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Abstract

Learning is one of our most adaptive abilities, allowing us to adjust our expectations about future events. Aberrant learning processes may underlie disorders such as anxiety, motivating the search for the neural mechanisms that underpin learning. Animal studies have shown that the neurotransmitter GABA is required for the computation of prediction errors, the mismatches between anticipated and experienced outcomes, which drive new learning. Given that evidence from human studies is lacking, we sought to determine whether these findings extend to humans. Here, in two samples of Caucasian individuals, we investigated whether genetically determined individual differences in GABA neurotransmission predict the P3 event-related potential, an EEG component known to reflect prediction error processing. Consistent with the results of animal studies, we show that a weighted genetic risk score computed from the number of *GABRB2* rs1816072 A alleles (associated with increased expression of the $GABA_A$ receptor β2 subunit gene) and the number of *ErbB4* rs7598440 T alleles (associated with increased GABA concentration) predicts optimal prediction error processing during aversive classical conditioning with both visual (Experiment 1, $N = 90$; $p = .010$) and auditory (Experiment 2; $N = 92$; $p = .031$) unconditioned stimuli. Our finding that optimal processing of aversive prediction errors is reduced in individuals genetically predisposed towards decreased GABA neurotransmission suggests a potential mechanism linking GABA and anxiety. Specifically, reduced GABA signalling via $GABA_A$ receptors could result in aberrant learning from aversive experiences and vulnerability to anxiety disorders.

Keywords: prediction error, classical conditioning, electroencephalography, GABA, polymorphism

1. Introduction

Learning allows us to associate neutral cues with subsequent important events, such as desirable or unpleasant consequences, enabling us to anticipate the occurrence of these important events and prepare for them. Important individual differences in both pathological and non-pathological behaviour have been explained by appealing to differences in the ability to associate events that regularly occur together (e.g., Corlett et al., 2007; Gradin et al., 2011; Kaufman et al., 2010; Soliman et al., 2010). In particular, individual differences in learning to fear stimuli that signal the occurrence of aversive events have been proposed to contribute to vulnerability to anxiety disorders (e.g., Beckers et al., 2013; Blechert et al., 2007; Duits et al., 2015; Lissek et al., 2005). According to this view, a propensity to learn to associate neutral cues with aversive outcomes, or an inability to update memories when these cues are no longer followed by aversive outcomes, could contribute to the development and maintenance of maladaptive anxiety responses.

Such individual differences in fear learning seem to be at least partially genetically determined (e.g., Lonsdorf et al., 2009; Raczka et al., 2011). Investigating genetic individual differences in fear learning could therefore lead to better identification of individuals who are at risk of developing an anxiety disorder. Furthermore, a better understanding of the relationship between genes and specific learning mechanisms could inform ways to enhance the efficacy of extinction-based therapies that rely on teaching the patient to extinguish, or attenuate, a fear memory. Yet, genetics in the field of fear learning is still in its infancy, as genes that affect several neurotransmitter systems have not yet been explored. Importantly, although it has long been known that gamma-aminobutyric acid (GABA) plays an important role in both anxiety and learning, human studies investigating the relationship between polymorphisms in GABA-related genes and fear learning are lacking. Here we present two experiments investigating the relationship between GABA-related polymorphisms, fear learning, and self-reported anxiety. Not only is this the first study to investigate the role of GABA-related polymorphisms in fear learning in humans, but we also aim to shed light on the specific mechanism through which GABA influences learning.

Individual differences in learning are typically studied in the laboratory using classical conditioning paradigms (Pavlov, 1927), in which the subject or participant is exposed to pairings of a neutral stimulus (the conditioned stimulus, CS) with an important event (the unconditioned stimulus, US). Computational models can guide the investigation of specific mechanisms that might generate individual differences in learning performance in such experiments (e.g., Cavanagh et al., 2010; Eisenegger et al., 2014; Krugel et al., 2009).

Associative models posit that experience with various events occurring together, or separately, changes the connections between the internal representations of these events. A CS signalling the occurrence of a US is assumed to become associated with it; subsequent presentation of the CS alone will retrieve the memory of the US via this shared connection. Most computational models of learning rely on prediction error to alter connections (e.g., Baetu et al., 2015; Rescorla & Wagner, 1972; McLaren & Mackintosh, 2002; Sutton & Barto, 1987). Prediction error is a formalisation of the concept of surprise and reflects the extent to which the US was not anticipated. Importantly, a connection between a CS and a US is updated only if the occurrence or non-occurrence of the US generates a prediction error, that is, if there is a discrepancy between the experienced and the expected US. This proposal has extensive empirical support from both animal and human studies (Eshel et al., 2016; Gradin et al., 2011; Miller et al., 1995; Morris et al., 2012; Schultz et al., 1997).

Animal studies have advanced our understanding of the neural networks that might compute prediction error (Fanselow, 1998; Kim et al., 1998; McNally et al., 2011). Although these models were developed for different species and different conditioning procedures, they share striking similarities, and are consistent with the general architecture illustrated in Figure 1. The architecture requires inhibitory neurotransmission for the computation of prediction error: this allows a negative feedback mechanism (④ in Figure 1) to suppress activity generated by a US if a preceding CS has already generated an expectation that the US would occur. Given that this is a critical component of prediction error processing, investigating the neurotransmitter system(s) involved in this computation would substantially further our understanding of the learning processes that give rise to pathological and non-pathological individual differences. One possible candidate is GABA, the most common inhibitory neurotransmitter in our nervous system (Steighart, 1995). Animal studies suggest that GABA plays an important role in learning (Botta et al., 2015; Jovasevic et al., 2015). Importantly, there is evidence that prediction error signalling during learning seems to involve GABA (Kim et al., 1998). Furthermore, a recent optogenetic study on mice has shown that GABA plays a causal role in the computation of prediction error (Eshel et al., 2015).

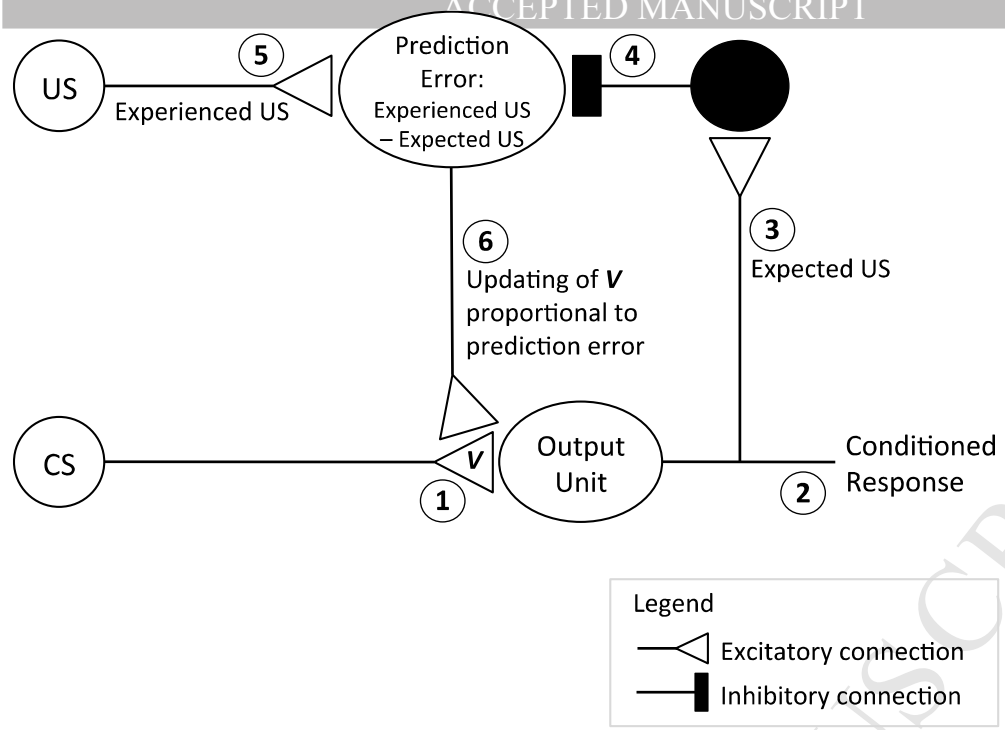

Figure 1. General neural architecture that might support conditioning. Sensory input generated by a CS activates connection $\boldsymbol{V}$ (1) that represents the strength of the CS-US association. If the CS has been previously followed by the US, then connection $\boldsymbol{V}$ is positive, generating activity in the Output Unit and a conditioned response (2). The Output Unit also activates an inhibitory unit (3). This negative feedback mechanism suppresses (4) activity in a Prediction Error (PE) unit that receives sensory input when the subsequent US finally occurs (5). Thus, when the PE unit is activated by the physical presence of the US, its activity is dampened if the CS has already generated an expectation of the US. The output of the PE unit is proportional to the difference between the Experienced US (5) and the CS-generated expectation of the US (3). The PE unit output alters $\boldsymbol{V}$ (6): if the output of the PE unit is large (the US was unexpected), then $\boldsymbol{V}$ will be strengthened, but if the output of the PE unit is small (the US was expected), then $\boldsymbol{V}$ will not be altered. Connection $\boldsymbol{V}$ (1) represents existing knowledge of the CS-US relationship, whereas the altering of this connection (6) represents learning.

This has important implications for our understanding of the possible disruptions in the neural mechanisms that underpin fear learning that could give rise to pathological behavior. If GABA neurotransmission is required to suppress processing of an aversive US when it is anticipated, then one of its important consequences is to reduce further strengthening of the association between the US and its immediately preceding CS. If this

mechanism fails, then a CS might generate a strong expectation of the US (② in Figure 1), but a lack of GABAergic neurotransmission would nevertheless result in further strengthening of the CS-US association (⑥ in Figure 1). As a consequence, both surprising and anticipated aversive USs would generate large prediction error signals that would strengthen fear memories. Thus, aberrant fear learning might be at least partially due to a diminished GABA-mediated negative feedback mechanism that is critical for the computation of prediction errors.

Although this theory might explain why aberrant fear learning occurs in anxiety disorders, which are typically characterised by reduced GABA neurotransmission, there is little evidence that the findings from the animal studies mentioned above extend to humans. Although $GABA_A$ receptors are involved in aversive processing in humans (Hayes et al., 2013), it is unclear whether GABA receptors are involved specifically in the processing of aversive prediction errors. We therefore present two experiments in which we investigated whether GABA is involved in processing prediction errors during aversive conditioning in humans. We tested whether two polymorphisms that affect GABA neurotransmission predict brain activity that has been previously associated with optimal prediction error processing. We used aversive classical conditioning procedures in which initially neutral visual stimuli (the conditioned stimuli; CSs) were paired with aversive pictures (Experiment 1) or a burst of 85-dB white noise (Experiment 2). As GABA has been shown to be involved in prediction error processing in both aversive (Kim et al., 1998) and appetitive (Eshel et al., 2015) conditioning procedures in animals, we expected to find similar effects with visual and auditory unconditioned stimuli. The second experiment was thus intended to be a replication of the first in an independent sample of participants, and using a different aversive conditioning procedure in order to increase the generalisability of our findings.

Our experimental designs enabled us to study prediction error processing as the aversive US should have been anticipated on some trials (low prediction error trials), whereas it should have been surprising on others (high prediction error trials). Optimal prediction error processing is said to occur when brain responses accurately reflect whether the aversive US was anticipated or surprising, thus closely matching the hypothesised prediction error. Using electroencephalography (EEG), prediction error processing was indexed by the amplitude of the P3, a posterior event-related potential that occurs 300-600 ms after the onset of the aversive US. Compared to expected USs, unexpected USs generate a larger P3 (Bellebaum & Daum, 2008; Philiastides et al., 2010; Yeung & Sanfey, 2004). Thus, an enhanced P3 component on high prediction error trials (i.e., when the US should be surprising) relative

to low prediction error trials (i.e., when the US should have been expected) would be indicative of enhanced prediction error processing. A larger P3 effect in those individuals with genotypes that are associated with increased GABA neurotransmission would support the hypothesis that GABA contributes to the computation of prediction errors.

To investigate genetic individual differences in GABA neurotransmission on prediction error processing, two common single-nucleotide polymorphisms in GABA-related genes were studied. The physiological effects of both polymorphisms have already been documented (Luykx et al., 2012; Marenco et al., 2011; Zhao et al., 2006).

The first polymorphism, rs7598440, is located on the *ErbB4* gene and is predictive of GABA concentration. The T allele has been shown to be associated with increased GABA concentration in human cerebrospinal fluid (Luykx et al., 2012) and in the dorsal anterior cingulate gyrus (Marenco et al., 2011). The *ErbB4* gene is of particular interest, as it codes for one of the ErbB receptor tyrosine kinases activated by neuregulin 1 (NRG1). It has been shown that NRG1 regulates long-term potentiation via ErbB4 (Pitcher et al., 2008). It does so by stimulating GABA release in parvalbumin-positive (i.e., GABAergic) interneurons. This has been shown in mice both pharmacologically – $GABA_A$ receptor antagonists suppressed the effect of NRG1 – and genetically – ablation of the *ErbB4* gene prevented NRG1 from increasing GABA neurotransmission and decreasing long-term potentiation (Chen et al., 2010).

The second polymorphism, rs1816072, influences the expression of the gene that codes for the $GABA_A$ receptor β2 subunit (*GABRB2*). The A allele has been shown to be associated with increased mRNA expression (Zhao et al., 2006), and is in strong linkage disequilibrium with rs1816071 (D' = .93; r^2 = .87), which has also been shown to be associated with gene expression (Zhao et al., 2006, 2009). The *GABRB2* gene is also a promising candidate, as $GABA_A$ receptors are involved in fear learning (Brown et al., 2012), and mRNA expression of the *GABRB2* gene changes following fear conditioning in mice (Jovasevic et al., 2015).

2. Methods

2.1 Participants

One hundred and two participants (48 males; age: *M* = 24.2, *SD* = 5.39, range 18-40 years) completed Experiment 1, and an independent sample of 98 participants (43 males; age: *M* = 24.4, *SD* = 4.76, range 18-40 years) completed Experiment 2. All participants provided a saliva sample for genotyping. Data collected from five participants in Experiment 1 and five participants in Experiment 2 were omitted from the EEG analyses because of excessive ocular

artefacts during the EEG recording (see below). Genotyping failed for seven of the remaining participants in Experiment 1, and one participant in Experiment 2, so the final sample for the analysis of genetic effects on the P3 component consisted of 90 participants (42 males) with an age range of 18–40 yrs (M = 24.2, SD = 5.64) in Experiment 1, and 92 participants (40 males) with an age range of 18–40 yrs (M = 24.5, SD = 4.79) in Experiment 2. Participants were recruited via a local classified advertisement and community website. Participants were eligible for the study if they were Caucasian, aged 18-40 yrs, had normal or corrected-to-normal vision, did not suffer from major medical or psychiatric conditions, were not taking medications that have sedative or stimulant actions, had not used medication that affects neurological function (e.g., antidepressants, sedatives, antipsychotics) over the past six months, were not suffering from drug or alcohol dependence and did not smoke more than five cigarettes per day. All participants provided informed, written consent and were paid a small honorarium to reimburse their time. Ethical approval was obtained from the University of Adelaide Human Research Ethics committee and all protocols were performed according to the Declaration of Helsinki (2008 version).

2.2 Genotyping

DNA extraction and genotyping were performed by the Australian Genome Research Facility, Ltd (AGRF). DNA for each participant was recovered from stabilised saliva samples using the manual prepIT system according to manufacturer's instructions (Oragene DNA (OG-500); DNA Genotek Inc, Ontario, Canada). DNA precipitates were resuspended for a minimum of 48 hrs before quantification by fluorimetry (QuantiFluor™ dsDNA System; Promega Corporation, Madison, Wisconsin, USA) in conjunction with a Gemini™ Spectramax XPS fluorescence microplate reader (Molecular Devices, LLC; Sunnyvale, CA, USA). DNA stocks were adjusted to a working concentration of between 10 and 50 ng/ul for subsequent genotyping.

The two polymorphisms were genotyped using the Sequenom iPLEX MassARRAY® platform according to the methods described by Gabriel et al. (2009). PCR and extension primers were designed using Sequenom Assay Designer v3.1. The following sequences of primers were used: rs1816072 (PCR-1: ACGTTGGATGGTGTTTAAGGGCCTGGATTC, PCR-2: ACGTTGGATGCTCAGAGGTCAAGATCACAC, extension primer: TTCCAATGGCAACTCTA), rs7598440 (PCR-1: ACGTTGGATGGAGTTTATGTTGCTCTGTGG, PCR-2: ACGTTGGATGAGGACTGCACCTGTTTGTTC, extension primer: TTGCTCTGTGGTTTAAGAAGTCC).

Seven samples failed genotyping quality control in Experiment 1. These samples were genotyped again, however failed once again. Further investigation isolated this to a single

batch collection, where low quality DNA was collected. Therefore, these missing samples are unlikely to influence genotype distribution, as the genotyping issue is independent to genotype.

2.3 Psychometric Testing and Demographic Information

Participants reported their age and sex, as well as their education level. In Experiment 1 they reported the highest level of education they had achieved on a scale that ranged from 1 to 6 (1 = no high school, 2 = high school, 3 = other certificate, 4 = trade certificate, 5 = certificate/diploma, 6 = bachelor degree or higher). In Experiment 2 they reported the number of years of education completed. To ensure that the different genotype groups had similar affective and cognitive functioning, participants also completed the Depression, Anxiety, and Stress Scale (Lovibond & Lovibond, 1995), as well as a series of computerised psychometric tests that assess reasoning ability, working memory, and processing speed.

In Experiment 1 reasoning ability was measured using an abbreviated version of Raven's Advanced Progressive Matrices (Bors and Stokes, 1998), and the Comprehensive Abilities Battery-Induction (CAB-I), a test of inductive reasoning (Hakstian and Cattell, 1975). Processing speed was estimated using Inspection Time (Nettelbeck, 2001) and a computerised version of Symbol-Digit Coding (McPherson and Burns, 2005). Working memory was measured using the Sentence Span (Lewandowsky et al., 2010) and the Dot Matrix test, also known as the Spatial Verification Span (Law et al., 1995).

In Experiment 2 reasoning ability was measured using the abbreviated version of Raven's Advanced Progressive Matrices (Bors and Stokes, 1998), processing speed was estimated using Simple- and Four-Choice Reaction Time, and working memory was measured using the Spatial Verification Span (Law et al., 1995).

General intelligence (g) was estimated in each experiment. We performed a principal components analysis on the scores of all cognitive tests (the principal component analysis was performed separately for each experiment given that different measures were used). Scores for the first unrotated component were used as an estimate of g (Jensen, 1998).

2.4 Conditioning Procedure in Experiment 1

Participants were seated approximately 70 cm in front of a computer monitor. Stimulus presentation was controlled by E-Prime software, version 2.0 (Psychology Software Tools, Pittsburgh, PA, USA). The conditioning procedure consisted of repeated pairings of four visual CSs with neutral or aversive pictures. The four CSs were pictures of white arrows pointing either up, left, right, or down, displayed on a black background. Fifty aversive pictures and 50 neutral pictures were selected from the Geneva affective picture database (Dan-Glauser &

Scherer, 2011), and served as the US pictures. These pictures have been previously rated in terms of valence (negative/positive) and arousal (dataset available from www.affective-sciences.org/researchmaterial). The 50 chosen aversive pictures have been given negative valence ratings (valence ratings < 20) and relatively high arousal ratings (arousal ratings > 66). The 50 neutral pictures were rated as relatively neutral (valence ratings range = 47 - 70) and non-arousing (arousal ratings < 30). These two sets of pictures were thus non-overlapping in terms of valence and arousal ratings.

Each trial began with a variable inter-trial interval (ITI) followed by the presentation of a CS. A US picture was presented upon the termination of the CS (Figure 2, left). On every trial, an aversive or a neutral picture was selected randomly from the relevant set. Two of the CSs were followed by an aversive picture on 75% of their presentations, and by a neutral picture on the remaining 25% of their presentations. Given that conditioning was identical for these CSs, they were analysed together and will be referred to as the aversive CSs. The other two CSs, referred to as the neutral CSs, were paired with an aversive picture on 25% of their presentations, and with a neutral picture on the remaining 75% of their presentations. Each CS was presented 128 times, for a total of 512 trials. For approximately half of the participants, the aversive CSs consisted of the left and right arrows and the neutral CSs consisted of the up and down arrows. These arrow-CS assignments were reversed for the remaining participants.

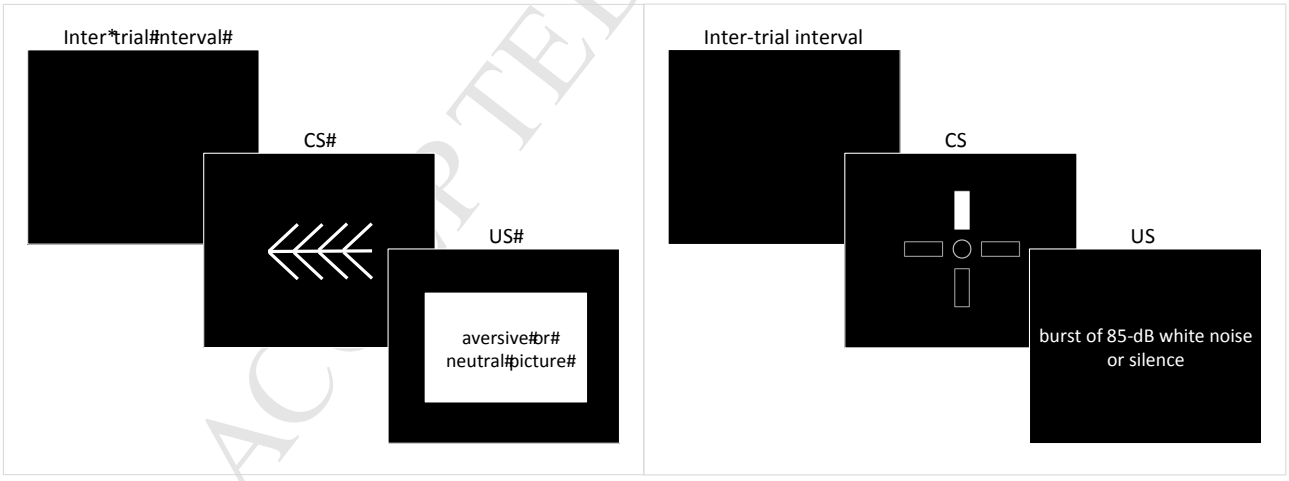

Figure 2. Conditioning procedures used in Experiments 1 (left) and 2 (right). Every trial began with an inter-trial interval followed by a CS (an arrow that pointed either left, right, up, or down in Experiment 1, or the layout of a cross with one of the arms highlighted in Experiment 2). The CS offset was immediately followed by the onset of the US (an aversive or a neutral picture in Experiment 1, or a black screen accompanied by a burst of white noise or silence in Experiment 2).

Continuous EEG and pulse from the left index finger were recorded during conditioning. The EEG data were used to analyse event-related potentials (ERPs) following the onset of the US that might reflect prediction error processing. The pulse data were used to analyse heart rate changes following the onset of the CSs, in order to determine whether the conditioning procedure was effective. CSs that signal aversive USs can elicit heart rate deceleration or acceleration, but only the latter component is thought to reflect a state of fear (Hodes et al., 1985; Hamm & Vaitl, 1996), and has been shown to increase over the course of conditioning (Hare & Blevings, 1975; Fredrikson, 1981). Thus, an increase in heart rate following the onset of the aversive CSs relative to the neutral CSs would indicate that conditioning took place.

Heart rate and ERP analyses have different requirements. Heart rate analyses require a CS duration in the range of seconds so that a change in heart rate can be detected. ERP analyses, on the other hand, do not require long stimulus durations, but they require many trials to be averaged in order to obtain a reasonable signal-to-noise ratio. In order to perform both types of analysis, the conditioning trials were divided into three phases. Phases 1 and 3 consisted of few relatively long trials (ITI duration ≈ 6 s, CS duration = 6 s, US duration = 1.5 s), whereas Phase 2 consisted of many fast trials (ITI duration ≈ 1.2 s, CS duration = 1 s, US duration = 1 s; see Table 1). The inclusion of many short trials in Phase 2 ensured that a reasonable signal-to-noise ratio was achieved for the ERP analyses, whereas the long trials in Phases 1 and 3 allowed us to measure the changes in heart rate triggered by the aversive and neutral CSs at the beginning and at the end of the conditioning procedure.

Heart rate was analysed for the Phase 1 and Phase 3 slow trials. The first two trials of each phase were excluded from these analyses because we have previously observed that the first two trials in Phase 1 and the unexpected switch to the longer CS duration in Phase 3 generate physiological responses to any stimulus. Pulse was recorded continuously using a Bionomadix wireless transmitter-receiver system (Biopac Systems Inc., Goleta, CA, USA). The pulse data was converted offline into beats-per-minute using AcqKnowledge version 4.3 (Biopac Systems Inc., Goleta, CA, USA). The heart rate was averaged into 0.5-second bins starting 1 second prior to CS onset up to 6 seconds post CS onset. The 1-second baseline period prior to CS onset was subtracted from every 0.5-second bin, allowing us to compare changes in heart rate following CS onset for the two types of CS.

Table 1. Conditioning trials and stimulus parameters in Experiment 1

	Phase 1	Phase 2	Phase 3
Number of trials of each type			
Aversive CS 1 – Aversive picture	3	90	3
Aversive CS 1 – Neutral picture	1	30	1
Aversive CS 2 – Aversive picture	3	90	3
Aversive CS 2 – Neutral picture	1	30	1
Neutral CS 1 – Aversive picture	1	30	1
Neutral CS 1 – Neutral picture	3	90	3
Neutral CS 2 – Aversive picture	1	30	1
Neutral CS 2 – Neutral picture	3	90	3
Parameters			
CS duration	6 s	1 s	6 s
US duration	1.5 s	1 s	1.5 s
ITI duration	6 s ± 100 ms	1.2 s ± 100 ms	6 s ± 100 ms

Notes. Aversive CS1 and Aversive CS 2 refer to the two aversive CSs, and Neutral CS 1 and Neutral CS 2 refer to the two neutral CSs. ITI = inter-trial interval. The duration of the ITI varied randomly between 5900 and 6100 ms in Phases 1 and 2, and between 1100 and 1300 in Phase 2. Within each phase, the trial types were randomly intermixed, and there were no obvious transitions between phases other than the changes in the duration of the stimuli.

2.5 Conditioning Procedure in Experiment 2

The same equipment and set-up was used in Experiment 2 except that the conditioning procedure was changed in several ways. Firstly, the aversive US consisted of a 0.7-s, 85-dB burst of white noise, and the CSs consisted of four rectangular white outlines on a black background distributed in the shape of a cross, with one of the outlines filled white (as in Figure 2, right). There were four CSs (labeled A-D), each represented by a different white rectangle (the upper, lower, left or right rectangle). The CS duration was set to 1 s throughout the experiment, and the ITI was approximately 1.5 s.

Secondly, the experimental design included reversals of some of the CS-US contingencies to increase the number of surprising US presentations and omissions. That is, whereas CSs A and B were paired on 87% and 13% of their presentations with the aversive US, respectively, the remaining two CSs, C and D, repeatedly changed their contingency with the US over 8 blocks of trials. That is, on odd-numbered blocks (blocks 1, 3, 5, and 7) CS C was

paired with the US on 87% of its presentations and CS D was paired with the US on 13% of its presentations, and these contingencies were reversed on even-numbered blocks (blocks 2, 4, 6, and 8) such that C and D were paired with the aversive noise on 13% and 87% of their presentations, respectively (see Table 2). Each CS was presented 15 times within each block, for a total of 120 trials with each CS. The transition between blocks occurred without warning so the sudden contingency changes should have caused participants to experience relatively frequent violations of their expectations throughout the experiment, which should have caused large prediction error signals. The purpose of including contingency reversals in the experimental design was to increase the number of high prediction error trials.

Finally, the effectiveness of the conditioning procedure was assessed behaviourally via pleasantness ratings rather than changes in heart rate. The reason for this change was two-fold: the CS-induced changes in heart rate revealed only a modest effect of conditioning in Experiment 1 (see below), likely due to the large variability in this measure, and secondly, heart rate measurements require longer trials, which increases the duration of the experiment. We therefore chose a quicker behavioural measure to reduce the duration of the experiment. After conditioning, participants were presented with each CS in random order and asked to rate its pleasantness on a scale ranging from 1 (least pleasant) to 6 (most pleasant). This procedure is similar to that used in other previous studies (e.g., Schumacher et al., 2015; Vansteenwegen et al., 1998).

Table 2. Conditioning trials and stimulus parameters in Experiment 2

	Blocks 1, 3, 5, 7	Blocks 2, 4, 6, 8
Number of trials of each type		
CS A – Aversive white noise	13	13
CS A – No noise	2	2
CS B – Aversive white noise	2	2
CS B – No noise	13	13
CS C – Aversive white noise	13	2
CS C – No noise	2	13
CS D – Aversive white noise	2	13
CS D – No noise	13	2
Parameters		
CS duration	1 s	1 s
US duration	0.7 s	0.7 s
ITI duration	1.5 s ± 100 ms	1.5 s ± 100 ms

Notes. CS A and CS B were consistently paired with the aversive noise on 87% and 13% of their presentations, respectively, in all trial blocks. CSs C and D were paired with the aversive noise on 87% and 13% of their presentations in alternating blocks of trials. ITI = inter-trial interval. The duration of the ITI varied randomly between 1400 and 1600 ms. Within each block, the trial types were randomly intermixed, and there were no obvious transitions between blocks.

2.6 EEG Recording and Analysis

Continuous EEG was recorded from tin electrodes embedded in a cap (Electro-Cap International, Ohio) from the Fz, Cz, C3, C4, and Pz scalp sites according to the International 10-20 system in Experiment 1, and from the Fz, F3, F4, Cz, C3, C4, Pz, P3, and P4 sites in Experiment 2. An additional active electrode was placed on the right earlobe, and all electrodes were referenced to the left earlobe with a ground located at AFz. Impedances were generally kept below 5 kΩ, and never exceeded 10 kΩ. A vertical and a horizontal electrooculogram (EOG) were recorded from electrodes placed above and below the left eye, and at the left and right outer canthi. EEG and EOG were recorded at a sampling rate of 1000 Hz and amplified using a BioNomadix wireless system (Biopac Systems Inc., Goleta, CA, USA). EEG data was filtered online with a 0.1-100 Hz bandpass filter, and EOG data was filtered online with a 0.005-35 Hz bandpass filter.

The data were further analysed offline using EEGLAB (Delorme & Makeig, 2004) and ERPLAB (Lopez-Calderon & Luck, 2014). EEG data was re-referenced to the average of the two earlobes and filtered using a 50-Hz notch filter and a 30-Hz low-pass filter (12 dB/octave). The continuous EEG was segmented into epochs ranging from 150 ms prior to US onset to 600 ms post US onset, and baseline corrected using the 150-ms pre-stimulus interval. Blinks and eye movements were detected using a function in ERPLAB that detects step-like artefacts in the vertical and horizontal EOG channels, as recommended by Luck (2014). Trials with such artefacts were rejected from further analyses. In order to maintain an acceptable signal-to-noise ratio, we included only participants who had more than 30 artefact-free trials in each of the four conditions (low positive, high positive, low negative, and high negative prediction error). Data from five participants in Experiment 1 and five participants in Experiment 2 were removed from the dataset because they did not reach this criterion.

The P3 component was measured as the mean amplitude between 300 and 600 ms post US onset at channel Pz. Mean amplitude was chosen because, unlike other measures, it is less sensitive to differences in the number of trials between conditions (Luck, 2014).

2.7 Computational Modelling to Estimate Prediction Error Magnitude

Trial-by-trial variations in theoretical prediction error were estimated for each participant using the Rescorla-Wagner model (Rescorla & Wagner, 1972). This model updates the connection between every presented CS, CS_X, and the US on every trial using Equation 1.

$$\Delta V_{CSx\text{-}US} = \text{Learning rate} * (\lambda - \Sigma V_{CS}) \quad [1]$$

$$= \text{Learning rate} * \text{Prediction error}$$

$V_{CSx\text{-}US}$ represents the strength of the connection between CS_X and the US, and $\Delta V_{CSx\text{-}US}$ represents the change in this connection's strength on a given trial. λ is proportional to the magnitude of the US. Larger or more potent USs are assigned a larger λ value than less potent USs; thus larger USs will support the acquisition of stronger CS-US connections. It is typical to use a λ value of zero when the US is absent on a trial, and a λ value of 1 when the US is present. Finally, ΣV_{CS} is the sum of the connection strengths of all CSs present on that trial, including CS_X, so ΣV_{CS} represents the extent to which the US is anticipated before its occurrence or omission. The term $(\lambda - \Sigma V_{CS})$ represents the magnitude of the prediction error, that is, the extent to which the magnitude of the US (λ) is different from the predicted US magnitude based on previous learning (ΣV_{CS}). Finally, the learning rate represents the combined salience of CS_X and the US, and varies between 0 and 1. This parameter governs the speed with which connections are changed: larger learning rates cause larger increases or decreases in connection strength on every trial if the prediction error term is not null.

The model captures the computation performed by the architecture shown in Figure 1. $V_{CSx\text{-}US}$ represents connection $\boldsymbol{V}$ ①, which determines the size of the conditioned responses ② (e.g., increases in heart rate). λ represents the Experienced US ⑤, ΣV_{CS} represents the Expected US ③, and the prediction error computation involves subtracting (via inhibitory connection ④) the Expected US ③ from the Experienced US ⑤ (i.e., $\lambda - \Sigma V_{CS}$). The learning rate parameter determines the proportion of the computed prediction error ⑥ that is used to update connection $\boldsymbol{V}$. The model can thus be used to simulate not only trial-by-trial increments or decrements in the strength of a CS-US connection, but also trial-by-trial variations in prediction errors (i.e., the output ⑥ of the Prediction Error unit in Figure 1). Simulations were run for each participant's sequence of trials. In all simulations, λ was set to 1 on trials with an aversive US, and 0 on trials with no aversive US. The value of λ does not change the pattern of estimated prediction errors as long as this value is positive and larger on US-present trials. However, the learning rate has an effect on the estimated prediction errors. A larger learning rate causes larger trial-by-trial changes in associative strength. As a consequence of this, the estimated positive prediction errors diminish more quickly on the first trials, and the trial-by-trial variations in prediction error are larger throughout conditioning. We therefore ran separate simulations with a range of learning rate values. The overall pattern of results, however, remained unaffected by the learning rate.

Given our simple experimental designs, all positive prediction errors occurred on trials with an aversive US, and all negative prediction errors occurred on trials in which the aversive US was omitted. Positive prediction errors occurred when the aversive US was not fully expected (i.e., $\Sigma V_{CS} < 1$). Negative prediction errors occurred when an aversive US was expected to some extent (i.e., $\Sigma V_{CS} > 0$), but it did not occur. We used a threshold of 0.3 to classify positive prediction errors as high (high positive prediction errors > 0.3) or low ($0 \leq$ low positive prediction errors ≤ 0.3). Similarly, we used a threshold of -0.3 to classify negative prediction errors as high (high negative prediction errors < -0.3) or low ($-0.3 \leq$ low negative prediction errors ≤ 0). These thresholds captured both large and small trial-by-trial variations in the magnitude of prediction error. Thus, these simulations allowed us to study dynamic changes in prediction error. Although the thresholds we chose are arbitrary, a different method of dividing the prediction errors that has been used in previous studies (comparing upper and lower tertiles) generated very similar patterns of results, including the genetic effects reported below. We report the analyses using thresholds because they maximise the number of trials used in the event-related potential analyses.

Finally, it is worth noting that other prediction error models such as those proposed by Esber and Haselgrove (2011), Le Pelley (2004) and Pearce and Hall (1980) resulted in a similar categorisation of trials into high and low prediction errors, so the Rescorla-Wagner model simulations are presented here given that it is the most parsimonious of these models.

2.8 Statistical Analyses

After classifying the trial-by-trial prediction errors using computational modelling, we estimated the mean amplitude of the P3 component for the four trial types (high and low, positive and negative, prediction error trials) for each participant, and computed an aversive prediction error score as the difference between the mean P3 amplitude on high and low positive prediction error trials. The simulations and analyses were repeated with different values for the learning rate parameter (0.2, 0.3, and 0.4; these values are in the range of previously reported learning rates, e.g., O'Doherty et al., 2003; Robinson et al., 2012). Because we obtained similar results with these different learning rate values and to simplify the statistical analyses, we performed a principal components analysis on the aversive prediction error scores obtained from the three simulations (the principal component analysis was performed separately for each experiment). Scores for the first unrotated component were used as an index of aversive prediction error processing.

To investigate the effect of the two polymorphisms on prediction error processing, the aversive prediction error index obtained via principal component analysis was regressed on the number of alleles associated with increased gene expression (the number of A alleles for the *GABRB2* polymorphism) or GABA concentration (the number of T alleles for the *ErbB4* polymorphism). The number of alleles for the two polymorphisms were simultaneously entered into a multiple regression model along with the potential confounding variables age and gender.

Finally, we computed both weighted and unweighted genetic risk scores for each participant. The unweighted genetic risk scores consisted of the number of alleles predictive of increased GABA neurotransmission (the number of A alleles for the *GABRB2* polymorphism, and the number of T alleles for the *ErbB4* polymorphism). The weighted genetic risk scores were the sum of the number of A *GABRB2* and T *ErbB4* alleles weighted by the effect size of that polymorphism (i.e., the regression coefficient obtained from a regression model that regressed the prediction error processing scores on that polymorphism only). Two sets of weighted genetic risk scores were calculated, using the effect sizes obtained from each experiment. This allowed us to use the data from each experiment as reference for the other experiment, so the weighted genetic risk scores calculated with the regression coefficients

obtained in Experiment 1 were used to predict the prediction error scores in Experiment 2, and vice versa.

3. Results

3.1 Genotype Distributions and Cognitive and Affective Measures

Tables 3 and 4 summarise the demographic information and performance on the measures of affective and cognitive functioning in the different genotype groups. The genotype distributions were in Hardy-Weinberg equilibrium in both experiments (Experiment 1 *ErbB4*: $\chi^2(2) = .198$, $p = .906$, *GABRB2*: $\chi^2(2) = .314$, $p = 855$; Experiment 2 *ErbB4*: $\chi^2(2) = 1.81$, $p = .404$, *GABRB2*: $\chi^2(2) = .035$, $p = .983$) and the two genotype distributions were independent (Pearson $r = .05$; $\chi^2(4) = 3.17$, $p = .529$ in Experiment 1, and Pearson $r = .00$; $\chi^2(4) = 2.03$, $p = .731$ in Experiment 2). Sex distributions were similar across the different genotype groups and there were no reliable differences in age or education level.

Although the scores on the Depression, Anxiety and Stress Scale were similar in the different genotype groups in Experiment 1, there were significant differences between the *GABRB2* genotype groups in Experiment 2, with the A allele being associated with lower scores. We further analysed these effects by pooling the data from the two experiments (since all participants completed the same questionnaire) and investigating the effect of the two polymorphisms in a regression model that included experiment, age and gender as potential confounding factors. These analyses revealed that the number of A *GABRB2* alleles did not significantly predict the overall scores on the Depression, Anxiety and Stress Scale [regression coefficient = -1.37, SE = .861, $p = .114$], but they did predict a decrease in the scores on the Anxiety subscale [regression coefficient = -.550, SE = .253, $p = .031$]. The number of T *ErbB4* alleles did not have a significant effect on either the overall scores nor on the subscale scores [minimum $p = .154$].

There were also a few significant differences in the performance on the cognitive functioning tests, particularly in Experiment 2. These differences arose because participants with genotypes associated with increased GABA neurotransmission performed better. However, controlling for the potential confounding effects of these variables did not change the pattern of results reported below. That is, including the scores on the Depression, Anxiety, and Stress Scale and the estimated general intelligence (*g*) scores in the regression models investigating the genetic effects reported below did not change the pattern of results.

Table 3. Demographics, scores on psychometric tests and heart rate index of conditioning for participants with genetic and EEG data in Experiment 1 (N = 90)

	GABRB2 rs1816072				*ErbB4* rs7598440			
	G/G	A/G	A/A	*p*	C/C	C/T	T/T	*p*
N	11	45	34		20	47	23	
Age (years)	24.91 (5.66)	24.71 (6.09)	23.26 (5.02)	*.267*	22.35 (4.56)	24.62 (5.76)	24.92 (6.11)	*.149*
Sex F:M	5:6	20:25	23:11	*.105*	11:9	23:24	14:9	*.634*
Education	3.55 (1.97)	4.07 (1.84)	3.82 (1.88)	*.929*	3.80 (1.94)	3.85 (1.79)	4.13 (1.98)	*.553*
Depression, Anxiety, and Stress Scale	11.09 (10.29)	8.04 (5.89)	11.09 (8.65)	*.470*	7.90 (6.64)	9.68 (8.01)	10.78 (7.94)	*.226*
Reasoning Ability								
Raven's Advanced Progressive Matrices	6.36 (1.91)	6.43 (2.83)	6.26 (2.45)	*.840*	6.16 (2.87)	6.60 (2.64)	6.04 (2.18)	*.837*
Comprehensive Abilities Battery-I	5.82 (2.44)	7.22 (2.51)	6.85 (2.18)	*.506*	6.70 (2.25)	7.09 (2.68)	6.74 (1.94)	*.987*
Working Memory								
Dot Matrix	41.09 (5.49)	43.27 (7.23)	42.88 (6.79)	*.635*	43.72 (6.08)	43.68 (7.08)	40.36 (6.57)	*.103*
Sentence Span	.71 (.12)	.70 (.20)	.74 (.13)	*.424*	.73 (.13)	.69 (.20)	.75 (.12)	*.586*
Processing Speed								
Symbol Digit Coding	83.27 (12.49)	81.84 (16.91)	84.15 (14.08)	*.689*	78.10 (13.28)	85.3 (15.34)	82.13 (16.34)	*.437*
Inspection Time	36.32 (20.63)	35.59 (10.50)	32.63 (7.63)	*.225*	37.13 (13.58)	34.96 (10.86)	31.25 (8.48)	*.084*
Estimated General Intelligence (*g*)	-.19 (.67)	.02 (1.16)	.04 (.92)	*.614*	-.10 (.85)	.10 (1.13)	-.12 (.92)	*.923*
Heart rate change in Phase 3								
Aversive CSs	.73 (9.29)	-.51 (7.23)	-.58 (6.23)	*.672*	.28 (9.43)	-.62 (6.57)	-.47 (5.95)	*.744*
Neutral CSs	-1.49 (9.16)	-3.20 (8.20)	.05 (6.12)	*.223*	.51 (6.97)	-3.45 (7.35)	-.29 (8.36)	*.820*
Aversive – Neutral CSs	2.23 (14.56)	2.69 (9.91)	-.63 (9.71)	*.238*	-.24 (12.95)	2.83 (10.18)	-.19 (8.57)	*.957*

Notes. The standard deviation of each mean is indicated in parentheses. *p* values indicate the effect of the polymorphism on each variable (a chi-square test was performed for gender, and one-way analyses of variance were performed for all other variables). The highest education level attained was scored on a scale that ranged from 1 to 6 (1 = no high school, 2 = high school, 3 = other certificate, 4 = trade certificate, 5 = certificate/diploma, 6 = bachelor degree or higher). Inspection time is measured in milliseconds; smaller values indicate faster processing speed. The heart rate change refers to the heart rate change in the last second of

stimulus presentation relative to a 1-second pre-stimulus baseline. Data on some of the cognitive tests was missing for a small number of participants due to technical errors: One participant was missing scores on the Raven's Advanced Progressive Matrices and Dot Matrix tests, a further two participants were missing scores on the Dot Matrix test, and another two participants were missing Inspection Time scores. Therefore, the general intelligence (*g*) factor could not be estimated for these five participants.

Table 4. Demographics, scores on psychometric tests and CS pleasantness ratings discrimination scores for participants with genetic and EEG data in Experiment 2 (N = 92)

	GABRB2 rs1816072				*ErbB4* rs7598440			
	G/G	A/G	A/A	*p*	C/C	C/T	T/T	*p*
N	11	42	39		13	50	29	
Age (years)	26.91 (4.81)	23.98 (4.59)	24.26 (4.91)	*.266*	24.00 (4.93)	24.80 (4.90)	24.03 (4.66)	*.839*
Sex F:M	7:4	23:19	22:17	*.870*	6:7	30:20	16:13	*.658*
Education (years)	14.27 (1.85)	14.81 (2.72)	14.21 (2.95)	*.623*	13.92 (1.80)	14.40 (2.83)	14.90 (2.90)	*.263*
Depression, Anxiety, and Stress Scale	15.27 (9.50)	12.26 (7.73)	9.79 (7.60)	*.032*	12.08 (10.46)	11.92 (7.93)	10.76 (7.12)	*.550*
Reasoning Ability								
Raven's Advanced Progressive Matrices	4.64 (3.07)	6.57 (2.43)	6.54 (2.87)	*.129*	5.77 (3.17)	6.14 (2.71)	6.90 (2.61)	*.168*
Working Memory								
Dot Matrix	30.27 (7.8)	38.81 (10.70)	39.26 (9.45)	*.040*	34.92 (9.11)	37.62 (10.56)	39.97 (9.89)	*.128*
Processing Speed								
Simple Reaction Time	333.98 (67.09)	307.50 (50.47)	304.04 (40.75)	*.136*	321.33 (67.46)	314.73 (52.92)	294.22 (25.84)	*.054*
Choice Reaction Time	537.24 (125.72)	489.74 (92.69)	511.58 (99.05)	*.891*	511.46 (87.35)	511.09 (112.09)	490.57 (82.45)	*.429*
Estimated General Intelligence (*g*)	-.71 (.94)	.12 (1.02)	.07 (.94)	*.095*	-.27 (1.14)	-.09 (1.07)	.28 (.74)	*.062*
CS Discrimination Score	6.28 (1.69)	6.98 (1.58)	7.67 (1.95)	*.012*	7.68 (2.18)	7.18 (1.88)	6.99 (1.48)	*.285*

Notes. The standard deviation of each mean is indicated in parentheses. *p* values indicate the effect of the polymorphism on each variable (a chi-square test was performed for gender, and one-way analyses of variance were performed for all other variables). Education was measured in years. Simple and choice reaction time are measured in milliseconds; smaller values indicate faster processing speed. CS pleasantness ratings were given on a scale that ranged from 1 (least pleasant) to 6 (most pleasant). See the Results section for the formula used to calculate the discrimination scores.

3.2 Behavioural Measures of Learning

The behavioural measures of learning (heart rate changes in Experiment 1 and pleasantness ratings in Experiment 2) indicated that conditioning had been successful in both experiments (Figure 3). In Experiment 1, heart rate changes in response to the aversive and neutral CSs were similar in Phase 1, but in Phase 3 the heart rate change in the last second of presentation was more positive for the aversive CSs than for the neutral CSs; $t(101) = 2.17$, $p = .032$. In Experiment 2, CS A, which had been paired with the aversive burst of white noise on 87% of its presentations, was rated as more unpleasant than CSs C and D, which had been paired with the aversive US on 50% of the trials; $t(97) = 4.33$, $p < .001$. Furthermore, CSs C and D were rated as more unpleasant than CS B, which had been paired with the aversive US on only 13% of its presentations; $t(97) = 2.14$, $p = .035$. There was no reliable difference between the ratings for CS C and CS D; $t(97) = .45$, $p = .657$.

We analysed the effect of the two polymorphisms on the behavioural indices of learning by computing a CS discrimination score in each experiment. For Experiment 1, the CS discrimination score was simply the difference between the heart rate change in the last second of presentation for the aversive and neutral CSs. For Experiment 2, the CS discrimination score was calculated as the sum of the pleasantness ratings for the four CSs multiplied by the proportion of trials on which these CSs were followed by the absence of the US (i.e., CS discrimination score = CS A rating × .13 + CS C rating × .5 + CS D rating × .5 + CS B rating × .87). There were no significant genetic effects on the CS discrimination scores in Experiment 1; minimum $p = .392$ (see Table 3). In Experiment 2 the number of A *GABRB2* alleles predicted larger CS discrimination scores [regression coefficient = .709, SE = .276, $p = .012$], but the number of T *ErbB4* alleles did not [regression coefficient = -.309, SE = .282, $p = .276$] (see Figure 4 and Table 4).

Note that the genetic effects reported above remained unchanged if the scores on the Depression, Anxiety, and Stress Scale and the general intelligence (g) scores were included as additional predictors in the regression models. The effect of the *GABRB2* polymorphism on the CS discrimination scores in Experiment 2 remained significant [regression coefficient = .690, SE = .293, $p = .021$], whereas that of the *ErbB4* polymorphism remained non-significant [regression coefficient = -.280, SE = .293, $p = .342$]. Both polymorphisms still failed to show a significant effect on the CS discrimination scores in Experiment 1 [minimum $p = .257$].

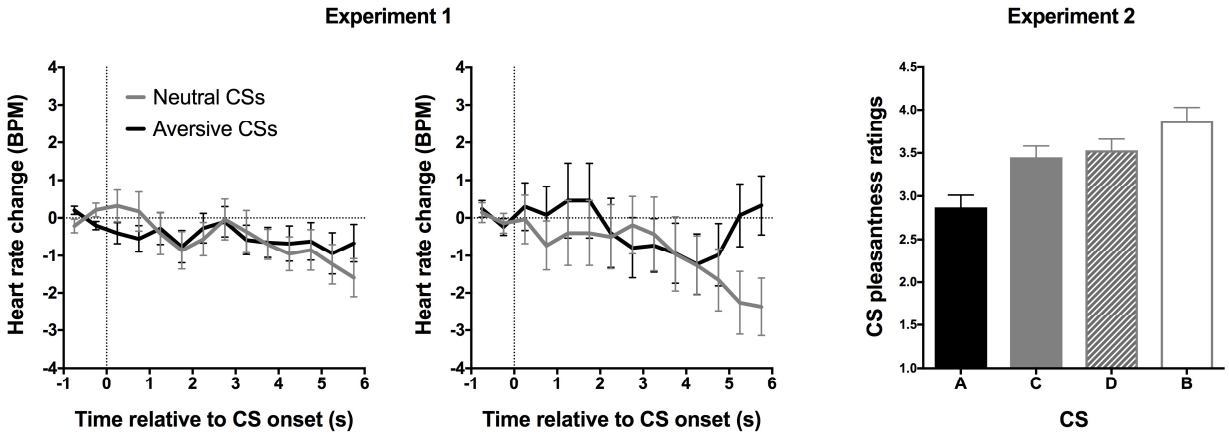

Figure 3. Behavioural measures of learning in Experiments 1 and 2. The first two panels show the mean heart rate change relative to a 1-second baseline period prior to CS onset for the aversive and neutral CSs in Phase 1 (left) and Phase 3 (middle) in Experiment 1. The CS duration on these trials was 6 seconds. Data is shown in 0.5-second bins from 1 second prior to CS onset to 6 seconds post CS onset. The right panel shows the mean CS pleasantness ratings in Experiment 2. The error bars represent standard errors.

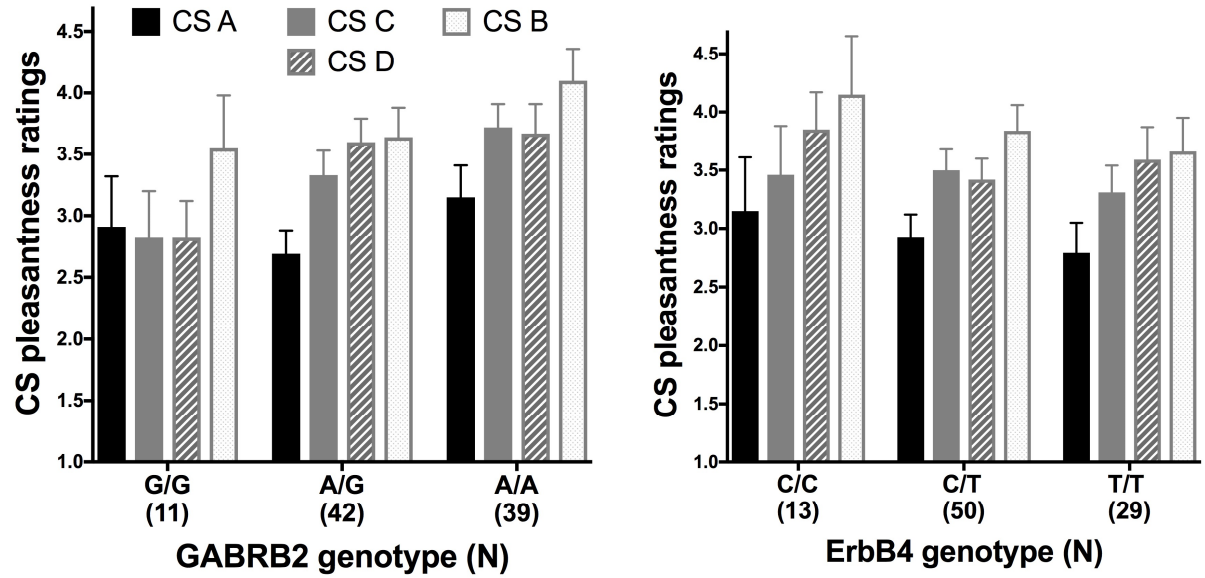

Figure 4. Genetic effects on CS pleasantness ratings in Experiment 2. Participants were grouped according to their *GABRB2* (left) or *ErbB4* (right) genotype. The sample size for each genotype group is shown in parentheses. Error bars represent the standard error of the mean.

3.3 Estimates of Prediction Error Processing

The first column of panels in Figures 5 and 6 show the trial-by-trial estimated prediction error for one representative participant's sequence of trials when the learning rate was 0.2, 0.3, or 0.4. Consistent with previous literature on prediction error processing, the mean P3 amplitude was larger for unexpected aversive USs (high positive prediction errors) than for expected aversive USs (low positive prediction errors; see the second column of panels in Figures 5 and 6). This effect was modest in Experiment 1 and did not reach statistical significance [0.2 learning rate: $t(96) = 1.94$, $p = .056$; 0.3 learning rate: $t(96) = 1.51$, $p = .133$; 0.4 learning rate: $t(96) = 1.04$, $p = .300$], but was much stronger in Experiment 2 [0.2 learning rate: $t(92) = 4.45$, $p < .001$; 0.3 learning rate: $t(92) = 5.50$, $p < .001$; 0.4 learning rate: $t(92) = 4.87$, $p < .001$].

As explained previously, we performed principal component analysis on the aversive prediction error scores obtained from the three simulations. The unrotated first principal component correlated strongly with the prediction error scores obtained from the three simulations (correlations ranged from .96-.98 in Experiment 1, and .92-.97 in Experiment 2) and accounted for a large proportion of variance (.94 in Experiment 1 and .91 in Experiment 2). We tested whether the two polymorphisms could predict the scores obtained from this principal component analysis (the aversive prediction error index, shown in Figure 7), taken to reflect each individual's capacity to process aversive prediction errors. The number of *GABRB2* A alleles predicted larger aversive prediction error scores in both Experiment 1 [regression coefficient = .324, SE = .157, $p = .043$] and Experiment 2 [regression coefficient = .332, SE = .151, $p = .031$]. There was no significant relationship between the number of *ErbB4* T alleles and the aversive prediction error scores in either experiment [regression coefficient = .239, SE = .148, $p = .111$ and regression coefficient = .115, SE = .154, $p = .456$ in Experiments 1 and 2, respectively].

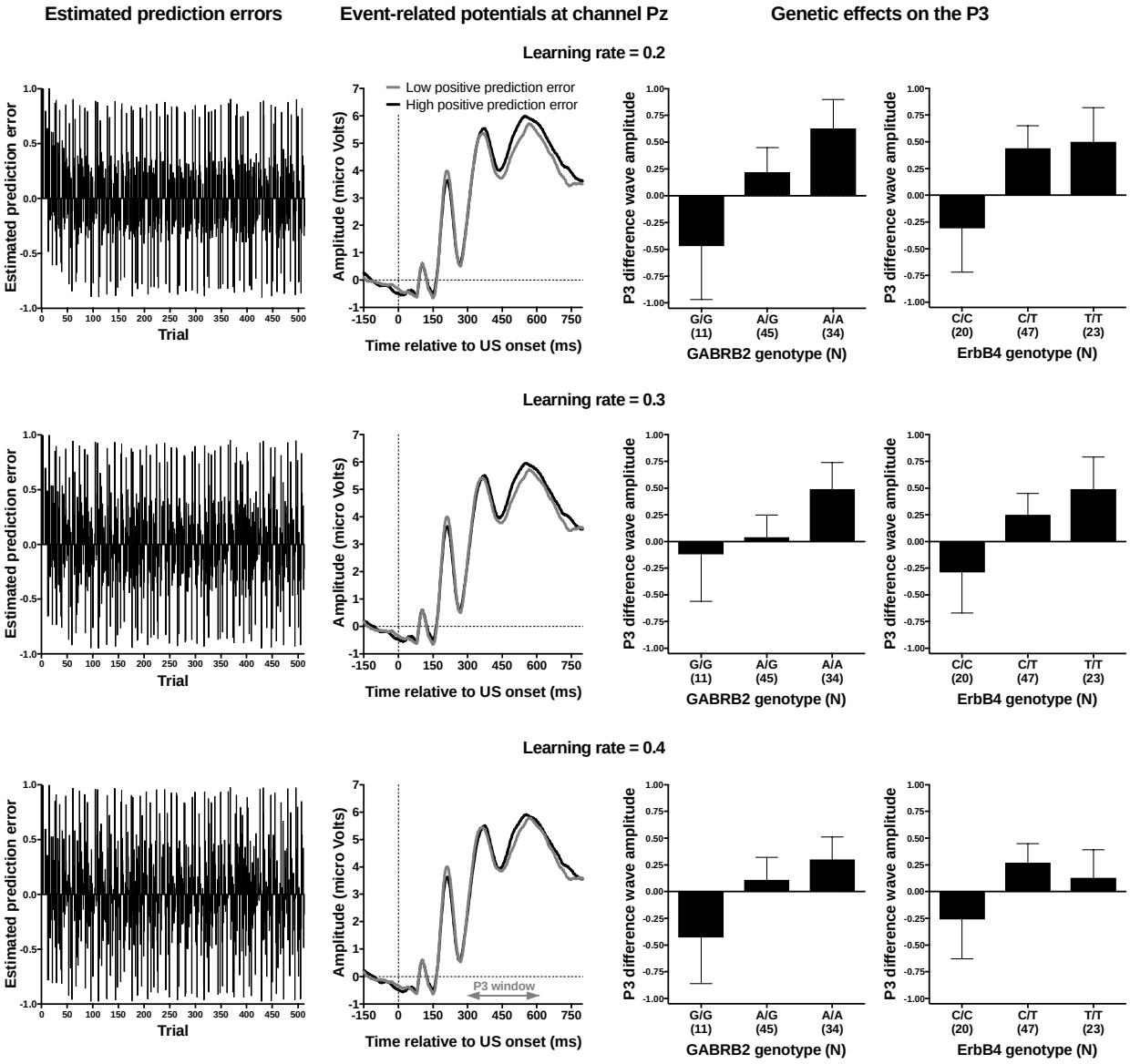

Figure 5. Genetic effects on prediction error processing in Experiment 1. **First column:** Example of trial-by-trial variations in prediction error magnitude estimated by the Rescorla-Wagner model for a representative participant. Data is shown for simulations using a learning rate of 0.2 (first row), 0.3 (second row) or 0.4 (third row). **Second column:** Event-related potentials at channel Pz following the onset of the aversive picture when the estimated prediction error was high (black line) or low (grey line). The trials were classified as high or low positive prediction error trials following simulations with the Rescorla-Wagner model; see method section for more details. The time window for the P3 component is shown in the lower graph. **Third and fourth columns:** Mean P3 amplitude difference between high and low positive prediction error trials for individuals with different *GABRB2* and *ErbB4* genotypes. The sample size for each genotype group is shown in parentheses. Error bars represent the standard error of the mean.

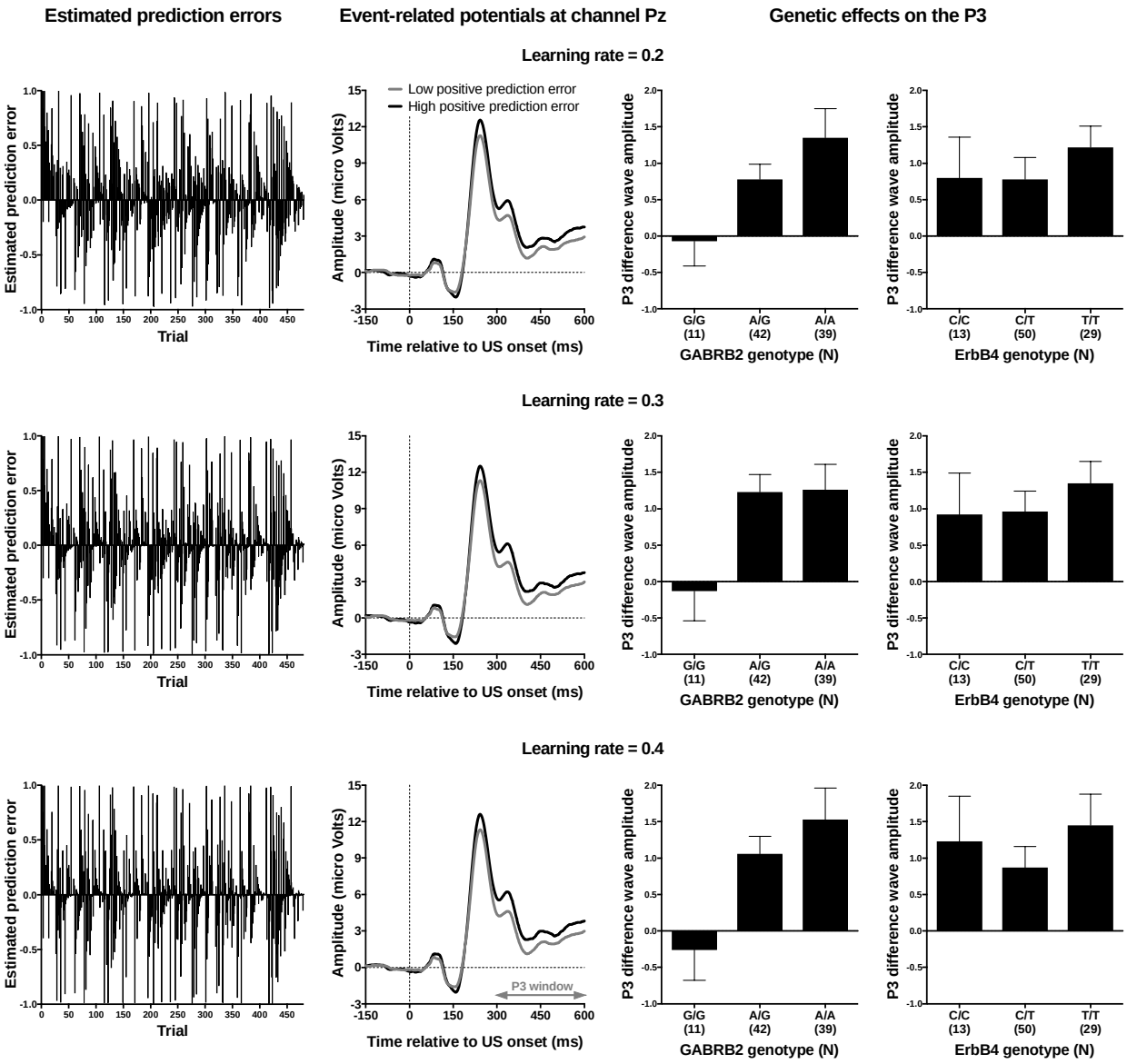

Figure 6. Genetic effects on prediction error processing in Experiment 2. **First column:** Example of trial-by-trial variations in prediction error magnitude estimated by the Rescorla-Wagner model for a representative participant. Data is shown for simulations using a learning rate of 0.2 (first row), 0.3 (second row) or 0.4 (third row). **Second column:** Event-related potentials at channel Pz following the onset of the aversive picture when the estimated prediction error was high (black line) or low (grey line). The trials were classified as high or low positive prediction error trials following simulations with the Rescorla-Wagner model; see method section for more details. The time window for the P3 component is shown in the lower graph. **Third and fourth columns:** Mean P3 amplitude difference between high and low positive prediction error trials for individuals with different *GABRB2* and *ErbB4* genotypes. The sample size for each genotype group is shown in parentheses. Error bars represent the standard error of the mean.

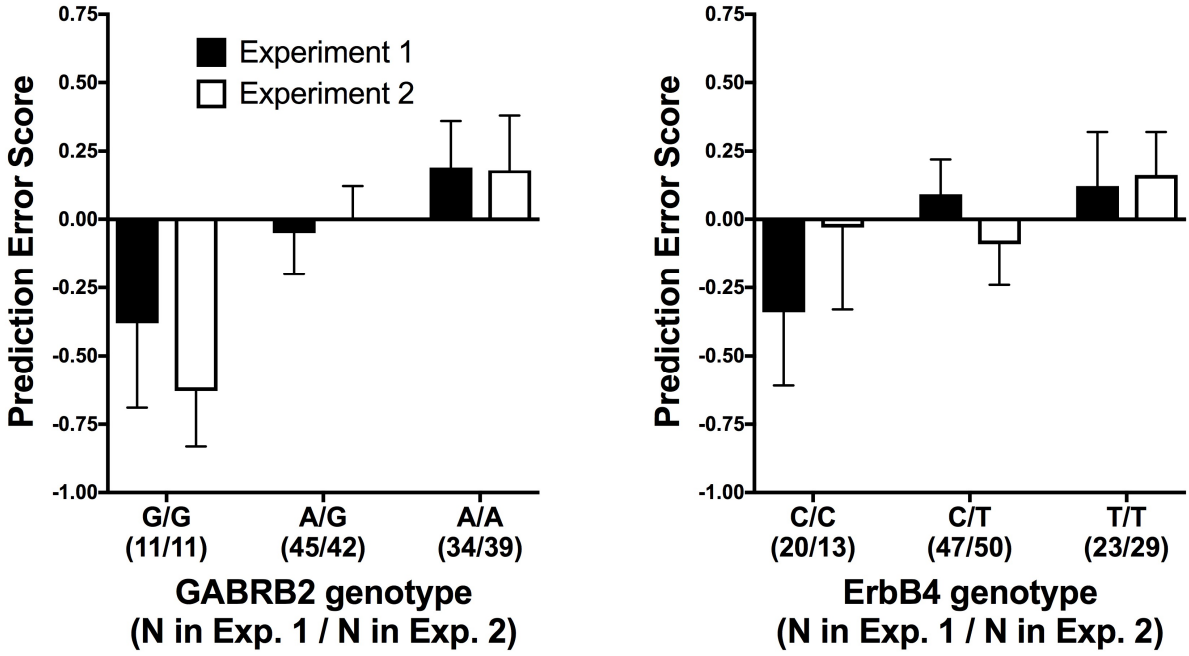

Figure 7. Genetic effects on the prediction error scores in Experiments 1 and 2. The prediction error scores were derived from a principal component analysis on the P3 difference wave amplitudes for all learning rate parameters; see method section for more details. Participants were grouped according to their *GABRB2* (left) or *ErbB4* (right) genotype. The sample size for each genotype group in each experiment is shown in parentheses. Error bars represent the standard error of the mean.

We also analysed the unweighted genetic risk scores (the number of *GABRB2* A and *ErbB4* T alleles), and the weighted genetic risk scores calculated using the regression coefficients obtained in each experiment, which are listed in Table 5. The prediction error scores were regressed on each genetic risk score, as well as age and sex. The unweighted genetic risk scores predicted the prediction error scores in both Experiment 1 [regression coefficient = .279, SE = .104, p = .009] and Experiment 2 [regression coefficient = .226, SE = .108, p = .039]. The weighted genetic risk scores computed with the regression coefficients obtained from Experiment 1 predicted the prediction error scores in both Experiment 1 – the reference set [regression coefficient = 1.14, SE = .419, p = .008] and Experiment 2 – the validation set [regression coefficient = .942, SE = .431, p = .031]. Similarly, the weighted genetic risk scores computed with the regression coefficients obtained from Experiment 2 predicted the prediction error scores in both Experiment 1 – the validation set [regression

coefficient = 1.10, SE = .420, p = .010] and Experiment 2 – the reference set [regression coefficient = .973, SE = .417, p = .022].

Controlling for the scores on the Depression, Anxiety, and Stress Scale and the general intelligence (g) scores did not change this pattern of results. The effect of the *GABRB2* polymorphism on the prediction error scores remained significant in both Experiment 1 [regression coefficient = .345, SE = .167, p = .042] and Experiment 2 [regression coefficient = .426, SE = .157, p = .008], whereas the effect of the *ErbB4* polymorphism still failed to reach the significance level in both Experiment 1 [regression coefficient = .237, SE = .160, p = .142] and Experiment 2 [regression coefficient = .191, SE = .157, p = .228]. The unweighted genetic risk scores still predicted the prediction error scores in both Experiment 1 [regression coefficient = .289, SE = .110, p = .010] and Experiment 2 [regression coefficient = .309, SE = .115, p = .009]. Both sets of weighted genetic risk scores significantly predicted the prediction error scores in both experiments [weighted genetic risk score using coefficients from Experiment 1: regression coefficient = 1.18, SE = .441, p = .009 and regression coefficient = 1.28, SE = .459, p = .006 for Experiments 1 and 2, respectively; weighted genetic risk score using coefficients from Experiment 2: regression coefficient = 1.15, SE = .443, p = .011 and regression coefficient = 1.29, SE = .442, p = .005 for Experiments 1 and 2, respectively].

Table 5. Percentage of variance in prediction error scores accounted for by each polymorphism and the genetic risk scores, and regression coefficients for each polymorphism used in the calculation of the weighted genetic risk scores

	Percentage of variance	Regression coefficient	Standard error
Experiment 1			
GABRB2 rs1816072 (number of A alleles)	3.27	.2726	.1581
ErbB4 rs7598440 (number of T alleles)	2.42	.2239	.1517
Unweighted genetic risk score	5.34		
Weighted genetic risk score (Experiment 1)	5.39		
Weighted genetic risk score (Experiment 2)	4.91		
Experiment 2			
GABRB2 rs1816072 (number of A alleles)	5.18	.3372	.1520
ErbB4 rs7598440 (number of T alleles)	.72	.1296	.1600
Unweighted genetic risk score	4.93		
Weighted genetic risk score (Experiment 1)	5.32		
Weighted genetic risk score (Experiment 2)	5.89		

Notes. The Weighted genetic risk score (Experiment 1) was calculated using the regression coefficients obtained from Experiment 1, and the Weighted genetic risk score (Experiment 2) was calculated using the regression coefficients obtained from Experiment 2.

3.4 Meta-Analysis of the Two Experiments

We combined the results of the two experiments using the 'metagen' function in the R package 'meta' (Schwarzer, 2007). A meta-analysis of the genetic effects on the prediction error scores revealed that the *GABRB2* polymorphism was a significant predictor of the prediction error scores [z = 2.79, p = .005], whereas the *ErbB4* polymorphism was not statistically significant [z = 1.63, p = .103] (see Figure 8). All genetic risk scores also significantly predicted the prediction error scores [unweighted: z = 3.10, p = .002; weighted using the coefficients from Experiment 1: z = 3.16, p = .002; weighted using the coefficients from Experiment 2: z = 3.18, p = .002]. Analysis of heterogeneity showed no significant differences between experiments for either predictor [maximum I^2 = 18%; $\chi^2(1)$ = .18, p = .669].

We also analysed the GABRB2 effect on the scores on the Anxiety subscale of the Depression, Anxiety, and Stress Scale. However, the effects in the two experiments were heterogeneous [I^2 = 86%; $\chi^2(1)$ = 7.14, p = .008]. As a consequence, the fixed and random effects models yielded different results: the GABRB2 effect was significant in a fixed effect model [z = -2.01, p = .044], but not in a random effects model [z = -.61, p = .542].

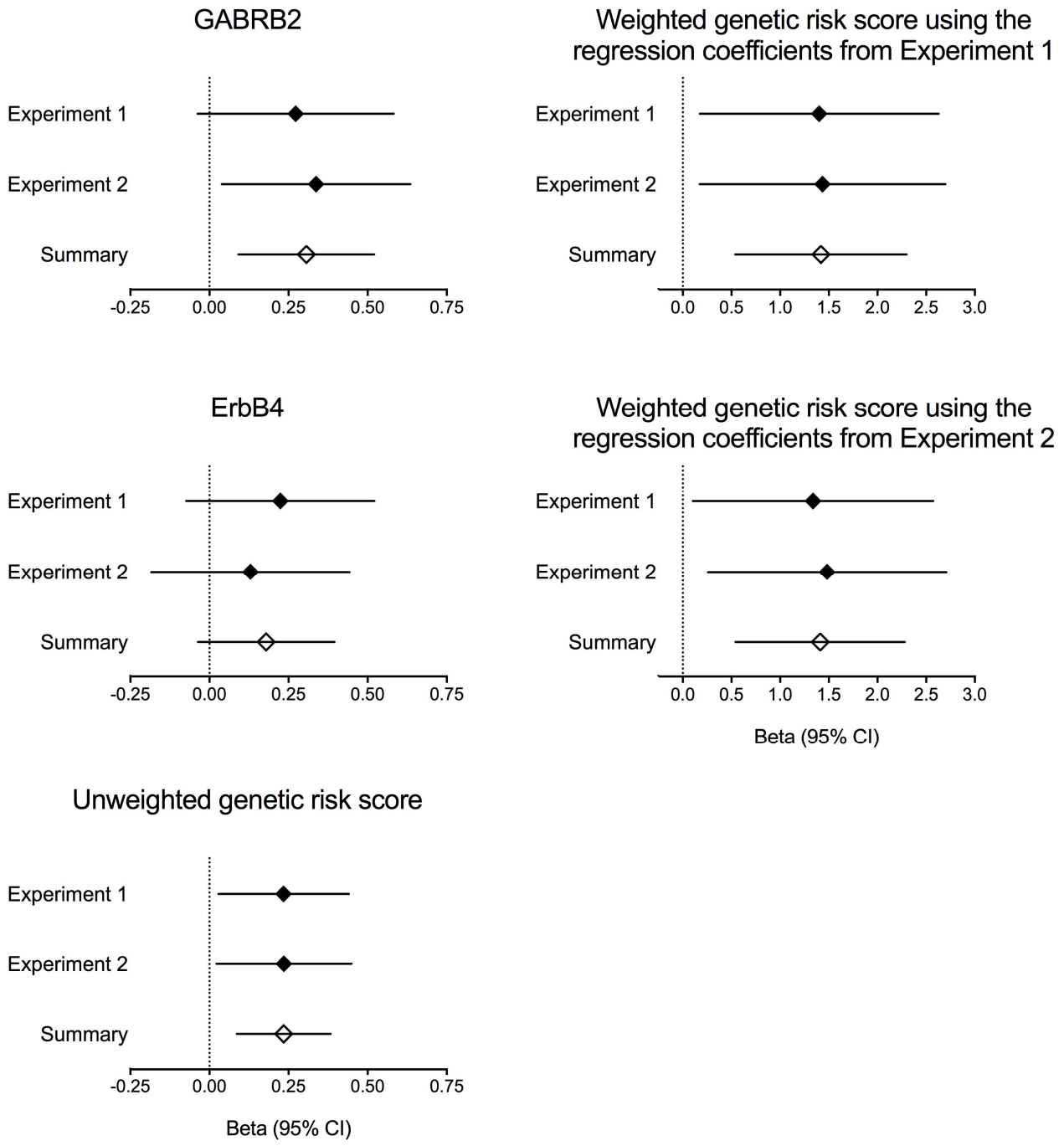

Figure 8. Meta-analysis of the genetic effects on the prediction error scores in Experiments 1 and 2. The forest plots show regression coefficients from simple regression models and their 95% confidence intervals, as well as the results of random-effects meta-analyses (note that in all cases the fixed-effects results were identical since the effects in the two experiments were not heterogeneous).

4. Discussion

We have investigated a potential mechanism through which GABA neurotransmission might affect learning: the computation or signalling of aversive prediction errors. The novel finding was that a genetic marker of increased GABA neurotransmission predicted larger brain responses to unexpected aversive USs relative to expected ones. That is, the number of A alleles for the *GABRB2* rs1816072 polymorphism (associated with increased expression of the $GABA_A$ receptor β2 subunit gene) predicted enhanced prediction error processing both when the unconditioned stimulus consisted of aversive pictures (Experiment 1) and a burst of 85-dB white noise (Experiment 2). This suggests that the effects of GABA are not modality specific, in line with animal studies that demonstrated the involvement of GABA in prediction error processing in both appetitive and aversive conditioning (Eshel et al., 2015; Kim et al., 1998). This enhanced prediction error processing was accompanied by an increased ability to discriminate the conditioned stimuli in Experiment 2, as well as lower scores on the Anxiety subscale of the Depression, Anxiety, and Stress Scale. Although the *ErbB4* rs7598440 polymorphism did not significantly predict the prediction error scores, its effect was in the expected direction in both experiments, as the number of T alleles, associated with increased GABA concentration, was associated with higher prediction error scores. We further combined the two polymorphisms into genetic risk scores. Because our sample included two separate cohorts, we were able to demonstrate that a weighted genetic risk score computed using the data from one (reference) experiment could predict the prediction error scores obtained in the other (validation) experiment. These analyses confirmed our hypothesis that the number of alleles associated with increased GABA neurotransmission is predictive of optimal prediction error processing.

Our findings are consistent with animal studies investigating the role of GABA in processing prediction errors in classical conditioning (Eshel et al., 2015; Kim et al., 1998). It has been suggested that fast-acting GABA neurons in the ventral tegmental area (VTA) might convey a temporally-precise signal that carries information about learnt CS-US contingencies, a signal that effectively conveys the expectation of the US (Cohen et al., 2012; Kim et al., 2010). This signal is thought to be critical for the computation of prediction error: GABA-mediated inhibition might reduce neural responses to USs that have already been signalled by a preceding CS, thus enabling the negative feedback mechanism illustrated in Figure 1. Cohen et al. (2012) suggested that drugs of abuse might cause sustained high prediction errors via their inhibition of VTA GABAergic neurons, which would then be unable to suppress prediction error signals for expected USs. This would result in sustained high prediction error

signals throughout learning, effectively boosting drug seeking. A similar argument can be proposed for aversive learning: lack of GABA-mediated inhibition would impair the computation of aversive prediction errors, such that, even after extensive CS-US pairings, the aversive US may nevertheless trigger large prediction errors. In other words, a lack of GABA-mediated inhibition would result in large prediction error signals regardless of whether the US was expected or not, which could result in aberrant fear learning. Our results showing that individuals with presumably reduced GABA neurotransmission have similar brain responses to high and low prediction error trials support this interpretation.

Others have proposed that $GABA_A$-receptor mediated inhibition in the basolateral amygdala controls emotional reactivity to electric footshock in rats, and hence might influence the amount of fear that can be acquired by conditioned stimuli (Van Nobelen & Kokkindis, 2006). That is, it is possible that GABA agonists such as muscimol disrupt fear conditioning by reducing the affective-motivational properties of the aversive US. This account bears some similarity to the account we have proposed here, according to which GABA mediated inhibition also reduces the effectiveness of the US to support new conditioning. However, we propose that it does so by reducing prediction error rather than attenuating the affective properties of the US.

Although we have focused our investigation on prediction error processing, evidence suggests that GABA is involved in several mechanisms that affect learning performance. For example, studies on pharmacological manipulations on rats have shown that $GABA_A$ receptors are involved in masking a conditioned response at test following learning from negative prediction errors (e.g., Garfield & McNally, 2009; Harris & Westbrook, 1998). Others have suggested that GABA modulates motivation (Reynolds & Berridge, 2002), selective attention (Gray et al., 1999; although see Harris & Westbrook, 1998, and Lawrence et al., 2003), or is involved in restoring the balance of cortical excitation and inhibition following learning (Barron et al., 2016). Given the various mechanisms through which GABA and other neurotransmitter systems might affect learning performance, careful investigations of specific potential mechanisms are needed for discovering their specific role(s) in learning. Here, we have demonstrated that GABA-related polymorphisms predict the integrity of one possible learning mechanism, namely processing of aversive prediction errors. As reductions in GABA neurotransmission are documented in mood and anxiety disorders (e.g., Gabbay et al., 2012; Rosso et al., 2013), our findings suggest a potential mechanism through which GABA deregulation might contribute to certain symptoms: reduced GABA signalling could result in

poor prediction error processing, resulting in aberrant aversive conditioning and excessive anxiety.

Finally, although our investigation focused on rs1816072 and rs7598440, other polymorphisms are known to influence gene expression and might therefore influence GABA signalling. For instance, Zhao et al. (2006, 2009, 2012) identified other polymorphisms that seem to influence *GABRB2* expression, in particular rs1816071 and rs187269. rs1816072 is in strong linkage disequilibrium with rs181071 (r^2 = .87, D' = .93), but only in moderate linkage disequilibrium with rs187269 (r^2 = .48, D' = .82). It would therefore be important replicate and extend our findings to these other polymorphisms, particularly rs187269. These investigations could be complemented by in vivo measurements of GABA concentration using magnetic resonance spectroscopy (Stagg, Bachtiar & Johansen-Berg, 2011), which would provide a converging method for elucidating the role of GABA in learning.

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