Developmental Psychology

Neonatal Neural Responses to Novelty Related to Behavioral Inhibition at 1 Year

Rebecca F. Schwarzlose, Courtney A. Filippi, Michael J. Myers, Jennifer Harper, M. Catalina Camacho, Tara A. Smyser, Cynthia E. Rogers, Joshua S. Shimony, Barbara B. Warner, Joan L. Luby, Deanna M. Barch, Daniel S. Pine, Christopher D. Smyser, Nathan A. Fox, and Chad M. Sylvester

Online First Publication, November 16, 2023. https://dx.doi.org/10.1037/dev0001654

AMERICAN PSYCHOLOGICAL ASSOCIATION

Developmental Psychology

ISSN: 0012-1649

https://doi.org/10.1037/dev0001654

Neonatal Neural Responses to Novelty Related to Behavioral Inhibition at 1 Year

Rebecca F. Schwarzlose[1], Courtney A. Filippi[2], Michael J. Myers[1], Jennifer Harper[1], M. Catalina Camacho[1], Tara A. Smyser[1], Cynthia E. Rogers[1, 3], Joshua S. Shimony[4], Barbara B. Warner[3], Joan L. Luby[1], Deanna M. Barch[1, 4, 5], Daniel S. Pine[6], Christopher D. Smyser[3, 4, 7], Nathan A. Fox[8], and Chad M. Sylvester[1, 4, 9]

[1] Department of Psychiatry, Washington University School of Medicine in St. Louis
[2] Department of Child and Adolescent Psychiatry, New York University School of Medicine
[3] Department of Pediatrics, Washington University School of Medicine in St. Louis
[4] Mallinckrodt Institute of Radiology, Washington University School of Medicine in St. Louis
[5] Department of Psychological and Brain Sciences, Washington University in St. Louis
[6] Section on Development and Affective Neuroscience (SDAN), Emotion and Development Branch, National Institute of Mental Health
[7] Department of Neurology, Washington University School of Medicine in St. Louis
[8] Department of Human Development and Quantitative Methodology, University of Maryland
[9] The Taylor Family Institute for Innovative Psychiatric Research, Washington University School of Medicine in St. Louis

Behavioral inhibition (BI), an early-life temperament characterized by vigilant responses to novelty, is a risk factor for anxiety disorders. In this study, we investigated whether differences in neonatal brain responses to infrequent auditory stimuli relate to children's BI at 1 year of age. Using functional magnetic resonance imaging (fMRI), we collected blood-oxygen-level-dependent (BOLD) data from $N = 45$ full-term, sleeping neonates during an adapted auditory oddball paradigm and measured BI from $n = 27$ of these children 1 year later using an observational assessment. Whole-brain analyses corrected for multiple comparisons identified 46 neonatal brain regions producing novelty-evoked BOLD responses associated with children's BI scores at 1 year of age. More than half of these regions ($n = 24$, 52%) were in prefrontal cortex, falling primarily within regions of the default mode or frontoparietal networks or in ventromedial/orbitofrontal regions without network assignments. Hierarchical clustering of the regions based on their patterns of association with BI resulted in two groups with distinct anatomical, network, and response-timing profiles. The first group, located primarily in subcortical and temporal regions, tended to produce larger early oddball responses among infants with lower subsequent BI. The second group, located primarily in prefrontal cortex, produced larger early oddball responses among infants with higher subsequent BI. These results provide preliminary insights into brain regions engaged by novelty in infants that may relate to later BI. The findings may inform understanding of anxiety disorders and guide future research.

Rebecca F. Schwarzlose https://orcid.org/0000-0002-8712-4449

This work was supported by the National Institute of Mental Health (R01MH113883 to Joan L. Luby, Christopher D. Smyser, Barbara B. Warner, Deanna M. Barch, and Cynthia E. Rogers; R01MH122389 to Chad M. Sylvester; T32MH014677-40 to Rebecca F. Schwarzlose; R21MH122976 to Nathan A. Fox; R01MH131584 and F32MH132185 to M. Catalina Camacho; and R00MH125878 to Courtney A. Filippi), the National Institute of Child Health and Human Development (K99HD109454 to Rebecca F. Schwarzlose), the National Institute of Mental Health Intramural Research Program Project (MH002782 to Daniel S. Pine), the Brain and Behavior Research Foundation (Young Investigator Grant 26735 to Chad M. Sylvester), and the Taylor Family Institute for Innovative Psychiatric Research. All authors report no potential conflicts of interest.

Rebecca F. Schwarzlose served as lead for visualization, writing–original draft, and writing–review and editing. Courtney A. Filippi served in a supporting role for investigation and resources. Michael J. Myers served in a supporting role for investigation and methodology. Jennifer Harper served in a supporting role for investigation and project administration. M. Catalina Camacho served in a supporting role for writing–review and editing. Tara A. Smyser served in a supporting role for supervision. Cynthia E. Rogers served in a supporting role for project administration and writing–review and editing. Joshua S. Shimony served in a supporting role for writing–review and editing. Barbara B. Warner served in a supporting role for supervision. Joan L. Luby served in a supporting role for supervision and writing–review and editing. Deanna M. Barch served in a supporting role for writing–review and editing. Daniel S. Pine served in a supporting role for conceptualization and supervision. Christopher D. Smyser served in a supporting role for funding acquisition, project administration, and writing–review and editing. Nathan A. Fox served in a supporting role for formal analysis, supervision, and writing–review and editing. Chad M. Sylvester served as lead for conceptualization, resources, and supervision. Rebecca F. Schwarzlose and Nathan A. Fox contributed equally to conceptualization. Rebecca F. Schwarzlose, Courtney A. Filippi, Nathan A. Fox, and Chad M. Sylvester contributed equally to methodology. Courtney A. Filippi and Joshua S. Shimony contributed equally to validation. Michael J. Myers and Jennifer Harper contributed equally to data curation. Rebecca F. Schwarzlose, Michael J. Myers, and Chad M. Sylvester contributed equally to formal analysis. Tara A. Smyser and Chad M. Sylvester contributed equally to project administration. Cynthia E. Rogers, Barbara B. Warner, Joan L. Luby, Deanna M. Barch, and Chad M. Sylvester contributed equally to funding acquisition. Cynthia E. Rogers and Christopher D. Smyser contributed equally to supervision. Daniel S. Pine and Chad M. Sylvester contributed equally to writing–review and editing.

Correspondence concerning this article should be addressed to Rebecca F. Schwarzlose, Department of Psychiatry, Washington University School of Medicine in St. Louis, 660 South Euclid, MSC 8134-70-2220L, St. Louis, MO 63110, United States. Email: schwarzlose@wustl.edu

Public Significance Statement

This study suggests that specific neural responses to novel sounds in the neonatal brain may be related to children's behavioral inhibition (BI), an early-life temperament characterized by increased vigilance to novelty, 1 year later. Neonatal brain areas producing these responses include regions of prefrontal cortex, temporal cortex, and subcortical structures including pons and amygdala. Given associations between BI and later anxiety symptoms, these findings may inform the understanding of neural antecedents of anxiety disorders.

Keywords: behavioral inhibition, neonate, anxiety, functional magnetic resonance imaging, auditory

Supplemental materials: https://doi.org/10.1037/dev0001654.supp

Anxiety disorders are the most common form of psychiatric illness, have widespread effects on human health, and often begin in childhood (Kessler et al., 2005; Ramsawh et al., 2009). Forty years ago, Jerome Kagan and colleagues characterized behavioral inhibition (BI), an infant temperament marked by vigilant responses to novelty (Coll et al., 1984; Kagan et al., 1984). Subsequent studies have linked BI to risk for anxiety disorders.(Chronis-Tuscano et al., 2009; Clauss & Blackford, 2012; Klein et al., 2010; Rosenbaum et al., 1991; Sandstrom et al., 2020; Tang et al., 2020) Uncovering the neural antecedents of BI can elucidate the biological bases of specific trajectories to anxiety disorders and support early identification and intervention.

In an attempt to understand the origins and correlates of BI, Kagan and colleagues incorporated both psychological and physiological measures (Kagan et al., 1987). Their pioneering work utilized measures such as heart rate, pupillary dilation, the brainstem auditory evoked response, and electroencephalography (EEG) to elucidate the physiological correlates of BI (Calkins et al., 1996; Kagan et al., 1987; McManis et al., 2002; Woodward et al., 2013). Through this work, they documented enhanced physiological arousal to novelty in children with BI and generated interest in its neural antecedents (Fox et al., 2022; Kagan et al., 1987; Reznick et al., 1986). Kagan and colleagues proposed that reactivity to novelty in early infancy gave rise to behaviors associated with BI through heightened subcortical neural responses to novelty, specifically in the amygdala and periaqueductal gray matter (Filippi et al., 2022; Fox et al., 2005; Kagan & Snidman, 1991; Kagan et al., 1987). However, direct support for this hypothesis remains limited, and to date no study has tested whether stimulus-evoked activity within specific brain areas in the neonatal period is related to later measures of children's BI. Closing this gap may uncover early correlates of BI, anxiety disorders, and other associated disorders.

A good deal of work has used EEG to examine brain activity associated with both BI and precursors of BI, namely, temperamental reactivity. This includes findings that EEG responses to deviant tones are related to temperamental distress (Marshall et al., 2009; Reeb-Sutherland et al., 2009). For example, infants identified with high negative reactivity at 4 months of age produced larger EEG responses to deviant stimuli in an auditory oddball task administered at 9 months of age, compared with those of infants with high positive reactivity or control infants (Marshall et al., 2009). Additionally, among children who were behaviorally inhibited as toddlers, those who produced enhanced EEG responses to deviant stimuli in adolescence were also more likely to have a lifetime history of anxiety disorders (Reeb-Sutherland et al., 2009). While these studies suggest that children with BI produce enhanced neural responses to infrequent or deviant stimuli, the relatively poor spatial resolution of EEG does not permit more specific conclusions about the anatomical locations and neural mechanisms underlying these differences.

In contrast, functional magnetic resonance imaging (fMRI) techniques have sufficient spatial resolution to specify brain regions and circuits in which neural activity is related to BI. Technical and practical advances in recent years have paved the way for conducting fMRI scanning of infants and informing our understanding of BI's neural correlates. For example, studies using functional connectivity (FC) metrics derived from fMRI of infants during sleep have identified associations between amygdala and prefrontal FC patterns and concurrent temperamental reactivity or subsequent BI (Filippi et al., 2021; Graham et al., 2016; Rogers et al., 2017; Sylvester et al., 2018). Yet, FC measures provide limited insight into how, why, or where neural responses to deviant stimuli may be related to BI. Since stimulus novelty and predictability affect stimulus-evoked responses in many brain areas (Kim, 2014), these insights require a technique like fMRI with high spatial resolution, paired with a novelty-related paradigm such as the auditory oddball paradigm, as well as measures of later BI. Incorporating these neural and behavioral measures affords new opportunities for elucidating the neural underpinnings of BI.

To take the first step toward this goal and establish its feasibility, we begin to characterize the developmental neurobiology of BI in relation to blood-oxygen-level-dependent (BOLD) responses to a modified auditory oddball paradigm in a small sample of sleeping neonates. Oddball responses in this sample and their relation to maternal trait anxiety were characterized in a prior publication (Sylvester et al., 2021). The current study is a planned analysis of these oddball fMRI data in relation to scores from a subset of the same infants 1 year later when they participated in a gold-standard observational assessment designed by Kagan and colleagues. To the best of our knowledge, this is the first study to relate neonatal stimulus-evoked fMRI data during an auditory oddball paradigm to subsequent BI. Results of this pilot study suggest that neonatal brain responses to novelty may be related to risk for BI, providing preliminary insights into developmental origins of anxiety disorders evident in the neonatal period.

Material and Method

Participants

Participant and recruitment information for this study have been published with results from initial analyses of the sample (Sylvester

et al., 2021). The current study comprises a follow-up of this work to analyze the neonatal data in relation to subsequent measures of BI. Participants with full-term deliveries (≥36 weeks gestational age at birth; $N = 45$) were recruited from a larger study, the Early Life Adversity, Biological Embedding, and Risk for Developmental Precursors of Mental Disorders (eLABE) study, which collected maternal assessments and neuroimaging data including structural MRI from neonates. See the online supplemental materials for full recruitment details and inclusion/exclusion criteria. The study and analyses were not preregistered. Oddball scans were performed in a separate visit soon after the structural scan (mean interim: 7.7 days, range: 0–18 days). Families that participated in the neonatal oddball scans were invited to return for the observational assessment of BI 1 year later. Of the original 45 participants, 27 completed the observational assessment when children were approximately 13 months of age (M_{age}: 13 months, range: 11–17 months; 33% male). Families who did not complete the observational assessment were lost because of transportation difficulties, failure to respond to multiple calls and emails, and COVID-related precautions that prevented in-person testing at the appropriate age. See Table 1 for demographic information about the full scan sample and the subset of participants with both scans and BI scores. There were no significant differences in sex, birth weight, gestational age at birth, area deprivation index (ADI), prenatal stress, maternal trait anxiety, and number of scan frames retained between infants who returned for the BI assessment and those who did not (Table S1 in the online supplemental materials). This study was approved by the Institutional Review Board at Washington University. Informed consent was obtained from parents on behalf of all infant participants.

Neonatal Imaging Acquisition

fMRI data were acquired for each neonate during natural sleep. In this adapted auditory oddball paradigm, the background noise of the scanner served as the expected stimulus, whereas 400-ms white noise bursts served as the oddball stimulus. To establish scanner noise as the expected stimulus, the first auditory oddball of each run was not played until 56 s after the run began. Unlike the regular repetition of scan acquisition sounds, the 23 subsequent oddball stimuli were played at irregular intervals (every 9–14 s) for the remainder of each run. We acquired between 2 and 10 same-day oddball runs for a total of 48–240 stimulus presentations per participant, depending on how well each infant tolerated the scan. See the online supplemental materials for full scan details.

Observational Assessment of BI at 13 Months

All participants who completed neonatal oddball scans were invited to return 1 year later to undergo an established observational assessment of BI that has been used extensively in prior work (Calkins et al., 1996; Coll et al., 1984; Fox et al., 2001). Twenty-seven participants completed this assessment, which comprised three episodes, each consisting of exposure to a different stimulus: a stranger, a robotic toy, and a tunnel. Two trained coders independently time-coded video footage of the assessments to produce continuous measures of percent time children spent close to the parent, their latency to interact with novel stimuli, and latency to vocalize for each episode. Intercoder reliability was high, as evidenced by high intraclass correlations (ICC) for proximity to parent (ICC = .93) and latency to interact or vocalize (ICC = .97). BI scores were computed from the meantime codes across the two raters as follows. After converting proximity to parent and latency measures for each episode to *z*-scores across the full sample, each participant's *z*-score measures were averaged with those from the same episode, generating stranger, robot, and tunnel subscores. The mean of these subscores generated the total BI measure used for our analyses. See the online supplemental materials for additional details.

In the 27 infants with both neonatal oddball data and BI assessments, observational BI scores were not significantly associated with any age or timing variables (e.g., gestational age at birth and ages at scans or assessment; see Table 2) or other relevant variables (i.e., birth weight, ADI, number of scan frames retained, or mean framewise displacement of retained frames; see Table S2 in the online supplemental materials).

Table 1
Information About Participants in the Full Sample (N = 45) and in the Subset (n = 27) With Both Neonatal Scan Data and Observational BI Assessment 1 Year Later

Sample characteristic	Full sample	*n* with available data	BI subset	*n* with available data
Sex, % (*n*)		45		27
Female	60 (27)		67 (18)	
Male	40 (18)		33 (9)	
Race/ethnicity		45	% (*n*)	27
Black	64 (29)		67 (18)	
White	36 (16)		33 (9)	
Hispanic/Latino/Latina	0		0	
Other characteristics, *M* (*SD*)				
National ADI	72.0 (22.5)	45	74.6 (19.4)	27
Income to needs	2.0 (1.6)	39	1.9 (1.6)	26
Birth weight (grams)	3,115 (488)	45	3,125 (469)	27
Gestational age at birth (weeks)	38.2 (1.0)	45	38.2 (1.0)	27
Age at neonatal fMRI scan (days)	28.1 (9.8)	45	27.9 (9.8)	27
Age at BI assessment (months)			13.0 (1.5)	27
Scan details, *M* (*SD*)				
Postcensored fMRI data (minutes)	30.8 (14.9)	45	32.5 (12.6)	27

Note. BI = behavioral inhibition; fMRI = functional magnetic resonance imaging; ADI = area deprivation index (national percentile).

Table 2
Information About Age and Timing Variables in Relation to BI Scores

Age variable	Range	*M* (*SD*)	Correlation with BI (*p*)
Birth/neonatal timing			
Gestational age at birth (weeks)	36–40	38.2 (1.0)	ρ = .04 (.85)
Age at oddball scan (days)	11–49	27.9 (9.8)	*r* = .28 (.15)
Postmenstrual age at oddball scan (weeks)	39–44	42.2 (1.2)	*r* = .25 (.21)
Time between T2 and oddball scan (days)	0–18	7.7 (4.5	*r* = −.17 (.40)
Timing of BI assessment			
Age at BI assessment (months)	11–17	13.0 (1.5)	ρ = .02 (.94)
Time between oddball scan and BI assessment (months)	10–16	12.2 (1.5)	ρ = −.10 (.62)

Note. BI = behavioral inhibition.

Therefore, these measures were not included as covariates in statistical analyses. BI observational assessment scores were not significantly associated with available parent-report measures of BI-like behavior at 12 months, Infant-Toddler Social and Emotional Assessment (ITSEA) inhibition to novelty subscale, ρ(18) = .16, *p* = .25, (Carter et al., 1999, 2003) or 8 months, Infant Behavior Questionnaire-Revised (IBQ-R) fear subscale, *r*(17) = .22, *p* = .19, (Gartstein & Rothbart, 2003) in the subsets of children with these scores; nor were the ITSEA inhibition to novelty scores at 12 months and IBQ-R fear scores at 8 months correlated with each other, ρ(13) = .03, *p* = .46. See the online supplemental materials and Table S2 in the online supplemental materials for additional information about parent-report measures and their relations with BI observational assessment scores.

Preprocessing and Statistical Analysis of fMRI Data

Preprocessing of fMRI data included correction of intensity differences due to interleaved acquisition, bias field correction, intensity normalization for each run to a brain-wide mode of 1,000, linear alignment within and across runs to compensate for rigid body motion, and linear registration of BOLD data to the Talairach 3 mm adult atlas using the T2-weighted images. An analysis of framewise displacement for the initial published report of these data found no significant difference in head motion at the onset of auditory stimuli than at other times in the scan across all *N* = 45 infants (*F* = 1.12, *df* = 39, 1,716, *p* = .28; Sylvester et al., 2021). Frames with displacement surpassing 0.9 mm were censored, based on evidence that this threshold is optimal for task-based analyses (Siegel et al., 2014). Frames from runs with fewer than 150 frames after censoring were additionally excluded. We retained a median of 2,425 frames (*SD* = 9.43), or 32.3 min (*SD* = 12.6), of postcensoring scan data for each infant with both neonatal scans and BI scores.

After preprocessing, BOLD data from auditory oddball runs were analyzed in Talairach volume space with a general linear model using in-house analysis software (code available upon request). The response to auditory oddballs was modeled without assuming a specific hemodynamic response function due to dramatic age-dependent changes in hemodynamic vascular coupling that take place over early infancy (Arichi et al., 2012; Issard & Gervain, 2018; M. Kozberg & Hillman, 2016; M. G. Kozberg et al., 2013; Zimmermann et al., 2012). This was carried out in a voxel-wise fashion with separate finite impulse response regressors for each of 40 frames (duration: 32 s) following onset of white noise bursts. Given that many anatomical and developmental features of neonatal hemodynamic responses remain undescribed, we followed the modeling approach of Sylvester et al. (2021) and analyzed a 32-s window to ensure that estimates of responses were not truncated (Sylvester et al., 2021). Censored frames were omitted in a framewise fashion when estimating BOLD response at each time point. To identify brain regions in which neonatal oddball response was associated with subsequent BI, we conducted an omnibus whole-brain repeated-measures analysis of variance with the following factors: oddball time point (corresponding to Frames 1–40 of the modeled oddball response), subsequent BI score, and oddball Time Point × BI interaction. BI score was handled as a continuous variable (see Figure S1 in the online supplemental materials for distribution). In this model, a Time Point × BI interaction indexes voxels in which BOLD responses to oddball stimuli were associated with BI. Regions producing oddball responses associated with later BI in this sample were identified based on this oddball Time Point × BI interaction. To correct the whole-brain error rate for multiple comparisons, criteria were derived based on study-specific autocorrelation parameters using 3dClustSim (Analysis of Functional NeuroImages [AFNI]; Cox, 1996; Cox & Hyde, 1994). With a voxel-wise significance threshold of $p < .0001$ ($z > 3.3$), a minimum cluster size of 28 voxels sharing at least one surface (i.e., nearest neighbor = 1) was needed to achieve a whole-brain cluster-wise error rate of $p < .01$. Due to COVID-related precautions, observational BI assessments were obtained from only 27 of the original 45 neonates scanned. Therefore, analyses to detect relationships between neonatal oddball responses and subsequent BI scores are reasonably powered to detect large effects ($f = 0.8$, expected power = 0.81) but poorly powered to detect moderate effects ($f = 0.5$, expected power = 0.32), as computed with a post hoc power analysis for an analysis of variance (ANOVA) interaction effect using G*Power Version 3.1 (Faul et al., 2007). Of 58 significant regions identified, 16 failed to pass additional quality control criteria (see the online supplemental materials). The remaining 42 regions were included in subsequent analyses. Comparison of these results with those from Sylvester et al. (2021) can be found in the online supplemental materials. Processed data are available upon request. As a negative control to ensure our analysis pipeline did not generate false positives, we used the same significance and size criteria to identify regions significantly associated with BI irrespective of stimulus timing (i.e., a main effect of BI in the omnibus ANOVA). Since existing hypotheses do not propose baseline or nontime-varying, differences in activity in neonates who go on to develop high BI, this parallel analysis should not identify any significant clusters.

To further characterize how neonatal oddball responses in these regions related to later BI scores, we used agglomerative hierarchical clustering to examine whether they exhibited shared patterns in their

associations with subsequent BI. To ensure that time courses could be averaged across regions within clusters for visualization and interpretation, we limited the analysis to regions that produced mean responses to oddball stimuli resembling a canonical positive BOLD response (i.e., regions with nonnegative maxima between 4 and 12 s poststimulus). These criteria excluded seven regions; the remaining 35 regions were submitted to the clustering analysis. Pearson correlations between BI and BOLD response were computed for each of the 40 modeled frames in each of the remaining 35 regions in which neonatal oddball response was associated with subsequent BI. These framewise correlations were then submitted to agglomerative hierarchical clustering using SPSS (IBM Corporation, 2020). Although BI was handled as a continuous variable in our analysis, the time courses in these regions were visualized separately for children with subsequent high or low BI based on a median split (Figure 2 and Figure S7 in the online supplemental materials) and restricted to the top and bottom quartile (Figure S7 in the online supplemental materials).

Results

Neonatal Brain Responses to Oddball Stimuli Associated With BI at 13 Months

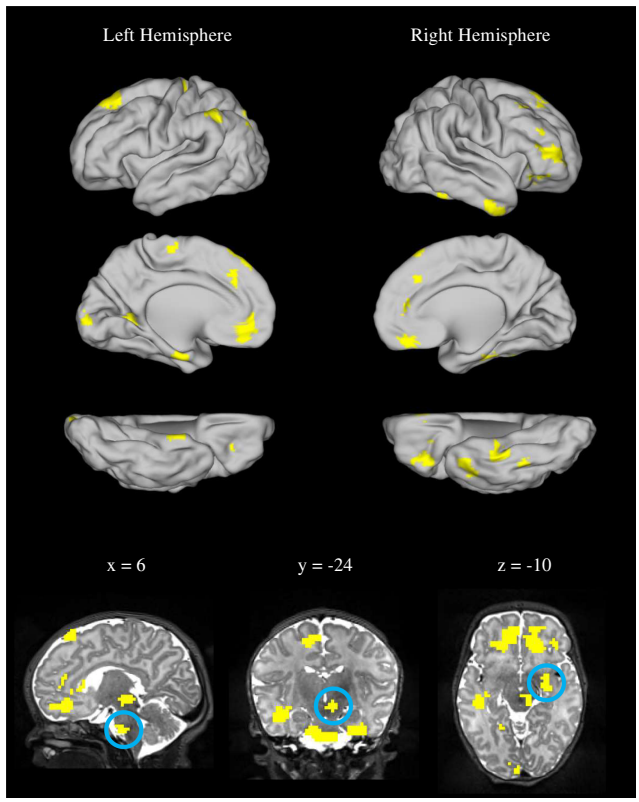
We identified 42 brain regions (Figure 1; Table S3 in the online supplemental materials) in which activity near birth in response to oddball auditory stimuli was associated with BI scores 1 year later. More than half of these regions were in the prefrontal cortex (PFC; $n = 23$, 55%) and fell primarily within brain areas assigned to the default mode network (DMN; $n = 9$, 21%), frontoparietal network (FPN; $n = 4$, 10%), or within medial/orbitofrontal PFC regions without network assignment ($n = 9$, 21%) as defined by adult parcellation (Gordon et al., 2016). Other identified regions included parietal regions of the DMN or FPN ($n = 2$, 5%) and unassigned regions of temporal cortex ($n = 7$, 17%). Subcortical regions were in bilateral pons ($n = 2$, 5%), right amygdala ($n = 1$, 2%), right midbrain ($n = 1$, 2%), and right cerebellum ($n = 2$, 5%). A parallel analysis of main effects of BI at 1 year, conducted to serve as a negative control, identified no regions significantly associated with subsequent BI score.

Patterns of Neonatal Brain Responses Related to BI at 13 Months

Hierarchical clustering was applied to framewise correlations between BOLD response and subsequent BI for the 35 regions associated with subsequent BI and submitted to clustering analysis. The clustering algorithm produced two clusters with distinct anatomical, network, and response profiles (Figure 2; Figures S3 and S4 and Table S3 in the online supplemental materials). One of these clusters comprised 14 regions, including subcortical regions ($n = 5$, 36%), unassigned regions of the temporal cortex ($n = 7$, 50%), and regions in the left parietal cortex ($n = 2$, 14%). Mean framewise correlation values between BI and oddball response in these regions revealed negative associations between BI and oddball BOLD response for time points early in the modeled response and positive associations for time points later in the modeled response. We call this cluster of regions the early-negative association group, reflecting the negative association between BOLD and BI in early frames of the modeled oddball response in these regions.

Figure 1

Regions in Which Neonatal Response to Oddballs Relates to BI at 1 Year



Note. Locations of regions in which oddball-related activity during the neonatal period is related to BI at 1 year are shown in yellow on the lateral, medial, and ventral cortical surfaces, as well as in slices (bottom row). Circles show locations of identified regions in the pons (left), midbrain (middle), and amygdala (right). BI = behavioral inhibition. See the online article for the color version of this figure.

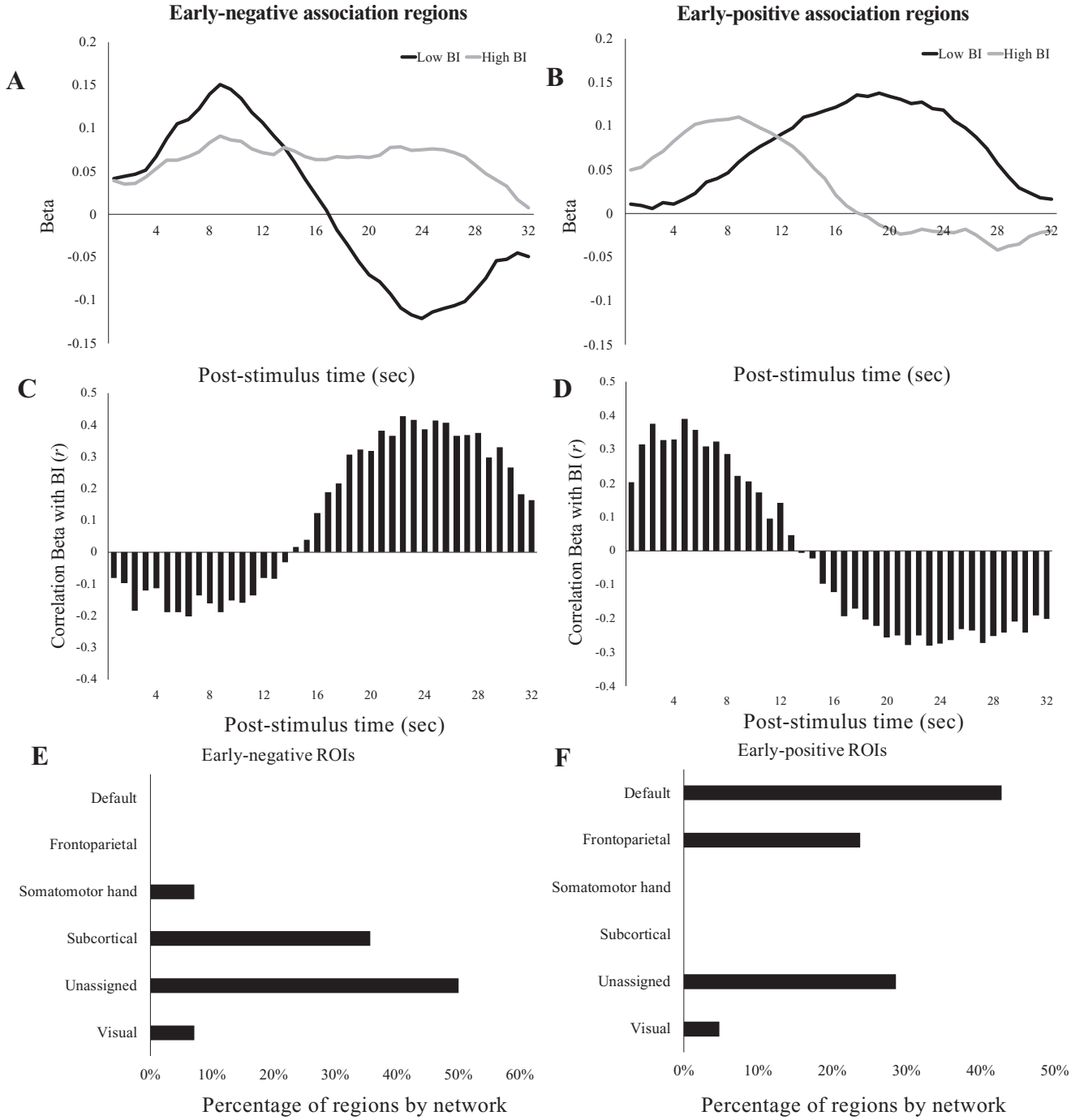
The other cluster comprised 21 regions primarily located in bilateral, medial, and superior regions of PFC. Most ($n = 14$; 67%) of these regions fell within the DMN and FPN networks, according to adult-derived network parcellations (Gordon et al., 2016). Others were in medial/orbitofrontal PFC lacking network assignment ($n = 6$, 29%) or near striate occipital cortex assigned to the visual network ($n = 1$, 5%). We call this cluster the early-positive association group because BI was positively associated with BOLD signal in early frames of the modeled oddball response in these regions.

Discussion

Results from this study suggest that newborns' subcortical and cortical responses to novel stimuli may relate to their subsequent levels of BI at 13 months of age, particularly in regions of PFC and subcortical structures. Overall, these findings are consistent with the hypothesis that individual differences in neural responses to novelty during infancy may relate to BI, a risk factor for anxiety disorders. At the same time, they specify anatomical and temporal features of

Figure 2

Distinct Response and Network Profiles in Two Sets of Brain Regions Associated with 1-Year BI by Group



Note. Panels A and B: Smoothed meantime courses are shown by set of brain regions and by subsequent BI (low BI in black, high BI in gray), shown here based on a median split for visualization purposes only. All statistical analyses treated BI as a continuous variable. Panels C and D: Mean framewise correlations between oddball BOLD response and subsequent BI scores are shown by group. Panels E and F: Distributions of identified regions are shown by network and group. BI = behavioral inhibition; BOLD = blood-oxygen-level-dependent; ROIs = regions of interest.

these individual differences that can inform predictions for larger studies and, if replicated, refine hypotheses about the neural antecedents of BI and anxiety disorders.

Among the regions identified by relations of neonatal oddball response to subsequent BI in this study are brain regions that featured heavily in the hypothesized neural origins of BI described by Kagan

and colleagues (i.e., amygdala; Kagan & Snidman, 1991; Kagan et al., 1987) and that prior studies have implicated in BI (i.e., PFC and amygdala; Filippi et al., 2021; Graham et al., 2016; Rogers et al., 2017). The current study found BI-associated differences in oddball-evoked responses in prefrontal areas, as well as in the right lateral amygdala. The prefrontal areas fell largely within adult-defined DMN and FPN, as well as unassigned regions of the ventromedial/orbitofrontal cortex. These regions are often implicated in tasks involving cognitive control, emotion regulation, prediction, and valuation (Delgado et al., 2008; Dosenbach et al., 2008; Klein-Flügge et al., 2022; Ochsner & Gross, 2005). Prior work has also linked neonatal FC of a medial prefrontal region of the DMN to parent-reported BI at 2 years of age (Sylvester et al., 2018).

In the context of these prior findings and the neurobiological hypothesis proposed by Kagan and colleagues, it is striking that the present analysis using different methods implicated many of the same brain regions in neural antecedents of BI. However, the oddball responses in these regions did not relate to BI in the straightforward fashion that Kagan and colleagues predicted (i.e., larger subcortical and specifically amygdala responses to oddball stimuli in children who go on to exhibit BI; Kagan & Snidman, 1991; Kagan et al., 1987). Rather, in subcortical regions including bilateral pons, midbrain, and right lateral amygdala, neonates who scored higher on BI at 13 months tended to produce diminished BOLD signal at early time points and enhanced signal at later time points, compared with low-BI children. Interpretation of these BOLD differences in relation to neural activity is complicated by the dramatic development of hemodynamic vascular coupling in the perinatal period, which includes changes in the directionality and timing of the BOLD response (Arichi et al., 2012; Issard & Gervain, 2018; M. Kozberg & Hillman, 2016; M. G. Kozberg et al., 2013; Zimmermann et al., 2012). Therefore, understanding the specific neural significance of BOLD magnitude and timing differences observed in the current study will require further research into the time course and regional specificity of hemodynamic maturation in neonates. Despite these challenges, the current findings broadly support Kagan and colleagues' hypothesis that BI is related to neurobiological differences present in the earliest days and weeks of life (Kagan & Snidman, 1991).

Several points should be considered when interpreting the current results. First, infants were sleeping during fMRI scanning and presentation of the auditory stimuli in this study. Although prior work demonstrates that considerable automatic subcortical and cortical processing of auditory stimuli takes place during sleep (Portas et al., 2000; Sylvester et al., 2021; Taga et al., 2018), some features of infants' responses to auditory oddballs may differ between sleep and wakefulness or across sleep stages. The current study did not collect data on sleep stage during scanning and so cannot determine whether the oddball response differed across sleep stages. This study also employed a modified version of the auditory oddball task that used ongoing scanner noise as the standard, in place of a standard tone. Future work is needed to assess whether the current results generalize to scan data collected with standard auditory oddball paradigms.

Strengths of the current study include a longitudinal design that permits BI assessment in the same children scanned as neonates, the use of fMRI to provide anatomic specificity for stimulus-evoked neonatal responses, and the use of an observational assessment to measure subsequent BI. Another strength is the demographic characteristics of the sample, which includes greater racial and socioeconomic diversity than most studies. Finally, relative to studies that limit analyses to a priori regions of interest, the current brain-wide analysis permitted the discovery of unexpected results while adjusting significance criteria to keep the false discovery rate low.

An important limitation of the study is its small sample size, which limits statistical power for finding modest effects and increases the likelihood of spurious findings due to sampling variability (Button et al., 2013). These concerns can be partially assuaged by the fact that a parallel analysis carried out as a negative control produced no significant results, indicating that our significance criteria and quality control measures are effectively reducing spurious findings. Nonetheless, future studies are needed to determine whether the current results replicate in larger samples. It is also important to note that the neonatal brain undergoes rapid maturational change. Although participant age at functional scan had a limited range (11–49 days) and age at scan was not significantly associated with later BI scores, age at scan is an important additional variable that may influence results. Future studies with larger sample sizes and smaller age ranges at scan are needed to clarify the impact of age on neonatal oddball response.

Finally, BI is typically conceptualized as a temperamental profile that applies to a minority of children (Kagan et al., 1989), whereas the current study analyzed BI as a dimensional trait subject to individual differences across participants in our sample. Although this dimensional approach is widely used to identify candidate brain–behavior relationships, including in studies investigating neural effects in relation to BI and negative reactivity (Filippi et al., 2021; Rogers et al., 2017; Sylvester et al., 2018), future work will be needed to determine whether the same brain–behavior associations are observed in studies specifically designed to sample children with an extreme temperamental profile of BI.

Taken together, the current study provides preliminary evidence that individual differences in specific neonatal responses to novelty present in the earliest weeks of life are related to children's subsequent development of BI. These findings provide new insights that can guide ongoing work into the neurobiological origins of BI and the development of anxiety disorders.

References

Arichi, T., Fagiolo, G., Varela, M., Melendez-Calderon, A., Allievi, A., Merchant, N., Tusor, N., Counsell, S. J., Burdet, E., Beckmann, C. F., & Edwards, A. D. (2012). Development of BOLD signal hemodynamic responses in the human brain. *NeuroImage*, *63*(2), 663–673. https://doi.org/10.1016/j.neuroimage.2012.06.054

Button, K. S., Ioannidis, J. P. A., Mokrysz, C., Nosek, B. A., Flint, J., Robinson, E. S. J., & Munafò, M. R. (2013). Power failure: Why small sample size undermines the reliability of neuroscience. *Nature Reviews Neuroscience*, *14*(5), 365–376. https://doi.org/10.1038/nrn3475

Calkins, S. D., Fox, N. A., & Marshall, T. R. (1996). Behavioral and physiological antecedents of inhibited and uninhibited behavior. *Child Development*, *67*(2), 523–540. https://doi.org/10.2307/1131830

Carter, A. S., Briggs-Gowan, M. J., Jones, S. M., & Little, T. D. (2003). The infant-toddler social and emotional assessment (ITSEA): Factor structure, reliability, and validity. *Journal of Abnormal Child Psychology*, *31*(5), 495–514. https://doi.org/10.1023/A:1025449031360

Carter, A. S., Little, C., Briggs-Gowan, M. J., & Kogan, N. (1999). The infant-toddler social and emotional assessment (ITSEA): Comparing parent ratings to laboratory observations of task mastery, emotion regulation, coping behaviors, and attachment status. *Infant Mental Health Journal*,

20(4), 375–392. https://doi.org/10.1002/(SICI)1097-0355(199924)20:4<375::AID-IMHJ2>3.0.CO;2-P

Chronis-Tuscano, A., Degnan, K. A., Pine, D. S., Perez-Edgar, K., Henderson, H. A., Diaz, Y., Raggi, V. L., & Fox, N. A. (2009). Stable early maternal report of behavioral inhibition predicts lifetime social anxiety disorder in adolescence. *Journal of the American Academy of Child & Adolescent Psychiatry*, *48*(9), 928–935. https://doi.org/10.1097/CHI.0b013e3181ae09df

Clauss, J. A., & Blackford, J. U. (2012). Behavioral inhibition and risk for developing social anxiety disorder: A meta-analytic study. *Journal of the American Academy of Child & Adolescent Psychiatry*, *51*(10), 1066–1075.e1. https://doi.org/10.1016/j.jaac.2012.08.002

Coll, C. G., Kagan, J., & Reznick, J. S. (1984). Behavioral inhibition in young children. *Child Development*, *55*(3), 1005–1019. https://doi.org/10.2307/1130152

Cox, R. W. (1996). AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research*, *29*(3), 162–173. https://doi.org/10.1006/cbmr.1996.0014

Cox, R. W., & Hyde, J. (1994). Software tools for analysis and visualization of fMRI data. *NMR in Biomedicine*, *10*(4–5), 171–178. https://doi.org/10.1002/(SICI)1099-1492(199706/08)10:4/5<171::AID-NBM453>3.0.CO;2-L

Delgado, M. R., Nearing, K. I., LeDoux, J. E., & Phelps, E. A. (2008). Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. *Neuron*, *59*(5), 829–838. https://doi.org/10.1016/j.neuron.2008.06.029

Dosenbach, N. U. F., Fair, D. A., Cohen, A. L., Schlaggar, B. L., & Petersen, S. E. (2008). A dual-networks architecture of top-down control. *Trends in Cognitive Sciences*, *12*(3), 99–105. https://doi.org/10.1016/j.tics.2008.01.001

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*(2), 175–191. https://doi.org/10.3758/BF03193146

Filippi, C. A., Ravi, S., Bracy, M., Winkler, A., Sylvester, C. M., Pine, D. S., & Fox, N. A. (2021). Amygdala functional connectivity and negative reactive temperament at age 4 months. *Journal of the American Academy of Child & Adolescent Psychiatry*, *60*(9), 1137–1146. https://doi.org/10.1016/j.jaac.2020.11.021

Filippi, C. A., Valadez, E. A., Fox, N. A., & Pine, D. S. (2022). Temperamental risk for anxiety: Emerging work on the infant brain and later neurocognitive development. *Current Opinion in Behavioral Sciences*, *44*, Article 101105. https://doi.org/10.1016/j.cobeha.2022.101105

Fox, N. A., Henderson, H. A., Marshall, P. J., Nichols, K. E., & Ghera, M. M. (2005). Behavioral inhibition: Linking biology and behavior within a developmental framework. *Annual Review of Psychology*, *56*(1), 235–262. https://doi.org/10.1146/annurev.psych.55.090902.141532

Fox, N. A., Henderson, H. A., Rubin, K. H., Calkins, S. D., & Schmidt, L. A. (2001). Continuity and discontinuity of behavioral inhibition and exuberance: Psychophysiological and behavioral influences across the first four years of life. *Child Development*, *72*(1), 1–21. https://doi.org/10.1111/1467-8624.00262

Fox, N. A., Zeytinoglu, S., Valadez, E. A., Buzzell, G. A., Morales, S., & Henderson, H. A. (2022). Annual research review: Developmental pathways linking early behavioral inhibition to later anxiety. *Journal of Child Psychology and Psychiatry*, *64*(4), 537–561. https://doi.org/10.1111/jcpp.13702

Gartstein, M. A., & Rothbart, M. K. (2003). Studying infant temperament via the Revised Infant Behavior Questionnaire. *Infant Behavior and Development*, *26*(1), 64–86. https://doi.org/10.1016/S0163-6383(02)00169-8

Gordon, E. M., Laumann, T. O., Adeyemo, B., Huckins, J. F., Kelley, W. M., & Petersen, S. E. (2016). Generation and evaluation of a cortical area parcellation from resting-state correlations. *Cerebral Cortex*, *26*(1), 288–303. https://doi.org/10.1093/cercor/bhu239

Graham, A. M., Buss, C., Rasmussen, J. M., Rudolph, M. D., Demeter, D. V., Gilmore, J. H., Styner, M., Entringer, S., Wadhwa, P. D., & Fair, D. A. (2016). Implications of newborn amygdala connectivity for fear and cognitive development at 6-months-of-age. *Developmental Cognitive Neuroscience*, *18*, 12–25. https://doi.org/10.1016/j.dcn.2015.09.006

IBM Corporation. (2020). *IBM SPSS Statistics for Windows* (Version 27.0) [Computer software]. https://www.ibm.com/

Issard, C., & Gervain, J. (2018). Variability of the hemodynamic response in infants: Influence of experimental design and stimulus complexity. *Developmental Cognitive Neuroscience*, *33*, 182–193. https://doi.org/10.1016/j.dcn.2018.01.009

Kagan, J., Reznick, J. S., Clarke, C., Snidman, N., & Garcia-Coll, C. (1984). Behavioral inhibition to the unfamiliar. *Child Development*, *55*(6), 2212–2225. https://doi.org/10.2307/1129793

Kagan, J., Reznick, J. S., & Gibbons, J. (1989). Inhibited and uninhibited types of children. *Child Development*, *60*(4), 838–845. https://doi.org/10.2307/1131025

Kagan, J., Reznick, J. S., & Snidman, N. (1987). The physiology and psychology of behavioral inhibition in children. *Child Development*, *58*(6), 1459–1473. https://doi.org/10.2307/1130685

Kagan, J., & Snidman, N. (1991). Infant predictors of inhibited and uninhibited profiles. *Psychological Science*, *2*(1), 40–44. https://doi.org/10.1111/j.1467-9280.1991.tb00094.x

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, *62*(6), 593–602. https://doi.org/10.1001/archpsyc.62.6.593

Kim, H. (2014). Involvement of the dorsal and ventral attention networks in oddball stimulus processing: A meta-analysis. *Human Brain Mapping*, *35*(5), 2265–2284. https://doi.org/10.1002/hbm.22326

Klein, M. H., Slattery, M. J., Goldsmith, H. H., & Kalin, N. H. (2010). Early risk factors and developmental pathways to chronic high inhibition and social anxiety disorder in adolescence. *The American Journal of Psychiatry*, *167*(1), 40–46. https://doi.org/10.1176/appi.ajp.2009.07010051

Klein-Flügge, M. C., Bongioanni, A., & Rushworth, M. F. S. (2022). Medial and orbital frontal cortex in decision-making and flexible behavior. *Neuron*, *110*(17), 2743–2770. https://doi.org/10.1016/j.neuron.2022.05.022

Kozberg, M., & Hillman, E. (2016). Chapter 10 - Neurovascular coupling and energy metabolism in the developing brain. In K. Masamoto, H. Hirase, & K. Yamada (Eds.), *New horizons in neuromuscular coupling: A bridge between brain circulation and neural plasticity* (Vol. 225, pp. 213–242). Elsevier. https://www.sciencedirect.com/bookseries/progress-in-brain-research/vol/225/suppl/C

Kozberg, M. G., Chen, B. R., DeLeo, S. E., Bouchard, M. B., & Hillman, E. M. C. (2013). Resolving the transition from negative to positive blood oxygen level-dependent responses in the developing brain. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(11), 4380–4385. https://doi.org/10.1073/pnas.1212785110

Marshall, P. J., Reeb, B. C., & Fox, N. A. (2009). Electrophysiological responses to auditory novelty in temperamentally different 9-month-old infants. *Developmental Science*, *12*(4), 568–582. https://doi.org/10.1111/j.1467-7687.2008.00808.x

McManis, M. H., Kagan, J., Snidman, N. C., & Woodward, S. A. (2002). EEG asymmetry, power, and temperament in children. *Developmental Psychobiology*, *41*(2), 169–177. https://doi.org/10.1002/dev.10053

Ochsner, K., & Gross, J. (2005). The cognitive control of emotion. *Trends in Cognitive Sciences*, *9*(5), 242–249. https://doi.org/10.1016/j.tics.2005.03.010

Portas, C. M., Krakow, K., Josephs, O., Armony, J. L., & Frith, C. D. (2000). Auditory processing across the sleep-wake cycle: Simultaneous EEG and fMRI monitoring in humans. *Neuron*, *28*(3), 991–999. https://doi.org/10.1016/S0896-6273(00)00169-0

Ramsawh, H. J., Stein, M. B., Belik, S.-L., Jacobi, F., & Sareen, J. (2009). Relationship of anxiety disorders, sleep quality, and functional impairment

in a community sample. *Journal of Psychiatric Research*, *43*(10), 926–933. https://doi.org/10.1016/j.jpsychires.2009.01.009

Reeb-Sutherland, B. C., Vanderwert, R. E., Degnan, K. A., Marshall, P. J., Pérez-Edgar, K., Chronis-Tuscano, A., Pine, D. S., & Fox, N. A. (2009). Attention to novelty in behaviorally inhibited adolescents moderates risk for anxiety. *Journal of Child Psychology and Psychiatry*, *50*(11), 1365–1372. https://doi.org/10.1111/j.1469-7610.2009.02170.x

Reznick, J. S., Kagan, J., Snidman, N., Gersten, M., Baak, K., & Rosenberg, A. (1986). Inhibited and uninhibited children: A follow-up study. *Child Development*, *57*(3), 660–680. https://doi.org/10.2307/1130344

Rogers, C. E., Sylvester, C. M., Mintz, C., Kenley, J. K., Shimony, J. S., Barch, D. M., & Smyser, C. D. (2017). Neonatal amygdala functional connectivity at rest in healthy and preterm infants and early internalizing symptoms. *Journal of the American Academy of Child & Adolescent Psychiatry*, *56*(2), 157–166. https://doi.org/10.1016/j.jaac.2016.11.005

Rosenbaum, J. F., Biederman, J., Faraone, S. V., Kagan, J., & Snidman, N. (1991). Further evidence of an association between behavioral inhibition and anxiety disorders: Results from a family study of children from a nonclinical sample. *Journal of Psychiatric Research*, *25*(1/2), 49–65. https://doi.org/10.1016/0022-3956(91)90015-3

Sandstrom, A., Uher, R., & Pavlova, B. (2020). Prospective association between childhood behavioral inhibition and anxiety: A meta-analysis. *Research on Child and Adolescent Psychopathology*, *48*(1), 57–66. https://doi.org/10.1007/s10802-019-00588-5

Siegel, J. S., Power, J. D., Dubis, J. W., Vogel, A. C., Church, J. A., Schlaggar, B. L., & Petersen, S. E. (2014). Statistical improvements in functional magnetic resonance imaging analyses produced by censoring high-motion data points: Censoring high motion data in fMRI. *Human Brain Mapping*, *35*(5), 1981–1996. https://doi.org/10.1002/hbm.22307

Sylvester, C. M., Myers, M. J., Perino, M. T., Kaplan, S., Kenley, J. K., Smyser, T. A., Warner, B. B., Barch, D. M., Pine, D. S., Luby, J. L., Rogers, C. E., & Smyser, C. D. (2021). Neonatal brain response to deviant auditory stimuli and relation to maternal trait anxiety. *American Journal of Psychiatry*, *178*(8), 771–778. https://doi.org/10.1176/appi.ajp.2020.20050672

Sylvester, C. M., Smyser, C. D., Smyser, T., Kenley, J., Ackerman, J. J., & Shimony, J. S. (2018). Cortical functional connectivity evident after birth and behavioral inhibition at age 2. *The American Journal of Psychiatry*, *175*(2), 180–187. https://doi.org/10.1176/appi.ajp.2017.17010018

Taga, G., Watanabe, H., & Homae, F. (2018). Developmental changes in cortical sensory processing during wakefulness and sleep. *NeuroImage*, *178*, 519–530. https://doi.org/10.1016/j.neuroimage.2018.05.075

Tang, A., Crawford, H., Morales, S., Degnan, K. A., Pine, D. S., & Fox, N. A. (2020). Infant behavioral inhibition predicts personality and social outcomes three decades later. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(18), 9800–9807. https://doi.org/10.1073/pnas.1917376117

Woodward, S. A., McManis, M. H., Kagan, J., Deldin, P., Snidman, N., Lewis, M., & Kahn, V. (2013). Infant temperament and the brainstem auditory evoked response in later childhood. In M. E. Hertzig & E. A. Farber (Eds.), *Annual progress in child psychiatry and child development 2002* (pp. 265–278). Routledge.

Zimmermann, B. B., Roche-Labarbe, N., Surova, A., Boas, D. A., Wolf, M., Grant, P. E., & Franceschini, M. A. (2012). The confounding effect of systemic physiology on the hemodynamic response in newborns. In M. Wolf, H. U. Bucher, M. Rudin, S. Van Huffel, U. Wolf, D. F. Bruley, & D. K. Harrison (Eds.), *Oxygen transport to tissue XXXIII* (Vol. 737, pp. 103–109). Springer New York. https://doi.org/10.1007/978-1-4614-1566-4

Received March 20, 2023
Revision received August 8, 2023
Accepted September 6, 2023 ■