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Original article

The effects of the *CACNA1C* rs1006737 A/G on affective startle modulation in healthy males

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ABSTRACT

Background: The *CACNA1C* rs1006737 risk A allele has been associated with affective psychoses and functional studies indicate that it is associated with increased hippocampal/amygdala activity during emotional face-processing. Here we studied the impact of the risk A allele on affective startle modulation.

Methods: Hundred and ninety-four healthy males stratified for their *CACNA1C* rs1006737 genotype (GG:111, GA:67, AA:16) were presented with 18 pleasant, 18 unpleasant and 18 neutral pictures with acoustic probes (104 dB) occurring during 12 pictures in each affective category. Baseline startle was assessed during blank screens. State mood was self-rated on arrival, pre- and post-test and the emotional valence and arousal of affective pictures at post-test.

Results: Relative to the other genotypes, risk A allele homozygotes presented with higher anxiety/negative affect at pre-test, reduced and exaggerated physiological responses to the pleasant and negative pictures respectively, negative affect with reduced arousal at post-test and rated the affective pictures as less arousing and inconsistently to their physiological responses (all $P < 0.05$). Sustained contextual negative mood predicted reduced baseline and affective startle reactivity in the AA group.

Conclusions: Healthy homozygous males for the risk A allele appear to have marked contextual sensitivity, affective reactivity akin to anxiety and depression and inefficient emotional appraisal. Our findings provide phenotypic detail of the *CACNA1C* AA genotype in non-symptomatic individuals, which suggest primary effects in emotional circuitry, consistent with previously documented alterations in hippocampal/amygdala processing.

1. Introduction

The *CACNA1C* gene codes for the pore-forming alpha 1C subunit of the L-type voltage-gated calcium channel, which couples transient activation of inward calcium current to transcriptional regulation and plays an important role in dendritic development, neuronal survival, synaptic plasticity, memory formation, learning and behavior [6]. The non-coding *CACNA1C* rs1006737 risk A allele has been associated with risk for bipolar disorder (BD) [25,69], major depression [29], schizophrenia [29,53,58,67] and schizotypal personality disorder [64]. Studies that try to understand gene effects in brain function [50] indicate that the same allele is associated with decreased gene expression [65], increased anxious/depressive/paranoid personality traits and psychopathology [21,63] and increased hippocampal/amygdala activity [7,38,74] with reduced corticolimbic/frontotemporal functional connectivity during emotional face-processing [73]. The gene may have pleiotropic effects possibly due to effects on hippocampal/amygdala and prefrontal circuits [7], which have been related to genetic risk for affective disorders [3,75] and schizophrenia [11].

This is a first attempt to test the effects of risk associated variation in this gene on affective startle modulation by IAPS (International Affective Pictures System) stimuli [42]. Startle is a

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defensive reflex and its magnitude increases with increased negative valence of the foreground stimuli and is attenuated during exposure to positive stimuli [9,15]. Consequently, this paradigm is useful in examining individual differences in affective reactivity of non-clinical populations [14] or alterations of affective reactivity in mood disorders [31]. While affective startle modulation is normal in schizophrenia [16,68], studies in severely depressed/anhedonic patients currently [2,40] or in remission [54], remitted bipolar patients and their unaffected 1st-degree relatives [27] or severely anxious patients with sustained dysfunction [49], suggest that blunted startle reactivity and affective modulation may be trait features of severe affective disorder phenotypes. In non-clinical individuals, reduced or non-detectable startle reactivity has been associated with temperament traits [28] or polymorphisms [55,61] relevant to affective disorders. As a risk gene for major depression and bipolar disorder, we thus hypothesized that the *CACNA1C* risk (A) allele would reduce baseline and affective startle reactivity in healthy subjects. Given the non-specific role of this polymorphism to psychopathology, the examination of its association with affective startle modulation may provide information regarding trans-diagnostic mechanisms.

2. Methods

2.1. Subjects and genotyping

Two hundred and twenty, right-handed males from the first wave of the LOGOS study, which was approved by the local Ethics Committee and has been previously described in detail [63], were randomly selected for startle assessment. All participants gave written informed consent. Genotyping was performed blind to phenotype measures as described elsewhere [63] with a 0.985 call rate. After exclusion of 26 startle non-responders, 194 subjects entered the study. These were of Caucasian ancestry on the basis of self-report, as well as STRUCTURE analysis [57] using a panel of ancestry informative unlinked markers, as described previously [62]. Genotype distributions were: GG:111, GA:67, AA:16, A/G allele counts were 99/289 and minor allele frequency (MAF) was 0.255. No deviation from Hardy-Weinberg Equilibrium was observed ($\chi^2 = 2.36$, $P = 0.13$).

2.2. Procedure

Subjects rated their mood (alertness, anxiety, discontentment) using visual analogue scales (VAS) [8] on arrival to the lab, immediately after the instructions in preparation for startle testing and after startle testing. They also rated the IAPS pictures for valence and arousal with the Self-Assessment Manikin (SAM) [41] at post-test.

2.3. Startle testing

Our equipment and set up have been described in detail previously [27]. All subjects were presented with 54 IAPS[1] pictures (18 pleasant, 18 unpleasant, 18 neutral), each presented for 6 s. Of these, 36 pictures (12/valence type) were accompanied by an acoustic probe (50-ms presentation of 104-dB white noise over 70-dB background noise running throughout the experiment). Of the 12 acoustic probes presented during each affective category, 6 were presented 3000-ms after picture onset and 6 probes were presented 4500-ms after picture onset to increase unpredictability of startle, while six pictures of each valence type were not accompanied with a startle probe. There were no differences in normative arousal ratings between pictures accompanied by different types of probes within the same affective category. Each picture was followed by a blank screen of 3–12 s (average 9 s) inter-picture interval. Subjects received further 15 acoustic probes during blank screens, which served as a control to calculate baseline startle. The experiment lasted about 35 minutes. EMG of the orbicularis oculi was recorded from the left eye. Trial exclusion and scoring criteria were identical to those used in previous studies [27,61]. Startle testing and scoring was blind to subjects' genotype. Startle magnitude responses were averaged across valence type and the mean magnitude scores for pleasant, neutral and unpleasant pictures entered the analysis. Startle magnitude differences (pleasant-neutral and unpleasant-neutral) were also calculated and analyzed. Baseline startle magnitude was defined as the mean startle magnitude across both probes during the blank screens.

2.4. Statistical analysis

Demographics, VAS-rated mood on arrival, and baseline startle were compared between the three genotypes with parametric or non-parametric comparisons as appropriate. Pre- and post-test VAS ratings were analyzed with separate 3×2 (genotype by occasion) repeated measures ANOVAs. Separate 3×3 (genotype by valence) repeated measures ANOVAs were used to analyze startle magnitude and SAM valence and arousal ratings of the IAPS stimuli. Greenhouse-Geisser corrections were used for main effects and interactions involving factors with more than two levels. For significant findings involving genotype, we report effect sizes (partial η^2) and observed power. Exploratory correlational analyses were also performed using parametric or non-parametric statistics as appropriate. Since group comparisons were planned and hypothesis driven, we did not consider Bonferroni correction of the threshold of statistical significance was necessary for startle data. However, alpha was set at 0.025 for VAS and SAM ratings.

3. Results

The genotype groups did not differ in demographics, baseline VAS-rated mood on arrival, or IQ (Table 1).

3.1. VAS-rated state mood

The three dimensions of the VAS state mood, rated immediately after instructions (pre-) and post-startle testing expressed as Δ scores, (differences from baseline mood on arrival) for each group are shown in Fig. 1. The ANOVAs revealed occasion main effects (higher at pre-test, lower at post-test) for anxiety and alertness [$F(1.191) = 37.9$, $P < 0.001$ and $F(1.191) = 18.3$, $P < 0.001$ respectively] but not for discontentment ($F < 1$). The risk allele homozygotes (AA) had a marked elevation of anxiety after the instructions, evidenced by a genotype by occasion interaction [$F(2.191) = 15.3$, $P < 0.001$, $\eta^2 = 0.136$, power = 0.99] and a genotype main effect [$F(2.191) = 7.3$, $P < 0.001$, $\eta^2 = 0.070$, power = 0.93] confirmed with Bonferroni post hoc tests. Discontentment was elevated throughout in the AA group [genotype main effect: $F(2.191) = 3.16$, $P < 0.05$, $\eta^2 = 0.031$,

[1] IAPS numbers for neutral pictures used in the experiment are: 2200, 5500, 5510, 7000, 7002, 7009, 7010, 7020, 7040, 7050, 7060, 7080, 7090, 7100, 7150, 7170, 7175, 7500; for the pleasant pictures: 1650, 2040, 2050, 2057, 2080, 2150, 2160, 4002, 4180, 4210, 4232, 4650, 4660, 7330, 8030, 8080, 8502, 8540; for the unpleasant pictures: 1030, 1111, 1270, 2120, 3051, 3062, 3063, 3064, 3100, 3102, 3140, 3150, 3210, 6242, 6570, 9050, 9405, 9810. Normative arousal ratings (mean ± SD) for the selected IAPS pictures were 5.5 ± 1.3 for pleasant, 2.7 ± 0.5 for neutral and 6.2 ± 0.7 for unpleasant pictures, thus yielding the usual V pattern whereby pleasant and unpleasant pictures are more arousing than neutral ones.

Table 1
Demographics and VAS-rated state mood on arrival (baseline) of the genotype groups (mean ± SD).

	GG	GA	AA	*P*-value
Demographics				
Sample size	111	67	16	
Age, years[a]	22.2 ± 4.0	23.3 ±4.5	23.6 ± 4.6	>0.1
IQ, Raven's raw score[a]	50.9 ± 9.8	50.7± 7.5	50.1 ± 8.5	>0.7
Smokers/non-smokers[b]	44/67	23/44	8/8	>0.5
Smokers: cigarettes/day	14.4 ± 7.0	14.3 ± 5.8	17.8 ± 4.7	>0.3
Baseline VAS-rated state mood				
Anxiety	2.67 ± 1.5	2.21 ± 1.7	2.52 ± 1.8	>0.5
Discontentment	2.16 ± 1.1	1.96 ± 1.3	2.12 ± 0.8	>0.6
Alertness	4.92 ± 1.0	4.96 ± 1.4	4.96 ± 1.0	>0.9

[a] Kruskal-Wallis comparison.
[b] χ^2 comparison, cone-way ANOVA.

power = 0.65], but this effect did not survive Bonferroni correction. The group by occasion interaction was not significant [F(2.191) = 1.1, $P > 0.1$]. Alertness dropped in the AA group at post-test to a greater degree than in the other genotypes, as confirmed with a significant group by occasion interaction [F(2.191) = 3.7, $P < 0.025$, $\eta^2 = 0.037$, power = 0.73]. Inclusion of age, smoking habit or IQ as covariates did not affect these results.

The pattern or correlations between pre- and post-testing mood dimensions is shown in Supplementary Table S1. Pre-test anxiety and pre- or post-test alertness correlated negatively in the GG and GA groups but positively in the AA homozygotes, suggesting non-anxious and anxious/tense arousal respectively. Pre-test anxiety in the AA group did not correlate with pre-test discontentment suggesting possibly dissociable underlying processes for contextual anxiety and negative mood. Post-test anxiety in this group correlated with pre- and post-test discontentment (but not anxiety) and may thus be better conceptualized as part of the negative affect experienced by AA individuals. Pre- and post-test alertness correlated positively in the GG and GA groups but not significantly so in the AA homozygotes suggesting that the high drop in post-test alertness in the latter group could be of a different nature to that observed in the other genotypes.

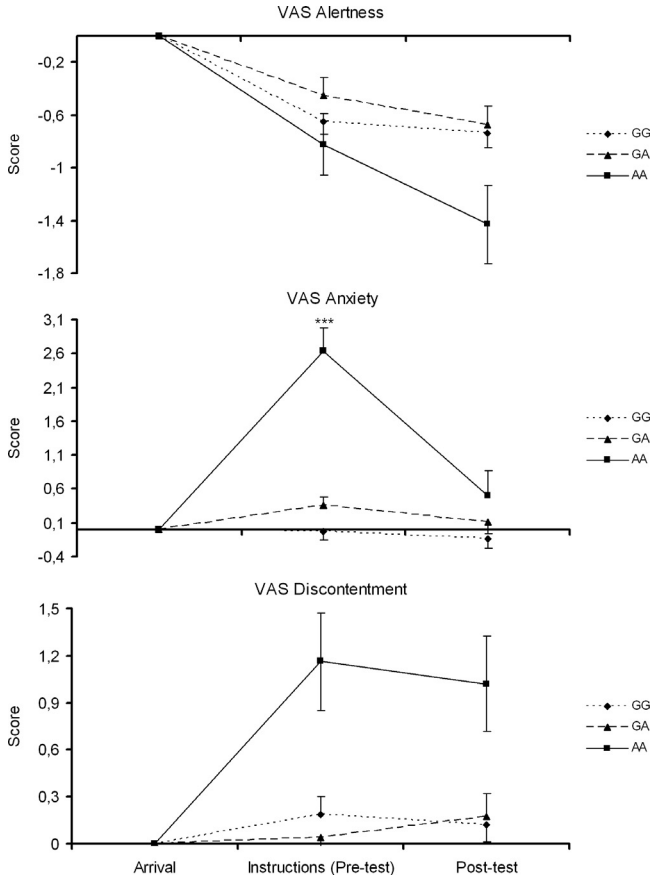

Fig. 1. VAS-rated mood (mean ± SEM) after instructions (pre-) and post-startle testing in the three genotype groups. Scores represent differences from baseline mood on arrival at the laboratory; ***$P < 0.001$.

3.2. Affective picture ratings

The affective picture valence ratings for the entire sample (mean ± SD) were 6.6 ± 1.0 (pleasant), 3.9 ± 1.4 (neutral), and 2.5 ± 1.1 (unpleasant) confirming the categorization of the pictures as pleasant, neutral, and unpleasant. The affective picture arousal ratings for the entire sample (mean ± SD) were 4.7 ± 1.7 (pleasant), 2.0 ± 1.2 (neutral), and 5.1 ± 1.7 (unpleasant) confirming the usual V pattern, where pleasant and unpleasant pictures are seen as more arousing than neutral ones. Fig. 2 shows the subjective valence and arousal ratings of all genotypes for the affective picture categories. ANOVA of the valence ratings showed an expected main effect of valence [F(2.382) = 349.9, $P < 0.001$], but not genotype [F(1.191) = 2.4, $P > 0.1$] or interaction [F(4.382) = 1.85, $P > 0.1$] effects. ANOVA of the arousal ratings showed an expected main effect of valence [F(2.382) = 198.3, $P < 0.001$] but not interaction [F(4.382) = 1.99, $P > 0.1$]. The genotype main effect was significant [F(1.191) = 2.95, $P = 0.05$, $\eta^2 = 0.028$, power = 0.70] due to reduced SAM arousal ratings of both positive and negative pictures by the AA homozygotes. This effect became non-significant ($P > 0.07$) when either post-test discontentment or post-test alertness were taken as covariates; the Preacher and Heys test of mediation showed that

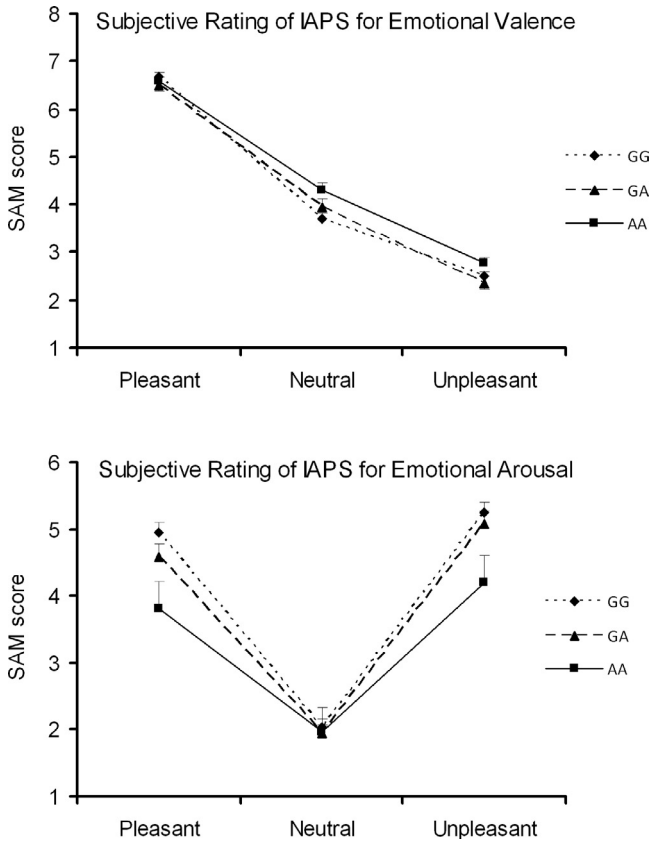

Fig. 2. Valence and arousal ratings (mean ± SEM) of the IAPS pictures assessed at post-testing with the Self-Assessment Manikin (SAM) in the three genotype groups.

both variables were significant mediators of the group main effect (discontentment: $t = -2.01$, $P < 0.05$; alertness: $t = 2.23$, $P < 0.05$), suggesting that the reduced arousal ratings of the pleasant and unpleasant IAPS by the AA homozygotes was attributable to their negative mood and low arousal state at post-test.

3.3. Baseline startle

Baseline startle magnitude (mean ± SD) was 90.3 ± 52.8, 82.5 ± 50.0, and 88.2 ± 60.0 for the GG, GA, and AA groups, respectively. Kruskal-Wallis ANOVA showed no group differences ($P > 0.5$). There were no effects of smoking status on baseline startle magnitude (Mann-Whitney $P > 0.4$). The correlations between baseline startle and VAS-rated mood were significant only within the AA group (Supplementary Table S2); pre-test alertness and anxiety predicted higher but pre-test discontentment and post-test discontentment and anxiety predicted lower baseline startle, altogether suggesting that in the AA group, sustained negative affect and distress expressed as high discontentment throughout and post-test anxiety may have operated to reduce startle reactivity on an individual level.

3.4. Affective startle modulation

Fig. 3 (top panel) shows startle magnitude across the three valence conditions for the three genotypes. Startle was linearly increased from pleasant to neutral to unpleasant pictures and the AA homozygotes had greater startle magnitude in the unpleasant condition. A 3 × 3 (valence by genotype) repeated measures ANOVA showed a main effect of valence [$F(2.382) = 24.5$, $P < 0.001$] and a significant interaction [$F(4.382) = 2.6$, $P < 0.03$, $\eta^2 = 0.026$, power = 0.80]. Fig. 3 (bottom panel) shows clearly that relative to the neutral condition, AA subjects had less attenuation in the pleasant and exaggerated reactivity in the unpleasant condition compared to the other genotypes. A 2 × 3 (valence by genotype) ANOVA of the Δmagnitude scores revealed a significant interaction [$F(2.191) = 3.03$; $P < 0.05$, $\eta^2 = 0.03$, power = 0.65] with post hoc tests confirming the significant startle increase of the AA group in the unpleasant condition compared to both other genotypes. The genotype main effect fell short of significance [$F(2.191) = 2.2$; $P = 0.09$] however, when the SAM arousal ratings for the pleasant IAPS stimuli were taken alone as the covariate, a significant genotype main effect was revealed [$F(2.190) = 3.5$; $P < 0.05$, $\eta^2 = 0.033$, power = 0.70]. No other covariates altered the results or revealed a significant genotype main effect. This suggests that the degree of startle attenuation in the pleasant condition covaried inversely with the way subjects scored in the SAM arousal ratings for the pleasant IAPS pictures only; indeed, exploratory correlations between Δ magnitude of startle attenuation and SAM arousal ratings for the pleasant IAPS confirmed a highly significant inverse relationship for the AA group (Supplementary Table S3), suggesting that the AA homozygotes with the lowest physiological reactivity (startle attenuation) to the pleasant pictures scored these pictures as most arousing. This inverse relationship extends to their SAM valence ratings as well (coefficient: −0.958, $P < 0.001$; data not shown in Supplementary Table S3). The relationship between SAM arousal ratings of the unpleasant IAPS pictures and startle potentiation to these pictures was significant only for the AA group and to the expected direction (Supplementary Table S3).

Exploratory correlations between VAS-rated mood and magnitude of startle potentiation/attenuation across the three genotypes, after controlling for baseline startle, are shown in Supplementary Table S3. Greater levels of startle potentiation to the unpleasant IAPS were predicted by higher alertness and lower pre-test anxiety in the GG homozygotes suggesting that this was an adaptive response related to non-anxious, post-instructions arousal. In the AA group, however, greater levels of startle potentiation and lower levels of startle attenuation to the unpleasant and pleasant IAPS respectively were predicted by higher pre-test alertness and anxiety, suggesting that affective startle reactivity in these individuals was related to their anxious, post-instructions arousal, thus supporting a maladaptive nature for the observed pattern of startle reactivity in this genotype. Post-test anxiety correlated negatively with both startle potentiation and attenuation in the AA group, suggesting that sustained distress expressed as high post-test anxiety relates to reduced affective startle reactivity to both affective stimuli in this genotype.

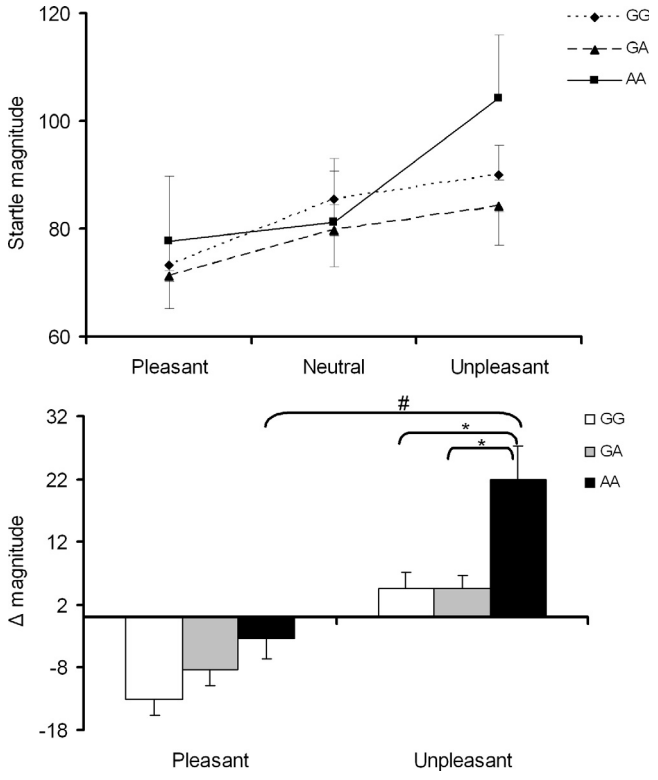

Fig. 3. Startle magnitude across pleasant, neutral and unpleasant pictures (top) and difference from neutral pictures (Δ scores, bottom) for the three genotype groups; #: significant group main effect (i.e. greater startle reactivity to the unpleasant and closer to zero reactivity to the pleasant pictures) when the SAM arousal ratings for the pleasant pictures entered the analysis as the covariate.

4. Discussion

This is the first investigation of the effects of the *CACNA1C* rs1006737 variant in affective startle modulation by IAPS stimuli, which revealed a rich and novel but complex set of data. Individuals homozygous for the risk A allele presented with a highly anxious arousal and negative affect to the experimental context, highly negative affect with much reduced arousal after the experiment, they had attenuated startle reactivity to the pleasant pictures, and rated the emotional IAPS pictures as less arousing. Contrary to prediction, they did not present with attenuated baseline startle or attenuated startle reactivity to the unpleasant pictures which in fact, was exaggerated; however, within the AA group only, sustained contextual negative mood predicted reduced baseline startle and startle reactivity to both affective valences. Our study suggests a recessive model for association of A allele with startle modulation in healthy individuals. These results cannot be attributed to demographic or IQ differences, smoking

habit or baseline state mood on the day. Although our study design does not allow us to measure directly the neural engagement, it is of note that IAPS pictures activate areas involved in emotion processing and regulation e.g. the amygdala, posterior hippocampus and ventromedial prefrontal cortex [1], which overlap significantly with regions of altered morphology or function in neuroimaging studies of *CACNA1C* rs1006737 risk allele carriers [7,38,56,74].

The significant post-instructions elevation of state anxiety and discontentment in AA homozygotes suggests that these individuals are sensitive to such contextual factors as verbal instructions, novelty and anticipation. This is in line with previous associations of the A allele with high neuroticism and introversion, personality traits that are long known to be sensitive to context conditioning [23,32]. This sensitivity is likely to include genetic components, and it is interesting in this respect that in the AA group, pre-test anxiety and (pre- or post-test) discontentment had dissociable profiles of correlations with startle reactivity (anxiety increasing and discontentment reducing startle reactivity – see discussion below) and the significant post-testing drop in alertness in AA individuals was unrelated to their pre-test alertness, in contrast to the other genotypes; this can hardly be a power issue given that most highly significant correlations were observed in the smallest AA group. Instead, it may signify potentially different mood and arousal neural mechanisms operating at post-test in this genotype; low arousal after mental stress has been described in depression [37], and indeed AA individuals may have been in a depression-equivalent state at post-test (sustained discontentment, anxiety, low arousal). Given that context sensitivity has been identified as a core characteristic of mood and anxiety disorders [30,48], the mapping of fear and contextual responses on amygdala/hippocampal processing [24,43,44] and the relevance of the latter to the effects of the risk *CACNA1C* genotype ([7,21] and discussion below), the contextual sensitivity of AA homozygotes is a potentially important finding which requires further exploration with more specific context manipulations.

The A allele homozygotes had lower levels of startle attenuation to pleasant IAPS, a pattern previously described in depressed patients [2,60], consistent with their general unresponsiveness to pleasant stimuli [59,70] and their clinical deficits in motivational behavior [4]. The exaggerated reactivity to the unpleasant IAPS observed in AA subjects is consistent with anxiety or negative affect [14,71] but also with the negative potentiation model of depression [10]; this is inspired by clinical observations of negative affect and cognitions in depressed individuals [5], the conceptualization of heightened affective negativity as a common distress factor of depression and anxiety disorders [13] and the observation that anxious personality traits are a common vulnerability factor of both anxiety and depression [66]; this model receives experimental support by neurocognitive evidence of enhanced fear conditioning [52], hyperactive aversive emotional responding [20,22] and increased defensive startle reactivity to personal threat in depression [33]. The above taken together suggest that healthy homozygous individuals for the risk A allele for major depression and bipolar disorder are sensitive to contextual aversion which leads to a reactivity pattern akin to a mixed anxious/depressed phenotype. This phenotype reflects the non-specific anxiety/depression psychopathology that often precedes the formal clinical disorders associated with this gene variant.

Our AA subjects did not present with the predicted blunted baseline startle and affective startle reactivity; this pattern has been described in severe anxiety and affective disorders [2,27,40,49,54] and is consistent with the emotion context insensitivity model of depression, which views depression as a state of adaptive disengagement from the environment that prevents any action [51]. One possible interpretation is that our healthy, non-symptomatic AA individuals could be relatively depleted for mood disorder susceptibility genes. More likely, however, is the possibility that the full blunted startle phenotype evolves as a consequence of clinical or subclinical episodes of affective illness which our young and healthy AA subjects did not have the chance yet to develop. On the other hand, the possibility should be considered that the observed lack of baseline startle attenuation in the AA group could be the result of heightened contextual anxiety operating to raise baseline startle from its hypothesized attenuated levels. This is supported by the positive correlation between pre-test anxiety/arousal and baseline startle in the AA group and previous findings of aversive context conditioning leading to increased and persistent startle increments especially in subjects reporting higher state anxiety [71]. Nonetheless, in keeping with our blunted startle reactivity hypothesis, and a hypothesized continuum from genetic risk status to the severe clinical (blunted) startle phenotypes, is that sustained negative affect (discontentment and post-test anxiety) within the AA group only was associated with reduced baseline and affective startle reactivity. The affectively modulated startle per se is not a heritable trait [72] but fear conditionability may well be [36]. Therefore, longitudinal examination is necessary to determine whether these physiological response profiles in our AA individuals precede disorder onset, as stable context-dependent dispositions in affective reactivity or evolve with time or discrete illness episodes from the current anxious/defensive to the more severe non-reactive startle phenotype. Future research should also evaluate the association and role of the A allele in disorders of context sensitivity and unstable affective reactivity such as borderline personality, some forms of anxiety disorders and PTSD for which there is some evidence for similar startle findings as those described here for our AA group [19,34,46]. The AA homozygotes may be at an increased risk for high affective or context reactivity, distinct from mood disorder susceptibility and this possibility cannot be entirely excluded.

We did not find significant differences in subjective valence ratings for the affective pictures, which indicates that genotype groups attributed similar emotional salience to these stimuli. However, compared to the other genotypes, the A allele homozygotes appraised the pleasant and unpleasant but not the neutral pictures, as less arousing and this specificity to the affective pictures argues against a general peculiarity in the way these subjects appraise along the arousal dimension. This pattern is consistent with low emotional arousal in depression [35], fits exactly with IAPS rating data from depressed patients [47] and was mediated by their depression-equivalent mood state post-testing (i.e. high discontentment-low alertness). This appraisal pattern would predict reduced startle reactivity (Δ scores) in the unpleasant condition for the AA group, which is at odds with their physiological findings. Moreover, the correlation pattern showed that the AA homozygotes who appraised the pleasant pictures as least arousing, had the most adaptive response i.e. the greatest startle attenuation, to those pictures, and vice versa which is counterintuitive. Given that the SAM ratings took place at post-test, these counterintuitive relationships in AA individuals could reflect an adaptive emotional regulation strategy i.e. control of their aroused emotion by minimizing its impact in their self-ratings; alternatively, it could reflect deficiencies in identification or expression of emotion in this genotype, consistent with high alexithymia in conditions associated with the *CACNA1C* risk A allele [12,26,39,45] and with different fMRI patterns of activation during positive or negative but not neutral IAPS pictures in high versus low alexithymia individuals [18]. These interpretations are not mutually exclusive and require further investigation; they could both reflect self-stabilization, consistent with the anxious and introverted profile of healthy male AA homozygotes [63] and an

anxious, low risk taking and self-stabilizing phenotype in *CACNA1C* knockout mice [17].

We found that in healthy males homozygocity for the *CACNA1C* risk A allele leads to context sensitivity and startle reactivity akin to depressed/anxious states with emotional appraisal difficulties, possibly of an adaptive self-stabilizing nature. These findings require replication while limitations of the study are the lack of females and the small number of AA individuals which raises the possibility of type I error, although the convergent nature of our findings argues against this. Our findings provide phenotypic detail of *CACNA1C* AA genotype at a pre-symptomatic illness stage, which further the understanding of the initial unspecific risk phenotype associated with this pleiotropic risk genotype. The current dataset has face validity primarily for affective disorders and constitutes a useful starting point for longitudinal designs in early intervention clinics.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eurpsy.2015.03.004.

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