Supplemental Section: Felsted et al., "Genetically determined differences in brain response to a primary food reward

Genotyping Procedure

The Oragene-DNA Self-Collection Kit (vial format) was used for the collection, preservation, and purification of DNA from saliva. After participants delivered saliva into the container, it was capped, resulting in releasing DNA-preserving fluid that mixes with the saliva. The DNA from each sample was extracted according to the manufacturer's instructions. The *Taq*IA single nucleotide polymorphism (SNP; rs1800497; Accession Number: NP_848605.1) region of the ANKK1 gene, located downstream from the DRD2 gene (Neville et al., 2004), was amplified for each genomic sample using polymerase chain reaction (PCR) techniques, run on an iCycler thermocycler (Bio-Rad), based on primer sequences specific for the sequence of interest (*Taq*IA SNP specific primers: forward 5'-CCCTTCCTGAGTGTCATCA-3', reverse 5'-CGGCTGGCCAAGTTGTCT-3'). The amplified PCR products were digested overnight with the *Taq*I restriction enzymes and analyzed using 3% agarose gel electrophoresis. Images of each processed gel were obtained using a cooled-CCD digital imaging system (ChemiDoc, Bio-Rad). The presence or absence of a given allele was established based on the predicted sizes of the PCR products. *Taq*IA allele status was determined as follows: a) for individuals without the A1 variant (A2/A2 genotype) two bands of predicted sizes 177 base pairs (bp) and 127 bp were observed, b) individuals with the A1/A2 genotype displayed three bands of predicted sizes 307 bp, 177 bp, and 127 bp, and c) individuals with the A1/A1 genotype showed a single band of predicted size 307 bp (Epstein et al., 2007).

Supplemental Table 1.

Eating Behavior			A1+	A1-
Dutch Eating Behavior Questionnaire	Emotional Eating – Total	F(1,24)=0.04, p=0.84	2.42±0.27	2.36±0.21
	Emotional Eating – Diffuse Emotions	F(1,24)=0. p=1	2.65±0.25	2.65±0.27
	Emotional Eating – Clearly Labeled Emotions	F(1,24)=0.08, p=0.78	2.32±0.29	2.22±0.19
	External Eating	F(1,24)=0.13, p=0.72	3.28±0.12	3.34±0.10
	Restrained Eating	F(1,24)=0.80, p=0.38	2.42±0.25	2.72±0.24
Three Factor Eating Questionnaire	Restraint	F(1,24)=0.33, p=0.57	7.69±1.52	9.0±1.84
	Disinhibition	F(1,24)=0.77, p=0.39	8.15±1.16	6.85±1.03
	Hunger	F(1,24)=0.37, p=0.55	6.77±1.27	5.77±1.16
Power of Food Scale	Food Available	F(1,24)=0.04, p=0.85	13.62±1.68	14.08±1.89
	Food Present	F(1,24)=0.01, p=0.93	17.54±1.84	17.31±1.87
	Food Tasted	F(1,24)=0.002, p=0.96	14.54±1.28	14.46±1.17
Binge Eating Scale		F(1,24)=0.36, p=0.55	12.54±2.31	10.77±1.99
Personality			**A1+**	**A1-**
Tridimensional Personality Questionnaire	Novelty Seeking 0	F(1,24)=2.68, p=0.11	18.08±1.25	14.69±1.75
	Novelty Seeking 1	F(1,24)=0.96, p=0.34	6.31±0.34	5.62±0.65
	Novelty Seeking 2	F(1,24)=0.29, p=0.60	2.31±0.52	1.92±0.53
	Novelty Seeking 3	F(1,24)=2.46, p=0.13	4.31±0.36	3.31±0.56
	Novelty Seeking 4	F(1,24)=3.20, p=0.09	5.15±0.47	3.85±0.60
	Harm Avoidance 0	F(1,24)=0.27, p=0.61	12.38±1.79	11.23±1.45
	Harm Avoidance 1	F(1,24)=0.41, p=0.53	2.92±0.65	2.38±0.58
	Harm Avoidance 2	F(1,24)=0.19, p=0.67	4.15±0.53	3.85±0.53
	Harm Avoidance 3	F(1,24)=0.45, p=0.51	2.31±0.67	2.92±0.68
	Harm Avoidance 4	F(1,24)=1.08, p=0.31	3±0.68	2.08±0.63
	Reward Drive 0	F(1,24)=0.63, p=0.44	19.69±1.08	20.77±0.92
	Reward Drive 1	**F(1,24)=4.72, p=0.04**	**3.69±0.25**	**4.38±0.22**
	Reward Drive 2	F(1,24)=0.47, p=0.50	5.77±0.50	5.31±0.49
	Reward Drive 3	F(1,24)=0.03, p=0.87	6.69±0.65	6.85±0.73
	Reward Drive 4	F(1,24)=2.72, p=0.11	3.54±0.35	4.23±0.27
Barrett Impulsiveness Scale (Version 11)	Total	F(1,24)=0.36, p=0.55	60.46±3.42	58.08±2.29
	Factor 1	F(1,24)=1.14, p=0.30	10±0.76	8.85±0.82
	Factor 2	F(1,24)=0.01, p=0.91	14.54±1.06	14.38±0.85
	Factor 3	F(1,24)=2.33, p=0.14	12.77±1.10	10.85±0.72
	Factor 4	F(1,24)=0.007, p=0.93	11.23±0.76	11.15±0.56
	Factor 5	F(1,24)=0.29, p=0.59	6.62±0.63	7±0.39
	Factor 6	F(1,24)=0.77, p=0.39	5.31±0.48	5.85±0.42

	Factor One	F(1,24)=0.21, p=0.65	15.31±0.79	14.69±1.13
	Factor Two	F(1,24)=0.02, p=0.89	21.15±1.38	21.38±0.93
	Factor Three	F(1,24)=1.08, p=0.31	24±1.70	22±1.07
BIS/BAS	BIS	F(1,23)=0.04, p=0.85	3.06±0.11	3.02±0.17
	BAS - Total	F(1,23)=2.00, p=0.17	3.28±0.12	3.08±0.08
	BAS - D	F(1,23)=1.49, p=0.24	2.98±0.20	2.69±0.14
	BAS - R	F(1,23)=0.02, p=0.88	3.63±0.11	3.62±0.06
	BAS - F	F(1,23)=2.94, p=0.10	3.15±0.16	2.81±0.13

Supplemental Table 2

Area or Contrast	Co-ordinate (# of voxels in cluster)	Z statistic	p value*
Main Effect of Milkshake			
(Milkshake – Tasteless)			
Left Insula Extending into Striatum			
Ventral Insular Cortex	-42, -3, -3 (493)	5.0	0.001
Dorsal Insular Cortex	-45, -9, 15	4.7	0.001
Putamen	-27, 3, 3	4.5	0.002
Right Insula Extending into Striatum/Amygdala			
Globus Pallidus	36, -12, -6 (254)	4.7	0.001
Putamen	27, -6, 3	4.5	0.002
Dorsal Insula	42, -9, 12	4.5	0.002
Amygdala	33, -3, -9	4.4	0.002
Right Anterior Dorsal Insula	36, 27, 3 (4)	3.2	0.02
Right Ventroposteromedial Thalamus	12, -21, 6 (85)	4.8	0.001
Left Ventroposteromedial Thalamus	-12, -15, 9 (68)	4.4	0.002
Left Operculum (Rolandic)	-63, 6, 15 (3)	4.3	0.002

Right Operculum (frontal)	63,12,24 (6)	3.9	0.003
	66, 6, 15 (10)	3.6	0.004

P values are FDR corrected across the entire brain unless indicated by *, in which case FDR correction is across the voxels within the region of interest.

Supplemental Table 3

Area or Contrast	Co-ordinate (# of voxels in cluster)	Z-statistic	p-value
Non Carriers (A1-)			
Milkshake - tasteless			
Left Operculum			
Rolandic	-66, -27, 24 (281)	5.3	0.002
Frontal	-51, -57, -18 (22)	3.4	0.05
Right Operculum			
Rolandic	69, -18, 27 (181)	4.3	0.01
Right Amygdala/Ventral Striatum			
Globus Pallidus	36, -15, -6 (769)	4.7	0.005
Amygdala/Pallidus	33, -6, -9	4.6	0.005
Left Amygdala/Ventral Striatum			
Amygdala/Pallidus	-33, -6, -6 (120)	4.3	0.01
Left Cerebellum	-24, -57, -24 (84)	4.3	0.01
	-15, -75, -27 (10)	3.5	0.03
Right Cerebellum	15, -57, -18	3.4	0.04
Pons	0, -18, -3 (15)	3.7	0.03

Medial Frontal Gyrus	33, 27, 36 (22)	3.5	0.04
Left Dorsal Insula	-39, -9, 18 (27)	3.4	0.04
Right Thalamus			
Mediodorsal	9, -21, 12 (80)	4.5	0.003*
Anterior	12, -6, 3	3.4	0.005*
Left Thalamus			
Mediodorsal	-9, -15, 9	4.1	0.003*
Right Midbrain			
Substantia Nigra	3, -15, -12 (51)	3.7	0.03*
Left Midbrain			
Substantia Nigra	-9, -24, -12 (4)	3.2	0.04*

P values are FDR corrected across the entire brain unless indicated by *, in which case FDR correction is across the voxels within the region of interest. Regions written in italics indicate anatomically distinct significant peaks within a cluster.

Supplemental Table 4

Area or Contrast	Co-ordinate (# of voxels in cluster)	Z-value	p-value
Carriers (A1+)			
Tasteless-milkshake			
Right Caudate	21, -18, 24 (8)	3.4	0.05* (one-tailed)
	-18, 18, 9 (4)	3.2	0.05* (one-tailed)

Thalamus	0, -12, 9 (3)	3.1	9.32* (n/s)
	-3, -21, 15	3.0	0.32*(n/s)
Midbrain	-3, 15, -12 (6)	3.2	0.15* (n/s)

* P values are FDR corrected across the across the voxels within regions of interest. Non significant trends are reported here because they help to explain the significant interaction between stimulus and genotype within the thalamus and midbrain. A one-tailed criterion significance was used for the caudate nucleus because we had a specific prediction about this region and the direction of the effect from our prior studies (Stice et al., 2008a; Stice et al., 2008b).

References Cited

Epstein LH, Temple JL, Neaderhiser BJ, Salis RJ, Erbe RW, Leddy JJ (2007) Food reinforcement, the dopamine D2 receptor genotype, and energy intake in obese and nonobese humans. Behav Neurosci 121:877-886.

Neville MJ, Johnstone EC, Walton RT (2004) Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. Hum Mutat 23:540-545.

Stice E, Bohon C, Veldhuizen MG, Small DM (2008a) Relation of reward from food intake and anticipated food intake to obesity: A functional magnetic resonance imaging study. J Abn Psychol 117:924-935.

Stice E, Spoor S, Bohon C, Small DM (2008b) Relation between obesity and blunted striatal respone to food is moderated by *Taq*IA A1 allele. Science 322:449-452.