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Cognitive and personality analysis of startle reactivity in a large cohort of healthy males

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ABSTRACT

Subjects with low/undetectable startle are usually excluded from startle studies but few reports not confounded by this factor, show reduced startle in healthy impulsive subjects, or clinical populations with disorders of affect and impulsivity but also in schizophrenia and its prodrome. We examined the relationship of startle reactivity including startle "non-responding" status to cognitive and affective personality traits in a large and ethnically/demographically homogeneous cohort of healthy males from the LOGOS study, Heraklion, Crete. Startle reactivity was monotonically related to sensitivity to reward (higher in "non-responders", lower in strong responders). In addition, "non-responders" had poorer strategy, working memory and sustained attention performance compared to responder tertile groups. More research in clinical and high risk populations is required to examine if low/undetectable startle reactivity is a valuable intermediate phenotype for disorders of affect and impulsivity. It is possible that the "non-responsive" status may capture disease related features such as executive dysfunction.

1. Introduction

The startle reflex is a ubiquitous, cross-species reflexive response to abrupt and intense stimulation. It consists of a rapid striated muscle contraction designed to facilitate the flight reaction and/or to move the organism away of an unexpected threat. The reflex is subserved by a well defined neural circuitry whereby afferents from the cochlea, trigeminal nucleus, and vestibular nuclei reach the caudal pontine reticular nucleus (PnC, the center of the reflex), which then projects to motor areas of cranial nerve nuclei and the spinal cord (Koch & Schnitzler, 1997). As the vestibular and trigeminal nuclei are also activated by vestibular and tactile stimuli, respectively, and the vestibulospinal tract converges with the reticulospinal tract in the spinal cord, a cross-modal processing of startle stimuli occurs at this level (Li, Steidl, & Yeomans, 2001). The startle reflex is straightforward to elicit, record and quantify in the animal and human laboratory using similar stimuli and techniques. The most common technique is startle elicitation following short and abrupt acoustic stimuli of high intensity (acoustic startle reflex – ASR), although visual or cutaneous stimuli have also been used successfully to elicit startle. In all modalities, the startle response is modulated by the duration, the intensity and the rise time of the stimuli. Although electromyographic recording of the activity of the orbicularis oculi muscle is the most employed technique for assessing the startle response, other effective procedures have also been suggested (e.g. eletrophysiologic recording of the eyelid and direct recording of eyelid movement; for review, see Berg & Balaban, 1999).

The ASR shows interesting forms of regulation, which are of great relevance to psychiatric research. For instance, the ASR to a sudden intense stimulus (pulse) can be inhibited by a preceding weak sensory stimulus (the prepulse), which is presented 30–500 ms before the startling stimulus (Graham, 1975), a phenomenon known as prepulse inhibition (PPI). PPI is observed with both discrete and continuous prepulses, it increases with higher prepulse duration and intensity and the optimal prepulse-pulse interval is around 100 ms for both humans and animals (for review see Blumenthal, 1999). PPI is thought to reflect "sensorimotor gating", a form of central nervous system inhibition wherein distracting sensory information is filtered out during the early stages

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of processing (Braff et al., 1978). Deficient PPI is a surrogate measure of psychosis in animal models and a candidate endophenotype for schizophrenia (Calkins et al., 2007) with significant applications in schizophrenia research (Roussos, Giakoumaki, Adamaki, & Bitsios, 2011a; Roussos, Giakoumaki, & Bitsios, 2009a; Roussos, Giakoumaki, Adamaki, Georgakopoulos, et al., 2011; Roussos et al., 2006). Prepulse facilitation (PPF) refers to the enhancement of startle magnitude when the inter-stimulus interval is either very short (<30 ms) or relatively long (>500 ms) (Graham, 1975). PPF at long inter-stimulus intervals is thought to reflect an orienting response to incoming information (Graham, 1980) and has been found deficient in schizophrenia patients (Ludewig, Geyer, & Vollenweider, 2003) and their unaffected siblings (Wynn et al., 2004). Also, the potentiation of startle magnitude by conditioned or contextual threat, has become a translational psychophysiological paradigm, particularly useful in the study of normal and pathological fear and anxiety in experimental animals, healthy human subjects and patients with psychiatric syndromes (Grillon & Baas, 2003). Finally, startle enhancement or attenuation when the reflex is elicited in the presence of affectively unpleasant or pleasant experimental conditions (usually pictorial stimuli) has been termed "affective startle modulation" (Lang, Bradley, & Cuthbert, 1990) and has become a prominent methodological tool in the study of human emotion and its disorders. This pattern of startle modulation has been observed regardless of the modality of the startle probe but the degree of modulation depends on laterality, the duration of the affective stimulus and the time point of startle stimulus delivery after picture onset (for review, see Bradley, Cuthbert, & Lang, 1999).

While the ASR regulation from early attentional processes and internal affective/motivational states has been exploited in fruitful translational psychiatric research over the last twenty years, relatively little research efforts have been allocated to startle reactivity or 'baseline' startle in the absence of any experimental manipulation. While startle magnitude shows very large between-subject variability, it is highly heritable (Anokhin, Heath, Myers, Ralano, & Wood, 2003; Hasenkamp et al., 2010) with considerable consistency within subjects across time (Larson, Ruffalo, Nietert, & Davidson, 2000). It is possible that individual differences in baseline startle reactivity may reflect activity of its regulatory brain structures (e.g. limbic) due to constitutional factors. Subjects with low/undetectable startle are usually excluded from startle studies but few reports not confounded by this factor (see Quednow et al., 2006 for a discussion of this point), showed reduced startle reactivity in healthy, high sensation seeking Asians (Swerdlow, Talledo, & Braff, 2005), and high novelty seeking Caucasian males (Roussos, Giakoumaki, & Bitsios, 2009). Interestingly, the low startle healthy Caucasian males in the study of Roussos, Giakoumaki, and Bitsios (2009) were carrying the long DRD4 variable number tandem repeat (L-DRD4 VNTR) polymorphism, which is implicated in sensitivity to reward and disorders with dysregulated affect and impulsivity (Roussos, Giakoumaki, & Bitsios, 2009). Consistent with the above, one study found reduced or entirely absent startle reactivity in psychopaths (Herpertz, Werth, Lukas, et al., 2001), while our group found reduced startle reactivity in remitted bipolar disorder (BD) patients and their unaffected siblings (Giakoumaki et al., 2010). One way of better understanding such constitutional factors underlying reduced startle reactivity in patient populations, is to examine cognitive and affective correlates of startle reactivity in healthy subjects devoid of the confounds of medication, presence of symptoms and the brain effects of chronic psychiatric illness. Learning about cognitive and emotional correlates of startle reactivity may advance our understanding of both startle reactivity and the disorders characterized by reduced startle. We therefore used our LOGOS cohort of healthy young males in Heraklion, Crete (Roussos, Giakoumaki, Adamaki, & Bitsios, 2011) to explore potential differences in personality traits and cognitive profile between subgroups with high, intermediate, low and blunt/undetectable acoustic startle reactivity. Based on the literature cited above, we hypothesized initially, that low reactivity subgroups would present with the highest levels of sensitivity to reward. Given that startle reactivity deficits may emerge with the onset of acute psychosis (Quednow et al., 2008) and reduced startle has also been found in schizophrenia patients (Quednow et al., 2006), we also hypothesized that low reactivity subgroups would present with reduced performance in measures of executive function.

Subjects with blunt/undetectable startle have been frequently characterized in the literature as "non-responders", as opposed to "responders" i.e. the rest of the population who present with some startle response. Informal reports estimate, somehow vaguely, that about 5–10% of the general population (and a higher percentage from clinical populations) are "startle non-responders", i.e. they exhibit startle responses on too few trials or none at all (Blumenthal et al., 2005), in the absence of any perceptual (i.e. auditory) impairments. However, studies vary substantially in the criteria used for the identification of startle "non-responders" (Table 1 in Supplementary data). These subjects are excluded from startle studies and it is hard to know whether they represent the low end of a continuum (weak responses which are lost in the EMG noise floor) or a qualitatively distinct population (genuine non-responding status). With the above considerations in mind, we used a fairly standard session (see Section 2 and Supplementary data) and equipment (San Diego, SR-LAB) similar to most research groups in the field, ensuring that recording environment, equipment setup, participant preparation, and data handling were all of sufficient sensitivity according to published guidelines (Berg & Balaban, 1999; Blumenthal et al., 2005) to allow for any possible very small responses to be detected. As in some previous studies (e.g. Csomor et al., 2009; Kumari et al., 2008), we set the criterion for response at 10 μV, the smallest detectable startle amplitude on our equipment. Subjects with zero response probability in the 12 pulse-alone trials were positively defined as "non-responders". Subjects with too few (1–3 out of 12) and sporadic responses which did not allow for calculation of a reliable startle were also defined as "non-responders" (see Section 2). Moreover, in order to confirm maximum sensitivity of the recording, we compared the EMG activity in the first 20 ms of recording between responders and "non-responders", to exclude the possibility that lack of startle in "non-responders" could be due to excessive noise occluding small responses. Finally, in additional analyses, we treated the "non responders" as a qualitatively separate group distinct from all other subjects clustered together as "responders", to ensure that potential differences in personality and cognition would not reflect peculiarities associated with the strong responders rather than the non-responders.

2. Methods

2.1. Participants

Participants were recruited from the first wave of the LOGOS (Learning On Genetics Of Schizophrenia Spectrum) study in Heraklion, Crete. The LOGOS project recruited 1149 randomly selected Greek Caucasian young male conscripts from the Greek Army (mean age 22.32 ± 3.78; range: 18–29), during its first phase between June 2008 and August 2010. The study took place between 9 am and 3 pm in the medical quarters of the Military Training Camp of Candidate, Supply Army Officers (S.E.A.P) in Heraklion, Crete. For this purpose, two adjacent rooms in the medical quarters were converted into laboratories. Following public presentation of the study's methods and goals in each consecutive series of new conscripts, all participants willing to volunteer, had a detailed information sheet and gave written informed consent before screening. All participants were tested on one single occasion at some point during their two months military training in this establishment.

All participants had been recently screened for current physical and mental health status by the army medical authorities and were physically healthy and free from any DSM Axis I disorders. However, they all underwent a review of their medical history, the Mini-International Neuropsychiatric Interview (M.I.N.I.) interview (Sheehan et al., 1998), urine toxicology and IQ testing with the Raven's Progressive

Table 1
Demographic and testing characteristics of response probability groups.

Response probability	Non-responders (n = 149)				Responders (n = 855)			
	0% (n = 130)	8–25% (n = 19)	p	50–66.7% (n = 75)	75–83% (n = 124)	91.7% (n = 149)	100% (n = 507)	p
Valid PA trials (out of 12)	0	1–3		6–8	9–10	11	12	
Amplitude μV (mean ± SD)	0	54 ± 14.9		56.6 ± 30.3	74.0 ± 40.1	103.1 ± 56.8	149.5 ± 76.5	<0.001
Baseline EMG (mean ± SD)[a]	10.42 ± 5.19	10.77 ± 5.72	>0.8	10.87 ± 10.74	11.43 ± 7.03	9.66 ± 5.17	9.34 ± 4.51	>0.1
Age[a]	21.4 ± 2.8	21.3 ± 3.1	>0.7	21.7 ± 3.1	21.6 ± 3.2	22.1 ± 3.5	22.5 ± 3.6	=0.052
Education	14.3 ± 2.2	14.3 ± 2.3	>0.9	14.4 ± 2.2	14.3 ± 2.4	14.4 ± 2.4	14.9 ± 2.5	<0.05
Raven raw score	49.4 ± 6.6	47.4 ± 8.0	>0.3	48.3 ± 8.5	49.4 ± 7.1	50.6 ± 7.1	50.0 ± 7.7	>0.2
Percentage smokers[b]	34.62%	52.63%	>0.2	40.0%	34.68%	47.65%	43.20%	>0.1
Smokers: cig per day[a]	14.9 ± 8.7	16.7 ± 7.5	>0.4	16.5 ± 8.6	16.0 ± 7.9	17.4 ± 7.8	16.8 ± 9.1	>0.7
SoT (winter to autumn)[b]	24/33/36/37	3/3/5/8	>0.6	13/21/18/23	26/28/29/41	39/33/26/51	111/113/110/168	>0.8

PA: pulse alone; SoT: season of testing.
[a] Non-parametric comparison.
[b] Chi square comparison.

Matrices. Eleven participants were on medication, 50 participants reported past history of alcohol/substance use, 36 participants reported a past history of closed head injury or neurological problems. Another 48 participants were excluded due to equipment failure or technical problems during startle/PPI recording. Therefore 1004 participants entered the study.

2.2. Procedures

The study was approved by the Ethics Committee of the University of Crete, the Executive Army Bureau and the Bureau for the Protection of Personal and Sensitive Data of the Greek State. Startle recording took place in a soundproof room between 09.30 am and 01.00 pm to avoid diurnal effects on startle reactivity (Miller & Gronfier, 2006). In agreement with the military premises administration, participants were free of military duties on the morning of the testing and had a good night's rest on the day before assessment. Individuals appearing or declaring that they were sleepy in the morning of the recording session would have to return another day for testing. There was a 3-day period on weapons' training per series of conscripts, during their 2–3 months stay in these premises. As weapons' training may be a common cause of temporary hearing threshold shift (Olszewski, Miłoński, Sułkowski, Majak, & Olszewski, 2005), recordings took place throughout the year except from the weapons' training days and the week immediately after. All participants had been instructed to maintain their normal patterns of caffeine and nicotine consumption until the morning of the experimental testing to avoid possible effects of caffeine (Andrews, Blumenthal, & Flaten, 1998) and nicotine (Cinciripini et al., 2006) withdrawal on startle. However, no participant was tested within 10 min of having smoked a cigarette to avoid an effect of nicotine during the testing session.

The study included recording of the acoustic startle reflex for testing of prepulse inhibition (PPI). Pulses consisted of 40 ms, 115 dB white noise bursts, and prepulses consisted of 20 ms, 75 and 85 dB white noise bursts, with instantaneous rise/fall time, over 70 dB background noise. Recording began with a 3 min acclimation period when only background noise was present. The recording period comprised 12 pulse-alone trials and 36 prepulse–pulse trials. Three lead intervals were used (30, 60, 120 ms; 6 trials/prepulse–pulse trial type). All trials were presented in pseudorandom order with the constraint that no two identical trials occurred in succession. The inter-trial interval varied between 9 and 23 s (average 15 s). The entire test session lasted approximately 15 min. All subjects were tested under identical conditions. The PPI data are out of the scope of this paper and will be published separately. The remainder of this paper is based on the 12 pulse-alone control startle-only trials, which were interspersed with the prepulse trials.

To increase the sensitivity of our recording procedure, the study followed the guidelines by Berg and Balaban (1999) and Blumenthal et al. (2005) for startle studies in humans. In detail, a commercially available electromyographic (EMG) startle system (EMG SR-LAB, San Diego Instruments, San Diego, CA, USA) was used to examine the eyeblink component of the acoustic startle response. This was used to deliver acoustic startle stimuli and record the EMG activity for 150 ms (sample interval = 1 ms) starting from the onset of the startle stimulus, whilst the raw data was stored for later application of rejection criteria and averaging. Acoustic stimuli were administered binaurally through headphones (model SONY MDR-V6). Electromyographic recordings were taken while subjects were seated comfortably in an armchair and instructed to relax but stay awake, with eyes open and fixated on a fixed point on the wall, 3.5 m in front of them. The eyeblink component of the startle reflex was indexed by recording EMG activity of the orbicularis oculi muscle directly beneath the right eye by positioning two miniature silver/silver chloride electrodes (one electrode was positioned 1 cm lateral to and 0.5 cm below the lateral canthus, and the other electrode was placed 1.5 cm below and slightly medial to the first electrode) filled with Signa Gel electrolyte paste (Parker Laboratories, Inc., New Jersey, USA) with a ground electrode behind the right ear on the mastoid (R < 10 kΩ); the external diameter of the electrodes was 13 mm. Before electrode placement and in order to minimize the impedance between skin surface and electrode gel, the area below the eye was cleansed as per Blumenthal et al. (2005) and Berg and Balaban (1999). Placement of the recording electrodes was such that both electrodes were equidistant from the center of the eye and as close to each other as possible but without overlapping, using adhesive collars (GV-ADH-RING/13 mm, GVB-geliMED, Bad Segeberg, Germany). EMG activity was band-pass filtered (100–1000 Hz) and a 50 Hz filter was used to eliminate the 50 Hz interference. Before recording, the signal was checked for noise artifacts and voluntary blinks were clearly distinguishable from the ongoing tonic activity of the muscle.

Before scoring and data analysis, all recordings were rectified and then submitted to a running average routine, which smoothed the digital signals recorded on each trial over 5 successive samples. Our smoothing procedure did not undermine the sensitivity of response detection, since the probability of detecting small or brief responses is known to decrease substantially only with time constants longer than 10 ms (Blumenthal, 1994). Following this, recordings were screened for spontaneous eyeblink activity and missing trials. Trials were excluded if excessive EMG activity (>20 μV) was observed during the first 20 ms of recording or when onset latencies (defined by a shift of 10 μV from the baseline value, occurring within 20–85 ms after the onset of the pulse stimulus) and peak latencies (the point of maximal amplitude occurring within 150 ms from the startling stimulus) differed by more than 95 ms as per Braff, Grillon, and Geyer (1992). The maximum absolute amplitude of the raw EMG data (i.e., the peak EMG value of the nonzero trials without subtracting the baseline average or the voltage at onset from that peak value) occurring in the 21–150 time window of the non-rejected trials was scored offline and stored for averaging and data analysis.

Before recording, all participants underwent a brief hearing test with an AC30 Clinical Audiometer (Kamplex Ltd, London, UK). All participants had <25-dB hearing thresholds at 0.5, 1, 2 and 4 kHz. Startle amplitude is presented in μV. On arrival to the testing room, following acclimatization, participants self-rated their moods and feelings on a 16-item visual analog scale (Bond & Lader, 1974). As there were no significant between-group differences, no further data are reported here.

2.3. Neuropsychological assessment

Participants underwent cognitive testing using the Cambridge Neuropsychological Test Automated Battery (CANTAB). Working memory and strategy formation was assessed with the Spatial Working Memory task (SWM), sustained attention and vigilance with the Rapid Visual Information Processing task (RVIP) and planning for problem solving was assessed with the Stockings of Cambridge (SoC). We also assessed verbal learning and memory [WMS-III Word Lists task (WL)], set-shifting and rule learning abilities [Wisconsin Card Sorting Test (WCST)], and emotional decision making [Iowa Gambling Task (IGT)]. For a detailed description of the tasks see Supplementary data.

2.4. Personality assessment

All participants were administered the Revised Eysenck Personality Questionnaire (EPQ-R), Cloninger's Temperament and Character Inventory (TCI), Spielberger's State-Trait Anxiety Inventory–Trait Scale (STAI-T), the Carver and White's Behavioral Inhibition/Behavioral Activation System (BIS/BAS) questionnaire, and the Schizotypal Traits Questionnaire (STQ). For a detailed description of the scales see Supplementary data.

2.5. Statistical analyses

Since we used 6 neuropsychological tasks, 5 personality scales and the VAS, alpha was set at 0.00416 (0.05/12). The statistical software SPSS 19.0 (SPSS Inc, Chicago, IL, USA) was used for the analyses. Following identification of startle "non-responders" (SNR group) we divided the startle responders into low, intermediate and high responder groups (LR, IR and HR), following a tertile split on baseline startle amplitude data. Therefore, we obtained 4 reactivity groups. We used non-parametric ANOVAs for between-group demographic comparisons since the

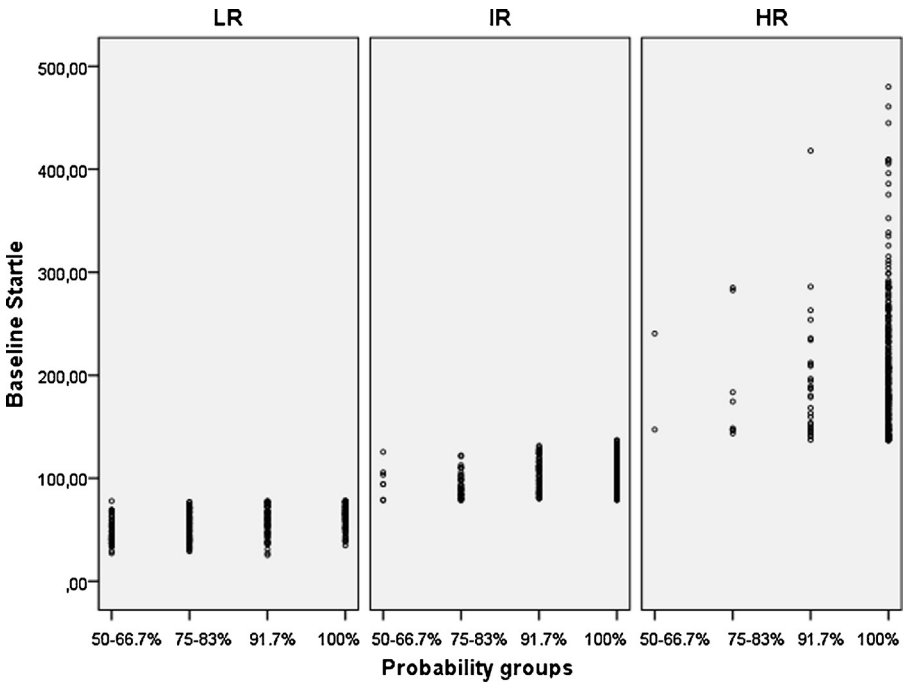

Fig. 1. Qualitative analysis of the relationship between response probability and startle responders' tertile group membership. HR: high-responders, IR: intermediate responders, LR: low-responders.

demographic variables were not normally distributed. We submitted scores on all personality questionnaires to principal component analysis (PCA) for the sake of data reduction and variables classification and compared the 4 reactivity groups on the resulting PCA personality factors. For each cognitive test used, multivariate analyses of variance (MANOVAs) were performed with selected key metrics as the dependent variables with group (4 levels) as the fixed factor. The selected metrics were: SWM: strategy, total between-errors, total within-errors and total double-errors; RVIP: A' B' and latency; WCST: categories achieved, Milner and Nelson perseverative errors; WL: accuracy and intrusion errors after immediate, short- and long-delay recall; SoC: problems solved correctly, mean moves, mean initial thinking time, mean subsequent thinking time; IGT: total money won and total cards picked from the safe minus total cards picked from the risky decks. Since the F test is robust to non-normality, if the latter is caused by skewness rather than by outliers, any outliers were previously identified and removed (i.e. in metrics RVIP B', intrusion errors with immediate recall in the WL and between errors in the SWM). For each MANOVA, the homogeneity of variances test revealed that all dependent variables exhibited equal levels of variance across the four groups. For each separate MANOVA, multicollinearity was ruled out since intercorrelations between its key selected metrics were acceptable (all rho coefficients < 0.7). Significant group effects from the multivariate tests (Pillai's trace), which met our alpha criterion, were followed up with "step down" univariate ANOVAs for each selected metric of the test. Finally, using an identical strategy as above, we also performed a dichotomous comparison between the SNR and the SR groups.

3. Results

The PPI data are out of the scope of this paper and will not be discussed here. The results below are based on the 12 pulse-alone control startle-only trials, which were interspersed with the prepulse trials.

3.1. Identification and description of "non responders" and responders based on response probability

All participants exhibited voluntary and spontaneous blink activity; however, 130 (13%) participants failed to exhibit a detectable response with our stimuli and equipment (cut-off criterion of 10 μV) in all trials (0 response probability) and were thus characterized as definite non-responders (SNR group). An additional 19 participants had only 1–3 sporadic responses in the pulse-alone trials used for the calculation of baseline startle (1 response: 7 participants; 2 responses: 10 participants, 3 responses: 2 participants). Calculation of a reliable baseline startle was impossible for these participants with minimal response probability (8–25%; group mean: 14.5%) under the fairly standard stimuli and equipment used for acoustic startle elicitation. There were no differences in demographics and baseline EMG activity from the definite non-responders and therefore, these participants were included in the non-responder group (Table 1). The remaining 855 participants were characterized as startle responders (SR group) (startle amplitude (mean ± SD): 123.03 ± 75.55; min: 25.36, max: 480). Table 1 also shows the breakdown of the SR group, in subgroups with different response probability. It is evident that startle amplitude was monotonically increased as a function of response probability (Kruskal–Wallis $x^2 = 272.9$, df:3, $p < 0.001$). The highest probability group was slightly but significantly older with more years of education than the 75–83% probability group. There were no other differences in demographics or baseline EMG activity between the SR probability groups.

Based on a tertile split of the baseline startle amplitude, the SR group was divided in Low, Intermediate and High Responder (LR, IR and HR) groups, in preparation for subsequent analyses (see below). Fig. 1 shows a qualitative analysis of the relationship between response probability and the SR tertile group membership. Interestingly, equal numbers of LR participants fell into each one of the four probability groups suggesting that within responders, low startle reactivity is not necessarily coupled to low response probability. High startle reactivity, however, was associated with high response probability although several HRs had low response probability.

3.2. Demographic comparison of SNR and SR reactivity (tertile) groups

The SNR and the three tertile startle reactivity groups (LR, IR and HR) were entered in the analyses. The HR group participants were older and were educated for longer than all other group members. There were no IQ or other demographic or baseline EMG differences

Table 2
Demographic and testing characteristics of startle reactivity groups ("non-responders" and responder tertile groups).

Group	SNR (n = 149)	LR (n = 285)	IR (n = 285)	HR (n = 285)	p
Startle amplitude μV (mean ± SD)[a]	0.0	55.5 ± 13.4	104.4 ± 16.0	201.6 ± 65.9	<0.001
Baseline EMG μV (mean ± SD)[a]	10.5 ± 5.3	10.1 ± 4.4	10.4 ± 5.3	10.7 ± 7.4	>0.3
Age[a]	21.4 ± 2.8	21.9 ± 3.3	21.5 ± 3.3	22.5 ± 3.8	<0.001
Education[a]	14.3 ± 2.2	14.5 ± 2.6	14.5 ± 2.4	15.2 ± 2.6	<0.001
Raven raw score[a]	49.2 ± 6.8	49.8 ± 7.5	49.7 ± 7.3	50.1 ± 8.1	>0.3
Percentage smokers[b]	37.58%	42.46%	42.11%	43.51%	>0.6
Smokers: cig per day[a]	15.4 ± 8.4	17.0 ± 8.8	17.6 ± 8.8	15.8 ± 8.1	>0.3
SoT (winter to autumn)[b]	26/36/41/46	65/70/70/80	61/67/58/99	63/58/60/104	>0.4

SNR: startle non-responders; LR: low responders; IR: intermediate responders; HR: high responders; SoT: season of testing.
[a] Non-parametric Kruskal–Wallis comparison.
[b] Chi square comparison.

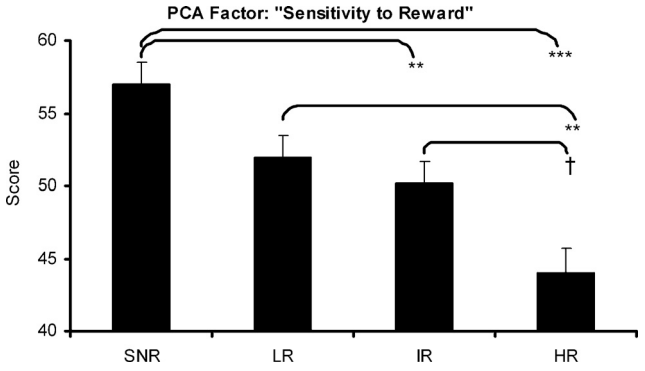

Fig. 2. PCA factor "sensitivity to reward" in the SNR and startle responder groups. Data were t-transformed ([PCA scoreX10) + 50] for graphical purposes. Columns represent group means and bars represent SEM. ***$p < 0.001$, **$p < 0.01$, †$p < 0.06$. SNR: startle non-responders, HR: high-responders, IR: intermediate responders, LR: low-responders.

(Table 2). In the entire group, years of education correlated strongly with age (rho = 0.85; $p < 0.001$), hence, age alone was chosen as the covariate in the subsequent statistical comparison between the four reactivity groups.

3.3. Principal component analysis of personality questionnaires

Our principal component analysis (PCA; Keiser–Meyer–Olkin = 0.829, $\chi^2 = 6973.62$, $p < 0.001$) revealed five personality factors ("Anxiety", "Psychosis", "sensitivity to reward", "Impulsivity", "Dependency to Social Reward" – Table 3) with eigenvalues >1.00, which explained 68.66% of the total variance.

3.4. Comparison of SNR and SR reactivity (tertile) groups along personality and cognitive performance

Separate univariate ANOVAs for each individual PCA factor revealed a significant group effect only for "sensitivity to reward" ($F = 8.76$; $p < 0.001$, partial $\eta^2 = 0.03$), which met our alpha criterion after univariate ANCOVA with age as a covariate ($p < 0.001$). Fig. 2 shows that "sensitivity to reward" followed a pattern of linear reduction from the SNR to the HR group. There were no differences in any other PCA personality factor or in the scores of the individual personality questionnaires (all p values > 0.1; data not shown).

The separate MANOVAs for each neuropsychological test revealed significant group effects of the multivariate tests only for SWM ($F = 2.8$; $p < 0.001$, partial $\eta^2 = 0.01$) and RVIP ($F = 4.38$; $p < 0.001$, partial $\eta^2 = 0.013$) which met our alpha criterion after taking age as a covariate (SWM $p < 0.004$, RVIP $p < 0.001$). Group effects at $p < 0.05$ were noticed for the IGT but this significance level did not reach criterion. There were no significant effects of the multivariate tests for SoC, WL and WCST ($p > 0.1$). The univariate ANCOVAs showed that the SNR group made more "between" errors ($p < 0.001$) with inferior strategy formation ($p < 0.002$) in the SWM task, had reduced sensitivity to target detection (lower A'; $p < 0.007$) and impulsive responding (higher B'; $p < 0.034$) in the RVIP test. These effects are shown in Fig. 3 which also shows that all SR groups were very similar to one another, with a tendency for better performance in the older HR group. Because the HR group included the highest number of participants with high (100%) response probability (Fig. 1), we checked whether our findings could be attributed to peculiarities associated with the strong responders, by removing 12 HR outliers or 50% HR participants with startle amplitude above the HR group median value. There was no change in the above stated results when HR participants were excluded from the analyses. The results remained robust even after the exclusion of the entire HR tertile, although personality and SWM group effects did not reach the alpha criterion set, most likely due to loss of power.

Finally, an identical series of analyses with response probability as the grouping factor (Table 1) and age as a covariate revealed significant group effects for "sensitivity to reward" and RVIP only. However, the post hoc tests revealed a different pattern compared to that seen in Figs. 2 and 3 when startle reactivity was used as the grouping factor; indeed, these group effects were driven by the highest probability group (less sensitivity to reward and better RVIP performance), with the SNR and the other responder groups being similar.

3.5. Dichotomous comparison (SNR vs SR group)

The SNR group was slightly but significantly younger ($p < 0.03$) compared to responders clustered in a single SR group, but the two groups did not differ in any other demographic variable or basal EMG activity (all p values > 0.1). Identical series of analyses to the above revealed a similar pattern of significant results for the multivariate tests for the SWM and RVIP (SNR worse than SR), which survived the alpha criterion after covarying with age (SWM $p < 0.003$; partial $\eta^2 = 0.016$ and RVIP $p < 0.001$; partial $\eta^2 = 0.023$). Group effects at $p < 0.02$ were noticed for the IGT but this significance level did not reach criterion. The univariate ANCOVA comparisons for SWM, RVIP and the personality PCA factor "sensitivity to reward" are shown in Table 4.

3.6. Correlations in the SR group

In the SR group, startle correlated positively with age (rho = 0.1; $p < 0.01$) and negatively with the PCA Factor "sensitivity to reward" (rho = −0.142; $p < 0.001$), the latter relationship surviving ($p < 0.001$) when the effect of age was partialled out. Startle also correlated positively with performance indices of RVIP (better target detection), Iowa Gambling (fewer risky decisions) and Wisconsin

Table 3
Principal component analysis of personality measures. Values represent factor loadings. Numbers in brackets represents the percentage of variance explained by the specific factor solution.

		Factor 1-"Anxiety" (25.70%)	Factor 2-"Psychosis" (15.68%)	Factor 3-"Sensitivity to reward" (11.99%)	Factor 4-"Impulsivity" (8.30%)	Factor 5-"Dependency to social reward" (6.99%)
STAI-T	STAI-T	0.802				
BIS/BAS	BIS	0.590				
	Reward responsiveness			0.881		
	Drive			0.845		
	Fun seeking			0.699		
EPQ	Psychoticism					−0.550
	Extraversion	−0.436				
	Neuroticism	0.720				
	Lie				−0.722	
TCI	Novelty seeking				0.831	
	Harm avoidance	0.806				
	Reward dependence					0.801
	Persistence	Factor solution < 0.5 and this variable was excluded from analysis				
	Self-directedness	−0.693				
	Cooperativeness					0.709
	Self-transcendence		0.796			
STQ	Magical thinking		0.850			
	Paranoid ideation		0.651			
	Unusual experiences		0.780			

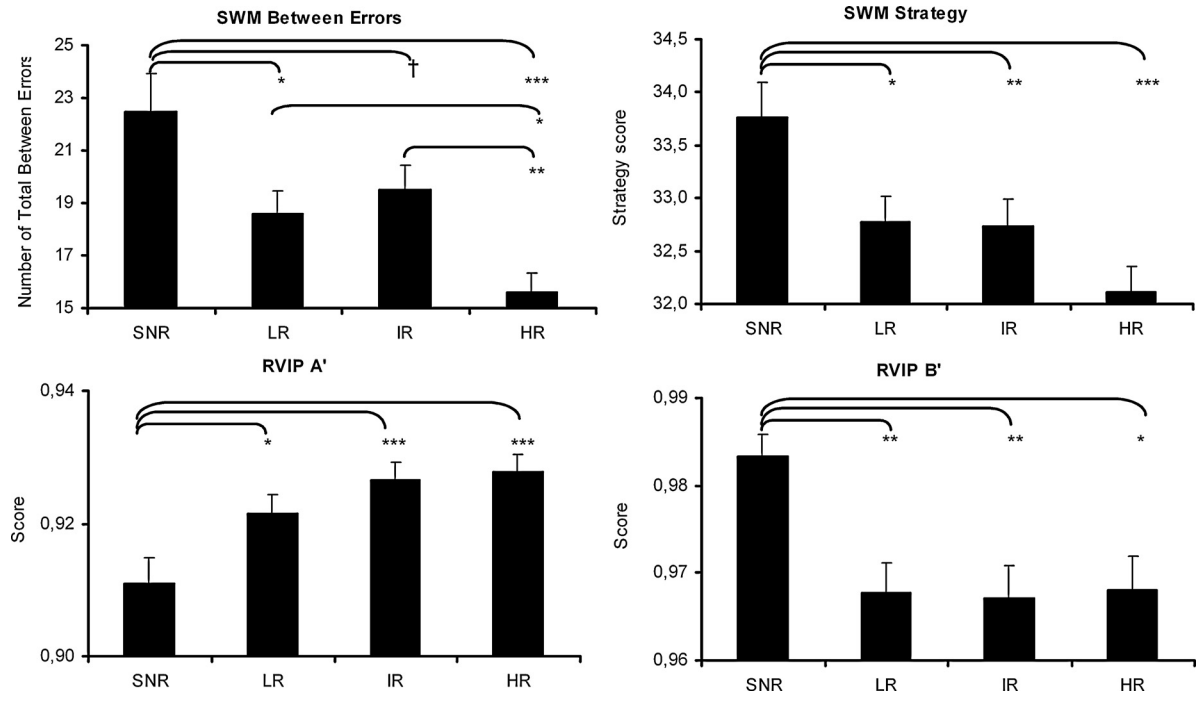

Fig. 3. Cognitive task performance in SNR and startle responder groups. Columns represent group means and bars represent SEM. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, †$p < 0.06$. SNR: startle non-responders, HR: high-responders, IR: intermediate responders, LR: low-responders.

Table 4
Mean (±SD) for personality and performance measures for cognitive tasks that have met alpha criterion ($p < 0.00416$) in MANCOVAs with group as the fixed factor and age as the covariate. P values are from follow up univariate ANCOVAs.

		SR ($n = 855$)	SNR ($n = 149$)	p
Personality	PCA "sensitivity to reward"	−0.120 ± 2.6	0.697 ± 1.9	0.003
Cognition	*Spatial working memory* (*CANTAB spatial working memory*)			
	Between Errors	17.88 ± 14.48	22.51 ± 17.15	0.002
	Strategy score	32.49 ± 04.05	33.77 ± 03.93	0.001
	Sustained attention (*CANTAB rapid visual information processing*)			
	A′	00.925 ± 0.046	00.911 ± 0.046	0.004
	B′	0.965 ± 0.09	0.983 ± 0.03	0.03

SR: startle responders, SNR: startle non-responders, PCA: principal component analysis, CANTAB: Cambridge Neuropsychological Test Automated Battery.

Card Sorting tasks (fewer errors) with rho values ranging from 0.074 to 0.086; however, these results did not survive when the effect of age was partialled out.

4. Discussion

In support of our hypothesis, we showed that reduced startle reactivity was associated with higher "sensitivity to reward", a PCA Factor derived from the Behavioral Activation System (BAS) scale (Carver & White, 1994), which measures Grey's Behavioral Approach System (Gray, 1994). This is a bio-behavioral system that regulates approach motivation and goal-directed behavior to attain rewards (e.g. Gray, 1994), with considerable evidence supporting its excessive activation as a vulnerability trait for bipolar spectrum disorders (e.g. Alloy et al., 2012). This was a linear relationship, the "non-responding" group occupying the high end of a continuum. Our findings replicate previous studies in healthy Asian subjects with high sensation seeking (Swerdlow et al., 2005) and in Caucasian males with high novelty seeking (Roussos, Giakoumaki, & Bitsios, 2009). Furthermore, we showed for the first time that reduced startle reactivity was associated with worse performance in measures of strategy and spatial working memory and reduced target detection with impulsive responding in a test of sustained attention. These effects were specific to the "non-responding" group, and persisted after removal of HR outliers, HR participants with a higher than group median startle or the entire HR group; effects also persisted in a dichotomous analysis comparing the "non-responders" to all responders clustered together in a single group. Therefore, these effects were associated with low/undetectable reactivity rather than peculiarities associated with the high responders. Our results do not seem to be attributed to IQ, smoking habit or season of testing while small age differences were controlled for.

While higher startle reactivity was associated with higher response probability, probability-based stratification did not discriminate well enough for performance differences in cognition and sensitivity to reward, possibly because response probability has less variability than startle amplitude. Another possibility may be that the two measures are the functional outcome of partially overlapping but dissociable and non-redundant neural circuits (Blumenthal & Berg, 1986). In favor of this view is the allocation of participants in reactivity and probability groups (Fig. 1), which showed decoupling of the two measures in a substantial number of participants. While we took every possible measure to ensure the highest possible sensitivity of recording, we cannot be certain as to whether some "non-responders" demonstrated a genuine lack of response or whether responses were smaller than our equipment's threshold (10 μV) or both. Specifically designed studies with parametric explorations that increase startle reactivity, such as more intense stimuli, darkness, different stimulus modalities or more sensitive EMG amplifiers are required to answer this question. Based on the baseline EMG activity in the first 20 ms of recording, and with our knowledge at present, we could state with some certainty that the difference in response probability was not due to excessive noise occluding small responses. Nevertheless, our definition of non-responders was rather conservative and most likely represented subjects frequently excluded from human acoustic startle reflex studies.

Our findings are novel and seem to suggest that healthy "non-responders" are in a continuum with startle responders with regards to impulsivity/reward sensitivity but they differ dichotomously in terms of working memory and sustained attention from all other responders. Notably, these differences were small (effect sizes explaining 1–3% of the total variance) and would not have been detected with small samples. A handful of studies that were not confounded by the exclusion of patients with very low startle reactivity, have found reduced startle reactivity in schizophrenia and prodromal psychosis patients and in patients with biporal disorder (BD; Giakoumaki et al., 2010; Quednow et al., 2006, 2008). Both these disorders are characterized by working memory and sustained attention deficiencies (Giakoumaki, Roussos, Pallis, & Bitsios, 2011; Phillips & Vieta, 2007). It is thus possible that our current definition of "startle non-responding" status may capture disease related features such as working memory/sustained attention impairments. It is interesting that in otherwise healthy males, reductions in such prefrontaly mediated functions emerge when startle reactivity lowers below a certain threshold (10 μV according to the present study).

Recent research addressing heritable variations in dopamine neurotransmission, shows that in healthy subjects, suboptimal prefrontal dopamine transmission is associated with reduced working memory (Giakoumaki, Roussos, & Bitsios, 2008; Mattay et al., 2003; Roussos, Giakoumaki, & Bitsios, 2009) and, as a result of downstream effects on limbic structures, lower baseline startle (Roussos, Giakoumaki, & Bitsios, 2009) and more resilience to negative mood (Drabant et al., 2006; Roussos, Giakoumaki, & Bitsios, 2009; Smolka et al., 2005; Weiss et al., 2007). A possible link, therefore, between reduced working memory, blunt startle reactivity and high sensitivity to reward as evidenced in the non-responder group may be the altered dopamine neurotransmission within frontal cortico-striato-limbic circuitry. Consistently, very low startle reactivity and high sensitivity to reward were phenotypic characteristics of healthy males carrying the long DRD4 variable number tandem repeat (L-DRD4 VNTR) polymorphism (Roussos, Giakoumaki, & Bitsios, 2009), implicated in disorders with dysregulated affect and impulsivity such as ADHD (Swanson et al., 2007), substance abuse (Kotler et al., 1997), and BD (Serretti & Mandelli, 2008). Finally, transgenic mice with a humanised version of the *FOXP2* gene, which controls corticobasal ganglia circuits (Enard et al., 2009; Lieberman, 2009) and is involved in the unique human ability for language (Fisher, Vargha-Khadem, Watkins, Monaco, & Pembrey, 1998; Gopnik & Crago, 1991; Lai, Fisher, Hurst, Vargha-Khadem, & Monaco, 2001; Marcus & Fisher, 2003; Vargha-Khadem et al., 1998) and cognition (Lieberman, 2009) present with higher startle and reduced sensitivity to novelty cues (Enard et al., 2009; Lieberman, 2009). It seems that the appearance of human cognitive ability enabled a reduction in impulsivity/approach behaviors and an increase in startle reactivity. These findings by Lieberman (2009) and Enard et al. (2009) indirectly validate our results and provide an interesting evolutionary perspective to the observed combination of reduced cognitive ability and high sensitivity to reward/blunt startle observed in our "non-responding" group. It is also interesting, that the high responder group had attained significantly more years of education and was the least sensitive to reward.

In the absence of perceptual impairments, which are highly improbable here, it could only be speculated that non detectable (i.e. <10 μV), low, intermediate or high startle reactivity may reflect respective differences in baseline excitability within the startle reflex circuitry. Studies on neurological patients have shown that lesions of the amygdala (Angrilli et al., 1996; Buchanan, Tranel, & Adolphs, 2004; Funayama, Grillon, Davis, & Phelps, 2001; Kettle, Andrewes, & Allen, 2006) and the orbitofrontal cortex (Angrilli, Bianchin, Radaelli, Bertagnoni, & Pertile, 2008) dramatically attenuate overall startle reflex magnitude, confirming the involvement of these brain areas in tonic startle reactivity in humans. Most notably, these neuroanatomic regions modulating the primary startle circuit overlap significantly with regions implicated in impulsivity in healthy subjects (Matsuo et al., 2009) and with the structural and functional neuroanatomy of disorders of affect and impulsivity such as BD (Haldane & Frangou, 2004), ADHD (Cocchi et al., 2012), violent or impulsive Psychopathy (Boccardi et al., 2011;

Contreras-Rodríguez et al., 2013; Sadeh et al., 2013), borderline personality disorder (De-Almeida et al., 2012; O'Neill & Frodl, 2012; Ruocco, Amirthavasagam, Choi-Kain, & McMain, 2013; Ruocco, Amirthavasagam, & Zakzanis, 2012; Wolf et al., 2012) and Substance Abuse (Koob, 2006; Koob & Volkow, 2010); in this context, it is important that reduced or entirely absent startle reactivity was found in adult impulsive psychopaths (Herpertz, Werth, Lukas, et al., 2001), children with ADHD and Conduct disorder but not ADHD alone (Herpertz, Wenning, et al., 2001), males with increased family genetic risk for alcoholism (Zimmermann, Spring, Wittchen, & Holsboer, 2004), while target detection impairments, increased sensitivity to reward and very low startle reactivity all seem to be trait characteristics of BD (Bora, Yucel, & Pantelis, 2008; Giakoumaki et al., 2010; Linke et al., 2012). One small study on females with borderline personality disorder found startle reactivity similar to controls (Herpertz & Koetting, 2005) but psychophysiological reactivity in this patient group was seriously confounded by symptom heterogeneity, state-dependent factors and small power (Rosenthal et al., 2008). Our results taken together with the literature cited above, suggest that blunt startle is associated with poor cognitive control over emotional processes in limbic structures, which could lead to emotional disorders under certain genetic and/or environmental circumstances. Given the high heritability (50–70%) of baseline startle (Anokhin et al., 2003; Hasenkamp et al., 2010), the current findings and the evidence cited above, it is tempting to suggest that low/undetectable startle reactivity might prove to be a valuable intermediate phenotype for disorders of affect and impulsivity. However, startle has been found to be reduced in non-affective psychoses such as schizophrenia and its prodrome; therefore, it is possible that the neuronal origin of low/undetectable reactivity is multifactorial. More specifically designed studies with patients and first degree relatives are required to test the relationship between baseline startle reactivity and disorders with primarily orbitofrontal/amygdala vs. primarily dorsolateral prefrontal cortex/basal ganglia pathology (e.g. non affective psychoses). Moreover, it seems critical for future studies to involve multi-modal assessment (for example, ASR along with visual, tactile/cutaneous startle etc.) for establishing the concept of the "non-responsive" phenotype, which would most likely predict a failure to demonstrate startle across modalities. Finally, future studies should include female populations, which might help in the clarification of the mechanisms involved in the current findings. Females have higher reactivity than males and therefore the likelihood for non-response may be lower. This is true regardless of mental health status (schizophrenia patients: Kumari, Aasen, & Sharma, 2004; healthy: Aasen, Kolli, & Kumari, 2005) and might be related to hormonal factors since postmenopausal women had significantly lower startle response than menstruating women (Bannbers, Kask, Wikström, & Sundström Poromaa, 2010).

Strengths of the present study include the use of a large, ethnically and demographically highly homogeneous sample, the relatively wide range of phenotypic assessment and the use of fairly standard equipment and methods for acoustic startle recording, under identical conditions for all participants. An important feature of the study was its setting; it was conducted in a military camp where behavioral activity and environmental stimuli were largely uniform for all participants, thus minimizing the between-subject influence of potential confounds such as psychosocial stressors, physical activity, sleep and dietary intake. Precisely for this reason, however, these results could not be generalized to the general population of this similar age, especially since no females were tested. On the other hand, our sample is representative of healthy young Greek Caucasian males because army training is mandatory in this country. As intermediate phenotypes in this cohort were selected with schizophrenia in mind, another limitation of this study was the relative lack of specific measures of emotional reactivity other than the affective personality traits.

In summary, we here present evidence replicating and extending previous findings on startle reactivity being monotonically and inversely associated with sensitivity to reward/impulsivity. We also present first-time evidence of low/undetectable startle reactivity association with reduced performance in working memory and sustained attention. These results were based on healthy males and blunt startle with high reward sensitivity in themselves cannot be taken to imply the presence of pathology. Under certain circumstances (e.g. combined with certain genes or environments), high reward sensitivity could even offer an advantage, if it affords resilience to adversity. Our findings help to understand constitutional factors underlying startle reactivity in health and should guide future research in those patient populations where startle reactivity is expected to be reduced or absent. It may be that low startle reactivity is a valuable intermediate phenotype of disorders of affect and impulsivity and that the "non responding" status captures more specific disease-related features such as executive dysfunction. Future research in high risk groups and patient populations should address these issues.

Conflict of interest

None.

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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.biopsycho.2013.09.005.

References

Aasen, I., Kolli, L., & Kumari, V. (2005). Sex effects in prepulse inhibition and facilitation of the acoustic startle response: Implications for pharmacological and treatment studies. *Journal of Psychopharmacology*, *19*(1), 39–45.

Alloy, L. B., Bender, R. E., Whitehouse, W. G., Wagner, C. A., Liu, R. T., Grant, D. A., et al. (2012). High behavioral approach system (BAS) sensitivity, reward responsiveness, and goal-striving predict first onset of bipolar spectrum disorders: A prospective behavioral high-risk design. *Journal of Abnormal Psychology*, *121*(2), 339–351.

Andrews, S. E., Blumenthal, T. D., & Flaten, M. A. (1998). Effects of caffeine and caffeine-associated stimuli on the human startle eyeblink reflex. *Pharmacology Biochemistry and Behavior*, *59*(1), 39–44.

Angrilli, A., Bianchin, M., Radaelli, S., Bertagnoni, G., & Pertile, M. (2008). Reduced startle reflex and aversive noise perception in patients with orbitofrontal cortex lesions. *Neuropsychologia*, *46*(4), 1179–1184.

Angrilli, A., Mauri, A., Palomba, D., Flor, H., Birbaumer, N., Sartori, G., et al. (1996). Startle reflex and emotion modulation impairment after a right amygdala lesion. *Brain*, *119*(6), 1991–2000.

Anokhin, A. P., Heath, A. C., Myers, E., Ralano, A., & Wood, S. (2003). Genetic influences on prepulse inhibition of startle reflex in humans. *Neuroscience Letters*, *353*(1), 45–48.

Bannbers, E., Kask, K., Wikström, J., & Sundström Poromaa, I. (2010). Lower levels of prepulse inhibition in luteal phase cycling women in comparison with postmenopausal women. *Psychoneuroendocrinology*, *35*(3), 422–429.

Berg, W. K., & Balaban, M. T. (1999). Startle elicitation: Stimulus parameters, recording techniques and quantification. In M. E. Dawson, A. M. Schell, & A. H. Böhmelt (Eds.), *Startle modification: Implications for neuroscience, cognitive science and clinical science* (pp. 21–50). USA: Cambridge University Press.

Blumenthal, T. D. (1994). Signal attenuation as a function of integrator time constant and signal duration. *Psychophysiology*, *31*(2), 201–203.

Blumenthal, T. D. (1999). Short lead interval startle modification. In M. E. Dawson, A. M. Schell, & A. H. Böhmelt (Eds.), *Startle modification: Implications for neuroscience, cognitive science and clinical science* (pp. 51–71). USA: Cambridge University Press.

Blumenthal, T. D., & Berg, W. K. (1986). Stimulus rise time, intensity, and bandwidth effects on acoustic startle amplitude and probability. *Psychophysiology*, *23*(6), 635–641.

Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology*, *42*(1), 1–15.

Boccardi, M., Frisoni, G. B., Hare, R. D., Cavedo, E., Najt, P., Pievani, M., et al. (2011). Cortex and amygdala morphology in psychopathy. *Psychiatry Research*, *193*(2), 85–92.

Bond, A. J., & Lader, M. H. (1974). The use of analogue scales in rating subjective feelings. *British Journal of Medical Psychology*, *47*(3), 211–218.

Bora, E., Yucel, M., & Pantelis, C. (2008). Cognitive endophenotypes of bipolar disorder: A meta-analysis of neuropsychological deficits in euthymic patients and their first-degree relatives. *Journal of Affective Disorders*, *113*(1–2), 1–20.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1999). Affect and the startle reflex. In M. E. Dawson, A. M. Schell, & A. H. Böhmelt (Eds.), *Startle modification: Implications for neuroscience, cognitive science and clinical science* (pp. 15718–15771). USA: Cambridge University Press.

Braff, D. L., Grillon, C., & Geyer, M. A. (1992). Gating and habituation of the startle reflex in schizophrenic patients. *Archives of General Psychiatry*, *49*(3), 206–215.

Braff, D. L., Stone, C., Callaway, E., Geyer, M. A., Glick, I., & Bali, L. (1978). Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology*, *15*(4), 339–343.

Buchanan, T. W., Tranel, D., & Adolphs, R. (2004). Anteromedial temporal lobe damage blocks startle modulation by fear and disgust. *Behavioral Neuroscience*, *118*(2), 429–437.

Calkins, M. E., Dobie, D. J., Cadenhead, K. S., Olincy, A., Freedman, R., Green, M. F., et al. (2007). The consortium on the genetics of endophenotypes in schizophrenia: Model recruitment, assessment, and endophenotyping methods for a multisite collaboration. *Schizophrenia Bulletin*, *33*(1), 33–48.

Carver, C. S., & White, T. L. (1994). Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: the BIS/BAS scales. *Journal of Personality and Social Psychology*, *67*(2), 319–333.

Cinciripini, P. M., Robinson, J. D., Carter, B. L., Lam, C., Wu, X., de Moor, C. A., et al. (2006). The effects of smoking deprivation and nicotine administration on emotional reactivity. *Nicotine and Tobacco Research*, *8*(3), 379–392.

Cocchi, L., Bramati, I. E., Zalesky, A., Furukawa, E., Fontenelle, L. F., Moll, J., et al. (2012). Altered functional brain connectivity in a non-clinical sample of young adults with attention-deficit/hyperactivity disorder. *Journal of Neuroscience*, *32*(49), 17753–17761.

Contreras-Rodríguez, O., Pujol, J., Batalla, I., Harrison, B. J., Bosque, J., Ibern-Regàs, I., et al. (2013). Disrupted neural processing of emotional faces in psychopathy. *Social Cognitive and Affective Neuroscience*, http://dx.doi.org/10.1093/scan/nst014

Csomor, P. A., Yee, B. K., Feldon, J., Theodoridou, A., Studerus, E., & Vollenweider, F. X. (2009). Impaired prepulse inhibition and prepulse-elicited reactivity but intact reflex circuit excitability in unmedicated schizophrenia patients: A comparison with healthy subjects and medicated schizophrenia patients. *Schizophrenia Bulletin*, *35*(1), 244–255.

De-Almeida, C. P., Wenzel, A., de-Carvalho, C. S., Powell, V. B., Araújo-Neto, C., Quarantini, L. C., et al. (2012). Amygdalar volume in borderline personality disorder with and without comorbid post-traumatic stress disorder: A meta-analysis. *CNS Spectrums*, *17*(2), 70–75.

Drabant, E. M., Hariri, A. R., Meyer-Lindenberg, A., Munoz, K. E., Mattay, V. S., Kolachana, B. S., et al. (2006). Catechol O-methyltransferase val158met genotype and neural mechanisms related to affective arousal and regulation. *Archives of General Psychiatry*, *63*(12), 1396–1406.

Enard, W., Gehre, S., Hammerschmidt, K., Hölter, S. M., Blass, T., Somel, M., et al. (2009). A humanized version of Foxp2 affects cortico-basal ganglia circuits in mice. *Cell*, *137*(5), 961–971.

Fisher, S. E., Vargha-Khadem, F., Watkins, K. E., Monaco, A. P., & Pembrey, M. E. (1998). Localisation of a gene implicated in a severe speech and language disorder. *Nature Genetics*, *18*(2), 168–170.

Funayama, E. S., Grillon, C., Davis, M., & Phelps, E. A. (2001). A double dissociation in the affective modulation of startle in humans: Effects of unilateral temporal lobectomy. *Journal of Cognitive Neuroscience*, *13*(6), 721–729.

Giakoumaki, S. G., Bitsios, P., Frangou, S., Roussos, P., Aasen, I., Galea, A., et al. (2010). Low baseline startle and deficient affective startle modulation in remitted bipolar disorder patients and their unaffected siblings. *Psychophysiology*, *47*(4), 659–668.

Giakoumaki, S. G., Roussos, P., & Bitsios, P. (2008). Improvement of prepulse inhibition and executive function by the COMT inhibitor tolcapone depends on COMT Val158Met polymorphism. *Neuropsychopharmacology*, *33*(13), 3058–3068.

Giakoumaki, S. G., Roussos, P., Pallis, E. G., & Bitsios, P. (2011). Sustained attention and working memory deficits follow a familial pattern in schizophrenia. *Archives of Clinical Neuropsychology*, *26*(7), 687–695.

Gopnik, M., & Crago, M. B. (1991). Familial aggregation of a developmental language disorder. *Cognition*, *39*(1), 1–50.

Graham, F. K. (1975). The more or less startling effects of weak prestimulation. *Psychophysiology*, *12*(3), 238–248.

Graham, F. K. (1980). Control of reflex blink excitability. In R. F. Thompson, L. H. Hicks, & V. B. Shryrkov (Eds.), *Neural mechanisms of goal directed behavior and learning* (pp. 511–519). New York: Academic Press.

Gray, J. A. (1994). Three fundamental emotion systems. In P. Eckman, & R. J. Davidson (Eds.), *The nature of emotion: Fundamental questions* (pp. 243–247). New York: Oxford University Press.

Grillon, C., & Baas, J. (2003). A review of the modulation of the startle reflex by affective states and its application in psychiatry. *Clinical Neurophysiology*, *114*(9), 1557–1579.

Haldane, M., & Frangou, S. (2004). New insights help define the pathophysiology of bipolar affective disorder: Neuroimaging and neuropathology findings. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *28*(6), 943–960.

Hasenkamp, W., Epstein, M. P., Green, A., Wilcox, L., Boshoven, W., Lewison, B., et al. (2010). Heritability of acoustic startle magnitude, prepulse inhibition, and startle latency in schizophrenia and control families. *Psychiatry Research*, *178*(2), 236–243.

Herpertz, S. C., & Koetting, K. (2005). Startle response in inpatients with borderline personality disorder vs. healthy controls. *Journal of Neural Transmission*, *112*(8), 1097–1106.

Herpertz, S. C., Wenning, B., Mueller, B., Qunaibi, M., Sass, H., & Herpertz-Dahlmann, B. (2001). Psychophysiological responses in ADHD boys with and without conduct disorder: Implications for adult antisocial behavior. *Journal of the American Academy of Child and Adolescent Psychiatry*, *40*(10), 1222–1230.

Herpertz, S. C., Werth, U., Lukas, G., Qunaibi, M., Schuerkens, A., Kunert, H. J., et al. (2001). Emotion in criminal offenders with psychopathy and borderline personality disorder. *Archives of General Psychiatry*, *58*(8), 737–745.

Kettle, J. W., Andrewes, D. G., & Allen, N. B. (2006). Lateralization of the startle reflex circuit in humans: An examination with monaural probes following unilateral temporal lobe resection. *Behavioral Neuroscience*, *120*(1), 24–39.

Koch, M., & Schnitzler, H. U. (1997). The acoustic startle response in rats – circuits mediating evocation, inhibition and potentiation. *Behavioural Brain Research*, *89*(1–2), 35–49.

Koob, G. F. (2006). The neurobiology of addiction: a neuroadaptational view relevant for diagnosis. *Addiction*, *101*(Suppl 1), 23–30.

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology*, *35*(1), 217–238.

Kotler, M., Cohen, H., Segman, R., Gritsenko, I., Nemanov, L., Lerer, B., et al. (1997). Excess dopamine D4 receptor (D4DR) exon III seven repeat allele in opioid-dependent subjects. *Molecular Psychiatry*, *2*(3), 251–254.

Kumari, V., Aasen, I., & Sharma, T. (2004). Sex differences in prepulse inhibition deficits in chronic schizophrenia. *Schizophrenia Research*, *69*(2–3), 219–235.

Kumari, V., Fannon, D., Geyer, M. A., Premkumar, P., Antonova, E., Simmons, A., et al. (2008). Cortical grey matter volume and sensorimotor gating in schizophrenia. *Cortex*, *44*(9), 1206–1214.

Lai, C. S., Fisher, S. E., Hurst, J. A., Vargha-Khadem, F., & Monaco, A. P. (2001). A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature*, *413*(6855), 519–523.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1990). Emotion, attention, and the startle reflex. *Psychological Review*, *97*(3), 377–395.

Larson, C. L., Ruffalo, D., Nietert, J. Y., & Davidson, R. J. (2000). Temporal stability of the emotion-modulated startle response. *Psychophysiology*, *37*(1), 92–101.

Lieberman, P. (2009). FOXP2 and human cognition. *Cell*, *137*(5), 800–802.

Li, L., Steidl, S., & Yeomans, J. S. (2001). Contributions of the vestibular nucleus and vestibulospinal tract to the startle reflex. *Neuroscience*, *106*(4), 811–821.

Linke, J., King, A. V., Rietschel, M., Strohmaier, J., Hennerici, M., Gass, A., et al. (2012). Increased medial orbitofrontal and amygdala activation: evidence for a systems-level endophenotype of bipolar I disorder. *American Journal of Psychiatry*, *169*(3), 316–325.

Ludewig, K., Geyer, M. A., & Vollenweider, F. X. (2003). Deficits in prepulse inhibition and habituation in never-medicated, first-episode schizophrenia. *Biological Psychiatry*, *54*(2), 121–128.

Marcus, G. F., & Fisher, S. E. (2003). FOXP2 in focus: What can genes tell us about speech and language? *Trends in Cognitive Sciences*, *7*(6), 257–262.

Matsuo, K., Nicoletti, M., Nemoto, K., Hatch, J. P., Peluso, M. A., Nery, F. G., et al. (2009). A voxel-based morphometry study of frontal gray matter correlates of impulsivity. *Human Brain Mapping*, *30*(4), 1188–1195.

Mattay, V. S., Goldberg, T. E., Fera, F., Hariri, A. R., Tessitore, A., & Egan, M. F. (2003). Catechol O-methyltransferase Val158-Met genotype and individual variation in the brain response to amphetamine. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(10), 6186–6191.

Miller, M. W., & Gronfier, C. (2006). Diurnal variation of the startle reflex in relation to HPA-axis activity in humans. *Psychophysiology*, *43*(3), 297–301.

Olszewski, J., Miłoński, J., Sułkowski, W. J., Majak, J., & Olszewski, S. (2005). Temporary hearing threshold shift measured by otoacoustic emissions in subjects exposed to short-term impulse noise. *International Journal of Occupational Medicine and Environmental Health*, *18*(4), 375–379.

O'Neill, A., & Frodl, T. (2012). Brain structure and function in borderline personality disorder. *Brain Structure and Function*, *217*(4), 767–782.

Phillips, M. L., & Vieta, E. (2007). Identifying functional neuroimaging biomarkers of bipolar disorder: Toward DSM-V. *Schizophrenia Bulletin*, *33*(4), 893–904.

Quednow, B. B., Frommann, I., Berning, J., Kühn, K. U., Maier, W., & Wagner, M. (2008). Impaired sensorimotor gating of the acoustic startle response in the prodrome of schizophrenia. *Biological Psychiatry*, *64*(9), 766–773.

Quednow, B. B., Wagner, M., Westheide, J., Beckmann, K., Bliesener, N., Maier, W., et al. (2006). Sensorimotor gating and habituation of the startle response in

schizophrenic patients randomly treated with amisulpride or olanzapine. *Biological Psychiatry*, *59*(6), 536–545.
Rosenthal, M. Z., Gratz, K. L., Kosson, D. S., Cheavens, J. S., Lejuez, C. W., & Lynch, T. R. (2008). Borderline personality disorder and emotional responding: A review of the research literature. *Clinical Psychology Review*, *28*(1), 75–91.
Roussos, P., Giakoumaki, S. G., Adamaki, E., & Bitsios, P. (2011). The influence of schizophrenia-related neuregulin-1 polymorphisms on sensorimotor gating in healthy males. *Biological Psychiatry*, *69*(5), 479–486.
Roussos, P., Giakoumaki, S. G., Adamaki, E., Georgakopoulos, A., Robakis, N. K., & Bitsios, P. (2011). The association of schizophrenia risk D-amino acid oxidase polymorphisms with sensorimotor gating, working memory and personality in healthy males. *Neuropsychopharmacology*, *36*(8), 1677–1688.
Roussos, P., Giakoumaki, S. G., & Bitsios, P. (2009a). A risk PRODH haplotype affects sensorimotor gating, memory, schizotypy, and anxiety in healthy male subjects. *Biological Psychiatry*, *65*(12), 1063–1070.
Roussos, P., Giakoumaki, S. G., & Bitsios, P. (2009b). Tolcapone effects on gating, working memory, and mood interact with the synonymous catechol-O-methyltransferase rs4818c/g polymorphism. *Biological Psychiatry*, *66*(11), 997–1004.
Roussos, P., Giakoumaki, S. G., & Bitsios, P. (2009c). Cognitive and emotional processing in high novelty seeking associated with the L-DRD4 genotype. *Neuropsychologia*, *47*(7), 1654–1659.
Roussos, P., Giakoumaki, S. G., Rogdaki, M., Pavlakis, S., Frangou, S., & Bitsios, P. (2006). Prepulse inhibition of the startle reflex depends on the catechol O-methyltransferase Val158Met gene polymorphism. *Psychological Medicine*, *38*(11), 1651–1658.
Ruocco, A. C., Amirthavasagam, S., Choi-Kain, L. W., & McMain, S. F. (2013). Neural correlates of negative emotionality in borderline personality disorder: An activation-likelihood-estimation meta-analysis. *Biological Psychiatry*, *73*(2), 153–160.
Ruocco, A. C., Amirthavasagam, S., & Zakzanis, K. K. (2012). Amygdala and hippocampal volume reductions as candidate endophenotypes for borderline personality disorder: A meta-analysis of magnetic resonance imaging studies. *Psychiatry Research*, *201*(3), 245–252.
Sadeh, N., Spielberg, J. M., Heller, W., Herrington, J. D., Engels, A. S., Warren, S. L., et al. (2013). Emotion disrupts neural activity during selective attention in psychopathy. *Social Cognitive and Affective Neuroscience*, *8*(3), 235–246.
Serretti, A., & Mandelli, L. (2008). The genetics of bipolar disorder: genome 'hot regions,' genes, new potential candidates and future directions. *Molecular Psychiatry*, *13*(8), 742–771.
Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., et al. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry*, *59*(Suppl 20), 22–33.
Smolka, M. N., Schumann, G., Wrase, J., Grüsser, S. M., Flor, H., Mann, K., et al. (2005). Catechol-O-methyltransferase val158met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. *Journal of Neuroscience*, *25*(4), 836–842.
Swanson, J. M., Moyzis, R. K., McGough, J. J., McCracken, J. T., Riddle, M. A., Kollins, S. H., et al. (2007). Effects of source of DNA on genotyping success rates and allele percentages in the Preschoolers with Attention-Deficit/Hyperactivity Disorder Treatment Study (PATS). *Journal of Child and Adolescent Psychopharmacology*, *17*(5), 635–646.
Swerdlow, N. R., Talledo, J. A., & Braff, D. L. (2005). Startle modulation in Caucasian-Americans and Asian-Americans: A prelude to genetic/endophenotypic studies across the 'Pacific Rim'. *Psychiatric Genetics*, *15*(1), 61–65.
Vargha-Khadem, F., Watkins, K. E., Price, C. J., Ashburner, J., Alcock, K. J., Connelly, A., et al. (1998). Neural basis of an inherited speech and language disorder. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(21), 12695–12700.
Weiss, E. M., Stadelmann, E., Kohler, C. G., Brensinger, C. M., Nolan, K. A., Oberacher, H., et al. (2007). Differential effect of catechol-O-methyltransferase Val158Met genotype on emotional recognition abilities in healthy men and women. *Journal of the International Neuropsychological Society*, *13*(5), 881–887.
Wolf, R. C., Thomann, P. A., Sambataro, F., Vasic, N., Schmid, M., & Wolf, N. D. (2012). Orbitofrontal cortex and impulsivity in borderline personality disorder: An MRI study of baseline brain perfusion. *European Archives of Psychiatry and Clinical Neuroscience*, *262*(8), 677–685.
Wynn, J. K., Dawson, M. E., Schell, A. M., McGee, M., Salveson, D., & Green, M. F. (2004). Prepulse facilitation and prepulse inhibition in schizophrenia patients and their unaffected siblings. *Biological Psychiatry*, *55*(5), 518–523.
Zimmermann, U., Spring, K., Wittchen, H. U., & Holsboer, F. (2004). Effects of ethanol administration and induction of anxiety-related affective states on the acoustic startle reflex in sons of alcohol-dependent fathers. *Alcoholism, Clinical and Experimental Research*, *28*(3), 424–432.