Supporting Information

Variation in TREK1 gene linked to depression-resistant phenotype is associated with potentiated neural responses to rewards in humans

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Supporting Methods

Participants

Functional MRI data were available for 32 participants. The ethnic composition of the sample included in the fMRI analyses was: 4 African-American, 1 American Indian/Alaska native, 3 Asian, 23Caucasian, 1 unknown or not reported. The SCID was not administered to one participant who did not return for the clinical interview session. A minority of participants reported past Axis I pathology during the SCID (MDD: $n = 1$; depressive disorder not otherwise specified: $n = 1$; binge eating disorder: $n = 1$; anorexia nervosa: $n = 1$; alcohol abuse: $n = 1$). Following the study, participants were debriefed regarding the task design and goals of the research.

Genotyping

A SNP tagging approach was used to examine common genetic variation within TREK1 (Chromosome 1, bp 213245508-213477059) ±10 kbp. All TREK1 SNPs ($n = 256$) contained in

the International HapMapProject Phase II B36 database were identified [The International HapMap Consortium, 2007]. The Tagger program [de Bakker et al., 2005] identified 33 SNPs which provided 99.6% coverage of TREK1±10 kbp polymorphisms with a minor allele frequency > 0.05 among Caucasian Europeans; SNPs tagged had a minimum r^2 of 0.8 with the tagging SNP (mean $r^2 = 0.95$). SNPs with a minor allele frequency of < 0.05 ($n = 1$) or successfully genotyped in fewer than 75% of participants ($n = 3$) were excluded. Due to *a priori* predictions regarding the four SNPs associated with antidepressant response [Perlis et al., 2008] and the limited sample size of the current study, only tests involving those SNPs are reported.

MID task

To increase the plausibility that participants had responded quickly enough on successful but not unsuccessful trials, target durations were slightly longer on "successful" trials (e.g., reward trials ending in gains) than on "unsuccessful" trials (e.g., reward trials ending in no change). Target durations on successful and unsuccessful trials were individually titrated and corresponded to the 85th and 15th percentiles of the RT distribution from a 40-trial practice session completed prior to scanning.

Finally, to increase task engagement, participants were told that good performance would yield an opportunity to complete a sixth "bonus" block including large gains and few penalties; all participants "qualified" for the bonus block. This combination of instructions and task parameters has been found to elicit motivated responding and activity in brain reward networks in two prior independent samples [Dillon et al., 2008; Pizzagalli et al., 2009].

Supporting Results

Entire Sample

RT

As expected, the main effect of *Cue* was highly significant, $F(2, 62) = 32.31$, $p < 0.001$. RT was fastest on reward trials (mean±SD: 325.64±50.76 ms), intermediate on loss trials (343.56±59.25 ms), and slowest on no-incentive trials (400.66±69.14 ms). Within-group *t*-tests revealed significant differences between the reward versus no-incentive comparison [$t(31) = -6.66$], the reward versus loss comparison [$t(31) = -4.43$] and the loss versus no-incentive comparison [$t(31) = -4.90$, all ps < 0.001]. Neither the main effect of *Block* nor the *Cue* x *Block* interaction was significant (ps > 0.25).

Affective Ratings

Cue-related ratings. A one-way repeated-measures ANOVA on cue-elicited valence ratings was significant, $F(2, 62) = 16.60$, $p < 0.001$. Follow-up paired *t*-tests revealed that valence ratings in response to reward (3.28±0.84), loss (2.28±0.74) and no-incentive (2.91±0.51) cues all differed significantly [reward vs. loss: $t(31) = 4.72$, $p < 0.001$; no-incentive vs. loss: $t(31) = 4.51$, $p < 0.001$; reward vs. no-incentive: $t(31) = 2.32$, $p = .033$]. A one-way repeated-measures ANOVA on cue-elicited arousal was also significant, $F(2, 62) = 4.58$, $p = .02$. This effect was driven by elevated arousal ratings for both reward (3.17±0.81) and loss (3.13±0.77) cues relative to no-incentive cues [reward vs. no-incentive: $t(31) = 2.41$, $p = 0.022$; loss vs. no-incentive: $t(31) = 2.33$, $p = 0.027$; reward vs. loss: $t(31) = 0.36$, $p = 0.72$].

Outcome-related ratings. A one-way repeated-measures ANOVA on valence ratings elicited by outcomes was highly significant, $F(2, 62) = 228.66$, $p < 0.001$. Differences in valence

ratings elicited by gains (4.41±0.55), penalties (1.47±0.57), and no change feedback on no-incentive trials (2.98±0.37) were all significant according to follow-up paired *t*-tests [gain vs. penalty: $t(31) = 17.85$, $p < 0.001$; gain vs. no change: $t(31) = 13.47$, $p < 0.001$; no change vs. penalty: $t(31) = 11.18$, $p < 0.001$]. The one-way repeated-measures ANOVA on outcome-elicited arousal was also significant, $F(2, 62) = 16.10$, $p < 0.001$. Within-group *t*-tests revealed that arousal ratings in response to gains (3.64±0.84) and penalties (3.45±0.95) were both greater than arousal in response to no change feedback (2.59±1.00) [gain vs. no change: $t(31) = 6.05$, $p < 0.001$; penalty vs. no change: $t(31) = 4.01$, $p < 0.001$; gain vs. penalty: $t(31) = 0.94$, $p = 0.36$]. In summary, the RT and ratings data indicated that the cues and outcomes elicited motivated behavior and the intended affective responses.

Whole-Brain Responses to Reward Cues and Gains

Results of the whole-brain reward cue minus no-incentive cue and gain minus no change feedback contrasts are presented in Tables S1 and S2, respectively.

Results by TREK1 Genotype

Demographics, DAT1/COMT, and questionnaires

Data are presented in Tables S3 through S6; one participant did not complete the BDI or MASQ, another did not complete the STAI or PANAS measures, and COMT data were unavailable for two participants. Importantly, there was no evidence for differential association of protective TREK1 alleles with possession of DAT1 9-R alleles or COMT met/met genotypes, which can influence dopamine levels and have been associated with reward responses in previous fMRI studies [e.g., Dreher et al., 2009].

RT

There was no evidence that variation in any SNP was associated with differences in reward-related RT (all effects involving *Genotype*, *p*s > 0.05).

Affective Ratings

There was little evidence that variation in SNPs influenced subjective responses to cues or outcomes. For cue-elicited valence and arousal ratings, no effect involving *Genotype* was significant for rs10494996, rs2841608, or rs12136349 (*p*s > 0.07). Similarly, cue-elicited valence did not differ by SNP sub-group for rs2841616 (*F*s < 1 for main effect of *Genotype* and *Genotype* x *Cue* interaction). By contrast, a significant main effect of *Genotype* was found for cue-elicited arousal ratings for this SNP, $F(1, 29) = 4.32$, $p = 0.047$. However, this reflected generally lower arousal ratings in the group with the protective variant (CC: 2.65±0.60) versus the group with the at-risk variant (CT/TT: 3.14±0.61), rather than an effect specifically related to reward processing. For outcome-elicited arousal and valence ratings, no effect involving *Genotype* was significant for any of the four SNPs (all *p*s > 0.08).

Caucasians

Basal Ganglia Outcome Analyses

Due to population stratification concerns, the basal ganglia ROI analyses examining responses to outcomes were repeated using data from Caucasian participants only ($n = 22$; 9 females; 21.96±3.73 years; mean education: 14.73±1.67 years). For the three SNPs with

significant effects on reward processing in the entire sample, the assignment of Caucasians into groups was as follows (rs10494996: AA/AG, $n = 8$; GG, $n = 14$; rs2841616: CC, $n = 8$; CT/TT, $n = 14$; 2841608: CC, $n = 9$; AA/AC, $n = 13$).

For all three SNPs, a stronger mean basal ganglia response to gains was observed in the group with the protective variant versus the group with the at-risk variant. As in the entire sample, group differences in response to no change or penalty feedback were minimal (Figure S1). However, the between-group test on responses to gains was marginal for rs2841608 [$t(20) = 1.89$, $p = 0.09$], and non-significant for rs10494996 [$t(20) = 1.76$, $p = 0.12$] and rs2841616 [$t(20) = 1.20$, $p = 0.24$].

Alternative Assessment of DAT1/COMT Interactions on Response to Gains

As reported in the main text, the combined influence of DAT1 and COMT genotypes was examined by coding DAT1 10-R or COMT val alleles as 0, and DAT1 9-R or COMT met alleles as 1, and then summing to form a composite score for each participant. This analysis was based on the results of Dreher et al. [2009], who found the strongest reward responses in multiple brain regions for 9-R met/met individuals. However, Yacubian et al. [2007] reported that ventral striatal responses to reward-predicting cues increased as a function of reward probability and magnitude in all participants except individuals with (1) both the DAT1 10/10 genotype and the COMT met/met genotype or (2) both the DAT1 9-R allele and the COMT val/val genotype. These individuals (i.e., 10/10 met/met and 9-R val/val) would receive intermediate composite scores on the continuous DAT1/COMT measure described in the main text (based on the findings of Dreher et al. [2009]), which would be problematic if in fact they exhibit especially weak basal ganglia reward responses (in line with the findings of Yacubian et al. [2007]). Thus,

we performed a secondary analysis that was based on the findings reported by Yacubian et al. [2007]. Specifically, we conducted a between-groups *t*-test comparing mean basal ganglia response to gains in individuals who did not show increased basal ganglia activation as a function of reward probability and magnitude in Yacubian et al.'s [2007] study (i.e., 10/10 met/met and 9-R val/val, $n = 8$) against all other genotype groups ($n = 21$). The test was not significant, $t(27) = 1.72$, $p = .10$. These results are again consistent with the conclusion that the association observed between TREK1 genotypes and gain responses did not simply reflect overlap with particular DAT1 and/or COMT genotypes.

Supporting References

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Table S1. Results of the *Reward Cue* minus *No-Incentive Cue* contrast in the entire sample (n = 32)

Location of Peak Voxel	x	y	z	Volume (mm^3)	Cluster-wise p-value
L Superior Frontal Gyrus	-6	3	47	3256	< .00001
L Globus Pallidus	-14	-7	3	3984	< .00001
R Head of Caudate	10	9	-1	3904	< .00001
R Precentral Gyrus	53	0	49	1032	.00184
R Middle Occipital Gyrus	38	-88	-1	712	.01813
L Cerebellum	-18	-43	-16	952	.00319
R Midbrain	4	-28	-6	664	.02619

Note. L = left, R = right. Coordinates are in Talairach space. Cluster-wise p-values reflect correction for multiple comparisons using Gaussian Random Field theory with a voxelwise threshold of $p < .001$; only clusters with cluster-wise p-values less than .05 are reported.

Table S2. Results of the *Gain* minus *No Change* contrast in the entire sample (n = 31)

Location of Peak Voxel	*x*	*y*	*z*	Volume (mm^3)	Cluster-wise p-value
L Insula	-38	16	-7	5656	< .00001
L Inferior Frontal Gyrus	-46	8	24	1952	.00001
R Inferior Frontal Gyrus	46	26	-6	33800	< .00001
L Dorsal Anterior Cingulate Cortex	-8	33	16	2368	< .00001
L Subcallosal Gyrus	-10	12	-13	2376	< .00001
R Gyrus Rectus	10	18	-14	648	.02629
L Posterior Cingulate Gyrus	-4	-25	32	840	.00606
L Caudate	-18	-4	20	1440	.00011
R Caudate	12	11	6	792	.00865
	18	-12	18	976	.00228
R Superior Frontal Gyrus	10	23	51	9184	< .00001
R Middle Temporal Gyrus	50	-34	1	4176	< .00001
	42	-61	-5	608	.03622
L Middle Temporal Gyrus	-48	-56	-8	1960	.00001
L Middle Occipital Gyrus	-24	-83	0	1992	< .00001
R Middle Occipital Gyrus	42	-86	5	1064	.00124
L Cerebellum	-14	-80	-36	3048	< .00001
Midbrain	2	-21	-8	1296	.00027

Note. See Table S1 for more detail.

Table S3. *Demographic, DAT1/COMT, and Questionnaire Data for rs10494996*

	At-Risk Variant (GG: *n* = 21)	Protective Variant (AA/AG: *n* = 10)	Statistic	p-value
Ethnicity (%Caucasian)	66%	80%	$\chi^2 = 0.59$	0.38
Age	21.20 (2.66)	21.95 (4.70)	$t = -0.57$	0.58
Gender	12 female, 9 male	4 female, 6 male	$\chi^2 = 0.80$	0.31
Education	14.43 (1.54)	14.60 (1.65)	$t = -0.28$	0.78
DAT1 (% 9-R carriers)	24%	30%	$\chi^2 = 0.14$	0.52
COMT (% met/met)	32%	30%	$\chi^2 = 0.30$	0.89
BDI-II	6.35 (10.11)	3.80 (3.79)	$t = 0.77$	0.45
STAI	38.35 (11.16)	32.10 (5.72)	$t = 1.66$	0.11
PANAS: Positive Affect	32.95 (4.51)	35.70 (6.90)	$t = -1.32$	0.20
PANAS: Negative Affect	**14.90 (5.35)**	**11.50 (1.84)**	$\mathbf{t = 2.56}$	**0.02**
MASQ GDA	17.35 (8.13)	17.20 (4.32)	$t = 0.05$	0.96
MASQ AA	20.60 (4.79)	19.30 (2.41)	$t = 0.80$	0.43
MASQ GDD	20.90 (9.80)	16.30 (4.37)	$t = 1.41$	0.17
MASQ AD	**54.70 (14.13)**	**40.15 (6.40)**	$\mathbf{t = 3.88}$	**0.001**

Note. With the exception of ethnicity, gender, and DAT1/COMT groups, data represent mean (*SD*). For categorical data, Fisher's exact test (2-sided) was used if any expected cell count was less than five. COMT data was unavailable for two participants.

Variables showing significant results are bolded. BDI-II: Beck Depression Inventory II (Beck et al., 1996); STAI: Spielberger State-Trait Anxiety Inventory (Spielberger et al., 1970); PANAS: Positive and Negative Affect Schedule (Watson et al., 1988); MASQ: Mood and Anxiety Symptom Questionnaire (Watson et al., 1995). For the MASQ, GDA = General Distress Anxiety; AA = Anxious Arousal; GDD = General Distress Depression; AD = Anhedonic Depression.

Table S4. *Demographic, DAT1/COMT, and Questionnaire Data for rs2841608*

	At-Risk Variant (AA/AC: $n = 21$)	Protective Variant (CC: $n = 10$)	Statistic	p-value
Ethnicity (%Caucasian)	62%	90%	$\chi^2 = 2.60$	0.12
Age	21.56 (4.01)	21.20 (1.53)	$t = 0.27$	0.79
Gender	13 females, 8 males	3 females, 7 males	$\chi^2 = 2.76$	0.10
Education	14.38 (1.69)	14.70 (1.25)	$t = -0.53$	0.60
DAT1 (% 9-R carriers)	24%	30%	$\chi^2 = 0.14$	0.52
COMT (% met/met)	25%	44%	$\chi^2 = 1.67$	0.45
BDI	6.35 (9.90)	3.80 (4.85)	$t = 0.77$	0.45
STAI	36.86 (10.99)	34.89 (7.75)	$t = 0.49$	0.63
PANAS: Positive Affect	34.33 (5.13)	32.78 (6.36)	$t = 0.71$	0.48
PANAS: Negative Affect	13.95 (4.72)	13.33 (5.05)	$t = 0.32$	0.75
MASQ GDA	18.40 (8.25)	15.10 (2.51)	$t = 1.22$	0.23
MASQ AA	20.85 (4.85)	18.80 (1.69)	$t = 1.29$	0.21
MASQ GDD	19.95 (8.08)	18.20 (9.87)	$t = 0.52$	0.61
MASQ AD	51.15 (14.72)	47.25 (12.28)	$t = 0.72$	0.48

Note. TREK1 genotype groups did not differ on any variable. See Table S3 for more detail.

Table S5. *Demographic, DAT1/COMT, and Questionnaire Data for rs2841616*

	At-Risk Variant (CT/TT: $n = 22$)	Protective Variant (CC: $n = 9$)	Statistic	p-value
Ethnicity (%Caucasian)	64%	89%	$\chi^2 = 1.98$	0.17
Age	21.32 (3.65)	21.74 (2.80)	$t = -0.31$	0.76
Gender	13 female, 9 male	3 female, 6 male	$\chi^2 = 1.70$	0.18
Education	14.36 (1.53)	14.78 (1.64)	$t = -0.67$	0.51
DAT1 (% 9-R carriers)	27%	22%	$\chi^2 = 0.09$	0.58
COMT (% met/met)	23%	57%	$\chi^2 = 4.26$	0.15
BDI	6.10 (9.72)	4.11 (5.01)	$t = 0.58$	0.57
STAI	36.91 (10.44)	34.50 (9.23)	$t = 0.58$	0.57
PANAS: Positive Affect	34.18 (5.06)	33.00 (6.76)	$t = 0.52$	0.61
PANAS: Negative Affect	13.91 (4.61)	13.38 (5.40)	$t = 0.27$	0.79
MASQ GDA	18.33 (8.01)	14.89 (2.80)	$t = 1.25$	0.22
MASQ AA	20.67 (4.80)	19.00 (1.66)	$t = 1.01$	0.32
MASQ GDD	19.57 (8.04)	18.89 (10.25)	$t = 0.20$	0.85
MASQ AD	50.76 (14.35)	47.72 (13.21)	$t = 0.54$	0.59

Note. TREK1 genotype groups did not differ on any variable. See Table S3 for more detail.

Table S6. *Demographic, DAT1/COMT, and Questionnaire Data for rs12136349*

	Protective Variant (CC: $n = 21$)	At-Risk Variant (CT/TT: $n = 10$)	Statistic	p-value
Ethnicity (%Caucasian)	76%	60%	$\chi^2 = 0.86$	0.30
Age	21.95 (3.93)	20.39 (1.44)	$t = 1.21$	0.24
Gender	9 female, 12 male	7 female, 3 male	$\chi^2 = 2.00$	0.15
Education	14.67 (1.71)	14.10 (1.10)	$t = 1.11$	0.28
DAT1 (% 9-R carriers)	24%	30%	$\chi^2 = 0.14$	0.52
COMT (% met/met)	37%	20%	$\chi^2 = 0.91$	0.69
BDI	4.38 (4.18)	8.11 (14.49)	$t = -0.76$	0.47
STAI	35.25 (8.72)	38.30 (12.53)	$t = -0.78$	0.44
PANAS: Positive Affect	34.75 (5.13)	32.10 (5.95)	$t = 1.27$	0.22
PANAS: Negative Affect	13.80 (4.37)	13.70 (5.66)	$t = 0.05$	0.96
MASQ GDA	16.33 (4.15)	19.56 (11.25)	$t = -0.84$	0.43
MASQ AA	20.00 (4.68)	20.56 (2.74)	$t = -0.33$	0.74
MASQ GDD	19.00 (8.07)	20.22 (10.16)	$t = -0.35$	0.73
MASQ AD	50.55 (14.14)	48.22 (13.89)	$t = .042$	0.68

Note. TREK1 genotype groups did not differ on any variable. See Table S3 for more detail.

Figure S1. Mean basal ganglia responses to gains, penalties, and no change feedback in Caucasian subjects only.