Cell Metabolism

Supra-Additive Effects of Combining Fat and Carbohydrate on Food Reward

Graphical Abstract



Highlights

- Fat and carbohydrate interact to potentiate reward independently of liking
- This is reflected in supra-additive responses in the striatum during food valuation
- Participants are able to estimate energy density from fat, but not carbohydrate
- Accurate estimation of energy density recruits a prefrontalfusiform gyrus circuit

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In Brief

DiFeliceantonio et al. show that foods containing fat and carbohydrate are more reinforcing than equicaloric foods containing primarily fat or carbohydrate. This effect is independent of liking and is reflected by supra-additive responses in the striatum during food valuation. This may be one mechanism driving overconsumption of highfat/-carbohydrate processed foods.



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Supra-Additive Effects of Combining Fat and Carbohydrate on Food Reward

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SUMMARY

Post-ingestive signals conveying information about the nutritive properties of food are critical for regulating ingestive behavior. Here, using an auction task concomitant to fMRI scanning, we demonstrate that participants are willing to pay more for fat + carbohydrate compared with equally familiar, liked, and caloric fat or carbohydrate foods and that this potentiated reward is associated with response in areas critical for reward valuation, including the dorsal striatum and mediodorsal thalamus. We also show that individuals are better able to estimate the energy density of fat compared with carbohydrate and fat + carbohydrate foods, an effect associated with functional connectivity between visual (fusiform gyrus) and valuation (ventromedial prefrontal cortex) areas. These results provide the first demonstration that foods high in fat and carbohydrate are, calorie for calorie, valued more than foods containing only fat or carbohydrate and that this effect is associated with greater recruitment of central reward circuits.

INTRODUCTION

Post-ingestive signals conveying information about the nutritive properties of food are critical for regulating ingestive behavior. Rats readily titrate the volume of food (i.e., portion size) they consume to hold daily caloric intake constant, indicating that rats eat for calories rather than portion (Adolph, 1947). Likewise, in humans, separate neural circuits respond to energy density compared with portion size of foods depicted in images (English et al., 2016).

Post-ingestive signals also act as powerful reinforcers in rodent models (Epstein and Teitelbaum, 1962; Miller and Kessen, 1952). In rodents, flavor-nutrient conditioning studies demonstrate that intragastric infusion of nutrients, but not saline, produces strong preferences for the flavor of a simultaneously consumed non-caloric flavored liquid (Holman, 1969; Sclafani, 2004) and these signals are both necessary and sufficient to sustain feeding via their effects on dopamine release in the striatum (de Araujo et al., 2008; Ren et al., 2010; Tellez et al., 2013a, 2013c). Accordingly, in humans, the tasteless and odorless carbohydrate maltodextrin, but not the non-caloric sweetener sucralose, conditions increased intake of sorbet (Yeomans et al., 2008), while the magnitude of the blood-oxygen-leveldependent (BOLD) signal in dopamine target areas to caloriepredictive flavors depends upon the increase in plasma glucose levels when the flavors are previously consumed with calories (de Araujo et al., 2013). Notably, neither sorbet intake nor BOLD response correlates with self-reported sorbet or flavor liking. Likewise, willingness to pay for food is associated with actual, but not estimated, caloric density and is reflected by BOLD response in the mesolimbic network (Tang et al., 2014).

Collectively, these findings suggest that post-ingestive signals regulate neural circuits in the dopaminergic meso-striato-prefontal system independently of other food characteristics that could influence reward, such as liking, sweetness, perceived energy density, and availability (e.g., portion size). This has important implications for understanding the human obesity epidemic because mesolimbic neural response to food cues correlate with obesity (Bruce et al., 2010; Feldstein Ewing et al., 2016; Stoeckel et al., 2008; Val-Laillet et al., 2011), genetic risk for obesity (Farooqi et al., 2007; Holsen et al., 2012; Rapuano et al., 2017), eating in the absence of hunger (Lawrence et al., 2012), food choice (Mehta et al., 2012; Schur et al., 2009), future weight gain (de Araujo et al., 2013; Demos et al., 2012; Geha et al., 2013; Kroemer et al., 2016), poorer performance on weight-loss trials (Murdaugh et al., 2012), and overfeeding (Cornier et al., 2007).

However, very little is understood about the mechanisms behind the generation of post-ingestive signals (in any species) and their regulation of mesolimbic circuits. One overlooked but

potentially important factor is the possibility that separate mechanisms evolved for fat and carbohydrate (Ritter and Taylor, 1990; Tellez et al., 2013a, 2013b). Although vagal afferent signals are critical for intra-gastrically administered lipids to increase extracellular striatal dopamine and promote appetitive behavior, dopamine release upon glucose infusion depends upon a yetunknown metabolic signal thought to be generated during the utilization of glucose as a cellular fuel (Ren et al., 2010; Tellez et al., 2013b). Further, in rodents, vagotomy disrupts the orexigenic effects of nutrient deprivation by blocking fatty acid oxidation, but not by blocking glucose utilization (Ritter and Taylor, 1990). There is also evidence for independent gut-brain pathways for fat and sugar (i.e., carbohydrate) reward in humans. People with a genetically derived deficiency in melanocortin-4 receptors (MC4Rs) exhibit increased preference for high-fat but reduced preference for high-sucrose foods (van der Klaauw et al., 2016), whereas variants in the hepatokine fibroblast growth factor 21 (FGF21) gene are associated with increased preference for sweet, but not fat, foods (Soberg et al., 2017).

The existence of independent sensing pathways for fat and carbohydrate is especially relevant when considering the human obesity epidemic. The modern food environment proffers up nutrients in doses and combinations that do not exist in nature. This contrasts sharply with our ancestors' diet composed mostly of woody plants and raw animal meat (Balter et al., 2012). Moreover, since hunter-gatherer societies eat single dietary components at a time (Berbesque et al., 2011), even when nuts and seeds, which contain some fat, were available, consuming multiple foods in a single meal would have been a rare occurrence. One exception would be fruits containing seeds high in fat and pulp high in carbohydrate. However, such plants are rare and are very high in fiber, which would have significantly reduced the rate of carbohydrate metabolism (Murray et al., 2001). Opportunities to consume fat and carbohydrate together certainly increased following the domestication of plants and animals and development of grain and dairy production (~12,000 years ago). However, this is recent in our evolutionary past and still significantly different than the processed foods of today. For example, the nutritional content of oats with a half cup of milk and honey is only 1 g of fat and 27 g of carbohydrates. Compare this with a donut of similar calories, which contains 11 g of fat and 17 g of carbohydrate.

Since physiology is shaped by natural selection in response to environmental pressures, it is possible that the simultaneous activation of fat and carbohydrate signaling pathways produces a potentiated or perhaps extra-physiologic effect to potentiate reward and render processed foods high in fat and sugar more rewarding, calorie for calorie, than foods high in only fat or sugar. Consistent with this suggestion, rodents tightly regulate total daily caloric intake and body weight (Adolph, 1947) when given access to fat alone or carbohydrate alone; however, when given unrestricted access to fat and carbohydrate they quickly gain weight (Beilharz et al., 2014, 2016; Johnson and Kenny, 2010), suggesting that it is the combination of these macronutrients that disrupts energy balance.

With this in mind, we set out to determine if palatable familiar foods high in fat and carbohydrate are more rewarding than similarly caloric, familiar, and liked foods high in only fat or only carbohydrate. To test this we used the Becker-DeGroot-Marshak auction (Becker et al., 1964) task, in which participants bid for snacks depicted in photographs, while BOLD response was assessed using fMRI. By using familiar snack items, we ensure that participants have had the opportunity to associate these foods with their nutritional properties via flavor-nutrient conditioning in the past and, based on this conditioning, the food images represent energy-predictive conditioned stimuli.

This enabled us to test the hypothesis that pictures associated with the post-ingestive effects of fat and carbohydrate are more reinforcing than those associated with the post-ingestive effects of primarily fat or carbohydrate. As predicted, we found that participants are willing to pay more for snacks with fat + carbohydrate, compared with fat or carbohydrate alone, and that this effect was reflected by response in the dorsal striatum (caudate and putamen) and mediodorsal thalamus. Unexpectedly, we observed that participants are very accurate at estimating the energy density of fat, but not carbohydrate or fat + carbohydrate foods, an effect that is reflected by response in the fusiform gyrus and its connectivity with ventromedial prefrontal cortex (vmPFC), anterior cingulate cortex, and cerebellum. These results are the first to demonstrate that foods high in fat and carbohydrate are more rewarding, calorie for calorie, than foods high only in fat or carbohydrate and that energy density estimation accuracy differs depending on macronutrient.

RESULTS

Our first step was to create a set of pictures of small snacks falling into one of three categories of macronutrient content: those with most of their calories coming from (1) fat, (2) carbohydrate, or (3) fat + carbohydrate, but varying equally within category for liking, familiarity, and caloric content (Table 1). Testing was performed in a pilot study (n = 56) that did not include participants from the main study (STAR Methods). Examples from each group are depicted in Figure 1A. Portion sizes were adjusted across macronutrient groups to equate caloric content across snacks so that there were examples of low, medium, and high caloric values in each category with overall mean calories across categories similar (Table 1). Portion size was not significantly different across groups and pictures were chosen to have equal object size, intensity, and complexity, although they differed slightly in contrast (Table 1). This resulted in 39 stimuli with 13 in each group.

Methods and analyses are described in detail in the STAR Methods section. In brief, all participants in the main study first rated these snacks for liking, familiarity, estimated energy density, and total calories shown. On a subsequent day, they arrived fasted to the laboratory and were fed a standard breakfast of 426 kcal from orange juice, cheddar cheese, whole-wheat toast, white toast, strawberry jam, and butter (described in Tang et al., 2014). They began the fMRI session 3 hr later. Prior to scanning, participants were given €5 and told they could bid between €0 and €5 against the computer to purchase snacks depicted in pictures presented during scanning. They were also told that one item will be selected at random for auction at the end of scanning. If the participant's bid was higher than the computer's bid, he or she was able purchase the item and receive the remainder of the €5 in cash. Otherwise, the participant received the entire €5 but did not get the item. Participants remained in a

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Table 1. Characteristics of Each Snack and Snack Image in the Three Macronutrient Groups								
	С	F	FC	Group Differences				
Carbohydrate (g per 100 g)	74.2 SEM ±3.75, 54.2–95.5	1.2 SEM ±0.57, 0-7.2	57.33 SEM ±2.87, 28.2-70.98	F(2, 36) = 193.7, p < 0.0001 ^a				
Fat (g per 100 g)	4.14 SEM ±1.44, 0–11.9	27.8 SEM ±2.8, 12.6-48.2	24.57 SEM ±1.61, 10.6–33.1	F(2, 36) = 39.49, p < 0.0001 ^a				
Calories shown (g)	128.5 SEM ±18.47, 27–267	128.5 SEM ±18.37, 32-267	128 SEM ±18.21, 29-256	F(2, 36) = 0.0003, p = 0.99				
Energy density (kcal per 100 g)	36.89 SEM ±13.76, 29.4–44.8	34.33 SEM ±30.45, 19.0-60.1	48.18 SEM ±22.67, 22.1-55.2	F(2, 36) = 9.99, p = 0.0004 ^a				
Portion size (g)	37.14 SEM ±6.176, 6.444–90.82	40.42 SEM ±8.553, 16.84-134.8	30.73 SEM ±7.987, 5.93-119.9	F(2, 36) = 0.401, p = 0.67				
Object size	0.24 SEM ±0.03, 0.07–0.41	0.25 SEM ±0.034, 0.12-0.53	0.24 SEM ±0.03, 0.12-0.28	F(2, 36) = 0.7863, p = 0.4632				
Intensity	25.02 SEM ±3.22, 4.66–49.12	22.34 SEM ±3.26, 6.586-44.6	30.17 SEM ±2.83, 14.45-48.66	F(2, 36) = 1.622, p = 0.2116				
Contrast	44.31 SEM ±5.344, 17.39–68.77	20.93 SEM ±2.028, 10.48-33.91	47.12 SEM ±3.941, 26.68–65.49	F(2, 36) = 12.87, p < 0.0001 ^a				
Complexity	0.08 SEM ±0.013, 0.025–0.17	0.05 SEM ±0.01, 0.23-0.134	0.09 SEM ±0.01, 0.02-0.15	F(2, 36) = 3.1, p = 0.0572				
Liking (scale)	10.23 SEM ±3.36, -6.76-37.85	16.96 SEM ±4.29, -26.57-32.85	20.40 SEM ±2.95, 2.58-40.08	F(2, 36) = 2.08, p = 0.1391				
Familiarity	71.80 SEM ±3.42, 54.36–87.52	72.65 SEM ±3.68, 39.48-87.56	73.34 SEM ±3.76, 44.93-85.66	F(2, 36) = 0.0458, p = 0.9553				
Estimated calories	48.26 SEM ±2.35, 30.35–59.33	58.59 SEM ±1.91, 46.25-69.19	58.12 SEM ±2.51, 46.46-81.12	F(2, 36) = 6.577, p = 0.0037 ^a				
Estimated energy density	64.1 SEM ±2.71, 48.29–77.9	72.34 SEM ±1.65, 57.23-82.99	80.5 SEM ±1.79, 63.42-88.05	F(2, 36) = 14.27, p < 0.0001 ^a				

For the snack images: object size is the proportion of non-white pixels, brightness is the differences between the mean luminance of all non-white pixels and the white background, contrast is the SD of the luminance of the non-white pixels, and complexity is the proportion of outline pixels to object pixels (Blechert et al., 2014b). Means (n = 39 pictures) with SEM are given with the range below each mean. C, carbohydrate; F, fat; FC, fat + carbohydrate.

^aSignificant p values (p < 0.05)

supervised setting following scanning, during which time they consumed the item, if one had been won. Under these circumstances the optimum strategy is to bid what one believes is the value of the item (Becker et al., 1964).

Behavioral Results

Between-Category Differences in Ratings and Energy Density

We first compared the snack macronutrient categories to test for differences in liking and estimated and actual energy density using one-way ANOVAs. By design, no differences were observed in caloric content ($F_{(2,36)} < 0.001$, p = 0.9; Figure 1B), familiarity ($F_{(2,36)} = 0.08$, p = 0.92; Figure 1C), or liking ($F_{(2,36)} = 1.94$, p = 0.073; Figure 1D). Fat + carbohydrate snacks were higher in energy density ($F_{(2,36)} = 9.99$, p = 0.0004; Figure 1E) and were accurately estimated by participants as being more calorically dense ($F_{(2,36)} = 8.73$, p = 0.0008; Figure 1F). Participants also bid slightly faster for these snacks ($F_{(2,36)} = 4.286$, p = 0.0214; Figure 1G).

Associations between Ratings and Energy Density

We next examined correlations between willingness to pay, energy density, estimated energy density, and liking using linear regressions. All regression p values are Bonferroni corrected for the total number of tests run (32). Replicating prior work by Tang et al. (2014), willingness to pay was highly associated with energy density ($r^2 = 0.225$, p < 0.001; Figure 2A) across all snacks. Importantly, willingness to pay was not correlated with estimated energy density in the full group ($r^2 = 0.070$, p > 0.9; Figure 2B) or in any subgroup. Taken together, this indicates that actual energy density is a better predictor of food reward than estimated energy density. Interestingly, participants could very accurately estimate the energy density for fat snacks ($r^2 = 0.688$, p = 0.016; Figure 2C). In contrast to fat snacks, participants were unable to accurately estimate energy density of carbohydrate ($r^2 = 0.012$, p > 0.9) and fat + carbohydrate snacks ($r^2 = 0.22$, p > 0.9; group × estimated energy density $F_{(1,37)} = 13.132$, p = 0.0008).

Willingness to pay was not related to portion size ($r^2 = 0.0009$, p > 0.9; Figure S1A). Participants could not estimate the number of calories shown in each snack portion ($r^2 = 0.211$, p = 0.82; Figure S1B). Liking ratings were also associated with energy density overall ($r^2 = 0.122$, p = 0.01; Figure S1D), but this was not significant in any individual group. Liking was not related to estimated energy density for the whole group ($r^2 = 0.0003$, p = 0.92; Figure S1E) or any individual group.

Unlike in prior work, where willingness to pay was unrelated to ratings of liking (Tang et al., 2014), we observed a strong positive





Examples of each macronutrient group are displayed in (A). Averages across macronutrient groups for the scanned participants in calories shown (B), familiarity (C), liking (D), energy density (E), estimated energy density (F), and reaction time (G). Following pre-testing on a separate day, participants viewed a fixation cross for approximately 9 s (H). A picture of a food item to be bid on was displayed for 5 s. Participants then had 5 s to make a bid on the item. They moved a trackball inside the scanner to move a cursor back and forth between 0 and 5 euros. After they submitted their response it remained on screen for the remainder of the 5 s. Data are represented as means \pm SEM. C, carbohydrate; F, fat. *p < 0.05.

correlation between liking and willingness to pay for all foods ($r^2 = 0.69$, p < 0.0001; Figure S1C) and in fat ($r^2 = 0.89$, p = 0.00003) and fat + carbohydrate ($r^2 = 0.70$, p = 0.011) categories, but not carbohydrate ($r^2 = 0.58$, p = 0.077). To test if energy density and liking are independent predictors of willingness to pay, we performed a mediation analysis by constructing models with both variables entered as predictors. Significance improved with both variables entered (p = 0.002 to <0.001), but each contributed uniquely to the effect, with energy density remaining a significant predictor when liking was included ($\beta = 0.207$, p = 0.03) and liking remaining a significant predictor

when energy density was included (β = 0.763, p < 0.001), indicating both predictors contribute independently.

Between-Group Differences in Willingness to Pay

Next, we generated a linear mixed effects model with bid as the outcome variable; subject as a random effect; and macronutrient group, true energy density, estimated energy density, liking, estimated portion calories, portion size, and calories shown in each picture as fixed effects to test our prediction that participants would pay more for the fat + carbohydrate foods compared with the fat or carbohydrate foods. As predicted, participants were willing to pay significantly more for foods



Figure 2. Relationships between Snack Characteristics and Participant Ratings

The relationship of willingness to pay with true (A) and estimated (B) caloric density is shown, with the all three macronutrient groups combined on the right and separated on the left. Estimated calories and true caloric density are shown to be related overall (C) and in the fat macronutrient group on the right. All tests are Bonferroni corrected for the total number of tests performed in this series (32). See also Figure S2. **p > 0.001.

Functional Neuroimaging

To test for regions sensitive to the rewarding potency of the foods and to determine if we could replicate prior work, we created a general linear model (GLM) in which bid amount for each trial was entered as a parametric modulator

with fat + carbohydrate compared with fat ($t_{(1,774)} = -5.024$, p < 0.0001; Figure 4A) or carbohydrate ($t_{(1,774)} = -4.9021$, p < 0.0001; Figure 4A) foods. Importantly, liking, actual energy density, and estimated energy density were in this model and therefore were adjusted for. To test if the effect was supra-additive, we generated a second model in which macronutrient categories were coded as containing fat or carbohydrate, with the other random and fixed factors identical to the previous model. The interaction term was significant ($F_{(1,773)} = 20.383$, p < 0.0001), showing that bids for fat + carbohydrate were greater than would be expected from summing the bids for fat and carbohydrate foods.

Although we controlled for liking in all analyses, fat + carbohydrate items were liked slightly, but not significantly, more, and liking and bid amount were highly correlated. We therefore took an additional step to verify that effects were not related to liking. More specifically, we re-ran all analyses after removing the two least liked items from the carbohydrate and fat categories and the two most liked items from the fat + carbohydrate category. In so doing, the fat category became most liked (carbohydrate = 7.655 ± 3.342 , fat = 17.12 ± 3.267 , fat + carbohydrate = 14.59 \pm 2.261), but there were still no significant differences in liking $(F_{(2,30)} = 2.674, p = 0.0854; Figure S2C)$. Categories were also still matched for calories shown ($F_{(2,30)} = 0.06$, p = 0.9417; Figure S2A) and familiarity ($F_{(2,30)} = 1.354$, p = 0.273; Figure S2B). Just as previously, fat + carbohydrate items were rated as more energy dense ($F_{(2,30)}$ = 8.235, p = 0.0014; fat + carbohydrate > C, $t_{(30)}$ = 1.89, p < 0.05; Figure S2D) and actually were more energy dense (kcal/g) (F_(2,30) = 6.12, p = 0.0055; fat + carbohydrate > C, t₍₃₀₎ = 2.82, p < 0.05; fat + carbohydrate > F, $t_{(30)}$ = 3.12, p < 0.01; Figure S2E). Reaction time did not differ significantly between groups in the subset ($F_{(2,30)} = 2.71$, p = 0.078; Figure S2F). Again, a linear mixed effects model identical to the one described above (and including energy density as a covariate) revealed that participants were willing to bid more for fat + carbohydrate items overall $(F_{(2,651)} = 8.161, p = 0.0003; fat + carbohydrate > C, F_{(1,651)} =$ 15.322, p < 0.0001; fat + carbohydrate > F, $F_{(1.651)}$ = 10.9797, p < 0.0001; Figure S2G). The supra-additive effect was also still present ($F_{(1.651)} = 15.771$, p < 0.0001).

controlling for energy density. This analysis produced responses in the right lingual gyrus ([8 -78 -4], z = 4.9, p < 0.00001; Figure 3), the anterior cingulate cortex ([-8 44 8], z = 3.66, p < 0.001; Figure 3), the orbitofrontal cortex ([-24 40 -26], z = 3.85, p < 0.0005; Figure 3), the frontal pole ([-21, 66, 2], z = 3.06, p < 0.001; Figure 3), and the anterior insula ([-32, 27, -7], z = 2.6, p < 0.001; Figure 3), largely replicating prior work (Tang et al., 2014). Without controlling for energy density, a similar network is observed, including the lingual gyrus ([8 -78 -4], z = 2.7, p < 0.001) and anterior cingulate cortex ([-8 38 8], z = 3.4, p = 0.002 and [6 30 24], z = 3.09, p = 0.002).

Next, to test our hypothesis that response in dopamine target areas would be greater for fat + carbohydrate compared with fat or carbohydrate foods during bidding, we created a GLM with estimated calories, true energy density, liking, and bid amount for each item in each group. This was done to control for factors that differed across group to ensure any effects seen were due to the bid amount alone. A contrast of fat + carbohydrate combination (FC) > fat + carbohydrate was modeled on the first level to account for within-subject variance. As predicted, response in the caudate and putamen was more strongly associated with bid amount for fat + carbohydrate compared with fat or carbohydrate alone (caudate, [14 - 4 20], z = 3.82, p < 0.001; Table 2; Figure 4C; putamen, [-28 -2 -2], z = 3.37, p < 0.001; Figure 4D). A similar effect was also observed in the thalamus ([-10 -12 18], z = 3.87, p < 0.001; Figure 4E). No significant effects for the reverse analysis (fat + carbohydrate > fat + carbohydrate) were found.

We next tested whether bid amount modulated connectivity with the striatum differentially as a function of macronutrient category by performing a psychophysiological interaction (PPI) analysis with the seed defined as a 5 mm sphere surrounding the caudate peak ([14 –4 20]). Group difference in bid amount modulated striatal connectivity were observed with insular cortex bilaterally ([38 –12 6], z = 2.719, p < 0.0001; [-40 –8 –10], z = 2.615, p < 0.0001; Figure 4F) and with the anterior medial temporal lobe (hippocampus and amygdala, [26 –2 –26], z = 2.718, p = 0.00106; Figure 4G), although this cluster was just above our threshold of p < 0.001. More specifically, striatal



Figure 3. Brain Regions Associated with Willingness to Pay

Brain areas whose BOLD activity is associated with willingness to pay, regardless of macronutrient group or true caloric density. ACC, anterior cingulate; OFC, orbitofrontal cortex.

connectivity with these regions is stronger when bidding for foods containing fat + carbohydrate compared with when bidding for food containing only fat or only carbohydrate.

In our behavioral analysis, we found participants were only able to accurately estimate the energy density of fat snacks, but not carbohydrate or fat + carbohydrate snacks (Figure 2A). To further confirm this relationship, we performed regressions such that beta values were generated for each subject for each snack type. Here, we controlled for portion size across groups to ensure it was not driving the effects. We found an overall group difference (Friedman's statistic = 9.1, p = 0.0106; Figure 5A) using a non-parametric test, as beta values were not normally distributed. Further, participants were better able to estimate the energy density of fat snacks over carbohydrate snacks (p = 0.0144) and over fat + carbohydrate snacks (p = 0.0228).

Having confirmed this relationship in the behavioral data, we sought to isolate the neural circuit reflecting the differences in the ability to estimate energy density of fat compared with carbohydrate and fat + carbohydrate by regressing brain response against estimated energy density of fat compared with carbohydrate and fat + carbohydrate foods. We found a negative relationship with estimated energy density in the fusiform cortex when viewing fat pictures, in contrast to a positive response when viewing carbohydrate or fat + carbohydrate pictures ([-26 -72 -8], z = 3.25, p < 0.00001; Figure 5B). A similar effect was observed in extrastriate cortex ([-20 -98 02], z = 3.84, p < 0.0001). To determine if there was differential connectivity with these visual sensory regions and areas of the mesostriato-prefrontal regions when estimating energy density for fat versus carbohydrate or fat + carbohydrate, we ran a PPI with the extracted time series from a 5 mm sphere around the fusiform gyrus peak ([-26 -72 -8]). This showed that estimated energy density increased fusiform connectivity with the vmPFC ([-10 38 -11], z = 3.02, p < 0.00001; Figure 5C), anterior cingulate gyrus ([-10448], z = 4.217; Figure 5C), and cerebellum ([26 - 68 - 17], z = 4.17; Figure 5D) when estimating the energy density of fat compared with the energy density of carbohydrate or fat + carbohydrate. This suggests that accurate estimates of energy density are associated with greater coupling of activity between the fusiform gyrus and the vmPFC, cingulate, and cerebellum.

DISCUSSION

Our study produced two novel findings that are relevant for understanding food choice. First, we demonstrate for the first time that foods containing both fat and carbohydrate are more rewarding, calorie for calorie, than those containing only fat or only carbohydrate, and we further describe a network of brain regions (caudate, putamen, and mediodorsal thalamus) underlying this effect. Second, we discovered, unexpectedly, that individuals are better able to estimate the energy density of fat compared with carbohydrate and fat + carbohydrate foods, with accurate estimations of energy density depicted in pictures of fatty foods associated with increased coupling of visual sensory areas with the vmPFC and cerebellum. Both findings support and extend work from animal models indicating that these two energy sources have distinct pathways for conveying nutritive value to the CNS to ultimately guide food choice and highlight the need to further understand the mechanisms driving the interaction of macronutrients on circuits regulating ingestive behavior.

Studies in mice show that the ability of ingested nutrients to produce dopamine release to drive reward depends critically upon their utilization as a cellular fuel (Tellez et al., 2013b, 2013c). Vagotomy blocks the orexigenic effects of the fatty acid oxidation blocker mercaptoacetate, but not of the orexigenic effects of the glucose utilization blocker 2-deoxy-D-glucose (Ritter and Taylor, 1990). This suggests that the gut-brain pathways driving fat and carbohydrate reward differ. Several observations from the current study support this contention.

First, our data suggest that the association between reward and energy density differs for fat and carbohydrate (Figure 2). For fatty foods, willingness to pay (our measure of reward) is tightly coupled to energy density and self-reported food liking. Although both food liking and energy density are associated with willingness to pay, mediation analyses indicate that these factors contribute independently to reward value. In contrast, for carbohydrate-containing foods, willingness to pay is positively related to self-reported food liking, but not energy density. Moreover, although individuals are very accurate at estimating the energy density of fatty foods, they are poor at estimating the energy density of carbohydrate-containing foods, which our fMRI data suggest results from differential engagement of the fusiform gyrus.

The fusiform gyrus is critical for encoding features of the visual world to enable object and place recognition. Our findings add to a growing literature suggesting it also contributes to the recognition and valuation of visual features of foods that convey information about energy density. There are numerous references to fusiform responses to food-related visual cues in the neuroimaging literature. Such responses are sensitive to the caloric value of the depicted food and to the internal state of the individual (Frank et al., 2010; Killgore and Yurgelun-Todd, 2005; Siep et al., 2009). Typically, they have been interpreted as part of a network of regions coding visual attention and/or food cue saliency. Accordingly, fusiform responses to high-calorie-food images predict inability to maintain weight loss (following a similar pattern as midbrain and striatum; Murdaugh et al., 2012), spontaneous fluctuations in the fusiform gyrus correlate

Density PPI						
Analysis	Peak Voxels	Z Value	Area	Contrast	Test Value	p Value
Bids	14 -4 20	3.82	caudate	FC > F and C	F _(2,57) = 221.7	p < 0.0001
				FC > C	t ₍₅₇₎ = 9.95	p < 0.0001
				FC > F	t ₍₅₇₎ = 21.05	p < 0.0001
	-28 -2 -2	3.37	putamen	FC > F and C	F _(2,57) = 40.01	p < 0.0001
				FC > C	t ₍₅₇₎ = 4.59	p < 0.0001
				FC > F	t ₍₅₇₎ = 8.94	p < 0.0001
	-10 -12 18	3.87	thalamus	FC > F and C	F _(2,57) = 116.2	p < 0.0001
				FC > C	t ₍₅₇₎ = 10.32	p < 0.0001
				FC > F	t ₍₅₇₎ = 14.8	p < 0.0001
Bid PPI	38 - 12 6	2.79	insula	FC > F and C	$F_{(2,57)} = 146.4$	p < 0.0001
				FC > C	t ₍₅₇₎ = 9.73	p < 0.0001
				FC > F	t ₍₅₇₎ = 17.05	p < 0.0001
	26 -2 -26	2.718	hipp/amyg	FC > F and C	F _(2,57) = 215.3	p < 0.0001
				FC > C	t ₍₅₇₎ = 9.88	p < 0.0001
				FC > F	t ₍₅₇₎ = 20.74	p < 0.0001
Est. ED	-26 -72 -8	3.25	fusiform	F > FC and C	F _(2,57) = 218	p < 0.0001
				F > C	t ₍₅₇₎ = 18.78	p < 0.0001
				F > FC	t ₍₅₇₎ = 17.92	p < 0.0001
	-20 -98 02	3.84	extrastriate	F > FC and C	F _(2,57) = 202.6	p < 0.0001
				F > C	t ₍₅₇₎ = 15.99	p < 0.0001
				F > FC	t ₍₅₇₎ = 18.59	p < 0.0001
Est. ED PPI	-10 38 -11	3.02	vmPFC	F > FC and C	F _(2,57) = 299.9	p < 0.0001
				F > C	t ₍₅₇₎ = 23.7	p < 0.0001
				F > FC	t ₍₅₇₎ = 6.51	p < 0.0001
	-10 44 8	4.217	Ant Cing	F > FC and C	F _(2,57) = 60.64	p < 0.0001
				F > C	t ₍₅₇₎ = 10.06	p < 0.0001
				F > FC	t ₍₅₇₎ = 1.15	p = 0.50
	26 - 68 - 17	4.17	cerebellum	F > FC and C	$F_{(2,57)} = 54.87$	p < 0.0001
				F > C	t ₍₅₇₎ = 10.45	p < 0.0001
				F > FC	t ₍₅₇₎ = 4.59	p < 0.0001

Table 2. ANOVA and t Test Results from Extracted PE Values for Bid Amount, Bid PPI, Estimated Energy Density, and Estimated Energy Density PPI

All p values are Bonferroni corrected.

Hipp/amyg, anterior temporal lobe, including the hippocampus and amygdala; extrastriate, extrastriate cortex; Ant Cing, anterior cingulate; Est. ED, estimated energy density.

with self-reported craving (Chen et al., 2017), and responses to food cues are greater in obese compared with healthy-weight youth (Allen et al., 2016; Janowitz et al., 2015) as well as in healthy weight individuals that carry the obesity predisposing variant of the FTO gene (Kuhn et al., 2016). Fusiform gyrus response to food pictures also covaries with degree of parental dietary restriction on children (Allen et al., 2016), as well as personal dietary restraint (Zhao et al., 2017), indicating that the fusiform gyrus is sensitive not only to the energetic properties and value of food pictures but also to the experience of food decisions where taste and energy density must be considered.

The mechanism by which striatal dopamine influences fusiform activity is unclear; however, a striatum to vmPFC to fusiform circuit is possible. Accordingly, we observe preferential connectivity between the fusiform gyrus and vmPFC when bidding for fat versus carbohydrate or fat + carbohydrate foods. The vmPFC plays a critical role in determining food choices based on comparing taste and health information (Hare et al., 2009) and the fusiform gyrus to vmPFC neural circuit is thought to integrate visual features of objects with the generation of value signals to drive choice (Lim et al., 2013). In a prior study, vmPFC response correlated with bid amount and actual, but not estimated, energy density (Tang et al., 2014). The pattern of activations we observe suggests that the fusiform gyrus-vmPFC circuit plays an important role in accurately estimating energy density from visual information. More specifically, estimates are accurate when there is a negative association between energy density and fusiform response (lower responses for higher energy density; Figure 5) coupled with increased functional connectivity with the vmPFC (and cerebellum). Collectively, these data suggest that the fusiform has access to the value of the nutritional properties of foods conveyed by visual information and that interactions between the fusiform and vmPFC are important in enabling more accurate estimates of energy density for fat compared with

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Figure 4. Effect of Macronutrient on Willingness to Pay and Associated BOLD Activity

Participants are willing to pay more for foods that contain fat and carbohydrate combinations than either macronutrient group alone (A). Foods receiving the highest and lowest bids are depicted in (B). BOLD signal in caudate nucleus (C), putamen (D), and thalamus (E) was more associated with willingness to pay in the fat and carbohydrate group than either alone. A PPI analysis showed that caudate connectivity with the bilateral insula was modulated by willingness to pay (F). The same is true of caudate activity with amygdala and hippocampus, although this bordered on significance at p = 0.00106 (G). Arrows point to the peak voxel in each cluster. Parameter estimates (PE) are a.u. and are represented as means \pm SEM. *p < 0.0001.

carbohydrate-containing foods. One possibility is that the postingestive signal conveying information about the nutritive value of fat is successfully integrated with visual information about foods to determine value, whereas the value of carbohydrates may derive primarily from other features, such as flavor or visual presentation; however, further research is needed to confirm this hypothesis. An alternative possibility is that the ubiquity of artificial sweeteners in modern diets degrades the association between carbohydrate-containing foods and energy density, resulting in an impaired ability to estimate calories (Davidson and Swithers, 2004). For example, in the gustatory modality, intensity ratings of sweetness are positively related to sugar content, but



the association is significantly stronger for raw and moderately processed foods compared with highly processed foods (van Dongen et al., 2012).

We also show, for the first time, that fat and carbohydrate interact to potentiate reward, by demonstrating greater brain response to, and increased willingness to pay for, equally caloric foods containing fat and carbohydrate compared with fat or carbohydrate alone. We further show that these effects are not accounted for by differences in food liking or energy density since the interactive effects of fat and carbohydrate survive the inclusion of these factors as covariates in statistical models. Finally, we rule out portion size as a contributing factor because portion size is unrelated to willingness to pay, which is consistent with a recent report showing different patterns of evoked brain response to food portion size and energy density (English et al., 2016).

Replicating prior work (Tang et al., 2014), irrespective of macronutrient category bid amount was associated with BOLD response in the anterior cingulate cortex and orbitofrontal cortex, which has also been shown to code food attributes (Suzuki et al., 2017). However, Tang et al. (2014) also reported associations between bid amount and energy density in the vmPFC and striatum, which we did not observe when considering all food items. Rather, we found that striatum (caudate and putamen/globus pallidus) was selectively engaged when bidding for foods that contain fat and carbohydrate, compared with fat or carbohydrate alone. This is of note because this area is thought to play an important role in the shift from goal-directed to habitual control over behavior, which is a fundamental characteristic of addiction (Belin et al., 2009).

Relatedly, dopamine release in the dorsal striatum is sensitive primarily to the nutritional, rather than the hedonic, properties of foods (Tellez et al., 2016), with evidence for caudate dopamine release in humans upon food consumption (Small et al., 2003) and aberrant caudate BOLD responses to fat/sugar foods associated with increased risk for weight gain (Stice et al., 2008; Sun et al., 2015). Moreover, using an operant task in which fractal cues are rewarded with snacks containing fat and carbohydrate, Tricomi et al. (2009) show that increasing response in the putamen/globus pallidus (overlapping with the putamen peak isolated here) is associated with the development of habitual responding. It is therefore possible that simultaneous striatal dopa-

Figure 5. Brain Areas Associated with Energy Density for Fatty Snacks

Individual participants' beta values from each group showing the relationship between true and estimated energy density controlling for portion size (A). BOLD activity associated with energy density differed between carbohydrate and fat groups in the fusiform gyrus (B). A PPI analysis showed connectivity modulated by estimated energy density between the fusiform and the vmPFC (C) and cerebellum (D). Arrows point to the peak voxel of the cluster. PEs are a.u. and are represented as means \pm SEM. *p < 0.0001.

mine release by lipids and carbohydrates (Tellez et al., 2013b, 2013c) synergizes to potentiate reward value, leading to alterations in motivation or altering

progression to habitual responding. That said, opioids (Bakshi and Kelley, 1993; DiFeliceantonio et al., 2012), amino acid transmitters (Faure et al., 2008; Richard and Berridge, 2011), endocannabinoids (Mahler et al., 2007; Williams and Kirkham, 1999), acetylcholine (Pratt and Kelley, 2005), and adenosine (Pritchett et al., 2010) have all been shown to alter food intake through actions at striatal sites and, in mice, a high-fat, high-sugar diet alters striatal glutamate, opioid, and dopamine transmission (Fritz et al., 2018). Regardless of the precise neurochemical mechanism, we propose that foods that are more reinforcing (as measured by willingness to pay) are more likely to lead to habit formation. If so, one mechanism by which the modern food environment may promote overeating is by combining fat and carbohydrate to potentiate reward and therefore facilitate the transition to habitual responding as is observed in drugs of abuse (Belin et al., 2013). This proposal is based on the fact that foods high in fat and carbohydrate rarely exist in nature; even breast milk is on average about 3.5% fat and 7% carbohydrate (Ballard and Morrow, 2013), whereas typical processed snack foods contain closer to 24% fat and 57% carbohydrates (Table 1).

Study Limitations

Although we ensured all stimuli were equally familiar across macronutrient groups, we did not explicitly measure frequency of consumption of each item. This distinction could be relevant given striatal activity of people who frequently consume ice cream is blunted in response to an ice cream-like milkshake (Burger and Stice, 2012) and repeated exposure to food decreases activity in dopamine neurons that project to striatal regions in response to that food (Schultz et al., 1993). Our effects were in the opposite direction: increases in striatal activity were associated with willingness to pay, but future studies should include a measure of frequency of consumption to control for this effect on striatal activity. Although our sample size was sufficient here, a future study should include more participants and expand the effect and food items to other regions of the world, outside of western Europe.

Concluding Remarks

The current findings provide the first evidence that the rewarding effects of fat and carbohydrates interact to potentiate reward

and engagement of neural circuits involved in habit formation and reward value. They also suggest that the association between energy density and reward is distinct for fat and carbohydrate, with energy density better appreciated at a cognitive level for fatty foods and acting with fatty food liking to determine reward. In contrast, liking, and not energy density, influences the value of carbohydrate-containing and fat + carbohydrate-containing foods. Portion size and estimated energy density, whether accurate or not, played little role in reward in our healthy-weight sample. However, individuals were better able to estimate the energy density of fatty foods compared with carbohydrate-containing and fat + carbohydrate-containing foods, an ability that depends on connectivity between the fusiform gyrus and the vmPFC. Overall, these results indicate that combining fat and sugar increases the reward value of foods independently of caloric load, liking, and portion size and disrupts the ability to accurately estimate the energy density of fatty foods.

These results imply that a potentiated reward signal generated by foods high in both fat and carbohydrate may be one mechanism by which a food environment rife with processed foods high in fat and carbohydrate leads to overeating.

STAR*METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- QUANTIFICATION AND STATISTICAL ANALYSIS
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SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and can be found with this article online at https://doi.org/10.1016/j.cmet.2018.05.018.

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AUTHOR CONTRIBUTIONS

Conceptualization D.M.S.; Investigation, G.C., S.E.T., and L.R.; Software, L.R. and A.G.D.; Formal Analysis, A.G.D.; Visualization, A.G.D.; Writing – Original Draft, A.G.D. and D.M.S.; Writing – Review & Editing, A.D. and G.C.; Supervision, M.T. and D.M.S.; Funding Acquisition, M.T.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR*METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Statistical maps of the human brain	NeuroVault Repository	HYELUVKG
Software and Algorithms		
GraphPad Prism 7	GraphPad Prism 7	7
Matlab2014b	2014b Release	2014b
FSL v5.0	FSL v5.0	5.0
MRIcron	MRIcron	N/A
Presentation	Neurobs	N/A
Other		
Butter	Rewe	REWE-8721
Cheddar Cheese	Rewe	PD2602784
Strawberry Jam	Rewe	PD7396228
Whole Wheat Bread	GoldenToast	Vollkorn Toast
White Bread	GoldenToast	Butter Toast
Orange Juice	Rewe	PD5051720

CONTACT FOR REAGENT AND RESOURCE SHARING

For further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Dana M. Small (dana.small@yale.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All procedures were approved by the ethics committee at the University of Cologne. Participants were recruited via flyers from the local community and university population. All participants reported having no known metabolic, neurologic, and psychiatric disorders. Fifty-six participants rated stimuli in a pre-testing session to validate the stimuli used. They had an average age of 25 (SEM \pm 0.39) and an average BMI of 22.6 (SEM \pm 0.2). They were Caucasian and 30 were male. For the fMRI portion of the study, 24 participants were recruited, but due to a technical error during scanning, data were discarded from 4. The resulting 20 participants were Caucasian with an average age of 27.5 (SEM \pm 0.98), and an average BMI of 23.29 (SEM \pm 0.56). Twelve were female.

METHOD DETAILS

Pre-testing, Stimuli Selection, and Scales

A selection of photographs was created in house to ensure stimuli would be familiar to the local population, others were drawn from an existing database (Blechert et al., 2014a). As described in the results section, stimuli were chosen to depict equi-caloric portions, be similarly liked, and familiar. Liking was rated on a vertical scale, translated to German from English (Lim et al., 2009). Familiarity, estimated energy density, and estimated portion calories were rated on horizontal scales.

Liking was rated on a vertical scale with "Staerkste Vorliebe, die vorstellbar ist" (most liked sensation imaginable) at the upper anchor point and "Staerkste Abneigung, die vorstellbar ist" (most disliked sensation imaginable) at the lower anchor point (Lim et al., 2009). Familiarity was rated on a horizontal scale with the prompt "Wie gut kennen Sie das Essen?" (How well do you know this food), "gar nicht gut" (not well at all) at the left anchor point and "sehr gut" (very well) at the right anchor point. Participants were asked calories per portion with the prompt "Wie viele Kalorien denken Sie hat hie hier abgebildete Portion?" (How many calories do you think are in the pictured portion?). The scale was bounded on the left by "wenige Kalorien" (few calories) and the right by "viele Kalorien" (many calories). Energy density of each item was asses by asking participants "Wie kalorienhaltig denken Sie ist das Essen generell?" (How caloric do you think the food is, in general). This scale was bounded on the left by "niedrig kalorisch" (low caloric) and "hoch kalorisch" (high caloric).

fMRI Session

Subjects entered the laboratory after an overnight fast. Blood was drawn to measure fasting insulin and glucose. Brain response was assessed 3 hours after a standard breakfast containing 426 kcals, designed to have low glycemic index and low protein content (Tang et al., 2014) on a 3T Siemens Prisma scanner while subjects performed the auction task. The auction task was presented using Presentation software (Neurobehavioral Systems, Berkeley, CA, USA, RRID: SCR_002521). In this version of the Becker- de Groot-Marschak task subjects are told they can bid between 0 and 5 euros for a displayed food item. At the end of the experiment, one food item will be chosen at random, if they bid more than the computer, they will receive that food item, but the bid will come out of their remuneration. Four 8-minute runs were performed on each participant. Over all runs, participants saw each of the 39 (13 from each category) snack items 3 times. They were presented in a random order for 5 seconds, participants were then given 5 seconds to bid. The ITI was 9 seconds (Figure 1H). Acquisition parameters for the echo planar images (EPI) were: TE=30ms, echo spacing=0.7ms, TR=2s, flip angle= 90°, voxel size= 2.8 mm x 2.8 mm x 2.8 mm, number of slices=33. Sets of identical images, but with opposite (posterior to anterior) phase encoding were collected to perform geometric distortion correction. A previously collected high quality T1 weighted anatomical image (TE=2.23ms, TR=2.3s, flip angle=8° voxel sixe= 0.9x0.9) was used for registration of functional images.

QUANTIFICATION AND STATISTICAL ANALYSIS

Behavioral Analysis

Linear regressions were performed on behavioral data using GraphPad Prism version 7.0.1, (GraphPad Software, La Jolla, California, USA, RRID: SCR_002798) and MATLAB 2014b (MathWorks, Natick, Massachusetts, USA, RRID: SCR_001622). All behavioral data were plotted using GraphPad Prism. Linear mixed effects models (LME) were performed using MATLAB 2014b. In Figures 1, 2, S1, and S2, the n=39 (the number of pictures rated), rather than the sample size. In all other figures n=20 (the number of participants). A description of the regression or GLM for each result can be found in the appropriate location in the results section. All data were treated as if they met parametric assumptions except for an ANOVA ran on beta weights from subject-wise regressions, as they were not normally distributed tested by the D'Agostino and Pearson test of normality (K2=12.11, p=0.0023).

fMRI Analysis

Preprocessing

First, the susceptibility-induced off-resonance field was estimated using the opposite phase encoded images and a correction applied using TOPUP (Andersson et al., 2003) implemented with FSL (Smith et al., 2004) (RRID: SCR_002823). Functional EPI images were analyzed using FSL-FEAT (fMRI Expert Analysis Tool 6.0) part of FMRIB's Software Library (FSL) (Smith et al., 2004). Using FSL-FEAT, images were brain extracted, slice-time corrected, motion corrected, high-passed filtered, smoothed (8 mm FWHM), and FILM pre-whitened. Images were registered to standard MNI space by first registering the EPI then to the T1-weighted anatomical, then finally to standard space.

First and Second Level Models

To replicate Tang et al. (2014), two GLMs with distinct parametric modulators were created. In GLM1 willingness to pay for each trial was entered as a parametric modulator orthogonalized with true energy density and in a second model, alone. They modulated the picture viewing period which was modeled as a 4 second box-car. Bidding period and head movement were also included as regressors of no interest.

To test our unique hypothesis, an additional GLM was created. This model contained estimated calories, true energy density, hedonic value, and bid amount for each item broken out by macronutrient group, resulting in 3 main onsets with 4 parametric modulators each. Picture viewing and bidding period were also included, as in the previous model. The contrast of combination greater than either macronutrient alone was modeled on the first level to account for within subject variance. Six head motion regressors were included as regressors of no interest.

To test brain correlates of energy density in fat versus carbohydrate groups, we created a final GLM containing energy density for all three groups and nuisance regressors. The other regressors were left out of the model as they did not differ between the fat and carbohydrate groups.

After first level modeling for all GLMs, the four runs were then combined for each participant using a fixed effects model. The resulting contrast maps were analyzed on a third level using Flame1 (Smith et al., 2004) variance estimation and whole brain cluster corrected at p<0.001, a significance threshold selected to reduce the risk of type I error (Eklund et al., 2016). Statistical maps are presented as z-scores of the t-statistic and are overlaid on the MNI template and displayed using MRIcroN (Rorden and Brett, 2000). Finally, analyses of variance (ANOVA) and Student's t-tests, where appropriate, were run on extracted parameter estimates using GraphPad Prism, post-hoc differences between groups were tested and the resulting p-values were Bonferroni corrected and reported.

Influence of Gender on Primary Findings

To tests the role of gender in our main effect of macronutrient content on bid amount, we added gender as a covariate to our linear mixed effects model. There was no main effect of gender in the model F(1,772)=0.22, p=0.6364 and the main effect of macronutrient was still significant (p<0.000001). Gender, however, did not improve model fit and appeared to decrease model fit (as indicated by AIC).

For our main neuroimaging findings of interest, we used a similar approach and entered gender as a covariate on the group level. Adding gender as a covariate does not alter the main effects.

DATA AND SOFTWARE AVAILABILITY

This accession number for the statistical maps of the human brain is NeuroVault: HYELUVKG.