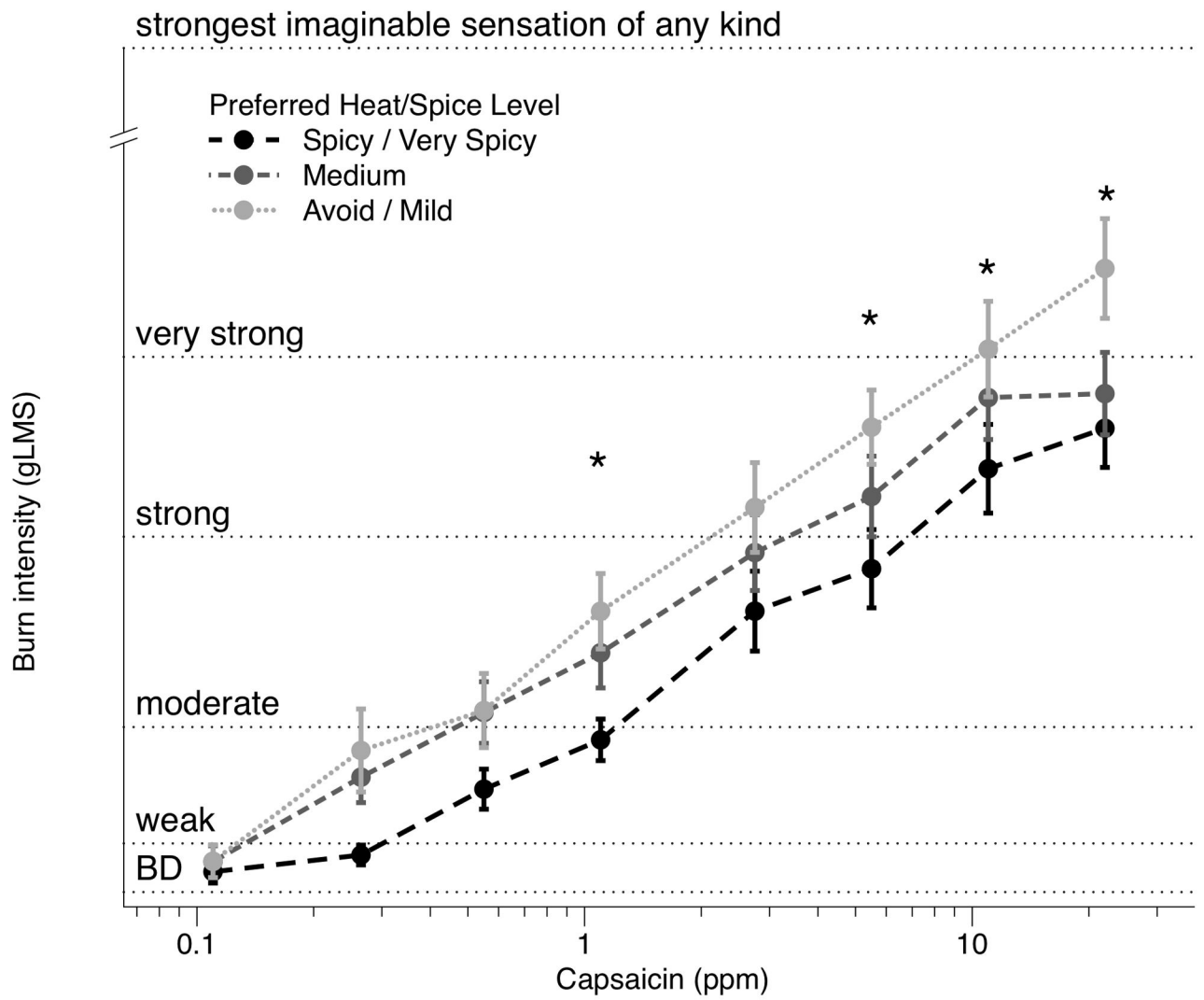


Figure 2.

(A) Same data as in Figure 1, but segmented by frequency of chili pepper consumption. The groups were formed using a median split of the summed annualized frequency for 5 different questions regarding pepper intake (hot sauce, chili peppers, habanero peppers, red pepper flakes, and spice mix containing chilies). (B) Group means and standard errors for hedonic ratings, segmented by intake frequency. See text for details of the ANOVA main effects and interactions. Stars indicate significant differences ($p < 0.05$) between groups at that concentration.



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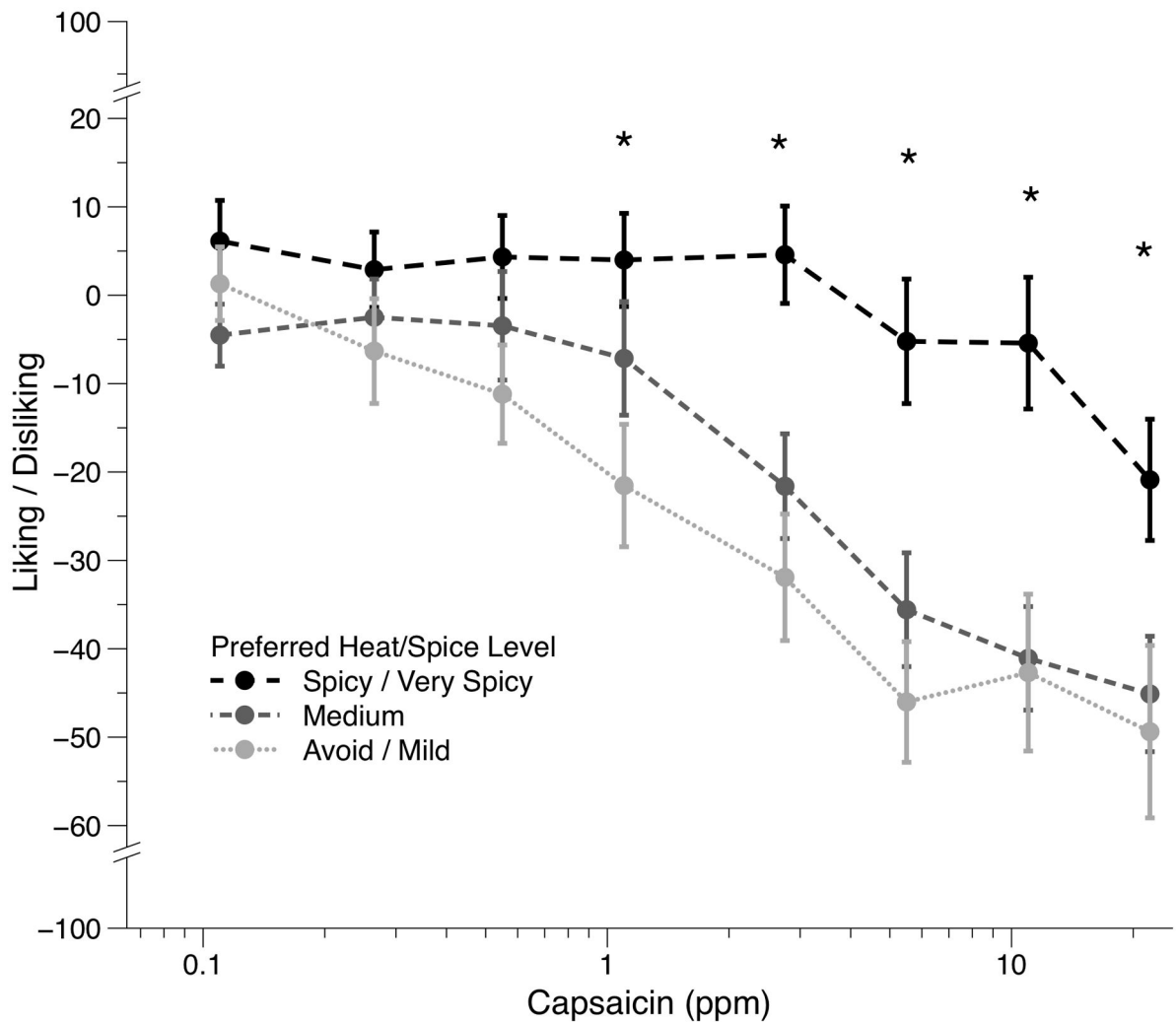


Figure 3. (A) Same data as in Figure 1, but segmented by stated (declared) preference for heat/spice level when ordering food at a restaurant: avoid/mild, medium, and spicy/very spicy. (B) Group means and standard errors for hedonic ratings, segmented by stated preference frequency. See text for details of the ANOVA main effects and interactions. Stars indicate significant differences ($p < 0.05$) between groups at that concentration.

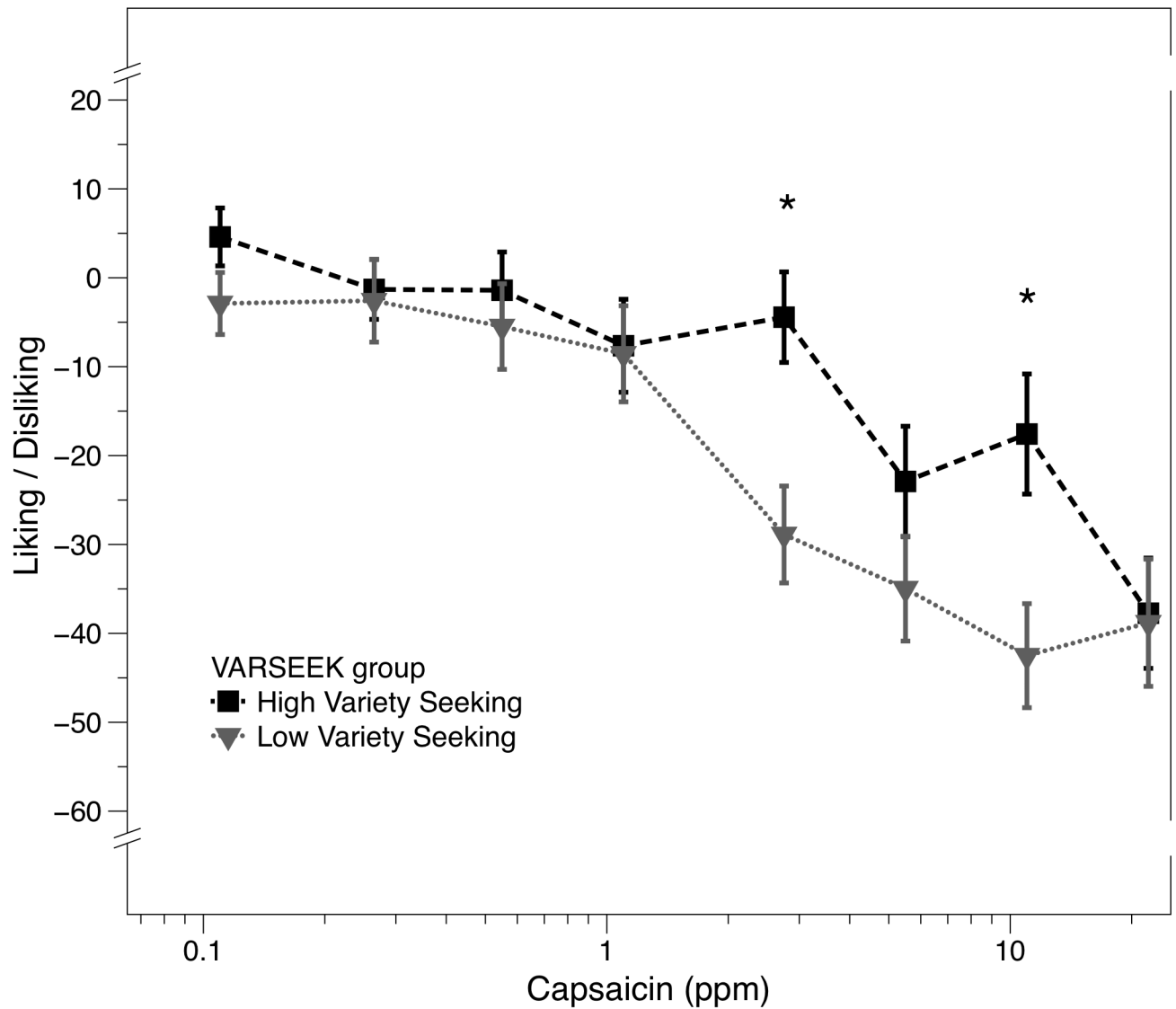


Figure 4. Group means and standard errors for hedonic ratings, segmented by VARSEEK scores. Groups were generated via median split of VARSEEK scores, a measure of food variety seeking and food adventurousness. See text for details of the ANOVA main effects and interactions. Stars indicate significant differences ($p < 0.05$) between groups at that concentration.

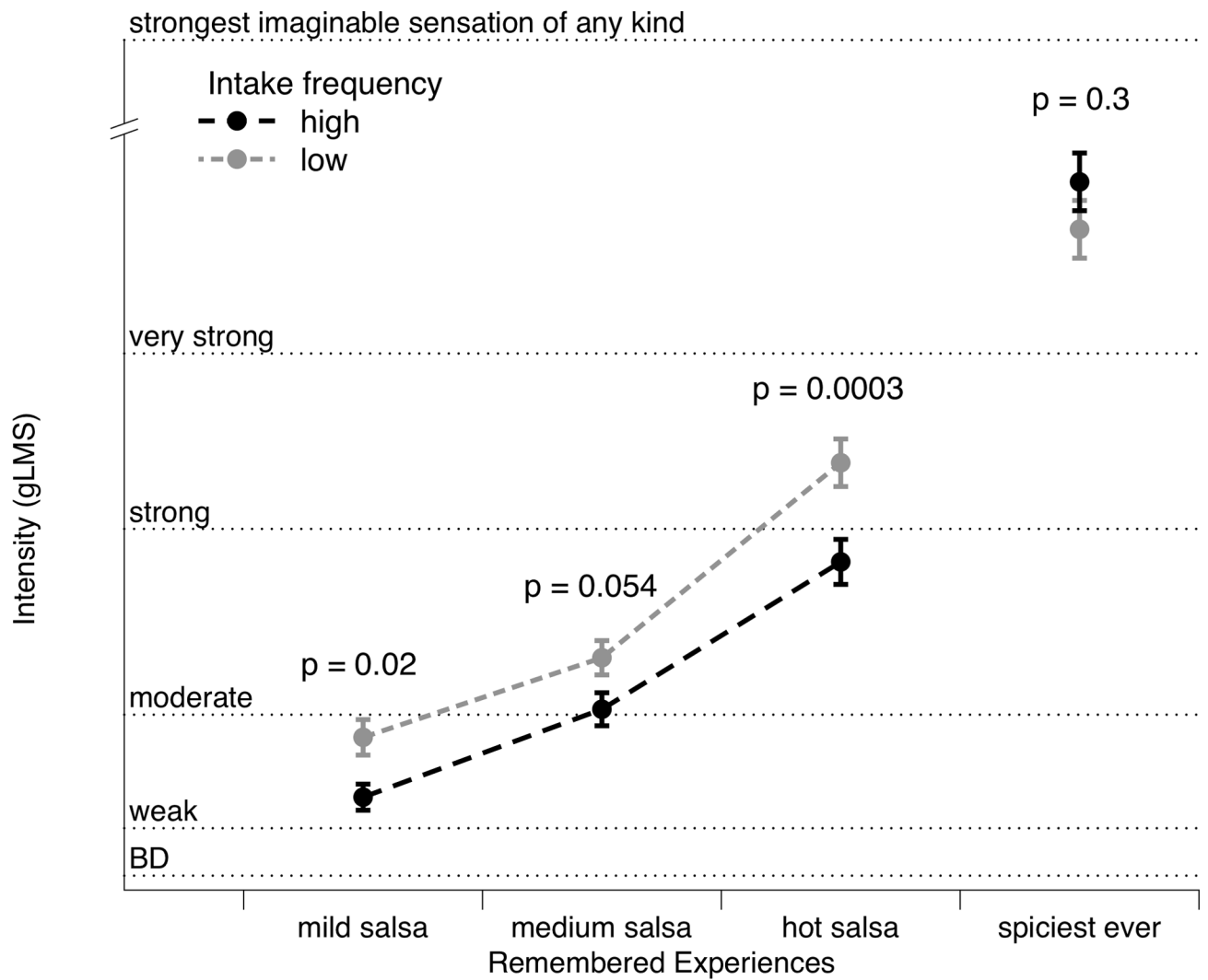


Figure 5. Participants rated the remembered intensity of mild, medium, and hot salsa, as well as ‘the spiciest meal or food you have ever experienced’ on a gLMS. Values are group means and standard errors, segmented by the same low and high intake groups used in Figure 2. For the salsa samples, there was a significant main effect of group, in ANOVA (see text), and the p-values for the individual comparisons are shown. Conversely, the mean intensity ratings for ‘spiciest ever’ did not differ by group.

Table 1

Characteristics of study participants

	<u>All Participants</u>	<u>Females</u>	<u>Males</u>
(n)	82	48	34
age	32.1±0.9	32.9±1.3	31.0±1.2
preferred spice level *	2.1±0.1	1.8±0.1	2.4±0.2
VARSEEK score	16.4±0.5	16.4±0.6	16.5±0.7
<u>Yearly Intake Frequency</u>			
hot sauce	55.5±11.1	44.2±9.7	71.4±23.1
chili peppers	42.0±12.5	34.2±15.0	53.0±21.7
habanero peppers	11.3±3.6	8.2±1.7	15.8±8.3
red pepper flakes	50.9±8.0	39.4±8.1	67.3±15.2
spice mix containing chilies	43.1±9.7	46.8±15.2	38.0±9.4
sweet snacks	185.6±20.0	199.6±28.6	165.8±26.6
salty snacks	195.6±18.9	213.3±27.0	170.5±24.6
fried foods	50.5±5.3	52.2±7.7	48.1±6.9
Ice cream/frozen yogurt	71.2±11.4	68.7±15.6	74.8±16.5

* Indicates significant differences in intake between males and females ($p < 0.05$).