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BIPOLAR DISORDERS

## Original Article

# Using affect-modulated startle to study phenotypes of pediatric bipolar disorder

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***Objectives:*** Affective neuroscience research that investigates core symptoms of pediatric bipolar disorder (PBD) may be effective in differentiating PBD phenotypes. The current study used affect-modulated startle to examine potential differences in reactivity to emotional stimuli (reward and punishment) in narrow and broad phenotype PBD and controls.

***Methods:*** Thirty children meeting DSM-IV bipolar disorder criteria (i.e. narrow phenotype PBD with defined manic episodes with elevated/expansive mood), 19 children meeting criteria for severe mood dysregulation (i.e. broad phenotype with chronic irritability, hyperreactivity, and hyperarousal), and 19 controls completed a lottery startle paradigm involving reward (money) and punishment (loud noise). Startle probes were presented during anticipation of the emotional stimulus, immediately following the presentation of the stimulus, or during return to baseline following the stimulus.

***Results:*** By self-report, patients and controls found the putative punishment to be preferable to the neutral condition. In the reward condition, patient samples reported greater arousal than did controls, but no between-group differences were found on the magnitude of startle response during the reward, punishment, or neutral conditions.

***Conclusions:*** The failure to find differences in affect-modulated startle between control children and those with narrow or broad PBD phenotypes speaks to the methodological challenges associated with studying reward mechanisms in PBD. Alternative paradigms that focus on different aspects of reward mechanisms are discussed.

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The controversy regarding the diagnosis of pediatric bipolar disorder (PBD) highlights both the limitations of diagnoses based solely on clinical presentation and the need for pathophysiological research that, eventually, will allow nosology to be brain-based. In particular, it is crucial that research focus on elucidating the pathophysiology of core symptoms thought to be most impairing and potentially most helpful in differentiating PBD phenotypes. A number of investigators have suggested that a critical trait of PBD may be increased reactivity to emotional stimuli relative to controls (1–3). In addition, reward-related paradigms are of particular interest in PBD because deficits in reward processes may be defining features of the disorder: the 'hyperhedonia' of mania may reflect heightened responsivity to reward and reduced responsivity to punishment, and conversely, the anhedonia of depression may reflect reduced responsivity to reward and heightened responsivity to punishment.

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Despite these somewhat straightforward theoretical deductions, there has been a surprising dearth of empirical investigations of reward mechanisms in PBD. In studies of adults with bipolar disorder (BD), there is some evidence for hyperresponsivity to reward, i.e. compared with depressed patients or controls, hospitalized manic patients selected outcomes with lower probability but greater potential reward on a decision-making task (1). However, other studies have reported no BD patient-control differences in responsivity to contingencies on gambling (4, 5) and decision-making (6) tasks. Thus, prior studies of reward mechanisms in adults with BD have had equivocal results. The one study that examined the question in children with PBD used a decision-making task with probabilistic monetary outcomes, and found that patients reported greater dissatisfaction with losing and greater satisfaction with winning, than did controls. However, this study failed to find any between-group differences in subjects' propensity for selecting risky outcomes (7).

The lack of research on reward mechanisms in PBD may reflect two primary challenges: controversy about the diagnosis of PBD in children, and methodological difficulties. The diagnostic controversy centers largely on the importance of elevated/expansive mood (versus irritability) as a symptom of mania, and on whether symptoms must be episodic, as opposed to chronic. In an effort to establish homogeneous groups and to facilitate pathophysiological research, we at the Intramural Research Program of the National Institute of Mental Health (NIMH) have suggested a classification system that differentiates children who clearly meet DSM-IV criteria for PBD (i.e. the narrow phenotype bipolar disorder, NP-BP) from those with severe mood dysregulation (SMD), characterized by chronic, non-episodic irritability and hyperarousal (i.e. the broad phenotype) (8, 9). Whereas NP-BP patients have a lifetime history of at least one distinct episode of mania or hypomania meeting DSM-IV duration criteria *and including elevated/expansive mood*, the broad phenotype, heretofore designated as SMD, have baseline irritable or angry mood, hyper-reactivity to negative emotional stimuli, and attention deficit hyperactivity disorder (ADHD)-like hyperarousal, but not sustained elation or grandiosity. Our hope is that establishing homogeneous diagnostic groups will facilitate pathophysiological research that will in turn aid in the establishment of an appropriate nosology for PBD.

The second major challenge of investigating reward mechanisms in children with PBD concerns methodological issues. To begin with, care must be taken in identifying which aspects of subjects' responses to contingencies will be studied. For example, should research focus on reward, punishment, or both? In addition, should research focus on the subject's response to a reward, or to the failure to receive an expected punishment (or vice versa, the subject's response to punishment or to the failure to receive an expected reward)? Another important methodological challenge is to identify paradigms that allow investigators to study children's responses to emotional stimuli validly and reliably in the laboratory. Paradigms must be sufficiently salient to provoke reactivity, while being sensitive to the ethical issues involved in pediatric research. In particular, the aversive nature of the punishment must not exceed the minimal risk standard of research with children (i.e. discomfort resulting from the paradigm must not exceed that ordinarily encountered in daily life). For example, the adult literature on reactivity to emotional stimuli finds that the most reliable psychophysiological responses are to the most aversive stimuli; not only may these be inappropriate for use with children, but also they may not even achieve the same response in children as in adults (10). An additional concern of research with children is that monetary rewards must be large enough to be salient, but not so large as to be coercive. Indeed, the appropriate amount of a monetary reward may vary developmentally, which can become problematic in studies that include children over a relatively large age span.

Behavioral paradigms designed to study responsivity to emotional stimuli in PBD must therefore have the potential to engage psychological processes relevant to the clinical differences between PBD phenotypes, assess responsivity to both reward and punishment, and to do so in a developmentally appropriate manner. The additional use of psychophysiological techniques allows investigators to supplement subjects' self-report and behavior with more direct measures of brain function. Although there are a variety of psychophysiological paradigms for studying reactivity to emotional stimuli, one technique that has received particular attention is affect-modulated startle, i.e. the use of startle probes in conjunction with positive or negative stimuli. The startle reflex consists of a series of involuntary muscle movements in response to an intense stimulus with sudden onset. Unlike traditional startle paradigms, affect-modulated startle involves pairing the startle probe with another stimulus of positive or negative valence, thus manipulating the environment in which the startle occurs. Prior studies suggest that negative stimuli heighten the startle response, whether the startle

occurs in anticipation of the stimulus or shortly after the stimulus is presented. In contrast, positive stimuli heighten the startle response if presented prior to the startle probe, but suppress startle if presented after the startle probe (10–19). Thus, the use of affect-modulated startle allows investigators to assess the saliency of given contexts and stimuli as well as the psychophysiological substrates of information processing and affective reactivity.

The nature of the relationship between psychopathology and affect-modulated startle varies greatly depending on the diagnosis. For example, adults with post-traumatic stress disorder (PTSD; 20–22), specific phobia (23, 24), and heightened fear (25) show more robust startle potentiation than do controls when the startle probe is paired with affective cues. In contrast, some studies have refuted these results (26) and other studies have not found a reliable relationship between affective startle modification and either depression (25), social anhedonia (27), or mood disorders as a whole (16). The few studies that investigated startle response in children found greater fear-potentiated startle in children with negative affect (28) and PTSD (29), but decreased startle in children with disruptive behavior disorder (30), when compared with controls. However, others have failed to document differences in fear-potentiated startle between children with ADHD (31) or temperamental inhibition (32) and controls. Thus, there is inconsistency in the research literature investigating the relationship between specific psychopathologies and affect-modulated startle.

To investigate affect-modulated startle in children with the broad and narrow phenotypes of PBD, we used a lottery paradigm designed by Skolnick and Davidson (18). In the lottery paradigm, startle probes are presented in conjunction with rewarding and punishing stimuli to assess reactivity to these emotional conditions. In particular, probes are presented at varying points in each trial to gauge if startle reactivity varies as a function of anticipation of the emotional stimulus, presentation of the emotional stimulus, or return to baseline following the emotional stimulus. Applying startle probes at specific time points during the emotionally arousing trial allows for an investigation of affective chronometry, or the time course of an emotional response (33). We predicted that greater NP-BP and SMD mood lability, when compared with controls, would be manifest in patients with greater anticipatory and feedback startle in the context of both reward and punishment. When comparing SMDs to NP-BPs on responsivity to negative stimuli, we predicted greater startle in SMD subjects, reflective of their characteristic hyper-reactivity to negative stimuli. In comparison, in response to positive stimuli, we predicted greater startle in NP-BP subjects, reflective of their capacity to exhibit elevated/expansive mood during manic episodes, a symptom not associated with SMD. We also hypothesized that NP-BP and SMD subjects, compared with controls, would show a slower return of the startle magnitude to baseline after trial completion. Additionally, comparing SMD and NP-BP patients, we hypothesized that the SMD sample would show a slower return to baseline than NP-BPs because the former have an impaired ability to suppress emotional outbursts.

## Materials and methods

### Participants

*Inclusion/exclusion criteria*. The NIMH Institutional Review Board (IRB) approved the two studies from which data were collected. Parents and children gave written informed consent/assent prior to participation. Subjects were recruited through advertisements placed on websites of relevant support groups and distributed at professional conferences, and a letter about the study was sent to child psychiatrists nationwide.

Children with NP-BP (N = 30) were enrolled in an ongoing neurocognitive and neuroimaging study at the NIMH. Inclusion criteria for NP-BP were: meeting DSM-IV criteria for BD, including a history of at least one hypomanic or manic episode meeting full duration criteria – i.e. lasting ≥4 days for hypomania or ≥7 days for mania – during which the child exhibited abnormally elevated and expansive mood and a total of at least three other DSM-IV criterion 'B' mania symptoms (34). Thus, these NP-BP patients met full DSM-IV criteria for both episode duration and abnormally elevated, expansive mood (35).

All SMD subjects (N = 19) were participants in an in-patient double blind, placebo-controlled study of lithium. Criteria for SMD differ from that of NP-BP in that the former have a chronic, rather than episodic, course of functionally impairing irritability, over-reactivity to negative emotional stimuli, and hyper-arousal, without elevated or expansive mood or decreased need for sleep (9). These children may represent a broad phenotype of pediatric bipolar-spectrum disorder or a distinct diagnostic group.

All subjects were between 7 and 17 years old at study enrollment. Exclusion criteria for both groups were: IQ < 70; severe pervasive developmental disorder, such as meeting DSM-IV criteria

for either Asperger's disorder or autism; psychosis that interfered with the child's capacity to understand and comply with study procedures; unstable medical illness (i.e. severe asthma); medical illness that could cause the symptoms of bipolar illness (i.e. multiple sclerosis, thyroid disease); pregnancy; or substance abuse within 2 months of the initial evaluation.

Diagnosis of BD was made using the Kiddie-Schedule for Affective Disorders-Present and Lifetime Version (K-SADS-PL) (36), a semi-structured diagnostic interview administered to parents and children separately by different clinicians. Diagnoses were based on best-estimate procedures (37) generated in a consensus conference of research staff led by two psychiatrists. Comorbid psychiatric diagnoses were also made using the K-SADS-PL and were assessed by inquiring about the presence of symptoms during a time of relative euthymia. SMD diagnoses were made using the criteria established by Leibenluft et al. (9), with all DSM-IV diagnoses made using the K-SADS. The SMD supplementary diagnosis module of the K-SADS was developed in collaboration with Joan Kaufman.

Control subjects (N = 19) had normal physical and neurological examinations, and both they and their first-degree relatives were free of current or past psychopathology. This was ascertained by a KSADS-PL interview to rule out psychopathology in the child and an interview of the parent to ascertain status of first-degree relatives.

To evaluate mood at the time of testing, graduate level clinicians with established inter-rater reliability administered the Children's Depression Rating Scale (CDRS) (38) and the Young Mania Rating Scale (YMRS) (39) to patients and their parents.

*Demographics*. Table 1 presents demographic data for the three groups. NP-BP, SMD, and control participants did not differ on sex (Pearson's chi-square $\chi^2 = 1.06$, $p = 0.59$), but an analysis of variance found a main effect of age [$F(2, 64) = 6.46$, $p = 0.003$], and *post-hoc* analyses found that SMDs were significantly younger than both controls ($p = 0.002$) and NP-BPs ($p = 0.044$). Among NP-BP participants, 76.7% (N = 23) met criteria for bipolar I and the remainder met criteria for bipolar II. The mean number of DSM-IV diagnoses in the NP-BP sample was 3.03 ± 1.47, and 83.3% (N = 25) had at least one diagnosis in addition to PBD. Among SMD participants, the mean number of DSM-IV diagnoses was 3.11 ± 0.99 and 100% of the sample had at least two DSM-IV diagnoses. See Table 1 for a list of the most common comorbid diagnoses in the patient samples.

Table 1. Group comparison of demographic variables

| | NP-BP | SMD | Control |
|---|---|---|---|
| N | 30 | 19 | 19 |
| Age (years) | 12.70 ± 2.67 | 11.06 ± 1.66 | 13.72 ± 2.07 |
| Male | 66.70 (20) | 73.70 (14) | 57.9 (11) |
| ADHD | 60.00 (18) | 84.20 (16) | – |
| MDD | – | 42.10 (8) | – |
| GAD | 40.00 (12) | 31.60 (6) | – |
| Separation anxiety | 10.00 (3) | 21.10 (4) | – |
| Social phobia | 10.00 (3) | 10.50 (2) | – |
| Simple phobia | 23.00 (7) | 5.30 (1) | – |
| Any anxiety | 60.00 (18) | 47.40 (9) | – |
| ODD | 30.00 (9) | 89.50 (17) | – |
| CD | 3.30 (1) | 0 (0) | – |
| CDRS | 23.17 ± 3.82 | 27.24 ± 5.56 | 18.73 ± 1.17 |
| YMRS | 3.76 ± 3.66 | 6.94 ± 5.21 | 0.13 ± 0.52 |

All diagnoses are current and based on DSM-IV criteria. Values are presented as % (N); CDRS and YMRS data = mean score ± SD. NP-BP = narrow phenotype bipolar disorder; SMD = severe mood dysregulation; ADHD = attention deficit hyperactivity disorder; MDD = major depressive disorder; GAD = generalized anxiety disorder; ODD = oppositional defiant disorder; CD = conduct disorder; CDRS = Children's Depression Rating Scale; YMRS = Young Mania Rating Scale.

None of the NP-BP or SMD subjects exceeded the CDRS cut-off score for depression (>40), or the YMRS cut-off score for hypomania (>12) or mania (>26). Our sample therefore can be considered euthymic at the time of testing (see Table 1 for group mean scores).

Regarding medication use, in the NP-BP sample, 93.33% (N = 28) of our patients were medicated at the time of testing, with a mean of 2.90 ± 1.30 medications per subject. The most common medications (not mutually exclusive) were mood stabilizers (73.30%, N = 22), antipsychotics (66.70%, N = 20), lithium (40.0%, N = 12), stimulants (36.70%, N = 11), and antidepressants (36.70%, N = 11). In our SMD sample, 89.47% (N = 17) had been removed from their medication for approximately 2 weeks prior to testing. The other two SMD subjects were medicated at testing: both were on stimulants, and another was additionally taking lithium.

## Procedure

*Physiological measures*. The lottery task was completed as part of a larger battery of psychophysiological testing. Subjects were comfortably seated approximately 75 cm from a desktop FlexScan T561 (Eizo Nanao Corporation, Hakusan, Japan) computer monitor with a 45-cm color display screen. Auditory stimuli were presented binaurally through headphones. Startle probes consisted of a white noise burst 50 ms in duration, 95 dBA, with

a nearly instantaneous rise time. Startle reflex eyeblink was measured using electromyographic (EMG) recordings of the orbicularis oculi muscle. Two disposable surface electrodes, placed 1 cm apart below the left eye, were situated symmetrically about the mid-pupillary line. Adequate placement and subject comfort was tested by obtaining EMG tracings of spontaneous and voluntary blinks. Impedance level was kept below 10 kOhms. The ground electrode was placed on the left wrist at an area of lowest impedance. Software from the James Long Company (EEG Analysis Program, Caroga Lake, NY, USA) allowed for digitization, editing of artifact, and analysis. EMG activity was amplified, high- and low-pass filtered (80–240 Hz), rectified, and then digitized using Snapshot-Snapstream acquisition software (HEM Data Corp., Southfield, MI) at a rate of 1000 Hz for 250 ms following startle stimulus onset. Response amplitude was quantified using the James Long EMG peak detection program (James Long Company, Caroga Lake, NY), with peak defined as the maximum change occurring within a window of 20–120 ms. Startle amplitude was represented by digitalized units amplified by $10^6$. Prior to the start of the experiment, subjects received nine startle probes spaced 18–22 s apart to allow habituation to a stable level of startle magnitude.

*Lottery paradigm*. The procedure for the lottery task follows that described by Skolnick and Davidson (18). On the computer screen, two rows of six blank spaces each appeared one above the other. Subjects were informed which type of trial was about to occur (i.e. reward, punishment, neutral; see description below). Subjects were told to select six digits using a keyboard placed before them. After the subject entered the sixth digit in the top row, these digits remained in place while the bottom row of digits began to rapidly change, giving the appearance of a spinning slot machine. Each of the six digits in the bottom row appeared to randomly stop spinning, but they were in fact programmed to match (or not match) the digits chosen by the subject. The number of matching digits determined the extent of reward or punishment the subject received.

The task was divided into four blocks, two of which constituted a 'chance of money' (reward) condition, and two of which constituted a 'chance of noise' (punishment) condition. Blocks 1 and 3 were 'chance of money' conditions and consisted of 27 trials each. Blocks 2 and 4 were 'chance of noise' conditions and consisted of 25 trials each. Subjects were also given 2–3 practice trials in each condition.

In the reward condition, subjects were informed that matching digits would result in monetary reward (chance of money), and that the reward amount varied based on the number of matching digits (matching one digit = \$0.05; two digits = \$0.10; three digits = \$0.20; four digits = \$0.50; five digits = \$2.00; six digits = \$5.00). Matching digits flashed in red, and the amount of money won was displayed in red in the center of the two rows of digits, along with the total winnings thus far. On neutral trials, subjects were told that they would not receive a monetary reward regardless of the number of matching digits (no chance of money). All subjects matched on half of both the reward and neutral trials. The order of matching and non-matching trials was randomized, as was the order of the number of matching digits. Each subject 'won' \$5.20 by matching one digit twelve times, two digits six times, three digits five times, four digits two times and five digits one time. Subjects never matched six digits.

In the punishment condition, subjects were informed that matching three or more digits would result in an aversive noise (chance of noise), and that the length of noise presentation increased as the number of matching digits increased. Matching three digits resulted in a 2-s noise, four digits resulted in a 3-s noise, and five digits resulted in a 4-s noise. Subjects never matched all six digits. Matching digits flashed in red, and the aversive noise was then presented. On neutral trials, subjects were told that they would not receive the aversive noise regardless of the number of matching digits (no chance of noise). Participants received the same number of matches and non-matches as in the reward trials. The order of matching and non-matching trials was randomized, as was the order and the number of matching digits.

For the timing of the startle probe, all trials were divided into four segments. The first two segments comprised the anticipatory period, which lasted 10 s, began immediately after the subject finished selecting his/her digits, and ended when the second row of digits stopping spinning. The anticipatory period was followed immediately by a 3-s feedback period, which began when the digits stopped spinning and included the subject being informed of the trial results (i.e. how many digits matched between his/her selection and the second row, and the amount of reward, punishment, or lack thereof). Following this, the inter-trial interval (ITI) lasted 6 s, during which the screen was blank and the subject awaited the commencement of the subsequent trial.

At specific times during each segment, a startle probe was presented. There were four different

times during each trial that a startle probe could be presented, but only one startle probe was presented per trial, with random assignment of when the probe occurred. The four times were: 5 or 9 s into the anticipatory period, 2.75 s into the feedback period (i.e. 11.75 s for the entire trial length), and 3 s during the ITI (i.e. 16 s for the entire trial length). Selection of probe presentation time, identical to the design validated by Skolnick and Davidson (18), allowed for optimal stimulus processing (i.e. 'spinning' numbers and feedback) prior to startle presentation. Only one probe was presented per trial so as to avoid potential contamination which may have occurred had multiple startle probes been presented in close succession on a single trial.

*Self-report mood*. To provide self-report mood data, subjects completed a paper and pencil rating of valence and arousal called the Self-Assessment Manikin (SAM) (10) at the conclusion of each block. The SAM consists of unlabeled line-drawings of human manikins along a 9-point scale, with extremes of happy/unhappy and calm/aroused at the ends of the scale. Subjects indicated their mood following the block as a whole for both reward and punishment conditions. In addition, after reward blocks, subjects indicated their mood after receiving the monetary reward and after not receiving the expected reward; after punishment blocks, subjects indicated their mood after receiving the noise and after not receiving the expected noise.

*Statistical procedures*. Due to the previously reported significant main effect of age, this variable served as a covariate on all analyses. To assess the impact of group (NP-BP, SMD, control), time (5, 9, 11.75, and 16 s), and condition (neutral, match, and no match) on startle, $3 \times 4 \times 3$ repeated measures MANCOVAs were conducted with group as the between-subjects variable and time and condition as the within-subjects variables. This MANCOVA was conducted with two dependent variables: raw startle scores, and $T$ score-transformed startle scores. Because there is considerable inter-subject variability in baseline startle magnitude, the use of within-subject designs for investigating affect-modulated startle has been recommended (40). Within-subject $T$ scores were thus calculated for each subject for each block in order to normalize the data and reduce the impact of outliers. First, $Z$ scores were calculated as [(subject's average startle for the entire block − the subject's raw startle on each trial)/the subject's standard deviation for the entire block]. Then, to avoid having positive and negative $Z$ scores, $T$ scores were calculated as [($Z$ score $\times$ 10) + 50] so that all scores were positive. All other data were analyzed according to trial and condition type, not block type, in an effort to avoid the possibility that the small number of blocks may reduce power and/or limit data interpretation.

Additional $3 \times 2 \times 2$ repeated measures MANCOVAs were conducted using percent change scores with group as the between-subjects variable and time and condition as the within-subjects variables. Percent change was calculated as [(baseline startle − 11.75 s startle/baseline startle) × 100] or [baseline startle − 16 s startle/baseline startle) × 100]. Baseline startle was the average of 5 and 9 s startle; this was done because both time periods occurred during the anticipatory period. Additional percent change comparisons were calculated to examine change in startle from feedback to the ITI period: [(11.75 s startle − 16 s startle/11.75 s startle) × 100] for both match and no-match conditions. Percent change was used to gauge the impact of reward and punishment on the change in startle from baseline to feedback and ITI periods as well as feedback to ITI. Percent change was calculated using both raw and $T$ score startle.

To examine self-report of affective responsivity to the task, a MANCOVA was conducted using SAM data. Statistical corrections were implemented where assumptions of sphericity or homoscedasticity were violated. The results are considered significant based on a two-tailed alpha ≤0.05. To minimize type I errors, the Greenhouse–Geisser procedure was applied when appropriate. All subsequent post-hoc comparisons employed the Tukey test. The data were analyzed using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA, 1995).

## Results

### Removal of punishment trials

Although it was our goal to examine the impact of reward and punishment on affect-modulated startle, a preliminary examination of our data led us to focus on the reward condition. Approximately midway through data collection, a preliminary analysis of SAM data revealed that the aversive noise paradigm was not having the desired punishment effect. Specifically, self-report data from 11 NP-BPs and 10 controls compared valence (happy/unhappy) for trials with, versus without, the aversive punishment noise. The results of a paired samples $t$-test found that the sample as a whole was significantly happier on trials *with* the aversive noise (3.40 ± 1.69) than without (5.00 ± 1.29;

$t = -3.33$, $p = 0.003$). This was true when NP-BPs and controls were examined separately as well. Given that our subjects deemed the punishment trials more enjoyable than the neutral (i.e. non-punishment) trials, the 'chance of noise' blocks were eliminated from the lottery paradigm and only the reward blocks (chance of money/no chance of money) were administered. Subsequent independent samples t-tests found no differences in startle response between subjects who experienced both reward and punishment trials and those who received only reward trials, so all subjects were included in the final sample when analyzing reward results.

### Mood self-report

A MANCOVA comparing arousal (calm/excited) and valence (happy/sad) levels in response to the task as a whole (now), as well as in response to reward (win) and failing to win reward found significant group differences on arousal for the block as a whole [$F(2, 53) = 4.00$, $p = 0.024$] and 'win' arousal [$F(2, 53) = 9.86$, $p < 0.001$]. *Post-hoc* analyses on arousal for the whole block found SMDs ($3.19 \pm 1.85$) to be significantly more aroused than controls ($5.00 \pm 1.56$; $p = 0.025$), with a trend for NP-BPs ($3.59 \pm 2.15$) to be significantly more aroused than controls ($p = 0.076$). *Post-hoc* analyses on 'win' arousal found both SMDs ($2.22 \pm 1.35$; $p < 0.001$) and NP-BPs ($2.78 \pm 1.62$; $p = 0.003$) to be significantly more aroused than controls ($4.53 \pm 1.64$).

### Startle and chance of reward trials

To examine the overall impact of the reward condition, paired-samples *t*-tests were compared with startle at specific time-points during the neutral condition to corresponding time-points during the reward condition. For the entire sample, startle (raw and *T*-score transformed) was significantly greater during the anticipatory period (9 s) of the reward condition, when compared with that in the neutral condition [raw: $t(2, 78) = 3.98$, $p < 0.001$; *T*-score: $t(2, 72) = 3.78$, $p < 0.001$]. All other time points were non-significant. The results followed the same pattern when each group was examined individually. Thus, startle during the later reward anticipatory period was greater than that in the neutral condition, but startle during feedback and ITI did not vary by condition nor did results vary between groups.

We next compared startle between our samples. The $3 \times 4 \times 3$ repeated measures MANOVA conducted with *raw startle scores* using group as the between-subjects variable, and time and condition as the within-subjects variables revealed no significant between-group differences: main effect group [$F(2, 64) = 1.27$, $p = 0.29$], group × time interaction [$F(6, 192) = 2.52$, $p = 0.08$], group × condition interaction [$F(4, 128) = 0.65$, $p = 0.60$], group × time × condition interaction [$F(12, 384) = 0.99$, $p = 0.44$] (see Fig. 1). The same $3 \times 4 \times 3$ repeated measures MANOVA conducted with *T*-scores similarly revealed no significant between-group differences. Subsequent $3 \times 2 \times 2$ repeated measures MANOVAs examining *percent change raw scores* (see Fig. 2) and *percent change T-scores* also produced the same pattern of non-significant results. Finally, $3 \times 2$ repeated measures

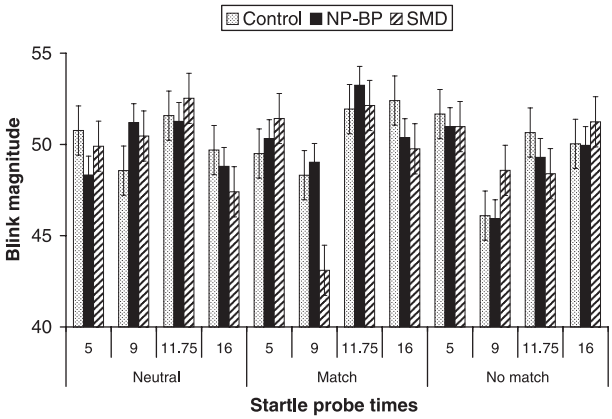


*Fig. 1.* Standardized (*T*-score) eyeblink magnitude: reward condition. Figure presents eyeblink response to startle probes in the neutral, match, and no-match condition of the reward trials. Results show that there were no significant differences between the three samples on startle across the affective manipulations of the lottery task. NP-BP = Narrow Phenotype Bipolar Disorder; SMD = Severe Mood Dysregulation.

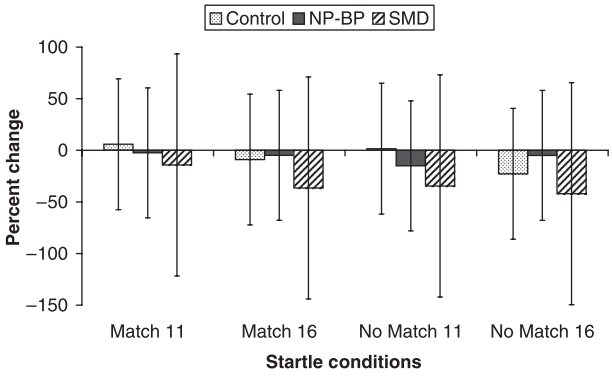


*Fig. 2.* Percent change startle: raw scores. Figure presents percent change from baseline in eyeblink response to startle probes in the match and no-match conditions. Results show that there were no significant differences between the three samples on change in startle from baseline to the feedback and inter-trial interval periods. NP-BP = Narrow Phenotype Bipolar Disorder; SMD = Severe Mood Dysregulation. Error bars represent standard deviation.

MANOVAs using *percent change raw score* and *percent change T-scores* specifically from 11.75 to 16 s revealed no significant between-group differences.

## Discussion

The current study sought to identify psychophysiological differences between different phenotypes of PBD by using affect-modulated startle to assess responsivity to emotional stimuli. We compared control children to those with DSM-defined PBD (NP-BP, i.e. discrete episodes of mania with elevated/expansive mood) and to those with SMD (i.e. chronic irritability, marked reactivity, and hyperarousal, but lacking episodic mania; broad phenotype) (9). Both NP-BP and SMD patient groups reported feeling more aroused by reward trials than did controls. However, we found no between-group differences on startle response, regardless of whether it was paired with reward or failure to achieve an expected reward, or if the startle occurred during the anticipatory or feedback period.

Our study has several limitations that should be noted. We stopped data collection on the punishment trials because the children did not find the 'aversive' noise punishing. Therefore, we were unable to assess possible between-group differences in affect-modulated startle in the context of punishment. Because our sample is small, there is the possibility of a type II error; however, if a larger study were to find significant between-group differences, the magnitude of such differences would likely be small. Our small sample size also prevents a thorough exploration of potential confounds, especially comorbid diagnoses and medication use. The impact of medication on startle is unclear, with some studies suggesting it dampens startle (41–43) and others reporting no impact (43–45). Of note, however, we failed to find differences in startle between controls and the NP-BP patients, most of whom were medicated, or the SMD patients, most of whom were medication-free.

It is problematic to conclude that the lack of between-group differences in startle suggests comparable responsivity to emotional stimuli between controls and PBD-spectrum patients. Instead, our results might reflect the methodological challenges of studying reward mechanisms in PBD. First, it is difficult to identify sufficiently aversive stimuli that comply with minimal risk research standards: our subjects reported enjoying the supposed aversive noise punishment. Similarly, in a prior study of responsivity to emotional pictures, pictures that were deemed aversive by girls and adults did not garner comparable ratings by boys (10). Thus, there is great gender and developmental variability in stimuli that will elicit a desired or expected response.

Identifying adequately rewarding stimuli is also a challenge. Although we used money in this task and failed to find group differences, we *have* found patient-control differences on another paradigm using money as the reward and punishment (46), as well as on a paradigm where subjects won and lost points without any monetary value (47). Nonetheless, it is certainly possible that, in children, faces or emotionally evocative social situations are more effective motivational situations than is money.

Finally, our lack of between-group differences in reward-modulated startle may indicate that startle paradigms are ineffective in examining the psychophysiological correlates of reactivity to emotional stimuli in children with PBD. This is supported to some degree by the fact that, while self-report data indicated that NP-BP and SMD subjects were in fact more excited and stimulated by the reward conditions than controls, this was not detected by differences in startle modulation. The utility of startle in the study of PBD is also called into question by previous work from our group which failed to find differences between children with PBD and controls in pre-pulse inhibition (PPI) of startle (48). Thus, we failed to find aberrant PBD startle in paradigms both with (affect-modulated) and without (PPI) co-occurring emotional stimuli.

A critical issue in the pathophysiology of PBD may be the introduction of, and/or changes to, contingencies. The current results, along with a previous study using a decision-making task that had a static relationship between stimulus and reward and/or punishment (money) (7), suggest that studying the mere presence or absence of reward and/or punishment may not be particularly informative with PBD. In contrast, research from our group suggests that children with PBD may have deficits in their ability to adapt to *changes* in stimulus–contingency relationships. Using a response reversal task (47), a paradigm that measures subjects' ability to learn and adapt to new reward-punishment contexts, and the affective Posner (46), a paradigm that assesses subjects' motor and psychophysiological responses to contingencies and frustration during an attention task, we have found that children with PBD are deficient in their ability to adapt their behavior appropriately in response to changing rewards and punishments. Therefore, rather than having deficits in their responses to reward or punishment *per se*, children with PBD may instead have deficits in their ability to adapt to changing reward mechanisms. Future

work examining the psychophysiological correlates of responsivity to reward and punishment in PBD might explore the issue of reaction to new or changing contingencies.

In sum, the current study failed to find differences in affect-modulated startle between control children and those with narrowly or broadly defined PBD. This lack of group differences speaks of the methodological challenges associated with studying reward mechanisms in PBD. Consistent with prior results of a study examining PPI in a PBD sample, it appears that startle paradigms may not have particular utility with this population. Instead, research that looks beyond simple response to reward and punishment, and instead examines more complex constructs such as adaptation to changing stimulus–contingency relationships, may prove useful when studying the relationship between reward mechanisms and PBD.

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