

ORIGINAL ARTICLE

CACNA1C risk variant affects reward responsiveness in healthy individuals

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The variant at rs1006737 in the L-type voltage-gated calcium channel (alpha 1c subunit) *CACNA1C* gene is reliably associated with both bipolar disorder and schizophrenia. We investigated whether this risk variant affects reward responsiveness because reward processing is one of the central cognitive-motivational domains implicated in both disorders. In a sample of 164 young, healthy individuals, we show a dose-dependent response, where the rs1006737 risk genotype was associated with blunted reward responsiveness, whereas discriminability did not significantly differ between genotype groups. This finding suggests that the *CACNA1C* risk locus may have a role in neural pathways that facilitate value representation for rewarding stimuli. Impaired reward processing may be a transdiagnostic phenotype of variation in *CACNA1C* that could contribute to anhedonia and other clinical features common to both affective and psychotic disorders.

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INTRODUCTION

Although the heritability of psychiatric illness is high, the genetic mechanisms that confer susceptibility remain relatively unknown.^{1,2} Recent genome-wide association studies (GWAS) have repeatedly identified a risk variant in the *CACNA1C* gene, which encodes an L-type voltage-gated calcium channel (alpha 1C subunit). The risk variant (rs1006737: A allele) is significantly associated with bipolar disorder^{3,4} and schizophrenia,^{5–8} supporting the hypothesis that *CACNA1C* contributes to the genetic overlap between these psychiatric disorders.

The rs1006737 risk variant has also been associated with several intermediate phenotypes such as neural activity during episodic^{9–12} and working memory,¹³ emotional regulation,^{14–16} and verbal fluency.^{17,18} Numerous studies also suggest the rs1006737 variant may affect components of cognition, such as logical/working memory^{19,20} and clinical symptomology.^{21,22} Furthermore, emerging evidence supports a role for rs1006737 in the neural processing of reward²³ and learning.²⁴ Considering that the *CACNA1C* variant appears to transcend diagnostic boundaries, we hypothesize that the risk variant may affect intermediate phenotypes that are associated with several psychiatric illnesses.

A growing body of research suggests that the neural circuitry supporting reward processing may be a suitable platform of study, as impaired reward function is repeatedly observed as a component of neuropsychiatric disorders.^{25–30} One promising candidate intermediate phenotype for several neuropsychiatric disorders is response bias, as measured by the probabilistic reward-learning task.³¹ The paradigm measures the development of response bias on the basis of a differential reinforcement schedule and indicates an individual's propensity to respond to reward. Reward responsiveness initially predicted an anhedonic phenotype,³¹ but has since been shown to be diminished in

patients with bipolar disorder³² and more general depressive phenotypes.^{33–35}

Reward responsiveness may also be modulated by dopaminergic innervation^{36,37} and interactions between stress and genes that influence the stress-axis.^{38–40} Additional evidence supports a role for gene variants that influence dopaminergic regulation.^{41,42} These associations are supported by findings that reward responsiveness during the task is moderately heritable.⁴³ However, no studies have looked at the potential association between GWAS-identified risk variants and reward responsiveness. We consider *CACNA1C* rs1006737 as a candidate variant to probe for effects in reward responsiveness considering previous literature suggests (a) *CACNA1C* is associated putative effects on reward/learning,^{23,24} (b) *CACNA1C* is associated with mood disorders at genome-wide level^{3,4,7} and (c) reward responsiveness is diminished as a component of these psychiatric illnesses.^{32–35} As the A allele at rs1006737 is overrepresented in neuropsychiatric illness, we anticipate that the genotype group with the A risk allele will show blunted reward responsiveness compared with individuals who do not carry a copy of the risk allele.

MATERIALS AND METHODS

Participants

Bangor site: 131 subjects were recruited from Bangor University and genotyped for the *CACNA1C* variant (rs1006737). Participants in this panel were recruited by advertisement, from among the University community (for example, students, employees) on the basis of the following self-reported criteria: western European descent; no experience of psychiatric/neurological symptoms or diagnoses in either themselves or first-degree relatives; no illegal (or recreational) substance use/dependence (excluding nicotine) and no alcohol abuse/dependence. Cardiff site: 34 Caucasian volunteers were genotyped for the *CACNA1C* variant (rs1006737). No participants reported any current mental illness⁴⁴ or use of psychotropic medication. The study was approved by the ethics committee of each University and written, informed consent was given by all participants

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Table 1. Descriptive statistics for CACNA1C rs1006737

| CACNA1C rs1006737 | AA | AG | GG | P |
|---------------------------------|----------------|----------------|----------------|-------|
| <i>Bangor site</i> | | | | |
| Genotype frequency ^a | N = 16 | N = 50 | N = 64 | 0.475 |
| Age ^b | 22.06 (±3.907) | 21.72 (±4.257) | 21.61 (±3.736) | 0.914 |
| Gender (M/F) ^c | N = 8/N = 8 | N = 27/N = 23 | N = 22/N = 42 | 0.097 |
| <i>Cardiff site</i> | | | | |
| Genotype frequency ^a | N = 7 | N = 12 | N = 15 | 0.475 |
| Age ^b | 24.14 (±2.911) | 24.50 (±4.056) | 25.13 (±7.100) | 0.919 |
| Gender (M/F) ^c | N = 2/N = 5 | N = 6/N = 6 | N = 7/N = 8 | 0.639 |
| <i>Combined sample</i> | | | | |
| Genotype frequency ^d | N = 23 | N = 62 | N = 79 | 0.217 |
| Age ^b | 22.70 (±3.698) | 22.21 (±4.315) | 22.23 (±4.717) | 0.899 |
| Gender (M/F) ^c | N = 10/N = 13 | N = 33/N = 30 | N = 29/N = 50 | 0.174 |

^aCACNA1C rs1006737 genotype frequencies between sample site tested with χ^2 . ^bGroup differences tested with one-way analysis of variance. ^cGender group differences tested with χ^2 . ^dGroup differences tested for Hardy–Weinberg equilibrium. Table shows means (except where noted; s.d. appear in parentheses).

before gDNA extraction and participation. A total of 164 healthy individuals were included in the study (sample demographics are described in Table 1). CACNA1C rs1006737 genotype frequencies did not significantly differ between sites ($\chi^2 = 1.488$, $P = 0.475$; Table 1). Genotype frequencies for rs1006737 did not deviate from Hardy–Weinberg equilibrium ($\chi^2 = 1.51$, $P = 0.217$; Table 1). On the basis of the effect size from a previous candidate gene–response bias study with a similar sample size,³⁸ we anticipate to see a small effect size (Cohen's $d = 0.38$). A power calculation⁴⁵ suggested that we had 78% power to detect an effect of this size for rs1006737 on response bias ($\alpha = 0.05$, one-sided).

rs1006737 SNP genotyping

Bangor site: genomic DNA was obtained from saliva using Oragene OG-500 saliva kits for 136 participants. Genotyping of rs1006737 was performed using the Illumina Golden Gate assay (Illumina, San Diego, CA, USA) using the BeadXpress platform, which allows high-throughput multiplex genotyping of SNPs. Assays were designed for the experiment using Illumina's Assay Design Tool (http://support.illumina.com/array/array_software/assay_design_tool.ilmn). SNP sequence: 5'-ACTTGCTCTATCAAAGTCTTGCTATCAATTACATAAGTTCATTCATCTCAGCCCGAA[A/G]TGT TTTCCAGAGCCGGAGACCTCACAGTGTCTCTCAGGACAGTACCTTCAGGTTTGA ATG-3'. Nucleic acid concentration was evaluated using PicoGreen assay (Life Technologies, Carlsbad, CA, USA). Golden Gate genotyping was performed according to manufacturer's protocols. Genotype calling and annotation were performed using GenomeStudio (Illumina). CACNA1C rs1006737 was not available for five individuals from the Bangor site.

Cardiff site: genomic DNA was obtained from saliva using Oragene OG-500 saliva kits for 37 participants. CACNA1C rs1006737 was genotyped using custom SNP genotyping arrays from Illumina (Illumina). Individuals were excluded for ambiguous sex, cryptic relatedness up to third-degree relatives by identity of descent, genotyping completeness < 97%, and non-European ethnicity admixture detected as outliers in iterative EIGENSTRAT analyses of an LD-pruned data set.⁴⁶ Thirty four of the 37 individuals included in the sample had genotype data available for rs1006737.

Experimental Procedure

To measure reward responsiveness, we used a line discrimination task with asymmetric reinforcement, closely modelled after that described in Pizzagalli et al.³¹ and Heerey et al.⁴⁷ Asymmetric reinforcement, in which correct responses to one stimulus receive more frequent rewards than correct responses to another, leads to the development of response bias by increasing participants' likelihood of reporting the more frequently reinforced stimulus.⁴⁸ It is hypothesized that individuals who develop greater levels of response bias are more responsive to rewards.³¹ Trials began with a fixation cross (500 ms), followed by the presentation of a cartoon face with no mouth. After 500 ms, either a short (22 mm) or long (24 mm) mouth appeared on the face. It was visible for 100 ms before disappearing. The face remained on screen until the participant responded with a button press indicating the presence of either the short or long

mouth. Following the response, participants saw a screen that either displayed feedback ('correct +5 pence') or remained blank (no-feedback trials) for 1750 ms. Participants completed three blocks of 100 trials. Both versions of the mouth appeared equally often in pseudo-random order such that there were no more than four successive trials of the same mouth. Participants received reward feedback on 40 correct responses per block. To induce a reward-related response bias in the task, we distributed the rewards asymmetrically across the mouths. The more frequently reinforced mouth received 30 rewards per block and the remaining 10 rewards occurred after responses to the other mouth. We used a pseudo-random reward schedule such that no more than three correct trials in a row received reinforcements. Participants never received feedback on incorrect trials. When positive feedback was scheduled for a trial and the participant answered incorrectly, the reinforcement was postponed until a later unreinforced, correct trial (of the same mouth length) occurred. The length (short or long) of the more frequently reinforced mouth was counterbalanced across participants. All trials where reaction times faster than 200 ms and slower than 3000 ms were removed from the analysis as previously described.⁴⁷ We measured the frequency of each participant's reward feedback schedule on the basis of the number of positive rewards ('correct +5 pence') they received. The maximum bonus was £6 and individuals who earned < £5 were excluded from the analysis ($n = 1$). The reward feedback schedule received did not significantly differ between gender ($F_{1,163} = 1.580$, $P = 0.210$); rs1006737 genotype group ($F_{2,163} = 0.180$, $P = 0.836$); across sample site ($F_{1,163} = 0.525$, $P = 0.47$) and did not correlate with age ($r = 0.084$, $P = 0.285$).

A post-task debriefing interview confirmed that no participants were aware of the reinforcement asymmetry. We used a standard signal detection analysis to calculate d' , a measure of discrimination accuracy [$d' = z(H) - z(F)$] and 'criterion,' the degree to which participants showed a bias towards the more frequently reinforced mouth ($c = -1/2[z(H) - z(F)]$).^{47,48} Please note that we reversed the values for criterion in the analysis (positive values represent a higher propensity for developing response bias), for ease of interpretation.

RESULTS

Effects of demographic factors

There were no site specific differences in participants' ability to discriminate between the mouths ($F_{1,163} = 1.999$, $P = 0.159$) or in the degree to which they developed response biases ($F_{1,164} = 1.095$, $P = 0.297$). Therefore, data from the two sites were combined. Moreover, data from men and women were analysed together, as there were no significant differences between gender groups in either discriminability (d') or criterion across the whole group (both P -values > 0.354) or within CACNA1C rs1006737 genotype groups (all P -values > 0.111). There was a significant positive association between age and discriminability at block 1 ($r = 0.155$, $P = 0.048$). Therefore age was entered as a covariate into a mixed-model analysis of covariance to assess the potential

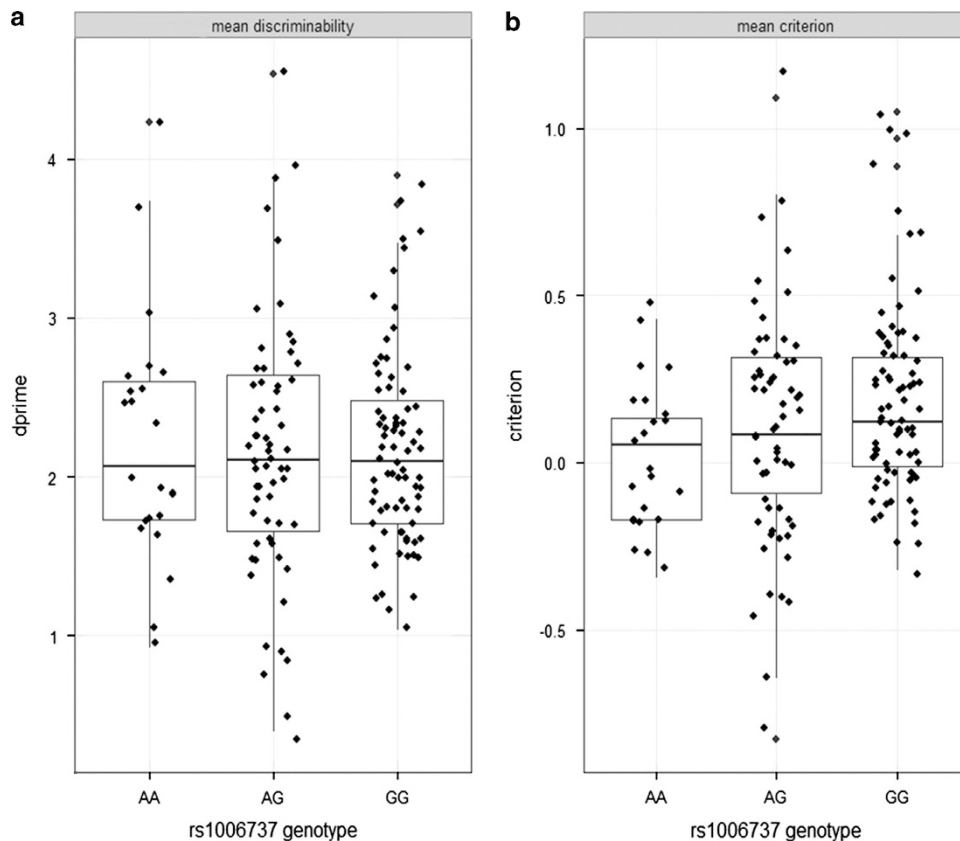


Figure 1. (a) Mean discriminability of long/short mouth types (d') between the *CACNA1C* rs1006737 genotype groups. (b) Differences in mean criterion (response bias) between the *CACNA1C* rs1006737 genotype groups. Horizontal lines represent median and represent 25th and 75th percentile. Note: positive values for criterion reflect an increase in response bias.

effects of *CACNA1C* rs1006737 on discriminability (d'). There were no associations between age and criterion within genotype groups (all P -values > 0.306). One additional individual's discrimination ability was above the expected frequency (GG rs1006737 genotype); however, removing this individual did not significantly affect any demographic or genetic analysis. There were no significant outliers for the criterion measure.⁴⁹ We therefore used mixed-model analyses of variance to compare criterion across task blocks (1–3) and genotype groups (AA=23; AG=62; GG=79). We also ran the same mixed-model analyses of variance for criterion using a dominant genetic model (AA/AG=85; GG=79) as previously described.^{9,23,24}

CACNA1C genotype effects

Participants performed the line discrimination task equally well, regardless of genotype group (additive genetic model: $F_{2,160}=0.006$, $P=0.994$; dominant genetic model; $F_{1,162}=0.000$, $P=0.995$; see Figure 1a). However, we observed *CACNA1C* genotype dependent differences in reward responsiveness, such that the risk allele was associated with diminished reward responsiveness (additive genetic model: $F_{2,162}=3.374$, $P=0.037$, $\eta_p^2=0.040$; dominant model: $F_{1,163}=5.656$, $P=0.019$, $\eta_p^2=0.034$; see Figure 1b). Furthermore, in the additive genetic model, there was a significant task block \times genotype interaction ($F_{2,161}=2.417$, $P=0.05$, $\eta_p^2=0.029$), suggesting that genotype differences in reward responsiveness were larger toward the end of the task (see Figure 2). One-way analysis of variance confirmed that the largest between group difference occurred in task block 3 ($F_{2,163}=3.810$, $P=0.024$, $\eta_p^2=0.045$). *Post hoc* independent-sample t -tests also revealed a dose-dependent response, in which the largest differences in mean response bias occurred between

the AA and GG groups (pairwise comparison; $p_{\text{corrected}}=0.011$; Cohen's $d=0.66$). Independent samples t -test using the dominant genetic model suggested that A risk allele carriers (AA/AG) also had pronounced deficits in reward responsiveness compared with the nonrisk (GG) genotype group ($P=0.019$; Cohen's $d=0.37$). On the basis of our smallest effect size (Cohen's $d=0.37$), we estimate we had 76% power to detect a significant main effect of rs1006737 on mean criterion (one-sided; $\alpha=0.05$).

CACNA1C genotype \times task block effects

To explore the block \times rs1006737 genotype interaction, we explored variations in response bias for each genotype group at each level. We calculated the change in criterion over the task (Δ response bias = block 3 – block 1). A one-way analysis of variance suggested that rs1006737 genotype related to an individual's propensity to develop response bias (additive model; $F_{2,163}=2.950$, $P=0.05$, which was driven by a difference between the AA and AG genotype $p_{\text{corrected}}=0.05$). We additionally performed *post hoc* one-sample t -tests to test whether the rs1006737 genotype groups (AA/AG/GG) response bias significantly differed from 0 at each block (1–3). The AA homozygous risk group did not begin with or develop any response bias at any block ($P>0.2$ in all cases). The AG heterozygous risk group showed a trend towards developing response bias (block 1: NS; block 2: $t=1.807$, $P=0.076$; block 3: $t=2.643$, $P=0.010$), but these results were not significant after correction for multiple comparisons. However, the GG homozygous group showed significant response bias during all three blocks (block 1: $t=4.446$, $P<0.001$; block 2: $t=3.996$, $P<0.001$ and block 3: $t=4.675$, $P<0.001$). This analysis indicates that the GG genotype showed response bias early in the task, the AG genotype group started to show a

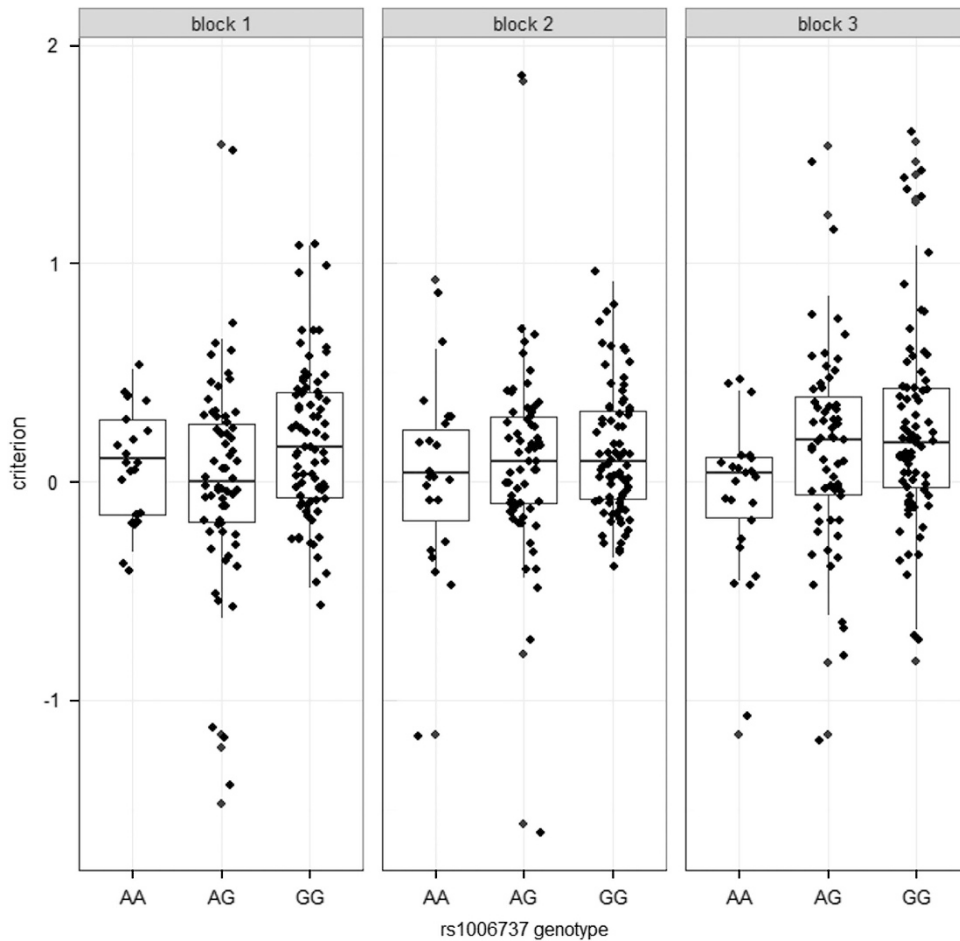


Figure 2. Data represent the criterion (response bias) for each of the three *CACNA1C* rs1006737 genotype groups (as Figure 1b), but split across the three experimental trial blocks. Horizontal lines represent median and represent 25th and 75th percentile. Note: positive values for criterion reflect an increase in response bias.

Δresponse bias in later trials, but in comparison, the AA risk group showed no evidence of developing response bias at any point throughout the paradigm (see Figure 2).

DISCUSSION

Previous research suggests that the *CACNA1C* rs1006737 risk variant is associated with heritable neuropsychiatric disorders.^{3,4,8,50} Reward responsiveness is potentially heritable⁴³ and has been shown to be disrupted in patients with bipolar and unipolar depression.^{32–35} Response bias is also blunted in remitted patients, suggesting that reward responsiveness may serve as trait marker.³³ We demonstrate for the first time that the *CACNA1C* risk variant (rs1006737: A allele) modulates an individual's propensity to respond to reward, without disrupting general task discrimination ability. It is an attractive feature of the reward-responsiveness paradigm that it allows for the investigation of cognitive biases in situations of normal discriminability, which makes it a particularly sensitive trait marker for healthy at-risk populations.

This finding adds to a growing body of literature suggesting that the *CACNA1C* variant may affect the neural mechanisms underlying reward processing and learning.^{23,24,51} Blunted reward responsiveness was seen across the whole task for AA genotype, the rs1006736 AG genotype group showed response bias towards the end of the task, whereas the GG nonrisk group showed reward responsiveness throughout the task. We suggest that rs1006737 'A' risk allele dose may contribute to psychopathology by affecting the rate of reinforcement-based learning, although

this would need to be formally tested in extended versions of the paradigm. This finding is comparable to previous reports where impairment in learning was most pronounced in rs1006737 A carriers at the beginning of the task.²⁴ These results offer novel insight into how *CACNA1C* may confer susceptibility to the neuropsychiatric illness characterized by reduced reward responsiveness and impairments in reward-based learning. The association between *CACNA1C* rs1006737 A allele and blunted response bias may point to a genetic basis for anhedonia, which is a symptom common to affective and psychotic disorders.^{27,32,52–54} Although evidence suggests that anhedonia is a residual trait underlying euthymic bipolar disorder,⁵⁴ it is less straightforward to align our findings with bipolar phenotypes associated with the hyperthymic state, as it is speculated that manic states could be associated with a potentiated rather than blunted response bias.³²

Although the genetic architecture of reward-related deficits and related clinical symptoms (such as anhedonia) remains unknown, reward responsiveness may be a promising neurobiological process that links novel risk loci (such as SNPs identified via genome-wide association studies) with core clinical symptoms. It could also emerge as a promising surrogate marker for treatment effects. A limitation of the present study was that we did not screen for nicotine use, which may have an interactive effect on reward responsiveness.⁵⁵ We suggest that the effect of the rs1006737 (and any other common variant) on reward responsiveness are likely to be small⁵¹ and thus, our sample may have been underpowered. We therefore recommend that our results should be treated with caution until replicated in larger,

independent samples.^{56–58} Additional studies could further help to verify how much variance in response bias associated with rs1006737 genotype. Furthermore, we cannot be sure whether rs1006737 is responsible for these effects or rather SNPs that are in linkage disequilibrium with the variant. Nevertheless, recent GWAS implicate *CACNA1C* as a suitable candidate for probing intermediate traits associated with multiple neuropsychiatric illness.⁵⁹ It is possible that additive variations within *CACNA1C*¹² or interactions with other genes⁶⁰ could further modulate reward responsiveness and explain larger proportions of variance.

In conclusion, our results suggest that the genome-wide identified psychiatric risk locus on *CACNA1C* (rs1006737) may affect an individual's ability to respond to reward. An attractive feature of the rs1006737 'A' risk allele is its association with increased *CACNA1C* mRNA expression in cortical tissue,¹⁶ which offers putative mechanistic insight into how the variant may affect neural circuitry. A growing body of knowledge implicates the *CACNA1C* gene product in animal models of reward processing,^{61–63} therefore translational animal models homologous to reward responsiveness⁶⁴ may also give further insight into the molecular mechanisms underlying the association between *CACNA1C* and reward responsiveness. Future studies could also explore the neural dynamics that support reward responsiveness to understand how *CACNA1C* may exert these effects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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