THE JOURNAL OF CHILD PSYCHOLOGY AND PSYCHIATRY

ACAMH THE ASSOCIATION FOR CHILD AND ADOLESCENT MENTAL HEALTH

Journal of Child Psychology and Psychiatry 55:7 (2014), pp 802–810 doi:10.1111/jcpp.12188

Neonatal brainstem dysfunction after preterm birth predicts behavioral inhibition

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Background: Behavioral inhibition (BI), the tendency to withdraw or exhibit negative affect when experiencing stressful situations, is a major risk factor for the development of social anxiety. However, neonatal biologic origins of this progression are still unknown. Click here to enter text.This study aimed to extend frameworks of behavioral inhibition by exploring empirically the central role of neonatal brainstem electrophysiologic functions in the development of social disengagement and BI. **Methods:** Sixty-six preterm neonates (means ±SD: gestation age = 33.1 ± 1.22 weeks, birth weight = 1775 + 346.7 g; 51% female) participated in a prospective longitudinal study. The infants were tested within the first 2 weeks of postnatal life using an auditory brainstem-evoked response test. Based on the typicality of the major ABR wave latencies, waves I, III and V, neonates were divided into two groups (compromised, CBSF- with at least one component ≥1.5 SDs from the mean for the respective gestation age; normal, NBSF, with all components within 1.5 SD around the mean), and were enrolled in a prospective longitudinal follow-up study. This report extends previous work from 4 m by testing responses to socioemotional challenges during the Separation–Reunion paradigm at 12 m. **Results:** Results show that infants with neonatal CBSF were more susceptible to be classified as BI at 12 m (age corrected for prematurity) than infants with NBSF (66% vs. 40%, respectively). The most striking symptom in the CBSF group was a disability to initiate self-regulatory activities in response to a socioemotional challenge, resulting in frequent passivity/dependency ($p < .001$). Statistical regression analysis revealed that face-to-face gaze engagement at 4 m moderates the risk related to neonatal CBSF for the emergence of BI at 12 m, but did not overturn the emergence of BI. **Conclusion:** Results support the hypothesis that neonatal brainstem dysfunction canalizes behavioral inhibition**.** These findings highlight, for the first time, the role of the early developing brainstem in later development of BI and in abilities to initiate self-regulatory behavior.
Keywords: Prematurity, temperament, infancy, neural development, motor inhibition.

Introduction

Behavioral inhibition (BI), the tendency to withdraw or exhibit negative affect when experiencing stressful situations, is present in its extreme form in about 15% of all children. BI has been described as a risk factor for psychopathology (Allan & Gilbert, 1997) and as a precursor to social and generalized anxiety disorder (Hirshfeld et al., 1992; Rosenbaum, Biederman, & Gersten, 1989). A recent report indicates that almost half of these inhibited children will eventually develop social anxiety disorder (Clauss & Blackford, 2012). Hence, an in-depth understanding of the early markers of BI may enable earlier identification of risk and boost the development of more effective preventative protocols.

Behaviorally inhibited children are typically shy, fearful and less likely to be spontaneous. Physiologically, they exhibit increased arousal responses, increased right frontal and dorsal cingulate activity as measured by EEG and differential activity in the amygdala in response to faces (Fox, Henderson, Marshall, Nichols, & Ghera, 2005). Compared with uninhibited children, children with BI have structurally abnormal ventral prefrontal cortices (Fox et al., 2005). Despite its considerable implications, little is known about the very early markers of BI during the first phases of development.

In early development, BI can be expressed through longer latencies to approach new situations, high proximity to parents, and negative affect expressed through vocal behavior (Kagan, Reznick, & Snidman, 1987). In-depth assessment of these behaviors depicts a temperamental link to self-regulation, executive control, and ability to cope with stress (Rothbart, Sheese, Rueda, & Posner, 2011). Recent models propose that the underlying mechanisms for BI involve aberrations in neonatal brainstem pathways, which can already be diagnosed in the perinatal period.

The role of the brainstem in social disengagement and BI

The vertical integrative model for self-regulation proposes a specific set of hypotheses regarding the brainstem's impact on self-regulation in social contexts and on social inhibition (Geva & Feldman, 2008). The model postulates a primary role for neonatal brainstem-related neural networks in canalizing the development of self-regulatory capacities (Geva & Feldman, 2008; Geva et al., 2011; Porges, 2010), suggesting their involvement in social disengagement and in BI (Geva et al., 2011).

Conflicts of interest statement: No conflicts declared.

Recent study showed that the integrity of brainstem pathways at the time of its maturational spurt during the last weeks of gestation is related to social engagement during the first postnatal months of life, thereby supporting the notion that humans are programed for social behavior at a period *preceding* social encounters, and suggesting that brainstem dysfunction may adversely impact fetal programing at this level (Geva et al., 2011). It is intriguing to understand how neonatal compromised brainstem function (CBSF) might unfold in time, and what specific clinical hallmarks might be associated with budding BI.

The vertical model delineates the developmental progression from the brainstem to the limbic and cortical systems, beginning with brainstem-mediated homeostatic processes, followed by limbic-controlled changes in attentional and emotional state, and culminating in higher level cortically mediated social executive functions (Geva & Feldman, 2008). The model presents the intriguing challenge of establishing a direct role of neonatal brainstem input in social behavior.

Brainstem compromise and avoidance

The effect of CBSF on BI may theoretically be twofold. First, it may affect the infant's primary voluntary means of regulating stress and their ability to regulate gaze behavior. Gaze has been firmly established as an indicator of self-regulation (Stifter & Braungart, 1995), and results from previous research demonstrate its role in infants (Hunnius & Geuze, 2004). Infants are able to control the perceptual input they receive by averting their eyes during arousing situations (Stern, 1974; Stifter & Moyer, 1991), whether positive (Stifter & Moyer, 1991) or negative (Field, 1984). Field (1981) used heart rate as a physiologic indicator during gaze aversion and observed increased rates before gaze aversion and normal rates afterward. Infants with CBSF have also been shown to be at risk for gaze-regulation difficulties (Geva & Feldman, 2008), thus drawing a possible link between the brainstem and gaze regulatory behavior in canalizing BI.

Second, neonatal CBSF may interfere with motivational aspects of BI by affecting the development of motor initiation abilities. Evidences suggest the involvement of brainstem in motor and regulatory behavior. For example, the lateral periaqueductal gray matter mediates regulation by activating coping strategies such as, vocalization, aggression, hypertension, and confrontation, whereas the ventrolateral columns control passive coping responses, such as freezing, immobility, hyporeactivity, hypotension, and bradycardia (Bandler & Shipley, 1994; Parvizi & Damasio, 2001). These functions may indicate the involvement of brainstem pathways in canalizing the motor activation aspects of BI. Hence, CBSF is hypothesized to affect responses to social stress by activating gaze avert behaviors and obstructing initiation of higher regulatory strategies.

Detecting CBSF

Due to its fatal consequences, research on structural brainstem damage is rare. However, the auditory brainstem-evoked response (ABR) is useful in detecting slight maturational delays in preterm-age neonates (Valkama et al., 2001). The ABR first appears around 30–33 weeks of gestation (Jiang, Brosi, Wu, & Wilkinson, 2009), a common age for preterm births. As ABR abnormalities quickly spontaneously recover, this period offers a sensitive window for testing premature neonates during a fundamental developmental period when myelination, axonal sprouting, and synaptic connections are still forming, influencing the integrity of the auditory pathways (Jiang et al., 2009; Krumholz, Felix, Goldstein, & McKenzie, 1985).

Evaluation of ABR in infants born preterm at the time of its emergence has proved to be fruitful for making predictions about social-engagement skills at 4 m. Recent study showed for the first time that preterm neonates who display perinatal CBSF are at a greater risk of exhibiting difficulties in regulating gaze during a face-to-face interaction at 4 m (Geva et al., 2011). This may extend BI research that concentrated so far mostly on infants born at term.

The objective of this research was to explore prospectively, if neonatal CBSF in infants born preterm affects the risk for BI at 12 m. The main hypotheses were that neonatal CBSF would increase the propensity for BI at 12 m and impede an infant's ability to initiate soothing strategies in response to socioemotional challenge. Moreover, to explore an avenue of developmental discontinuity, we hypothesized that an infant's reactivity in face-to-face interaction during early infancy would moderate the relationship between neonatal CBSF and BI, thereby highlighting a potential user-friendly preventative intervention to limit risk for BI and social anxiety.

Materials and Methods

Participants

A subsample of sixty-six premature infants [gestation age (GA) = 33.1 weeks, birth weight (BW) = 1775 g, 51% female], who were recruited from a Level III Neonatal Intensive Care Unit (NICU) at Sheba Medical Center, Ramat-Gan, Israel for a prospective longitudinal study, took part in the current research. None of the participants were diagnosed with intraventricular hemorrhage or periventricular leukomalacia on a cranial ultrasound. To minimize external sources of socioemotional stress, inclusion criteria included mothers at least 21 years old who lived with the infants' fathers. None reported use of psychoactive drugs or psychiatric medication during pregnancy or after birth, and all were rated as middle-class according to Israeli standards (Harlap, Davies, Grower, & Prywes, 1977). The sample's ethnicity was predominantly Caucasian, and the subsample's primary neonatal characteristics,

Table 1 Demographic characteristics of the participating groups

	CBff	NBSF	Significance
Fetal			
Fetal distress (%)	32	22	NS
Neonatal			
Females (%)	42	58	NS
Gestation age (weeks)	32.925 ± 0.232	33.224 ± 0.120	NS
Birth weight (g)	1721 ± 65.129	1810 ± 56.980	NS
NBRS score	2000 ± 0.388	2.375 ± 0.352	NS
VLBW (%)	15	18	NS
Days in NICU	27.962 ± 2.458	29.675 ± 2.130	NS
NB at discharge	5.640 ± 0.244	6.135 ± 0.355	NS
Infancy			
BSID-II PDI (4 m)	86.190 ± 2.558	88.132 ± 2.352	NS
BSID-II MDI (4 m)	87.842 ± 2.842	89.405 ± 2.665	NS
BSID-II behavioral (4 m)	54.294 ± 5.666	66.500 ± 5.018	NS
Parental			
Maternal depression at birth (BDI)	5.652 ± 0.802	5.936 ± 0.945	NS
Maternal anxiety at birth (STAI)	33.652 ± 2.099	32.516 ± 1.485	NS
Maternal depression at 12 m (BDI)	5.187 ± 7.287	4.389 ± 3.791	NS
Maternal anxiety at 12 m (STAI)	36.000 ± 13.995	31.950 ± 7.023	NS
Maternal education (years)	15.130 ± 0.549	16.154 ± 0.356	NS
Paternal occupation (%)[a]	94	100	NS
Maternal age	32.708 ± 5.086	34.615 ± 5.914	NS
Familial			
Number of siblings	1.920 ± 0.230	1.800 ± 0.144	NS
Social support[b]	30.120 ± 1.765	28.424 ± 1.963	NS
Previous pregnancies	1.231 ± 0.305	1.150 ± 0.257	NS

CBSF, compromised brainstem function; NBSF, normal brainstem function, NBRS, Neurobiologic Risk score (Brazy et al., 1993); VLBW, Very low birth weight (<1500 g); NICU, neonatal intensive care unit; NB, Neurobehavioral score; BSID-II, Bayley Scales of Infant Development-II (Bayley, 1993); PDI, Psychomotor Developmental Index; MDI, Mental Developmental Index; BDI, Beck's Depression Scale (Beck, Steer, & Carbin, 1988); STAI, Stait-Trait Anxiety Inventory-II (Spielberger, 1989).
[a]Full-time occupation.
[b]Social Support interview (Crockenberg, 1981).

GA, BW distribution, Neurobiological Risk score (Brazy, Goldstein, Oehler, Gustafson, & Thompson, 1993) and familial variables were not different from those in the previous report (Geva et al., 2011).

This study was approved by Sheba Medical Center's institutional review board, and parental informed consent was obtained prior to participation. To ensure detection of CBSF, children were tested for ABR in the hospital within the first 2 weeks of life, prior to its expected rapid recuperation (Geva et al., 2011). Follow-up tests ensured that all participants were within typical range of hearing. At 4 and 12 m (ages adjusted for prematurity), the group underwent tests to measure reactions to social stress. ABR status remained unknown to both experimenters and families to prevent potential bias. The sample was divided into two groups based on neonatal ABR wave latencies; normal brainstem function (NBSF, $n = 40$), and compromised brainstem function (CBSF, $n = 26$). Groups were comparable on prenatal, neonatal, and familial variables (Table 1).

Auditory brainstem-evoked responses in the neonatal period

Participating neonates underwent a bedside ABR measurement as soon as medically permitted (mean age at test = 2.1 ± 1.2 weeks) to test for CBSF. The test was conducted by a trained audiologist using the Biologic Navigator Pro (model 907, FDA approved). Three to four consecutive runs of one thousand twenty-four 100-μs square wave monaural rarefaction clicks, 75 dB hearing level, at a 10.1-Hz rate, were presented to the left auditory canal using microinsert earphones to minimize the risk of a collapsed ear canal (Jiang, Brosi, & Wilkinson, 2006). Recording was initiated after the infant was calm, post midmorning feed, without sedation, using surface gold-plated electrodes [vertex (active), ipsilateral mastoid (referent) and middle forehead (ground)]. Impedance levels were maintained<5 kΩ. Data were digitized at 50-μs intervals for 12-ms sweeps and averaged to produce the ABR wave form (Jiang et al., 2006). Further details on ABR processes are described by Geva et al. (2011). ABR dysfunction was classified on the basis of wave I–V brainstem transmission times (BTT) with normal functioning set as mean ±1.5 SDs for postconception age of the Wave-I, Wave-III and Wave-V BTTs, and I-III, III-V and I-V intervals. Examples of an NBSF case and CBSF case are presented in Figure 1, depicting a prolonged BTT, mostly due to a prolonged Wave III-V interval in the CBSF case relative to the NBSF case.

Behavior response paradigm at 4 m

At 4 m (age-adjusted for prematurity; mean = 57.25 ± 1.818 weeks), infants underwent the Behavior Response Paradigm at the lab (Garcia-Coll et al., 1988). The paradigm comprises of a series of infant–experimenter interactions with three varying levels of social load: Level one, nonsocial - an experimenter holds a toy in front of their face while interacting with the infant; Level two, slightly social - the experimenter wears a mask while interacting with the infant; and Level three, direct social - the experimenter interacts with the infant directly with a completely uncovered face. Recorded measures for this procedure included infant's latency to engage in gaze, time to disengage, and time between gaze shifts from the stimulus to the experimenter [Further details (Geva et al., 2011)].

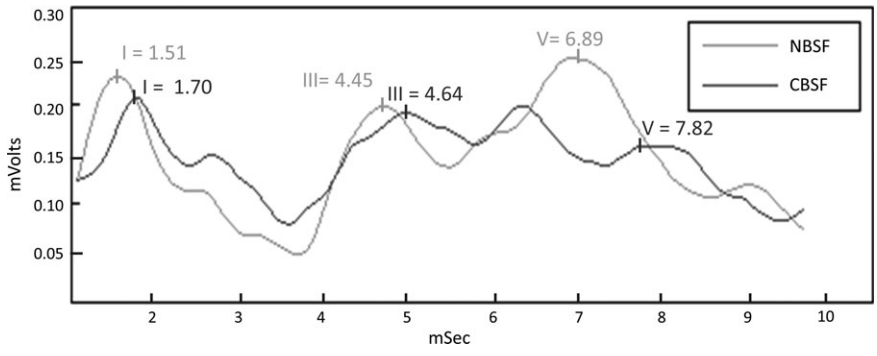

Figure 1 Example of a normal and an atypical prolonged ABR record. CBSF = compromised brainstem function, NBSF = normal brainstem function

Infant Behavior Questionnaire [IBQ (Rothbart, 1981)] at 9 m

This widely used questionnaire for evaluation of temperament was filled by parents through a structured interview (Clauss, Cowan, & Blackford, 2011). The questionnaire comprises of 6 scales (activity level, fear, distress to limitations, soothability, smiling and laughter, and duration of orienting), which are then clustered into three broad dimensions of surgency/extraversion, negative affectivity, and orienting regulation.

Separation–Reunion at 12 m

The Separation–Reunion paradigm (Ainsworth, Blehar, Waters, & Wall, 1978) is a well-defined and validated method that assesses a child's attachment to their parent. In this study, it was used during a follow-up session in the laboratory at 12 m (mean = 12.919 ± 0.753 m) to assess children's coping with a mild social stressful situation (Ainsworth et al., 1978). The paradigm began with a 3-min warm-up period, during which time a selection of toys was available in a room and the infant–parent dyad was instructed to play. This was followed by a period of separation, during which the parent left the room for 3 min, while the child remained in the presence of a noninteracting person. Finally, barring any distress or crying, in which case the separation period was cut short, the parent returned for a reunion session lasting another 3 min, and engaged with the infant.

Audiovisual recordings of the separation and reunion phases were later microcoded in 5-s intervals. Interrater reliability was computed on 6% of randomly selected records using two independent raters. First, BI factors adapted from a widely used Separation–Reunion methodology were coded (Kagan et al., 1987). Measures included the children's latencies to vocalize, cry, and touch objects during separation and reunion, and time spent in proximity to their parent during reunion (Cohen's Kappa coefficient = 0.92). A composite BI measure was created by compiling an average of these measures standardized scores (mean = .006, Range = −0.55–0.85). These scores are comparable with those reported using other cohorts ($t = -.040$, $p < .968$, NS; Calkins & Fox, 1992). The mean was then used for dividing the cohort to BI and non-BI groups (Calkins & Fox, 1992).

Additional coded variables pertained to the motor and affective activity patterns during separation, and joint gaze patterns during reunion. Motor activity variables during separation included: wandering around the room, characterized by aimless movements in the testing area while avoiding contact with toys or objects (Kappa = 0.94); waiting near the door, defined by periods of sitting or standing next to the door without using toys or objects, and apparently waiting for his/her mother's return (Kappa = 0.78); and inactivity, defined by a lack of movement or use of objects, during which time the child sat in place and did not interact with his/her surroundings (Kappa = 0.92).

Affective activity measures included: use of transitory objects, meaning that the child interacted in some way with a toy or object in the room (Kappa = 0.83); self-soothing, defined by the use of toys, objects, or their own self to achieve calmness, usually by placing the object in his/her mouth (Kappa = 0.84); and frantic movements, classified as quick, agitated movements indicative of distress (Kappa = 1).

During reunion, joint gaze measures were based on whether the mother and child simultaneously looked at a certain toy in the room (Kappa = 0.67), or whether the child attended to the objects on his/her own while the mother looked elsewhere (Kappa = 0.62).

To maintain consistency in analysis of the dependent factors, two standardized composite scores were created: (a) representing initiation strategy, comprised of periods spent waiting near the door during separation and gazing at objects during reunion; (b) reflecting passivity/dependency, comprised of periods of inactivity during separation and of joint-attention during reunion.

Results

Manipulation check

Preliminary repeated measures analyses showed that as expected, all infants displayed more distress during separation than during reunion, independent of ABR. During separation, infants displayed more negative affect ($F = 39.320$, $df = 1,63$, $p < .001$, $\eta^2 = 0.384$), showed a tendency to cry more often ($F = 30.771$, $df = 1,63$, $p < .001$, $\eta^2 = 0.328$), and were more likely to wander in the room ($F = 4.331$, $df = 1,63$, $p < .05$, $\eta^2 = 0.064$). During the reunion interval, they displayed more neutral affect ($F = 45.678$, $df = 1,63$, $p < .001$, $\eta^2 = 0.420$) and were more frequently inactive ($F = 20.813$, $df = 1,63$, $p < .001$, $\eta^2 = 0.248$), silent ($F = 24.997$, $df = 1,63$, $p < .001$, $\eta^2 = 0.284$), or engaged in neutral vocalizations ($F = 4.944$, $df = 1,63$, $p < .05$, $\eta^2 = 0.073$). These results indicate that the introduction of this mild socioemotional challenge did activate the infant's self-regulation system.

CBSF predicts BI and initiation difficulties

A chi-square analysis was conducted to test whether the presence of neonatal CBSF is related to an

increased risk for BI at 12 months. The analysis supported the main hypothesis and yielded a moderate effect size for CBSF ($\chi^2 = 4.062$, $df = 1,64$, $p < .044$). It showed that more than 66% of the infants who were diagnosed with CBSF as neonates were classified in the BI group, while 60% of neonates categorized as NBSF were later classified as non-BI (Cohen's $d = 0.77$, effect size $r = 0.36$, OR = .35, RR = .58). Interestingly, the propensity of females, VLBW and fetal distress were similarly distributed in the CBSF-BI and the other groups (CBSF-BI: 41% females, $\chi^2 = 0.75$, $p < .49$; 18% VLBW, $\chi^2 = 0.007$, $p < .642$; 31% with fetal distress, $\chi^2 = 0.69$, $p < .500$). Multivariate analysis of variance with ABR as the independent variable underscored proximity to parent as the most characteristic feature of the BI construct in this cohort ($F = 5.842$, $p < .05$, Cohen's $d = 0.605$, effect size $r = 0.29$; Figure 2).

To further understand the particular characteristics of the infants' behavior, and to clarify how neonatal CBSF influenced self-regulatory behaviors at 12 months of age, ANOVAs were performed to compare the initiation of motor, affective, and gaze patterns as a function of brainstem integrity (Figure 3).

Results showed that CBSF is related to decreased motor initiation responses, such that infants with neonatal CBSF were more likely to remain inactive during separation from their mothers ($F = 12.466$, $df = 5,60$, $p < .001$, $\eta^2 = 0.188$) and those with NBSF were more prone to initiate the regulatory response of waiting at the door ($F = 13.190$, $df = 5,60$, $p < .001$, $\eta^2 = 0.196$). These findings remained consistent after controlling for gender, GA, and temperamental activity level, which did not explain additional variance. No differences were found when comparing affective activity (Figure 3).

Compatible with the study's hypothesis, analysis revealed ABR main effects on infant's gaze patterns during reunion. Children with neonatal CBSF engaged in joint-attention to objects more than the

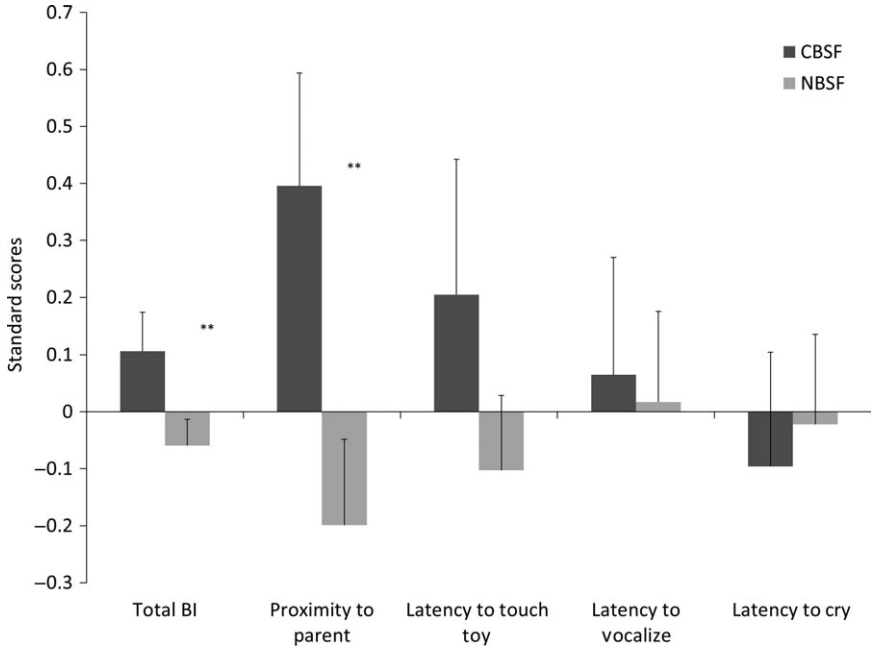

Figure 2 Behavioral inhibition behaviors as a function of neonatal compromised brainstem. CBSF = compromised brainstem function, NBSF = normal brainstem function, BI = Behavioral inhibition, *$p < .05$

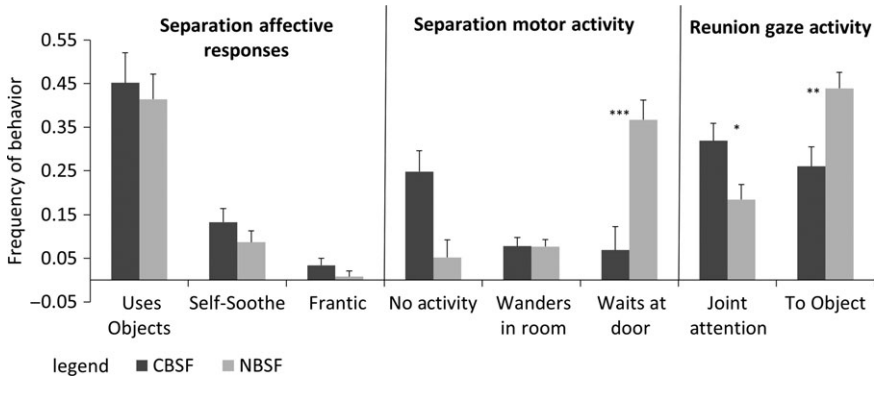

Figure 3 Affective, gaze, and motor responses as a function of neonatal compromised brainstem function. CBSF = compromised brainstem function, NBSF = normal brainstem function, *$p < .05$, **$p < .01$, ***$p < .001$

Table 2 Multivariate analysis of covariance summary (*F* values and significance) explaining self-regulation strategies in response to social challenge at 1 year of age using neonatal auditory brainstem response (ABR) and temperament measures

	ABR	BI	Gestation Age	Activity level	Gender	ABR*Gender
Passivity/dependency	17.007***	1.517	0.464	0.103	0.848	0.574
Initiation strategies	14.007***	1.118	0.015	0.284	1.873	0.568
JA SS	8.802**	0.409	0.265	0.083	3.201	0.349
Gaze to object SS	7.203*	13.187***	2.521	7.041*	1.024	0.663
Waits at door SS	6.975*	3.027	2.025	2.637	0.986	0.025
Inactivity SS	6.810*	4.636*	2.074	0.046	0.224	0.001

BI, behavioral inhibition; SS, Standard Score; JA, Joint Attention.
*$p < .05$; **$p < .01$; ***$p < .001$.

NBSF group ($F = 4.875$, $df = 5,61$, $p < .05$, $\eta^2 = 0.081$), suggesting a greater need for parental aid following stress; whereas infants with NBSF had a higher tendency to attend to objects independently ($F = 7.740$, $df = 5,61$, $p < .01$, $\eta^2 = 0.123$), possibly attesting for their ability to employ attention to objects to self-regulate following stress. These factors remained significant after controlling for gender, GA, and activity level (Figure 3).

To address the relationship between ABR and BI, standard composite scores were calculated for initiation strategy using 'waits at door' and 'gazes at object' behaviors (mean $\pm$SD = -0.1190 ± 1.32), and for passivity/dependency using 'inactivity' and 'joint-attention' behaviors (mean $\pm$SD = 0.4 ± 0.333). These measures were derived from previously determined ABR-sensitive factors. Analyses of covariance with BI, activity level, GA, and gender showed ABR main effects on the initiation strategies composite score ($F = 14.007$, $df = 6,54$, $p < .001$, $\eta^2 = 0.230$), and on each subfactor [waits at door ($F = 6.975$, $df = 6,53$, $p < .05$, $\eta^2 = 0.132$), and gazes at object ($F = 7.203$, $df = 6,53$, $p < .01$, $\eta^2 = 0.135$)], illustrating that infants with CBSF showed lower levels of initiation behavior than those with NBSF (Table 2) even after controlling for BI.

An ABR effect was also observed for the passivity/dependency composite factor ($F = 17.007$, $df = 6,54$, $p < .001$, $\eta^2 = 0.266$), and its two subvariables [inactivity ($F = 6.810$, $df = 6,53$, $p < .05$, $\eta^2 = 0.129$), and joint attention ($F = 8.802$, $df = 6,53$, $p < .01$, $\eta^2 = 0.181$)], demonstrating that infants with CBSF exhibited higher passivity/dependency qualities than those with NBSF (Table 2). BI composite score main effects were observed for the inactivity subfactor ($F = 4.636$, $df = 6,53$, $p < .05$, $\eta^2 = 0.092$) and the 'gazes at object' subfactor ($F = 13.187$, $df = 6,53$, $p < .001$, $\eta^2 = 0.223$). Gender, GA, and temperamental activity levels did not explain additional variance in this analysis.

Social engagement moderates BI risk

To examine the sensitive first months of life prior to BI stabilization, the role of early social engagement in the progression of neonatal CBSF, and the development of BI, a regression analysis was performed.

Table 3 Regression model predicting behavioral inhibition, using the neonatal auditory brainstem response (ABR) and early measures of social attention

	β	Sig. $F\Delta$	R^2
ABR	−.336	**.021**	.113
Gestation age	−.118	.414	.126
Face-to-face	.280	**.046**	.205
IBQ NA	.048	.730	.207

IBQ NA, Infant behavior questionnaire, negative affectivity scale (Rothbart, 1981); Face-to-Face, latency to engage in face-to-face interactions.

BI predictive factors included GA, neonatal ABR, latency to engage gaze in face-to-face interaction at 4 m, available for 92% of the cohort, and IBQ negative affect at 9 m. Results showed that ABR and latency to engage in face-to-face interaction contributed in predicting BI emergence, such that infants with CBSF and difficulty to engage in face-to-face interactions at 4 m were more likely to be diagnosed with BI at 1 year of age. Importantly, prematurity and negative affect were insignificant in this model, providing support for the second hypothesis (Table 3). Finally, the interaction between very low BW and CBSF, previously reported to affect social engagement at 4 months (Geva et al., 2011), did not affect the risk for BI at 12 m.

Discussion

Recent theoretical models highlight the potential role of neonatal brainstem input in mediating socioemotional regulatory abilities to secure adaptive psychiatric functioning (Geva & Feldman, 2008; Porges, 2010). Previous works describe a relationship between ABR latencies and introversion–extroversion tendencies in adults and in older children (Allan & Gilbert, 1997; Dix, Meunier, Lusk, & Perfect, 2012), but still little is known about the maturational trajectory of the ABR function and the relationship between its integrity at birth and temperamental traits early in development prior to their stabilization. Current findings, for the first time, draw a link between neonatal brainstem compromise in preterm infants, and the development of BI, shedding light onto the development of this

temperamental characteristic. These bear both theoretical and clinical/diagnostic implications.

Theoretically, these findings strengthen our understanding of the origins of BI and social anxiety. The framework examined in this study suggests a primary role for brainstem pathways early in the development of social stress regulation. Findings delineate a direct relationship between physiologic brainstem integrity at birth and BI risk through a prospective longitudinal follow-up study. Evaluating neonatal ABR at the very early age was fruitful in predicting BI risk, such that 66% of infants with neonatal CBSF were later clinically diagnosed with socioemotional deficits in the form of BI. Previous animal models demonstrated that medial brainstem input contributes to affective regulation (Merker, 2007; Panksepp, 2005). Findings may point to an evolutionary basis, providing evidence for the role of neonatal brainstem input in regulating socioemotional responses in human neonates.

To explore elements that may mediate the relationship between brainstem compromise and BI, we tested two factors: motor initiation and gaze regulation. Results noted a relationship between neonatal CBSF and difficulty in initiating regulatory behaviors. Such that children with CBSF did not initiate motor activity during socioemotional challenge (i.e., separation); and were less likely to initiate gaze activity independently to help alleviate a social stressor (i.e., reunion). Specifically, they remained passively inactive and hardly approached the door during separation to be closer to the parent or to increase the likelihood of being heard; and they showed difficulty initiating independent engagement with objects as a regulatory step to calm during the stressful separation. Upon reunion, children with CBSF showed a greater likelihood to engage in joint attention toward objects with their parent, rather than attend to objects independently. Findings highlight initiatory difficulties, specifically initiation of motor activity as a potential mediating mechanism of BI phenomenology. This deficit has not previously been associated with brainstem development in human infants. These findings may compliment frameworks that highlight the motivational avoidance component of BI (Davidson, 1992; Fox, 1991).

Passivity and dependency symptoms are characteristics of multiple psychopathologies, including major depression and chronic stress. These symptoms are themselves debilitating, yet, little is known about how they develop. The effect of CBSF on passivity/dependency presented in this study remained significant after controlling for well-established factors known to affect these tendencies, namely, BI (Rothbart et al., 2011), prematurity (Geva, Eshel, Leitner, Valevski, & Harel, 2006), activity levels (Calkins & Fox, 1992), and gender (Allan & Gilbert, 1997). Passivity has been thought of as a secondary reaction of children to maternal depression (Dix et al., 2012). The current finding, in a population of nondepressed mothers, offers a potential mechanism for passivity as a primary symptom rather than a secondary one that is directly related to infant's brainstem dysfunction. This may trigger further research on the role of brainstem pathways in other psychiatric diagnoses that involve anxiety and passivity.

The second factor linking brainstem and BI was that of gaze regulation. We previously showed that early brainstem dysfunction was related to difficulty in engaging in social face-to-face interactions early in development (Geva et al., 2011). Here, we examined how these difficulties in face-to-face gaze behavior at 4 m, and concurrent temperamental negative affectivity moderate the initial risk for BI. Results showed that CBSF accounted for the most variance in BI, while early gaze behavior in social interactions accounted for additional variance. This may suggest a brainstem-gaze regulation-BI path and attest to the importance of dyadic joint-attention activity as an effective self-regulation strategy for this risk group (Geva & Feldman, 2008).

This model also highlights the importance of late gestational age, at which time the brainstem develops. Importantly, neonatal brainstem susceptibility, and not low BW or prematurity, increased the risk for BI, highlighting the notion that BI does not result from immaturity at birth, but rather from a specific neural susceptibility at this fragile age. Moreover, findings also point to infancy as a sensitive period for moderating this susceptibility through early dyadic social encounters.

The current socioemotional findings pertained mostly to nonverbal social expressions (gaze, proximity, touch). Importantly, a verbal component may be considered. Deviant ABR were reported to predict impaired social interactions, particularly in the context of language disorders and a risk for autism (Cohen et al., 2013). Future study may explore further verbal outcome of neonatal CBSF.

Pending replication, two other potential clinical outcomes may be postulated. The first pertains to social anxiety. Current reports underscore parental anxiety and depression as important risk factors for BI. This project pinpoints increased risk for BI even in infants of healthy parents. In view of increased risk for social anxiety associated with BI, an exploration of childhood social anxiety may be warranted as these children mature (Allan & Gilbert, 1997; Rosenbaum et al., 1989). Finally, an increased risk for a mild autistic phenotype is conceivable, following reports of prolonged absolute ABR waves and interpeak latencies in young children with autism (Ari-Even Roth, Muchnik, Shabtai, Hildesheimer, & Henkin, 2011; Rosenhall, Nordin, Brantberg, & Gillberg, 2003).

Conclusion

This study extends current neurobiologic models of BI in four ways: First, the neural susceptibility involved in BI: The current findings shed light on the effects of neonatal CBSF on later psychiatric development, suggesting that neonatal brainstem functions canalize BI**.** Most previous BI research has concentrated on the HPA axis. This report demonstrates involvement of an understudied component, one that encompasses the brainstem in the development of BI. Second, age: Current data enable detection of BI during infancy using neonatal ABR. It proposes a biologic trajectory beginning with late-gestation brainstem development and leading to BI. Third, an understudied risk group: Most developmental work on BI has concentrated on infants born at term. The results introduce the possibility of a screening method that can be used in preterm neonates for detecting risk of later emerging BI. Fourth, underscoring early infancy as a sensitive period in moderating BI, specifically, pointing to dyadic regulatory skills as an effective factor to limit BI risk. Incorporating face-to-face gaze interaction in future interventions with infants at risk may support the development of mechanisms for coping with socioemotional challenges to reduce the risk of social anxiety within this population.

Acknowledgements

This project was partially funded by the Israel Science Foundation grant #1518 awarded to Ronny Geva. We thank the participating families, the medical team at the Sheba Medical Center Department of Neonatology and the members of the Developmental Neuropsychology lab at the Gonda Brain Research Center, particularly Lea Kurtzman and Hagit Yaron. Portions of this study were presented at the XVIII Biennial International Conference on Infant Studies, Minneapolis, June 2012; and at the Biennial meeting of the Society for Research in Child Development, Seattle, April 2013. The authors have declared that they have no potential or competing conflicts of interest.

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Key points

- Neonatal compromised brainstem functions increase the risk for behavioral inhibition.
- Neonatal brainstem compromise impedes the development of initiation abilities, resulting in passivity/dependency.
- Social engagement during the early months ameliorates effects of neonatal brainstem compromise and moderates the severity of the initial risk for BI.
- Early detection of brainstem dysfunctions may enable neonatal detection of behavioral inhibition risk in the preterm population.

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Accepted for publication: 11 November 2013
Published online: 28 December 2013