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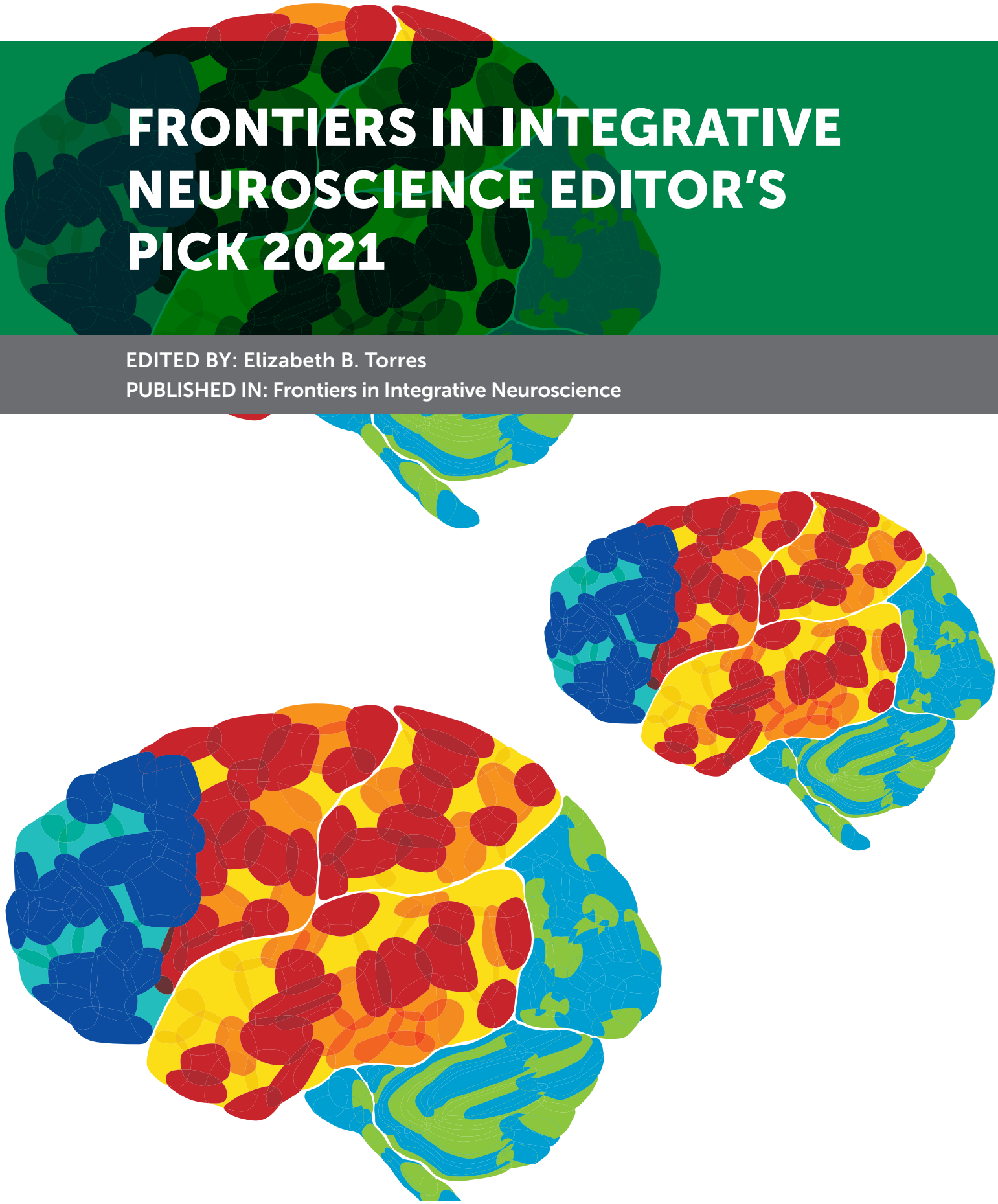

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FRONTIERS IN INTEGRATIVE NEUROSCIENCE EDITOR'S PICK 2021

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ORIGINAL RESEARCH
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White Matter Microstructure Associations of Cognitive and Visuomotor Control in Children: A Sensory Processing Perspective

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Objective: Recent evidence suggests that co-occurring deficits in cognitive control and visuomotor control are common to many neurodevelopmental disorders. Specifically, children with sensory processing dysfunction (SPD), a condition characterized by sensory hyper/hypo-sensitivity, show varying degrees of overlapping attention and visuomotor challenges. In this study, we assess associations between cognitive and visuomotor control abilities among children with and without SPD. In this same context, we also examined the common and unique diffusion tensor imaging (DTI) tracts that may support the overlap of cognitive control and visuomotor control.

Method: We collected cognitive control and visuomotor control behavioral measures as well as DTI data in 37 children with SPD and 25 typically developing controls (TDCs). We constructed regressions to assess for associations between behavioral performance and mean fractional anisotropy (FA) in selected regions of interest (ROIs).

Results: We observed an association between behavioral performance on cognitive control and visuomotor control. Further, our findings indicated that FA in the anterior limb of the internal capsule (ALIC), the anterior thalamic radiation (ATR), and the superior longitudinal fasciculus (SLF) are associated with both cognitive control and visuomotor control, while FA in the superior corona radiata (SCR) uniquely correlate with cognitive control performance and FA in the posterior limb of the internal capsule (PLIC) and the cerebral peduncle (CP) tract uniquely correlate with visuomotor control performance.

Conclusions: These findings suggest that children who demonstrate lower cognitive control are also more likely to demonstrate lower visuomotor control, and vice-versa, regardless of clinical cohort assignment. The overlapping neural tracts, which correlate with both cognitive and visuomotor control suggest a possible common neural mechanism supporting both control-based processes.

Keywords: DTI, cognitive control, visuomotor control, sensory processing dysfunction, attention

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INTRODUCTION

Historically, individual differences in cognitive control and visuomotor control have been studied as distinct processes, however growing evidence suggests that these two domains may be interrelated (Diamond, 2000; Rasmussen and Gillberg, 2000; Brandes-Aitken et al., 2018). Here we define cognitive control as the mental processes of attention, working memory and goal management (Anguera and Gazzaley, 2015) and visuomotor control as the processes by which an individual integrates visual-perception and fine motor coordination systems (Schultz et al., 1998). Research has demonstrated that both cognitive and visuomotor control are a fundamental for academic and socioemotional development (Davidson et al., 2006; Dziuk et al., 2007; Luna et al., 2010; MacDonald et al., 2013; Sumner et al., 2016).

With respect to the domain of cognitive control, attention is an especially important process that supports adaptive development (Davidson et al., 2006; Luna et al., 2010). The ability to focus on specific stimuli while ignoring distractions over sustained periods of time is necessary to effectively process, encode and retain relevant information from the environment (Zanto et al., 2011). These processes have particularly important implications in academic and social contexts (Kenworthy et al., 2009; Stevens and Bavelier, 2012). Furthermore, goal management is the cognitive foundation for goal-directed behavior such as planning and problem solving (Levine et al., 2000; Lustig et al., 2009). Likewise, visuomotor challenges have broad implications for everyday life, from deficiencies in handwriting (Fuentes et al., 2010; Kushki et al., 2011; Johnson et al., 2013; Rosenblum et al., 2016) to academic performance and self-perception (Feder and Majnemer, 2007; Cahill, 2009).

Cognitive control and visuomotor control deficits are common to many neurodevelopmental disorders (Pennington and Ozonoff, 1996; Kaiser et al., 2015), including those who have attention deficit/hyperactivity disorder (ADHD; American Psychiatric Association. Task Force on DSM-V, 2013) and developmental coordination disorder (DCD; American Psychiatric Association. Task Force on DSM-V, 2013). A high behavioral comorbidity of attention and visuomotor control deficits exists within ADHD and DCD populations (approximately 50%; Kadesjö and Gillberg, 1999; Pitcher et al., 2003), suggesting that these processes may be interrelated (Diamond, 2000; Anguera et al., 2010; Brandes-Aitken et al., 2018). Notably, individuals with broadly-defined sensory processing dysfunction (SPD; Ahn et al., 2004; Gowen and Hamilton, 2013; Craig et al., 2016), a disorder characterized by challenges with sensory modulation, discrimination and sensory-based motor challenges (Miller et al., 2007) also show overlapping challenges related to cognitive control and visuomotor control (Ahn et al., 2004; Anguera et al., 2017; Brandes-Aitken et al., 2018). The compelling possibility exists that abnormal sensory integration accompanies deficits in both cognitive control and visuomotor control, with shared neural underpinnings given the overlapping neural architecture that connects thalamic sensory centers to frontal cortical regions (Mori et al., 2008). Indeed, previous SPD research has demonstrated decreased integrity in sensory-rich thalamocortical tracts that correlate with parent-reported inattention (Owen et al., 2013) and proprioception abilities (Chang et al., 2016).

There are a number of compelling data-driven findings that would support the aforementioned hypothesis. To begin with, a frontal cortico-striatal-thalamic network has been suggested to support behavioral-inhibitory processes, including proactive cognitive and motor processes (Jahanshahi et al., 2015). Along those same lines, the prefrontal cortex and motor cortex are anatomically adjacent and share vast reciprocal interconnections, likely contributing to the observed phenotypic overlap (Barbas and Pandya, 1987; Burman et al., 2014). The use of distinct neuroimaging techniques [electroencephalography (EEG) and functional near-infrared spectroscopy (fNIRS)] has revealed patterns of neural coherence and activity within the prefrontal cortex during cognitive-motor tasks indicative of such frontal regions facilitating visuomotor performance (Hatakenaka et al., 2007; Gentili et al., 2013, 2015).

These findings agree with structural architecture work suggesting that integrity within specific neural tracts can predict cognitive control and visuomotor control abilities. More specifically, research has found that tracts which terminate in the frontal cortex are commonly associated with attention and executive functions (Luppino et al., 1993; Ashtari et al., 2005; Pavuluri et al., 2009; de Luis-García et al., 2015; Ursache and Noble, 2016). Interestingly, these frontal-related tracts also play a role in visuomotor control (Steele et al., 2012; Langevin et al., 2014). However, it should be noted that there are a number of other regions that have been associated with supporting each process. For example, posterior parietal and cerebellar regions are neural areas specifically associated with visuomotor abilities (Martin, 2005; Paulin, 2008; Zwicker et al., 2012a; Koziol et al., 2014; Song et al., 2015) while superior parietal regions are associated with higher-order cognitive abilities (Sylvester et al., 2003; Collette et al., 2006).

Based on these previous findings, we hypothesize that specific white matter tracts, identified using diffusion tensor imaging (DTI), would correlate with either cognitive control, visuomotor control, or both. For example, given that the anterior limb of the internal capsule (ALIC), anterior thalamic radiation (ATR), and superior longitudinal fasciculus (SLF) share connections within the frontal lobe, we would expect their tract integrity to correlate with performance on both cognitive control (Luppino et al., 1993; Ashtari et al., 2005; Pavuluri et al., 2009; de Luis-García et al., 2015; Ursache and Noble, 2016) and visuomotor control (Steele et al., 2012; Langevin et al., 2014) tasks. Conversely, integrity of the superior corona radiata (SCR), which has been associated with dual-task processing (Seghete et al., 2013), is predicted to show associations with cognitive control abilities (Koenigs et al., 2009; Stave et al., 2017). Finally, the integrity of the cerebral peduncle (CP), the posterior thalamic radiation (PTR), and the posterior limb of the internal capsule (PLIC), classically found to be associated with sensory and motor information

transmission (Martin, 2005; Paulin, 2008; Zwicker et al., 2012a,b; Koziol et al., 2014), is predicted to correlate only with visuomotor function.

Here, we were interested in exploring the interrelation of cognitive control and visuomotor control processes across children with SPD and typically developing controls (TDCs). Given that our previous work has already established group-based differences in attention, visuomotor control and fractional anisotropy (FA; see Owen et al., 2013; Chang et al., 2014, 2016; Anguera et al., 2017; Brandes-Aitken et al., 2018), this article aims to take a phenotypic-first approach to assess brain behavior-relations, more generally. To do so, we intentionally pooled both the SPD and TDC groups together in our analysis to optimize variable dispersion. In the current analysis, we first assessed the behavioral association between directly assessed cognitive control and visuomotor control performance. Second, on a subset of our participants that completed the neuroimaging portion of the study, we examined the neural networks associated with cognitive and visuomotor control performance using a common measure of white matter microstructure, FA. We hypothesized that: (i) cognitive control and visuomotor control performance would be associated with each other; (ii) white matter tracts that share connections with the frontal lobe would be associated with both cognitive control and visuomotor control; and (iii) white matter tracts that are primarily connected to superior parietal regions would correlate with cognitive control, whereas tracts which primarily connect the brainstem and posterior parietal regions would correlate with visuomotor control ability. Assessing control from a neural architecture vantage will clarify how and why these two control deficits often co-exist, and elucidate the underlying neural basis of these co-occurring control processes.

MATERIALS AND METHODS

Demographics

Participants were recruited from the UCSF Sensory Neurodevelopment and Autism Program (SNAP) clinic, SNAP research registry, and local online parent groups. We recruited a total of 62 participants between 8 years and 12 years of age: 37 children with SPD (16 female; age 10 ± 1.4 years) age and gender matched with 25 TDC children (12 female; age 10.5 ± 1.3 years). All participants successfully completed the visuomotor control battery, 35 SPD and 24 TDC children completed all cognitive control tasks, and 27 SPD and 16 TDC children provided usable DTI data for the current analysis. See **Table 1** for complete demographic information. This study was carried out in accordance with the recommendations of "APA Ethics Guidelines." The protocol was approved by the "UCSF Institutional Review Board."

TABLE 1 | Demographics.

	SPD (*N* = 37)	TDC (*N* = 25)
Age, M ± SD (range)	10 ± 1.4 (8.0–12.9)	10.5 ± 1.3 (8.0–12.8)
Gender, N		
Female	16	12
Male	21	13
Handedness, N		
Right	35	24
Left	2	1
IQ, M ± SD		
NVIQ	110.8 ± 16.0	115.3 ± 11.0
VIQ	116.0 ± 14.7	123.8 ± 11.0
Ethnicity, N		
Caucasian	24	14
Asian	0	2
African American	0	2
Mixed Ethnicity	12	2
Unknown	1	5

Screening Procedures

Inclusion criteria for the SPD cohort was based on the widely used sensory assessment, the Sensory Profile, a parent report questionnaire (Dunn, 1999). All children in the SPD cohort had a community diagnosis of SPD and a score on the Sensory Profile in the "Definite Difference" range (<2% probability) in one or more of the sensory domains (auditory, visual, oral/olfactory, tactile, vestibular, or multisensory processing). All children were administered The Social Communication Questionnaire (SCQ) to screen for any additional social communication challenge that might meet diagnostic criteria for Autism Spectrum Disorder (ASD). Any participant who scored above 15 points was administered the Autism Diagnostic Observation Schedule, Module 3 (ADOS; Lord et al., 1989) and excluded if they met ASD criteria on the ADOS. Two participants scored above SCQ threshold and were administered an ADOS in which they scored as non-spectrum and were subsequently included in our SPD group for analysis. One subject from the SPD group was excluded for scoring above 15 points on the SCQ and declining an ADOS assessment. Participants were assigned to the TDC group if they did not meet ASD cut-off on the SCQ, ADHD cut-off on the Vanderbilt (Wolraich et al., 2003) or SPD cut-off on the Sensory Profile. In addition, participants were included in the TDC cohort only if they did not have community neurological or psychiatric diagnoses.

Seven children in our SPD cohort were on medication for attention/impulsivity symptoms and/or emotional regulation, one child in each group was on medications for allergies. All children were required to be on a stable dose of prescribed medications for at least 6 weeks prior to participating. Exclusion criteria were premature delivery (<37 weeks), brain malformation or injury, movement disorder, bipolar disorder, psychotic disorder, hearing impairment, or Perceptual Reasoning Index (PRI) score <70 on the Wechsler Intelligence Scale for Children—Fourth Edition (Wechsler, 2003).

Cognitive Control Assessments

Selective/Sustained Attention

Test of Variables of Attention (TOVA)

We administered the test of variables of attention (TOVA; Greenberg et al., 1996) to assess sustained attention in our participants. The TOVA has demonstrated an estimated 85%

sensitivity as a predictor of ADHD (Schatz et al., 2001). It is a 23-min, fixed interval, visual continuous performance task administered on a laptop computer. Participants are instructed to respond to a visual stimulus (white square) appearing in the top edge (target stimuli) of the computer screen and to ignore the stimuli when it appears at the bottom edge (non-target stimuli) of the computer. The stimuli appear for 100 ms every 2 s. The assessment is broken up into two parts measuring sustained attention (target stimuli appears in 22% of trails) and impulsivity (target stimuli appears in 77% of trials). Here, we assessed response time (RT) from the sustained condition, in line with previous work using this measure in related populations (Anguera et al., 2017).

Goal Management

Project: EVO™ (EVO)—MULTI

Project: EVO™ (EVO) is proprietary software developed by Akili Interactive Labs, specifically designed as a medical device to assess cognitive and visuomotor control. EVO was developed from the principles of a previous cognitive intervention known as NeuroRacer (Anguera et al., 2013) but modified for iOS mobile compatibility. The EVO assessment includes three tasks: perceptual discrimination, visuomotor tracking, and multitasking by performing each aforementioned task simultaneously (Goal Management Task).

Here, we measured performance on the goal management task. In this assessment, the player simultaneously completes a perceptual discrimination task while also performing a visuomotor tracking task. In the perceptual discrimination component of the assessment, the user taps the iPad screen for correctly colored target stimuli while ignoring distracting targets. In the visuomotor tracking component of the assessment, the player navigates their EVO character through a dynamically moving environment by moving the iPad with the goal of avoiding the walls and obstacles. EVO incorporates adaptive psychometric staircase algorithms to ensure that comparisons between individuals reflect actual differences and not testing-based disparities. EVO changes its level of difficulty in a dynamic, trial-by-trial basis until the participant is performing at ∼80% rate of accuracy (Klein, 2001; Leek, 2001; García-Pérez, 2013). This approach also helps mitigate against any biases of age-related slowing, instrumentation, or ceiling/floor effects, thus finding an individualized level of performance that is specific to each child. The EVO assessment takes approximately 7 min. Here we specifically focused on performance during the multitasking condition to avoid redundancy with our other attentional measures. Here, we assessed EVO multitask RT in line with previous work (Anguera et al., 2017).

Visuomotor Assessments

Visuomotor Integration

Beery Visuomotor Integration (VMI)

We administered the Beery Visuomotor Integration (VMI; Beery and Beery, 2004) to assess coordinated visual perception and motor coordination abilities. The Beery VMI is a pencil and paper task requiring the participant to draw copies of 30 geometric forms which increase in complexity. A trained administrator scores the copied items as either correct or incorrect based upon the criteria listed in the Beery VMI Scoring Manual (Beery and Beery, 2004). A participant can score between 0 and 30 on this assessment.

Visuomotor Coordination

Beery Visuomotor Coordination

We administered the Beery VMI Motor Coordination Subtest (Beery and Beery, 2004) to assess fine motor control abilities. The Beery motor control subtest is a pencil and paper task requiring the participant to trace the interior of 30 geometric forms which increase in complexity, without crossing over the shape's border. A trained administrator scores the traced items as either correct or incorrect based upon the criteria listed in the Beery Motor Coordination Subtest Scoring Manual (Beery and Beery, 2004). A participant can score between 0 and 30 on this assessment.

Visuomotor Tracking

EVO—Navigation

As a part of the EVO assessment, participants complete a visuomotor tracking task requiring them to tilt the iPad to navigate their character through a dynamically moving road while avoiding walls and obstacles. Here we analyzed performance on the navigation only assessment to measure visuomotor performance without the added cognitive load of the simultaneous perceptual discrimination task (see "EVO—MULTI" section in Cognitive Control Methods).

Like EVO—Goal Management, EVO—Navigation also follows an adaptive algorithm by changing the level of steering difficulty, on a second-by-second basis, until the participant is performing at ∼80% rate of accuracy (Klein, 2001; Leek, 2001; García-Pérez, 2013). The final navigation score is calculated as a function of the number of seconds it takes the EVO character to move forward a single unit—this movement is stymied by hitting walls and obstacles while steering. The EVO-Navigation score (which reflects visuomotor tracking performance) is the primary metric of interest for this study.

Diffusion Tensor Imaging

DTI Acquisition

MR imaging was performed on a 3T Tim Trio scanner (Siemens, Erlangen, Germany) using a 12 channel head coil. Structural MR imaging of the brain was performed with an axial 3D magnetization prepared rapid acquisition gradient- echo (MPRAGE) T1-weighted sequence (TE = 2.98 ms, TR = 2,300 ms, TI = 900 ms, flip angle of 90°) with in-plane resolution of 1 × 1 mm on a 256 × 256 matrix and 160 1.0 mm contiguous partitions. Whole-brain diffusion imaging was performed with a multislice 2D single-shot twice-refocused spin echo echo-planar sequence with 64 diffusion-encoding directions, diffusion-weighting strength of b = 2,000 s/mm^2, iPAT reduction factor of 2, TE/TR = 109/8,000 ms, NEX = 1, interleaved 2.2 mm-thick axial slices with no gap, and in-plane resolution of 2.2 mm × 2.2 mm on a 100 × 100 matrix. An additional image volume was acquired with no diffusion weighting (b = 0 s/mm^2). The total diffusion acquisition time was 8.7 min. Structural MRI for all children was reviewed by PM, a board certified pediatric

neuroradiologist. No structural anomalies or other clinically significant findings were identified.

DTI Pre-processing

The diffusion-weighted images were corrected for motion and eddy currents using FMRIB's Linear Image Registration Tool (FLIRT1) with 12-parameter linear image registration (Jenkinson et al., 2002). All diffusion-weighted volumes were registered to the reference $b = 0$ s/mm^2 volume. To evaluate subject movement, we calculated a scalar parameter quantifying the transformation of each diffusion volume to the reference. Children were excluded from analysis if their brain imaging had artifact and/or median relative displacement between volumes greater than 2 mm, where a volume represents a single diffusion directional measurement of the entire brain. A heteroscedastic two-sample Student's *t*-test verified that there were no significant differences between the SPD and TDC cohort with respect to movement, during the DTI scan ($p > 0.05$). The non-brain tissue was removed using the Brain Extraction Tool (BET2). FA was calculated using FSL's DTIFIT at every voxel, yielding an FA map for each subject. Out of the original sample of 62 children, 44 were including in the final DTI analysis.

Region of Interest DTI Analysis

Tract-Based Spatial Statistics (TBSS) in FSL (Smith et al., 2006) was used to co-register and skeletonize the diffusion maps for each subject in order to perform voxel-wise comparisons along the white matter skeleton. First, each subject's FA map was non-linearly registered to each other subject's FA map to identify the most representative FA map as a registration target. The registered maps were then averaged and skeletonized to the center of the white matter. Next, each subject's FA data was projected onto this mean skeleton to obtain skeletonized FA maps per subject. Tract regions of interest (ROIs) were created according to The Johns Hopkins University (JHU) ICBM-DTI-81 White-Matter Labeled Atlas (Wakana et al., 2004). *A priori* ROIs were selected based upon existing literature suggesting white matter connections associated with cognitive and visuomotor control. ROI selection was restricted to the ALIC, the ATR, the CP, the PLIC, the PTR, the SCR, and the SLF. As right and left hemisphere ROI tracts were highly correlated ($r \geq 0.62$, $p \leq 0.001$), right and left tract FA values were averaged for each participant.

Statistics

Analyses were performed in the R environment (R Core Team, 2013). To minimize highly influential data points, we removed values ± 3 SD away from the total mean in our behavioral assessments. Under this threshold, one individual was removed from the selective/sustained attention task and one individual was removed from the VMI task. To assess global cognitive control and visuomotor control, we generated composite scores that incorporate performance from each individual test into their functional domain. We first transformed scores from each direct assessment to z-scores (mean = 0, SD = 1 to keep scaling uniformed) using all participants' data. Cognitive control composite scores were constructed by averaging z-scores calculated from the RT of the TOVA and EVO Goal Management Tasks. The cognitive control z-scores were reverse scored such that higher values were associated with better performance. The visuomotor composite score was constructed for each child by averaging z-scores calculated from the Beery VMI and EVO Navigation total scores. To assess for relationships between behavioral performance and DTI we constructed general linear model (GLM) regressions for each DTI tract including cohort as a factor to control for any confounding cohort effects. *P*-values for each set of regressions correlations were corrected for multiple comparisons using False Detection Rate (FDR; Benjamini and Hochberg, 1995) methods at a *p*-value threshold of $p \leq 0.05$.

RESULTS

Group Differences

To assess for cohort differences in demographics, we constructed *t*-tests for age and nonverbal IQ differences and chi-square difference tests for gender. Results demonstrated that the SPD and TDC cohorts did not significantly differ in age ($t_{(60)} = -1.43$, $p = 0.16$) nonverbal IQ ($t_{(58)} = -1.21$, $p = 0.23$), or gender ($\chi_{(1)} = 0.0$, $p = 1.0$). Because there were no group-based demographic differences, we did not include these variables in subsequent analyses. We also constructed *t*-tests for cognitive control composite and visuomotor composite scores to test for group-based differences. Results indicated that the SPD group scored significantly lower than the TDC group in both cognitive control ($t_{(57)} = -2.48$, $p = 0.016$) and visuomotor control ($t_{(60)} = -2.82$, $p = 0.006$). Given the group differences which exist for cognitive and visuomotor control, we included cohort assignment as a covariate in all further analyses.

Behavioral Correlations

To assess whether performance on the cognitive control composite was associated with performance on the visuomotor control composite we constructed GLM regressions, controlling for cohort. Results demonstrated a significant, positive correlation between cognitive control and visuomotor control ($t_{(56)} = 3.46$, $p = 0.001$, $\beta = 0.41$; see **Figure 1**), after accounting for cohort effects.

Neural Correlations

To assess for associations between our behavioral measure of cognitive and visuomotor control and FA values in white matter tracts of interest, we analyzed GLM regressions (controlling for cohort effects) first between composite scores (see **Table 2**) and secondarily within each cognitive and visuomotor task (see **Table 3**).

Results from this analysis revealed significant, positive associations between the cognitive control and visuomotor control composite scores and multiple DTI FA tracts (see **Figure 2**) including the anterior thalamic tracts of the ALIC and ATR and the long association fibers of the SLF. White matter microstructure of the SCR was positively associated with cognitive control performance and FA in the CP and PLIC was

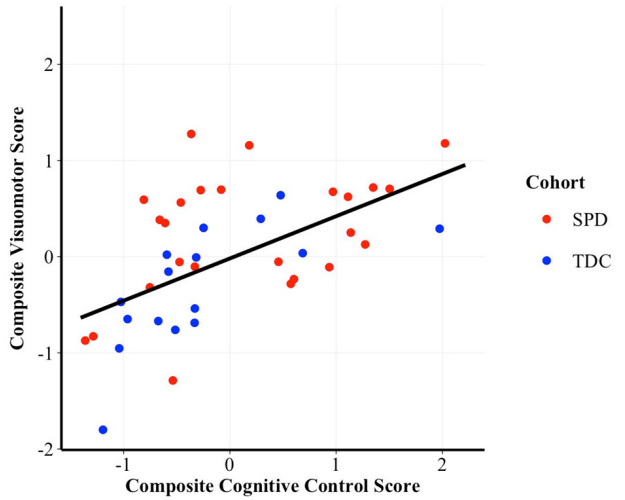

FIGURE 1 | Scatterplot of the correlation between cognitive control and visuomotor control composite scores. Higher scores are indicative of better performance. $\beta = 0.41$, $p = 0.001$. SPD, Sensory Processing Disorder; TDC, Typically Developing Controls.

positively correlated with visuomotor control performance. The white matter microstructure of the PTR did not significantly correlate with either cognitive or visuomotor control composite scores.

The sustained/selective attention task (TOVA) showed significant, positive associations with FA in the ALIC and ATR and trend level positive associations with FA in the SCR and SLF. Similarly, the goal management task (EVO—MULTI) demonstrated significant positive associations with the ALIC, ATR, SCR and the SLF. In the visuomotor coordination domain, results demonstrated a significant relation between performance on the VMI task (Beery VMI) and FA in the ALIC, ATR, CP and PLIC and a trending relation with FA in the SLF. The visuomotor coordination (Beery Motor Subtest) and the visuomotor tracking task (EVO—NAV) did not demonstrate any significant brain-behavior associations.

TABLE 2 | Association between composite cognitive and visuomotor control tasks and white matter tract integrity.

	Cognitive control (β ± Std. Error) *N* = 42	Visuomotor control (β ± Std. Error) *N* = 44
ALIC	0.41 ± 0.14*	0.42 ± 0.13*
ATR	0.40 ± 0.14*	0.41 ± 0.13*
CP	0.19 ± 0.15	0.30 ± 0.14*
PLIC	0.17 ± 0.15	0.38 ± 0.13*
PTR	0.25 ± 0.16	0.23 ± 0.15
SCR	0.36 ± 0.15*	0.20 ± 0.15
SLF	0.40 ± 0.14*	0.38 ± 0.14*

*β value regression coefficients are reported to assess for strength of the relationship after controlling for cohort assignment. ALIC, Anterior Limb of the Internal Capsule; CP, Cerebral Peduncle; PLIC, Posterior Limb of the Internal Capsule; PTR, Posterior Thalamic Radiation; SCR, Superior Corona Radiata; SLF, Superior Longitudinal Fasciculus. *Indicates FDR adjusted p-value ≤ 0.05 for regression co-efficient.*

DISCUSSION

This study aimed to explore the domains of cognitive and visuomotor control across children with and without SPD. The main objective of this study was to assess the existence of a common set of control-related neural tracts which may support both cognitive and visuomotor control processes. Results from this study indicate that, indeed, cognitive control and visuomotor control are behaviorally associated, and have shared and divergent neural tracts that contribute to the variability in this relationship. This finding is valuable given that both cognitive control and visuomotor control are predictive of academic readiness (Cameron et al., 2012); thus targeting these control deficits with interventions and utilizing brain based metrics for assessing change will be instrumental in ongoing work to support children with neurodevelopmental challenges.

Cognitive Control and Visuomotor Control Associations

The outcomes from our behavioral regressions indicate that there is a relationship between cognitive and visuomotor control, such that children who struggle in one domain are more likely to struggle in the other, regardless of cohort assignment. These findings add to a growing literature positing a concordant cognitive and visuomotor control behavioral model (Diamond, 2000; Brandes-Aitken et al., 2018). Another possibility which should be considered is that cognitive control and visuomotor control are not interrelated, but that children with impaired attention and motor control are more globally impaired in all domains. However, we find this to be an unlikely explanation given that children with neurodevelopmental disorders often show unique phenotypic profiles such that a child who struggles in some domains are not necessarily burdened in all domains (Simonoff et al., 2008; Wåhlstedt et al., 2009).

Common and Distinct Neural Elements of Cognitive and Visuomotor Control

To further substantiate the framework of a shared control system network supporting both visuomotor and cognitive control, we constructed GLM regressions controlling for cohort effects between our *a priori* DTI tracts and behavioral performance on direct assessments. These regression analyses revealed a common set of neural tracts, the ALIC, ATR, and SLF that supported performance on both cognitive control and visuomotor control composite scores. Thus, we propose that these fibers compose a putative set of control-related neural tracts, capable of supporting both cognitive control and visuomotor control.

This control network consists of two frontothalamic projection tracts, the ALIC and ATR, and a long cortical association tract, the SLF. The frontothalamic contribution to visuomotor functioning, can be interpreted from multiple perspectives. First, the ALIC and ATR largely subsume fibers to the prefrontal cortex, an area which enables us to store items in our working memory (Curtis and D'Esposito, 2003; Funahashi, 2006; Zanto et al., 2011), cognitively manipulate information (Rougier et al., 2005; Kim et al., 2011), inhibit

TABLE 3 | Associations between individual cognitive and visuomotor control tasks and white matter tract integrity.

	Selective/sustained attention (*n* = 40)	Goal management (*n* = 39)	Visuomotor integration (*n* = 43)	Visuomotor control (*n* = 44)	Visuomotor tracking (*n* = 40)
ALIC	0.43 ± 0.15*	0.44 ± 0.15*	0.43 ± 0.14*	0.33 ± 0.15	0.09 ± 0.15
ATR	0.42 ± 0.15*	0.37 ± 0.15*	0.40 ± 0.14*	0.35 ± 0.15	0.08 ± 0.15
CP	0.24 ± 0.16	0.18 ± 0.16	0.37 ± 14*	0.15 ± 0.16	0.11 ± 0.15
PLIC	0.17 ± 0.16	0.19 ± 0.16	0.49 ± 0.13*	0.19 ± 0.15	0.11 ± 0.15
PTR	0.24 ± 0.17	0.15 ± 0.17	0.26 ± 0.16	0.28 ± 0.16	−0.06 ± 0.16
SCR	0.33 ± 0.15^	0.37 ± 0.15*	0.07 ± 0.16	0.05 ± 0.16	0.31 ± 0.14
SLF	0.35 ± 0.15^	0.43 ± 0.15*	0.27 ± 0.15^	0.28 ± 0.15	0.21 ± 0.15

*β value regression coefficients are reported to assess for strength of the relationship after controlling for cohort assignment. ALIC, Anterior Limb of the Internal Capsule; CP, Cerebral Peduncle; PLIC, Posterior Limb of the Internal Capsule; PTR, Posterior Thalamic Radiation; SCR, Superior Corona Radiata; SLF, Superior Longitudinal Fasciculus. Attention is assessed with the TOVA, goal management is assessed with EVO-MULTI, visuomotor integration is assessed with the Beery VMI, and visuomotor tracking is assessed with EVO-Nav. *Indicates FDR adjusted p-value ≤ 0.05 for regression co-efficient. ^Indicates FDR adjusted p-value ≤ 0.10 for regression co-efficient.*

inappropriate responses (Madsen et al., 2010; Sharp et al., 2010), and attend to relevant information (Desimone and Duncan, 1995; Schafer and Moore, 2011; Squire et al., 2013). It is plausible that visuomotor control relies upon these frontal lobe mechanisms (Diamond, 2000). Similarly, the prefrontal cortex shares neural pathways to the premotor cortex (Barbas and Pandya, 1987; Burman et al., 2014) and pre-supplementary motor area (pre-SMA; Luppino et al., 1993; Tanji, 1994), both of which are included in the frontal lobe. The pre-SMA is thought to be involved in movement preparation, coordination, and decision making (Matsuzaka et al., 1992; Nachev et al., 2008; Wilson et al., 2014) and the premotor cortex is believed to support and prepare for sensory guided movement (Passingham, 1985; Chouinard and Paus, 2006). Therefore, the inter-wirings between the prefrontal cortex, pre-SMA, and premotor cortex are likely explaining some of the overlap in behavioral challenges between cognitive and visuomotor control previously reported in children with sensory processing and neurodevelopmental challenges (Brandes-Aitken et al., 2018).

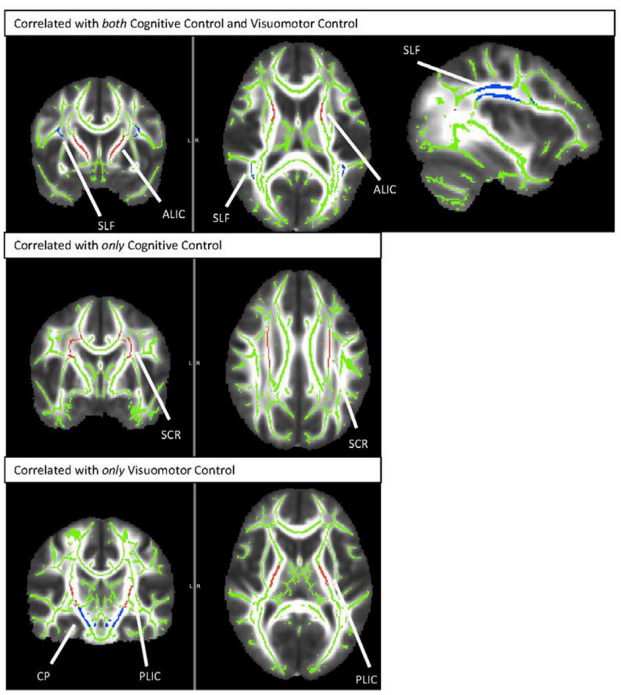

FIGURE 2 | TBSS rendering of the tracts demonstrating correlations with cognitive control and/or visuomotor control. SLF, Superior Longitudinal Fasciculus; ALIC, Anterior Limb of the Internal Capsule; SCR, Superior Corona Radiata; CP, Cerebral Peduncle; PLIC, Posterior Limb of the Internal Capsule.

The SLF, which bridges the aforementioned frontal regions to the parietal regions, has historically predicted attention (Pavuluri et al., 2009; Cortese et al., 2013; de Luis-García et al., 2015) and visuomotor control (Langevin et al., 2014; Biotteau et al., 2016) in separate studies. Here, we demonstrate a similar effect but augment this finding in the literature by assessing both domains in the same sample of children, to more clearly compare these processes. To further understand why this set of neural tracts is associated with performance on both cognitive control and visuomotor control, it is important to consider their relationship from a neurocognitive perspective. It could be posited that the ALIC and ATR relay incoming sensory information through the thalamus to the frontal cortex (including the prefrontal, supplementary motor and premotor cortex) and from there, the SLF bridges connections to the primary motor cortex and posterior cortical regions which have historically been associated with visuomotor functioning (Steele et al., 2012; Sripada et al., 2015; Biotteau et al., 2016).

While our analyses revealed shared white matter tracts which correlated with both control processes, we also discovered some tracts which were uniquely associated with either cognitive control or visuomotor control. Specifically, our regression analysis demonstrated an association between visuomotor control and the CP, a tract which includes fibers from the corticospinal tract to the internal capsule, and the PLIC, a region which conducts sensory input from the thalamus to the posterior cortex and back to the CPs. In the context of visuomotor control, both pathways are often cited as critical to supporting coordinated motor function (Martin, 2005; Paulin, 2008; Zwicker et al., 2012a; Koziol et al., 2014; Song et al., 2015). Likewise, cognitive control performance was found to be associated with white matter microstructure in the SCR which connects the internal capsule to frontal-parietal cortical regions. Given that the cognitive control composite score incorporated multiple domains of attention and executive

function, it is likely that both frontal and parietal regions support assayed performance (Sylvester et al., 2003; Collette et al., 2006). The PTR, which was included in our tracts of interest group, did not correlate with either cognitive or visuomotor control. It is possible that the lack of association suggests that although this tract is important for some aspects of sensory processing, it is not critical to cognitive or visuomotor control in children.

Within each individual task, the TOVA assessment of sustained/selective attention showed trend-level associations with FA in the ALIC and ATR, which is in line with the vast majority of literature connecting attentional control to the frontal cortex (Desimone and Duncan, 1995; Knight et al., 1995; Dimitrov et al., 2003; Sharp et al., 2010; Schafer and Moore, 2011). In addition, results demonstrated that FA in the ALIC, ATR, SCR and the SLF were associated with goal management abilities. Goal management processes draw on higher-order cognitive control and perceptual reasoning skills (Levine et al., 2000; Salthouse, 2005; Anguera and Gazzaley, 2015). Given that successful goal management depends on multiple interacting cognitive domains, it is likely supported by more wide spread cortical and sub-cortical regions including thalamo-frontal and parietal tracts (Herath et al., 2001; Nebel et al., 2005; Collette et al., 2006).

In the visuomotor domain, our results demonstrated that VMI performance was significantly associations with FA in the ALIC, ATR, the PLIC, and the CP. These findings are consistent with the literature connecting white matter integrity in the CP and PLIC to motor control output (Martin, 2005; Paulin, 2008; Koziol et al., 2014). Both the CP and PLIC are subsumed by fibers that connect the primary motor cortex with the cerebellum which is believed to support smooth motor execution (Shibasaki et al., 1993; Sanes and Donoghue, 2000; Wing, 2000; Chouinard and Paus, 2006). The significant association between VMI and white matter microstructure of the ALIC and ATR, which projects to frontal regions, suggests that the same frontal-dependent control abilities that support attention and inhibitory control may also support visuomotor control. Moreover, the close anatomical proximity and neural interconnections between the prefrontal cortex and the premotor and supplementary motor cortex highlight importance of frontal terminating white matter fibers in supporting visuomotor abilities. These findings suggest that evaluations of dyspraxia could benefit from the inclusion of cognitive control measures in addition to motor assessments to guide essential elements of remediation (Smits-Engelsman et al., 2001; Furuya et al., 2015). Surprisingly, the visuomotor coordination and visuomotor tracking assessment did not show significant correlations with the selected ROIs. The visuomotor coordination task followed similar directionality to the VMI task, but failed to reach significance. This could suggest that it is the integration aspect over the coordination component of visuomotor abilities that are supported by the selected tracts. Alternatively, given our relatively small sample size and increased variability within the visuomotor coordination task, our regressions may have been underpowered to detect true associations. Moreover, this visuomotor tracking task is substantially different from the latter two tasks and requires individuals to visually track a dynamic stimulus while integrating feedback to correct their performance. The possibility exists that these complex processes are not captured by structural white matter integrity within the tracts analyzed. Moreover, previous literature studying visuomotor tracking have primarily demonstrated associations using functional imaging methods (Brown et al., 2004; Grafton et al., 2008; Kashiwagi et al., 2009), which may capture its variability over neural architecture.

Collectively, the observed brain-behavior associations within this sample of children with rich variability in sensory, attention, and motor domains, suggest one possible theory of a unified neural network that supports and integrates sensation/perception processes with inhibition-based cognitive and visuomotor abilities. Specifically, the reported white matter microstructural findings are in line with previous research identifying a frontal cortico-thalamic circuit that is involved in both goal-directed cognitive and motor control (Jahanshahi et al., 2015). The ATR, ALIC, and SLF constitute an important part of the structural architecture within this circuit that connects the thalamus to various regions within the frontal cortex. Within this network, the striatum, thalamus and subthalamic nucleus, mediate connections from the basal ganglia (an inhibitory control center) to various regions within the frontal cortex (including prefrontal and motor regions). This unifying inhibitory-based neural framework provides a neural explanation for the concurrent overlap observed between sensory processing integration abilities and cognitive control and visuomotor control processes. Given that the sample composition within this study has allowed for optimal variation within the domains of sensory processing, cognitive control and visuomotor control, we were offered a unique opportunity to investigate the sensory integration origins of cognitive and visuomotor control overlap. While these findings offer preliminary support of this idea, future experimental research is needed to confirm whether distinct structural and functional networks underlie observed comorbid cognitive, visuomotor and sensory modulation challenges in children with neurodevelopmental disorders.

This study offers a biological explanation for why there is strong but not absolute concordance between ADHD and DCD (Rasmussen and Gillberg, 2000). Further research investigating the theory of a synchronized control system is warranted to inform future invention research. Specifically, findings from the current study offer preliminary evidence that interventions targeting visuomotor control networks could have a positive influence on cognitive control processes, and vice-versa. Existing cognitive control intervention studies have demonstrated some promising outcomes in children that extend to benefits in academic performance and intellectual abilities (Shalev et al., 2007; Jaeggi et al., 2011; Cortese et al., 2015). Similarly, visuomotor control-related intervention efforts have been explored in occupational therapy research trials with subjects showing promising improvements in multiple domains including, sensory experiences, academic achievement and motor-coordination abilities (Humphries et al., 1990; Mandich et al., 2001; Polatajko and Cantin, 2005). Furthermore, research using an integrated attention and visuomotor training program

has demonstrated pronounced effects on both cognitive and visuomotor domains in children (Anguera et al., 2017) and older adults (Anguera et al., 2013). Collectively, childhood remediation efforts aimed at both cognitive and visuomotor control may have the potential to support a positive developmental trajectory.

LIMITATIONS

While these findings offer new information to the literature, there are several limitations to this study that merit further investigation. First, we are limited by our relatively small sample size. This study would benefit by increasing our sample of children with SPD given that we could conduct regressions within each group instead of across groups which would minimize the group heterogeneity. Furthermore, many of these interpretations would be better supported following the use of temporally-locked functional neuroimaging research (e.g., EEG or fNIRS) to directly test these claims. Finally, current efforts are limited by lack of ecological validity and would be improved by including classroom-based measures of correlates of cognitive and visuomotor control (i.e., teacher reports, academic scores). Bridging this research from the lab to a school-based environment is a critical next step to inform intervention methods and benefits.

CONCLUSION

In summary, the current study demonstrates behavioral overlap of cognitive and visuomotor control across children with and without sensory process dysfunction. Further, the outcomes from these results support a proposed set of common neural white matter tracts which explain the strong but not complete concordance between cognitive and visuomotor control. This work emphasizes the importance of assessing children with neurodevelopmental disorders with these overlapping control abilities in mind rather than treating diagnostic labels as standalone conditions.

AUTHOR CONTRIBUTIONS

AB-A contributed to the data curation, formal analysis, and writing of the manuscript. JA contributed to the study conceptualization, data curation, methodology, and writing of the manuscript. Y-SC contributed to the methodology and data processing. CD contributed to the analysis and writing of the manuscript. JO contributed to the data processing, visualization, and supervision of analysis. AG contributed to study conceptualization and methodology. PM contributed to the study conceptualization and study supervision. EM contributed to the study conceptualization, investigation, funding acquisition and writing of the manuscript.

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The Effect of Dual Task on Attentional Performance in Children With ADHD

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Attention-deficit/hyperactivity disorder (ADHD) is a common psychiatric disorder without validated objective markers. Oculomotor behavior and executive motor control could potentially be used to investigate attention disorders. The aim of this study was to explore an oculomotor and postural dual task in children with ADHD. Forty-two children were included in the study, gathering children with ADHD (n = 21) (mean 8.15 age ± years 0.36) and sex-, age-, and IQ-matched typically developing children (TD). Children performed two distinct fixation tasks in three different postural conditions. Eye movements and postural body sway were recorded simultaneously, using an eye tracker and a force platform. Results showed that children with ADHD had poor fixation capability and poor postural stability when compared to TD children. Both groups showed less postural control on the unstable platform and displayed more saccades during the fixation task. Surprisingly, in the dual unstable platform/fixation with distractor task, the instability of children with ADHD was similar to that observed in TD children. "Top-down" dys-regulation mediated by frontal-striatal dysfunction could be at the origin of both poor inhibitory oculomotor deficits and impaired body stability reported in children with ADHD. Finally, we could assume that the fact both groups of children focused their attention on a secondary task led to poor postural control. In the future it could be interesting to explore further this issue by developing new dual tasks in a more ecological situation in order to gain more insight on attentional processes in children with ADHD.

HIGHLIGHTS

- Children with ADHD showed poor fixation capability when compared to TD children.
- "Top-down" dys-regulation mediated by frontal-striatal dysfunction could be at the origin of both poor inhibitory oculomotor deficits and impaired body stability reported in children with ADHD.

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- Both groups of children focused their attention on the visual fixation task leading to poor postural control.
- In the future it could be interesting to develop new dual tasks in an ecological situation in order to gain more insight on attentional processes in children with ADHD.

Keywords: eye movements, postural control, dual-task, cerebellum, fixations identification

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) consists of several abnormal patterns such as inattention, hyperactivity and impulsivity. In Western countries 5% of children are affected by this disorder (American Psychiatric Association, 2013).

In the literature it has been shown that children with ADHD present several deficits in executive motor control and inhibitory abilities (Willcutt et al., 2010). Motor control deficit could be related to a deficit in the integration and processing of different sensory inputs and of visual and vestibular inputs (Wang et al., 2003). A smaller cerebellar vermis which is implicated in the processing of sensory information, as well as in postural control, has been associated to ADHD disorder (Castellanos et al., 1996).

Several studies showed also alterations in the thalamus (Ivanov et al., 2010) and in the cerebellum (Valera et al., 2007) of ADHD patients. Moreover Durston (2003) showed in an fMRI study that children with ADHD presented abnormalities in morphology and functioning of frontostriatal brain circuits. More recently, Hong et al. (2017), using resting state fMRI, studied age-related brain network differences between ADHD patients and typically developing subjects (TD). These authors showed an atypical development in ADHD patients' brain regions, particularly of left middle temporal gyrus, left inferior frontal gyrus and left insular gyrus. Recall that all these cortical and subcortical networks are involved in performing eye movements and (Leigh and Zee, 2015); indeed poor eye movements performance and inhibitory capabilities had been observed in children with ADHD (see review from Rommelse et al., 2008).

In the domain of eye movements, a fixation task, with or without distractors, involves a strong inhibitory control mechanism. It consists in the ability to maintain an image on the fovea in order to perceive it. It had been also demonstrated that active visual fixation involves a distributed circuitry including frontal eye fields (Goldberg et al., 1986), posterior parietal cortex (Mountcastle et al., 1981; Shibutani et al., 1984), and brain stem structures (Munoz and Wurtz, 1992). Moreover when a distractor is introduced, the activity of the visual fixation system is reduced and the difficulties to maintain engagement to the previous location of the fixation increase (Reilly et al., 2008). Based on these findings, in the present study we wonder to explore further eye movements control, particularly the inhibition capabilities of children with ADHD by testing two different types of fixations calling for different attention load: a simple fixation and a more complex fixation with distractors. Recall that the response inhibition task is known to be a specific task able to discriminate between subjects with ADHD and TD subjects (Tamm et al., 2004), consequently we could expect to find a different oculomotor behavior between ADHD and TD children.

Our aim was to explore postural stability during a dual task (fixation and postural capability) in a group of children with ADHD compared to control children. Several studies from ours and other research groups reported that postural stability is not under an automatic control but it can be influenced by attentional resources, particularly when the secondary task is a complex task (Blanchard et al., 2005; Palluel et al., 2010; Legrand et al., 2013). More recently, our group reported poor postural control during fixation as well as poor quality of fixation itself in children with ADHD (Bucci et al., 2014). However the quality of fixation during simple sitting condition as well as the quality of fixations during a complex fixation with distractor task has not been studied.

It is well known that since attention is involved in the execution of eye movements (Rizzolatti et al., 1987; Deubel and Schneider, 1996) as well as in postural stability (Woollacott and Shumway-Cook, 2002), oculomotor and postural systems are mutually influenced; however, in children with ADHD such relationship could be different given to their poor inhibition/attentional capabilities (Bush, 2010). Indeed, in line with the U-shaped non-linear interaction model of Huxhold et al. (2006) the secondary task executed during a postural task could affect in a different way postural stability that is increasing or decreasing postural sway depending on the complexity of the secondary task. Based on this model, we expect to find an effect of the secondary oculomotor task (fixation task) on postural parameters. Such effect could be different in children with ADHD with respect to TD children according to the amount of attention focused on the secondary oculomotor task. In other way, when the secondary oculomotor task demands less attentional resources (i.e., simple fixation task) we could observe in both children groups tested similar postural as well as oculomotor performance; in contrast, when the secondary oculomotor task is more difficult (i.e., fixation with distractor task) it demands an high attentional load that could decrease postural performance more in children with ADHD than in TD children.

MATERIALS AND METHODS

Participants

Clinical Characteristics

Twenty-one children with ADHD (mean 8.15 age ± years 0.36) and twenty-one IQ- and age-matched typically developing children (TD) children (mean age 8.68 ± years 0.34) were

recruited at the Child and Adolescent Psychiatry Department, Robert Debré Hospital (Paris, France).

All subjects were evaluated by trained child psychiatrists. The diagnosis of ADHD according to DSM-5 criteria (American Psychiatric Association, 2013) was carried out using the Kiddie-SADS-EP (Goldman et al., 1998). During the general interview related to diagnosis, the presence of psychiatric comorbidities was systematically screened for. The severity of the ADHD symptoms was estimated by the ADHD Rating Scale-parental report (ADHD-RS). All children with ADHD were also assessed using the Wechsler scale (Wechsler Intelligence Scale for Children, fourth edition), the Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI, Beery, 1997) and the Motor Assessment Battery for Children (MABC) (Henderson and Sugden, 1992).

To confirm the absence of ADHD, controls were systematically interviewed. In order to be included in our study, controls needed to have a total score $\leq$10 on the ADHD-RS (Dickson et al., 2011) and a neurological examination in the normal range.

Moreover IQ was evaluated in controls using two subtests, one related to verbal ability (the similarities test) and the other related non-verbal abilities (matrix reasoning test).

The score on these two tests was not significantly different between the two groups of children. The clinical characteristics of children with ADHD and controls are reported in **Table 1**.

The investigation adhered to the principles of the Declaration of Helsinki and was approved by our Institutional Human Experimentation Committee (Comité de Protection des Personnes CPP, Ile de France V, Hôpital Saint-Antoine).

After the nature of the procedure had been explained, a written informed consent was obtained from the participants and their parents.

Visual Conditions

All children performed two visual tasks paradigms. In the first one, named *simple fixation* (see **Figure 1A**), the child was invited to fixate on the target appearing in the center (filled white circle subtending a visual angle of 0.5°) of the black screen during 30 s. In the second one, named *fixation with distractors* (see **Figure 1B**), the child had to maintain fixation on the central target and to inhibit saccades toward the distractors. The distractor was a white smile target (of 0.5°) appearing for a random duration from 500 to 2000 ms and calling for horizontal saccade amplitudes from 5° to 20°. The distractor was presented during the *fixation with distractor* trail (30 s), in total 8 distractors were presented during each *fixation with distractor* trial. Instructions were given to the child to try to fixate the central target as better as possible (in the *simple fixation* task) and to try to fixate the central target as better as possible without looking the distractors (in *the fixation with distractor* task).

TABLE 1 | Clinical characteristics of ADHD children and typically developing children (TD) tested.

	TD *N* = 21	ADHD *N* = 21
Clinical data		
Age (years)	8.68 ± 0.34	8.15 ± 0.36
ADHD-RS		
ADHD-RS total score	4 ± 0.8	37 ± 2
ADHD-RS inattention subscore	–	17.9 ± 1.4
ADHD-RS hyperactivity/impulsivity subscore	–	18.3 ± 1.7
Wechsler scale (WISC-IV) scores		
Verbal Comprehension subscale	–	99.1 ± 3.3
Perceptual Reasoning subscale	–	93.6 ± 3.5
Working Memory subscale	–	86.6 ± 3.0
Processing Speed subscale	–	90.9 ± 2.7
Similarity test	10.06 ± 0.4	9.9 ± 0.6
Matrix reasoning test	10.14 ± 0.5	9.7 ± 0.5

Postural Conditions

The experimental sessions took place in a dark room. Three postural conditions were performed (see **Figure 1**).

Simple sitting condition: Children were asked to sit in a comfortable chair. Their head was stabilized by a forehead and chin support while their eye movements were recorded.

Complex standing on stable platform condition: Children were asked to stand upright on the Framiral® stable platform with their arms along their body and their feet on the footprints.

Complex standing on unstable platform condition: Children were asked to stand upright on the Framiral® unstable platform with their arms along their body and their feet on the footprints.

Eye movements and body sway were recorded simultaneously.

For these three postural conditions, the PC monitor, in which the two visual tasks were presented, was placed 60 cm away and adjusted at each child's eye level (see **Figure 1**). Each visual task (*simple fixation* and *fixation with distractor*), respectively for each of the three postural conditions tested was performed twice, with the order of each trial defined randomly. The number of saccades made during fixation task and the postural measures from the same visual and postural conditions tested for each child were averaged.

Eye Movement Recording

Fixation performance was recorded by the Mobile EBT Tracker (SuriCog), a CE-marked medical eye-tracking device. The Mobile EBT is equipped with cameras that capture the movements of each eye independently. The frequency of recording was set up to 300 Hz and system precision was 0.25°. There was no obstruction of the visual field with this recording system.

Postural Recording

The excursions of the center of pressure (CoP) were recorded with Multitest Equilibre (Framiral®, Grasse, France), also called Balance Quest. This device consists in a platform which can be stable or unstable. In case of unstable condition the platform can be moved by oscillations allowing forward and backward translations, with a constant linear velocity which may vary from 0.03 to 0.07 m/s with a frequency of 0.25 Hz.

The displacement of the CoP was sampled at 40 Hz and digitized with 16-bit precision. Postural recording

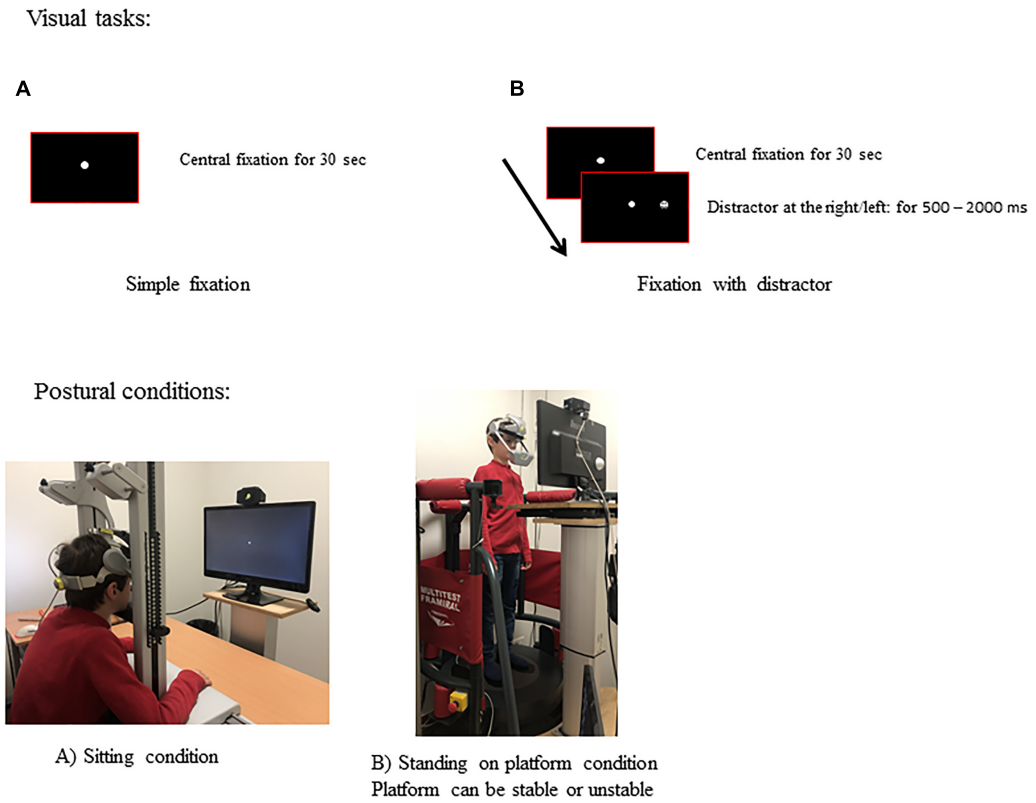

FIGURE 1 | Experimental set-up. Visual tasks and postural conditions used. Temporal arrangement of *simple fixation* **(A)** and of *fixation with distractor* **(B)** task. Example of a child in sitting condition **(A)** and in standing on platform condition **(B)**. Note that in standing on platform condition, than platform was stable and unstable (it was moved by oscillations allowing forward and backward translations).

was performed in stable and unstable platform conditions. The duration of each postural condition was of 30 s. Visual task started at the same time of postural recording lasting 30 s.

Data Analysis

Calibration factors for each eye were determined from the eye positions during the calibration procedure (see Bucci and Seassau, 2012) done before the visual task. Eye movement analyses were performed using the MeyeAnalysis software, which automatically determined the onset and the end of each saccade by using a built-in saccade detection algorithm, and visually inspected and verified by the investigator. For both visual conditions (simple fixation and fixation with distractor) we counted the number of saccades done during the paradigm. All saccades $\geq 2°$ were counted given that it is well known that micro-saccades are normally of smaller amplitude (for more details, see Tiadi et al., 2016). The time looking outside the fixation target in teh two visual tasks was also evaluated.

Postural control performance was evaluated using the surface area of the CoP (in cm^2) and the mean velocity (mm/s). The surface of the CoP was calculated corresponding to the area of an ellipse encompassing 90% of all CoP data point excursions.

Statistical Analysis

After testing the normality and homogeneity of variance assumptions on eye movements results a three-way ANOVA with children (ADHD and TD), visual task (fixation and fixation with distractor), and postural condition (sitting, standing stable, and standing unstable) as factors was run. In contrast, on postural measure two-way ANOVA was run with children (ADHD and TD), visual task (*simple fixation* and *fixation with distractor*) and postural condition (standing stable and standing unstable) as factors. Note that postural stability was not measured during the sitting condition. When necessary, Bonferroni *post-hoc* comparisons were employed. Finally, the Student paired *t*-test was also done comparing the surface area and in the mean velocity of the CoP of the two postural conditions (stable and unstable platform) for the two groups of children. Analyses were performed using the Statistica software the GLM (Advanced Linear Models) software and the level of significance was kept at 0.05.

RESULTS

Eye Movements

Figure 2A shows the number of saccades made by the two groups of children in the two different visual tasks during the three

postural conditions. The ANOVA showed that during fixation tasks children with ADHD made significantly more saccades compared to TD children [$F_{(1,41)} = 94$, $p < 0.0001$].

ANOVA failed to show a significant effect of visual task and of postural condition [$F_{(1,41)} = 2.44$, $p = 0.1$ and $F_{(1,41)} = 2.36$, $p = 0.1$, respectively], but it reported a significant postural and visual task interaction [$F_{(2,82)} = 4$, $p < 0.05$]. *Post hoc* tests showed that both groups of children made a larger number of saccades in the simple fixation task during standing condition on the unstable platform with respect to both visual tasks in simple sitting condition ($p < 0.02$ and $p < 0.003$, respectively). Moreover, during the standing condition on the unstable platform, both groups made more saccades than in the standing condition on the stable platform ($p < 0.004$).

ANOVA also showed significant group and visual task interaction [$F_{(1,41)} = 15.070$, $p < 0.0001$]. *Post hoc* tests showed that the number of saccades in both visual tasks was higher for children with ADHD when compared to TD children (both $p < 0.0001$). Moreover, *post hoc* tests showed that children with ADHD made more saccades during the simple fixation task than during the fixation with distractors task ($p < 0.002$).

Figure 2B shows the time looking outside the fixation target or at the distractor for the two groups of children tested. ANOVA showed a significant effect of group [$F_{(1,41)} = 18.24$, $p < 0.0004$]: children with ADHD looked more time outside the fixation target. ANOVA failed to show any significant effect of visual task and of postural condition.

Postural Control

Concerning postural conditions, ANOVA showed a significant effect of group on the surface of the CoP [$F_{(1,41)} = 20.86$, $p < 0.0001$]. The surface of the CoP was significantly

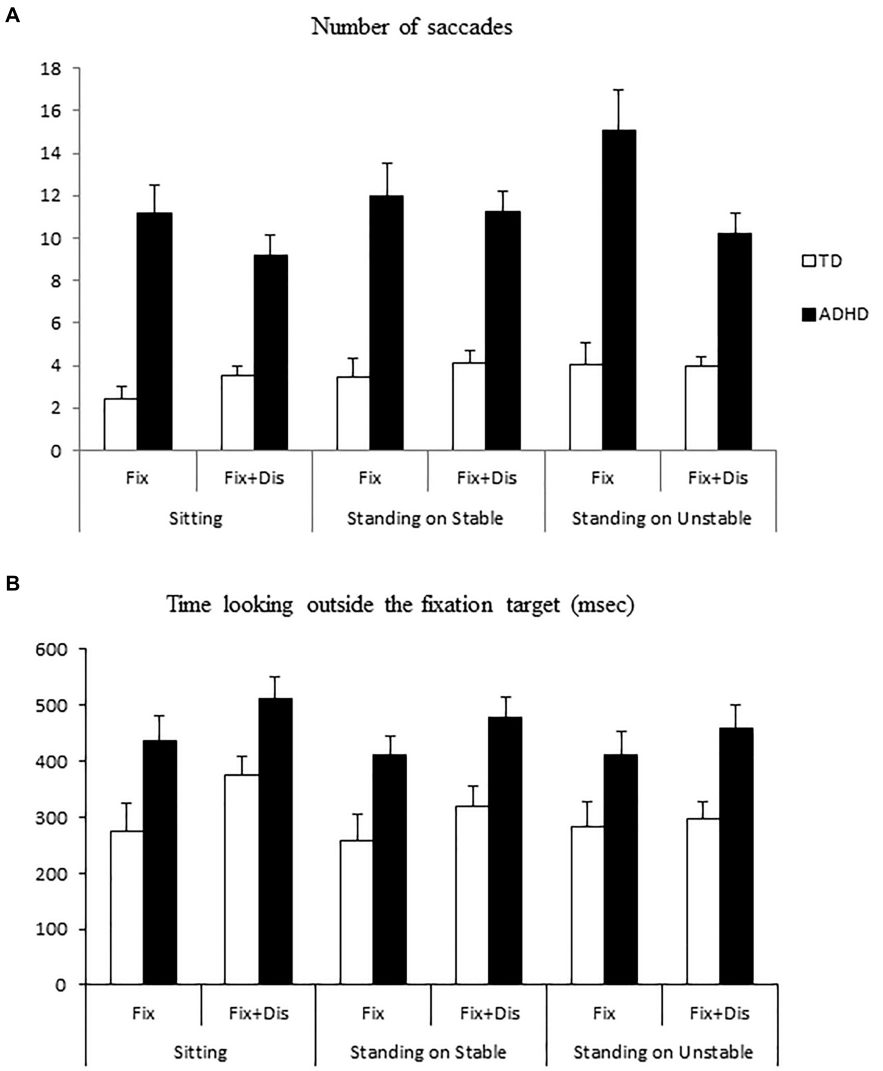

FIGURE 2 | (A) Means and standard deviations of number of saccades in three different postural conditions (sitting, standing on stable, and unstable platform) in typically developing and attention-deficit/hyperactivity disorder (ADHD) children. **(B)** Means and standard deviations of the time looking outside the fixation target or at the distractor in typically developing and ADHD children.

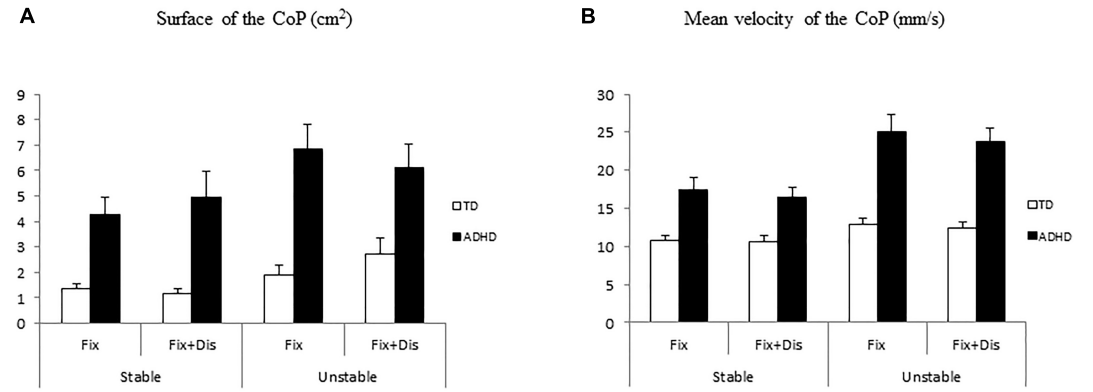

FIGURE 3 | Means and standard deviations of surface **(A)** and mean velocity **(B)** of the CoP in two different postural conditions (standing on stable and unstable platform) in typically developing and ADHD children.

larger in children with ADHD than in TD children (see **Figure 3A**). Moreover, ANOVA showed significant effect of postural condition [$F_{(1,41)} = 8.18$, $p < 0.006$], with the surface of the CoP significantly larger when standing on the unstable platform than on the stable platform; in contrast ANOVA failed to show any significant effect of visual task [$F_{(1,41)} = 0.31$, $p = 0.5$].

Finally, ANOVA showed significant group, postural and visual task interaction [$F_{(1,41)} = 5.6$, $p < 0.002$]. *Post hoc* tests showed that ADHD children in simple fixation task while standing on the unstable platform had larger surface of CoP with respect to all the other conditions reported in children with ADHD as well as in TD children (all $p < 0.0001$). Moreover, *post hoc* test showed that the surface of the CoP for children with ADHD in the fixation with distractor task in the unstable postural condition was significantly larger with respect to TD children (all $p < 0.04$) in all but one condition (fixation with distractor on the unstable platform); surface of the CoP of children with ADHD was also significantly larger than those reported in this group of children in simple fixation task on the stable platform ($p < 0.0002$).

Concerning mean velocity of the CoP (see **Figure 3B**), ANOVA showed a significant effect of group: ADHD children had larger mean velocity in contrast to TD children [$F_{(1,41)} = 32.04$, $p < 0.0001$]. Moreover, ANOVA showed significant effect of postural condition: standing on the unstable platform the mean velocity was larger than standing on the stable platform [$F_{(1,41)} = 41.76$, $p < 0.0001$], while it failed to show any significant effect of visual task [$F_{(1,41)} = 3.22$, $p = 0.08$].

Finally, ANOVA showed group and postural condition interaction [$F_{(1,41)} = 16.19$, $p < 0.0001$]. *Post hoc* tests showed that children with ADHD standing on the stable platform had larger mean velocity than TD children in the same postural condition ($p < 0.008$). The mean velocity in children with ADHD standing on the unstable platform was also significantly larger with respect to TD children in both postural conditions and to children with ADHD standing on the stable platform (all $p < 0.0001$).

Finally, we wonder to explore further whether the increase of the surface area and of the mean velocity of the CoP was more important in ADHD group compared to TD group. For TD children both the surface area and the mean velocity of the CoP were significantly larger in unstable condition ($t = -2.56$, $p < 0.01$, and $t = -2.78$, $p < 0.01$, respectively), while for children with ADHD the mean velocity of the CoP only was significantly larger in unstable condition ($t = -5.6$, $p < 0.0001$) but the surface area of the CoP was similar in both stable and unstable condition ($t = -1.9$, $p = 0.6$).

DISCUSSION

The aim of this study was to explore oculomotor and postural dual tasks in children with ADHD.

The present study reports that: (i) children with ADHD have poor fixation capabilities when compared to TD children; (ii) children with ADHD show more difficulties during simple fixation visual task than in fixation with distractors task; (iii) children with ADHD show poor postural stability compared to TD children; (iv) all children had more difficulty standing on the unstable platform (the surface area of the CoP was significantly larger); and (v) both groups of children made more saccades during simple fixation on the unstable platform than in the sitting condition; interestingly, on the unstable platform during the simple fixation task, children with ADHD were significantly more unstable than TD children, while in fixation with distractor task, the surface of the CoP was similar for the two groups of children. Each finding will be discussed below.

Poor Fixation Capabilities in Children With ADHD

Our results show that children with ADHD made more saccades than TD children, particularly during the simple fixation task. These findings are in line with the literature and suggest that children with ADHD have a difficulty of inhibitory control mechanism (Gould et al., 2001; Feifel et al., 2004; Bucci et al., 2017). A high number of saccades during fixation could suggest that these children have difficulty to maintain fixation, which is consistent with a failure of "top-down" regulation mediated by frontal-striatal dysfunction (Gould et al., 2001) given that these

regions are in fact implicated in visual fixation activity (Wurtz and Goldberg, 1989).

Indeed, neurophysiological studies on saccades have shown that Frontal Eye Fields has two principal connections with the Superior Colliculus, namely a direct pathway (fronto-tectal) and an indirect one via basal ganglia (Hikosaka et al., 2000). Based on these findings, we suggest that children with ADHD could have abnormalities in this cortical/central network. Indeed, in a meta-analysis report based on fifty-five fMRI studies (Cortese et al., 2012), hypoactivation has been observed in frontoparietal and ventral attention networks in children with ADHD when compared to controls.

Furthermore, in this study we observed that children with ADHD made more saccades during the simple fixation task compared to the fixation with distractors task; one could make the hypothesis that this could be due the fact that children with ADHD were looking more at the distractor leading to make less saccades in this specific visual condition, but this was not the case given that the time spended by looking outside the fixation target was similar in both visual tasks (simple fixation as well as fixation with distractors). This finding is quite surprising and it is in contrast to our pervious hypothesis, according to which children with ADHD having poor inhibitory capabilities (Feifel et al., 2004; Bucci et al., 2017) could make more saccades in the fixation with distractor task with respect to the simple fixation task.

We could explain this finding by the fact that children with ADHD are able to orient their attention to the more difficult visual task (as the fixation with distractor). Indeed, according to the multiple-resources model of attention (Wickens, 1991) child would use in a different way attentional resources depending on the type of the task he/she has to perform. In more details, if the two tasks are of similar type (i.e., visual information), as in fixation with distractor task in which the child has to fixate a target and at the same time to avoid to fixate the distractors, he/she has difficulties to focus attention on one of the two visual tasks because they interfere each other. In contrast, children with ADHD who have attentional and inhibitory deficiencies could focus better their attention in such conditions where the interference level and attentional load is higher leading to a better performance of this visual task (fixation with distractor task). In other words, if attentional demanding is high, children with ADHD could increase their attentional performance; this finding need to be tested further by using dual task of similar type with higher level of interference (i.e., attention).

Poor Postural Performance in Children With ADHD

In this study, we have found that children with ADHD have a poorer postural control than that of TD children. This is also showed by the fact that the surface area only and not the mean velocity of the CoP was similar in children with ADHD independently to the postural condition (stable/unstable). Such finding could suggest that children with ADHD make more muscular effort (shown by high velocity of the CoP) to try to reduce their body sway.

Our finding is in line with previous studies. Recall that motor deficiencies occur in 30–50% of children with ADHD (Goulardins et al., 2017); ADHD is frequently associated with poor gross and fine motor control capabilities (Piek et al., 1999; Wang et al., 2011; Papadopoulos et al., 2014) and our group (Bucci et al., 2014, 2016) as well as other groups (Zang et al., 2002; Wang et al., 2003; Buderath et al., 2009) showed postural instability in ADHD children compared to control children. In line with these studies, we can suggest a deficit in sensory processing for body stability in central structures. More recently, Kim et al. (2017), evaluating postural and gait balance as well as functional connectivity of brain regions controlling balance, reported that children with ADHD showed disturbances of balance and posture that could be associated to decreased brain connectivity in the premotor cortex. Particularly, they showed an alteration of connectivity from the cerebellum to the middle frontal gyrus and medial frontal gyrus in children with ADHD with respect to control children.

Dual Task Effect

Firstly, our study reported that both groups of children showed a better quality of fixation when they were sitting than when they were on the unstable platform; this could be due to the fact that when postural load is high, children shift their attention on postural control, leading to poor fixation performance. However, we also found that when both tasks (oculomotor and posture) became more difficult (fixation with distractor on unstable platform) the instability in terms of the surface of the CoP of children with ADHD was similar to that of TD children. This finding is in line with the model of U-shaped non-linear interaction of Huxhold et al. (2006), according to which secondary task can influence postural stability differently. However, this could also be due to the "ceiling effect": the dual task requires a high level of attention resources that cannot be allocated to posture by both groups of children. This finding is in line with the study of Shorer et al. (2012), showing that the effect of dual task on postural performance was similar in children with ADHD and TD children. Moreover, Manicolo et al. (2017) explored the dual task effect of walking and they did not observe any difference in gait performance during a dual cognitive task. Based on all these studies, we could assume that both groups of children (ADHD and TD) prioritized the fixation task over the postural task that is more difficult in unstable condition, in agreement with the U-shaped non-linear interaction model of Huxhold et al. (2006).

Note, however, that small head movements could occur during stable/unstable postural conditions and that unfortunately, we do not have already a system able to measure at the same time both head and eye movements. In the future it could be interesting to explore further this issue by developing a new dual task in a more ecological situation by recording eye/head/body movements in order to gain more insight on how children with ADHD can focus their attention.

Finally, the evaluation of oculomotor and postural dual task could be considerated as a potential biomarker allowing a better discrimination between subjects with ADHD with respect to

the subjects with neurodevelopmental disorders (for example with autism spectrum disorder, ASD). In fact subjects with ASD showed more executive dysfunctions such as planification and flexibility (Hill, 2004), consequently we could expect that children with ASD would perform this type of dual task worse than children with ADHD. However further studies need to be done in order to better define the salient characteristics of each of these two diseases.

CONCLUSION

Our data suggest that children with ADHD have difficulty of inhibitory control mechanism and poor postural stability, probably due to a failure of "top-down" regulation mediated by frontal-striatal dysfunction.

Finally, we could assume that both groups of children focused their attention on an oculomotor task, leading to poor postural control.

In the future it could be interesting to test whether postural training could improve attentional performance in children with ADHD.

AUTHOR CONTRIBUTIONS

MB acquired funding and conceptualized, supervised, wrote, and reviewed and edited the manuscript. SC and MR curated the data, analyzed formal, and reviewed and edited the manuscript. MS and EA curated the data and reviewed and edited the manuscript. JB and RD reviewed and edited the manuscript.

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Visuo-Haptic Exploration for Multimodal Memory

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When faced with a novel object, we explore it to understand its shape. This way we combine information coming from different senses, as touch, proprioception and vision, together with the motor information embedded in our motor execution plan. The exploration process provides a structure and constrains this rich flow of inputs, supporting the formation of a unified percept and the memorization of the object features. However, how the exploration strategies are planned is still an open question. In particular, is the exploration strategy used to memorize an object different from the exploration strategy adopted in a recall task? To address this question we used iCube, a sensorized cube which measures its orientation in space and the location of the contacts on its faces. Participants were required to explore the cube faces where little pins were positioned in varying number. Participants had to explore the cube twice and individuate potential differences between the two presentations, which could be performed either haptically alone, or with also vision available. The haptic and visuo-haptic (VH) exploratory strategies changed significantly when finalized to memorize the structure of the object with respect to when the same object was explored to recall and compare it with its memorized instance. These findings indicate that exploratory strategies are adapted not only to the property of the object to be analyzed but also to the prospective use of the resulting representation, be it memorization or recall. The results are discussed in light of the possibility of a systematic modeling of natural VH exploration strategies.

Keywords: haptic, vision, active exploration, bimodal perception, perception and action

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INTRODUCTION

Humans are very good at recognizing objects and inferring their properties by integrating information coming from multiple sensory channels and also from motor commands. This is particularly true for haptic perception, which depends on cutaneous and kinesthetic (proprioceptive) inputs, related to the position and the force applied by the limbs used to touch, but also on the exploration strategies adopted, e.g., the velocity of the exploratory movements or the way the object is handled (Lederman and Klatzky, 2009). Recognizing an object through haptic exploration is, therefore, a multimodal ability, which requires also short term memory and a strategy to collect information (Fernandes and Albuquerque, 2012). As a result, it enables us to gather an approximate estimate of a wide range of object properties, as the weight, the size, the volume of the object at hand, together with the force needed to manipulate it. Notwithstanding this apparent complexity, it comes to us as very naturally and we use it commonly, for instance when we have to recognize the keys or a coin in a pocket or a purse.

Haptic exploration entails the acquisition of stimulus properties both in parallel, through the integration of inputs derived from the different effectors, as the fingers, the palm, the two hands; and in sequential manner, integrating information of different parts of the explored objects acquired over an extended period of time. Hence, the strategy adopted when touching or actively manipulating the object is crucial in determining the representation of the object as a whole. Starting from the seminal work by Lederman and Klatzky (1987), many authors have shown that haptic movements are specific and directed to feature evaluation, both for 3D and 2D objects (Hatwell et al., 2003; Kappers and Douw, 2011). Lederman and Klatzky (1987) have provided a systematic classification of the hand movements adopted during haptic exploration. Blindfolded participants could touch and freely explore objects of daily use to assess certain specific features. Through movement analysis and video annotation, the authors defined six stereotyped movements, each specific to determine a particular information about the object: lateral motion (texture), pressure (hardness), contour following (shape), unsupported holding (weight), enclosure (global shape), static contact (temperature). Hence, beyond individual differences, it is possible to find general models of exploratory movements tailored to extract a specific feature. For instance, to judge object texture, the majority of people will tend to touch and move on the surface, although with individual variations—e.g., using a single finger or the whole hand. Human haptic exploration is therefore specifically planned as a function of the property of interest to be extracted, at least after 8–10 years of age (Cirillo et al., 1967; Hatwell, 2003).

Also vision can be considered an exploratory procedure, since it offers an alternative way to explore the object, instead of or in conjunction with haptics (Klatzky et al., 1993). Vision and haptics seem to have similar uni-sensory object processing, potentially supported by common neural substrates. Indeed, cortical areas traditionally considered as specialized for visual processing have been proven to be functionally activated during the corresponding haptic tasks (Lacey et al., 2007). In particular, the lateral occipital cortex (LOC), an object-selective region in the visual pathway, responds consistently also to haptic stimuli (Amedi et al., 2002; Stilla and Sathian, 2008) qualifying it as an area processing geometric shape information independently of the sensory modality used to acquire it (Amedi et al., 2002). These neuroimaging findings are also confirmed by case studies and virtual lesions studies, which indicate the LOC as necessary for both haptic and visual shape perception (Lacey and Sathian, 2014). Additionally, several parietal regions have demonstrated visuo-haptic (VH) responses, as the anterior intraparietal sulcus (aIPS) and the postcentral sulcus (PSC; Stilla and Sathian, 2008). These commonalities would lead to a shared multisensory representation enabling cross-modal object recognition (Lacey et al., 2007).

Though having some similarities, the two modalities are also complementary, as they are not equally efficient in the perception of the different properties of an object. The haptic modality is more appropriate for material properties as hardness, weight or texture, whereas for geometric, spatial properties the visual information seems to be richer and more economical (Hatwell et al., 2003; Gori et al., 2010). Moreover, haptics seem determinant in the development of size perception, whereas vision is crucial in orientation estimation (Gori et al., 2008).

Some differences exist between the two modalities also in the context of memory. Even though perceptual representations can be formed that are sufficiently abstract to permit sharing or exchange across vision and haptics (Easton et al., 1997), haptic working memory is characterized by a more limited and more variable capacity than visual working memory (Bliss and Hämäläinen, 2005). However, long term memory is preserved similarly for objects studied visually and haptically. In particular, when participants are tasked with a recognition test both immediately and after 1 week, the recognition is best for visual study and test, but also haptic memory is still apparent after a week's delay (Pensky et al., 2008; Hutmacher and Kuhbandner, 2018).

Although it is widely recognized that the strategy of manual and visuo-manual exploration varies as a function of the object property of interest (Lederman and Klatzky, 1987; Hatwell et al., 2003; Kappers and Douw, 2011), it is less clear whether exploration is also prospectively tailored to the use that will be made of the information acquired. Indeed, shape perception is characterized by two different dimensions: *shape encoding*, which includes shape features extraction, online construction and storage of mental representation and *shape matching*, which foresees evaluation of shape features in reference to a stored representation and decision-making (Miquée et al., 2008). The question is whether exploration adaptively changes as a function of its main goal, be it *encoding* or *matching*.

From the few studies which have dissociated these two cognitive processes (Stoeckel et al., 2003; Miquée et al., 2008), it emerges that each stage of haptic shape perception activates a unique set of brain areas. Only a subset of them, those lining the IPS, are recruited throughout the task for encoding, maintaining in memory, and deciding on the shape of tactile objects (Rojas-Hortelano et al., 2014).

Rojas-Hortelano et al. (2014) in particular, used fMRI to measure cortical activation of participants performing a haptic shape discrimination task in which they had to decide whether two objects presented sequentially had the same shape or not. The first exploration of object shape bilaterally engaged the somatosensory, motor, premotor and parietal areas and the primary visual cortex. During the delay phase separating the presentations of the two objects, when participants had to maintain the shape of the first object in short-term memory, the prefrontal cortex (PFC) was active, together with the premotor and the lateral parietal cortices. A control experiment demonstrated further that only the areas in the posterior parietal cortices were specifically engaged in maintaining the short term memory of object shape (and not for instance its temperature). The presentation of the second object, in addition to the mechanisms of exploration and shape encoding that are active also for the first one, engages decision-making processes such as objects comparison and the generation of a decision about whether they are different or the same. These processes recruit a network of frontoparietal areas that include the medial

premotor, the right ventrolateral PFC, and the parietal cortices bilaterally, with only the left premotor and the bilateral parietal cortices being specifically engaged for shape-related decision-making processes.

Given the very different neural processing supporting the different phases of shape perception, it might be expected that also the corresponding exploratory behaviors might differ as a function of the sub-goal currently addressed, be it encoding or matching. Indeed, the imaging results by Rojas-Hortelano et al. (2014) suggest that the decision-making process starts as soon as the second object is presented. This could imply that the hand movements used to explore the second object could be aimed not at obtaining its general shape but to obtain information to directly evaluate whether the objects are different or the same, i.e., to support the decision.

This hypothesis could not be thoroughly tested in the above-mentioned studies due to the strong experimental constraints associated with the neuroimaging investigation. Miquée et al. (2008) describe a significant decrease in exploration time for haptic encoding and recall (called "reference" and "comparison" shapes, respectively) of 2D shapes explored with a single finger. The traces of the finger scanning paths show that the global movement patterns are similar in the two phases, but the number of scanning cycles (i.e., finger passages) was reduced in the matching phase. Moreover, the similarity in response times during trials where the two objects were different or equal suggests that the participants made their decision after a thorough exploration of the second object and not as soon as they detected a salient difference between the shape being explored and the memorized reference shape. Rojas-Hortelano et al. (2014) who investigated haptic perception of 3D shapes did not analyze participants' object motion and fixed also the exploratory time for both encoding and matching phases.

It is, therefore, still to be assessed whether our exploratory behaviors change when we explore an object to encode it in our memory or to recall a memorized information and perform a comparison.

As mentioned above, addressing this question is complex, as currently the characterization of the movements adopted during haptic exploration is performed mainly through lengthy manual annotations by human observers. This approach, beyond being fatiguing for the observers, might also be at risk of missing important exploratory behaviors due to drop in attention or to limitations in visibility (Jansen et al., 2015). It is, therefore, necessary to increase efficiency and reproducibility through new methods for automatic classifications (Jansen et al., 2013, 2015). To this aim, we adopted a novel tool, iCube, a cube which measures its orientation in space and the location of the contacts on its faces and communicates this information wirelessly to a computer. The cube is of about 5 cm side, with 16 tactile cells per face and weighs about 150 g. The main novelty with respect to previous tools is the possibility to investigate haptic exploration with a sensorized object that can be also freely moved in space. The small size and weight allow for a natural manipulation and the sensorization avoids the need for *post hoc* annotations. Although a direct mapping between the data from the cube and the exploratory procedures defined by Lederman and Klatzky, 1987 is not straightforward, as it is not possible to infer exactly the relative motion of the different parts of the body (e.g., of different fingers) with respect to the object, this tool can help augmenting the analysis of exploratory procedure with information about rotation, number and the temporal dynamics of the exerted touches.

With this new tool, here we aim at assessing whether haptic and VH exploratory procedures change during *shape encoding* and *shape matching* tasks. To do so, we positioned little raised pins on the surface of the cube in varying number, in a dice-like configuration, and asked subjects to explore twice the cube, either with vision or while blindfolded, to determine whether the pins configuration varied or not.

Traditionally in same/different haptic tasks, the objects used have different shapes, and as a consequence, the first exploration tends to be slower, as it is necessary to find the contours on which the hands will move. To avoid this potential confound, in this experiment the shape to be explored was kept exactly the same between explorations (a cube) and was well known to participants even before the experiment, with only the pattern of pins on the faces varying. Hence, subjects could decide *a priori* a fixed "cube-exploration" strategy and replicate it during all manipulations, to facilitate their guess.

Conversely, our results show that the exploratory procedures differ significantly when exploration is performed to memorize the cube structure with respect to when it is instead aimed at recalling a previous memorized structure to perform a comparison.

MATERIALS AND METHODS

Subjects

Seventeen subjects took part in the experiments described here [seven males, 10 females, age: 23 ± 2 (SD) years]. All participants but one were right-handed and all were naïve to the goal of the experiment. The research protocol was approved by the Regional Ethical Committee (Comitato Etico Regione Liguria—Sezione 1), all participants provided their written informed consent, received a compensation of 10 euro and followed the same experimental procedure.

The Device

The measurement tool used in this study was a sensorized cube designed at IIT, called iCube, which measured its orientation in space and the location of the contacts on its faces and communicated this information wirelessly to a computer. The cube was of about 5 cm side, with 16 cells per face and a weight of about 150 g (see **Figure 1**). Touch sensing was based on a set of Capacitive Button Controllers (CY8CMBR2016) developed by Cypress Semiconductor Corporation. These were based on Multi Touch technology, enabling detection of simultaneous touches and supported up to 16 capacitive cells ($6 \times 6 \times 0.6$ mm), which could be organized in any geometrical format, e.g., in matrix form. Each face of iCube was made with one of these boards. Their sensitivity, i.e., the smallest increase in capacitance that could be detected clearly as a signal, was set

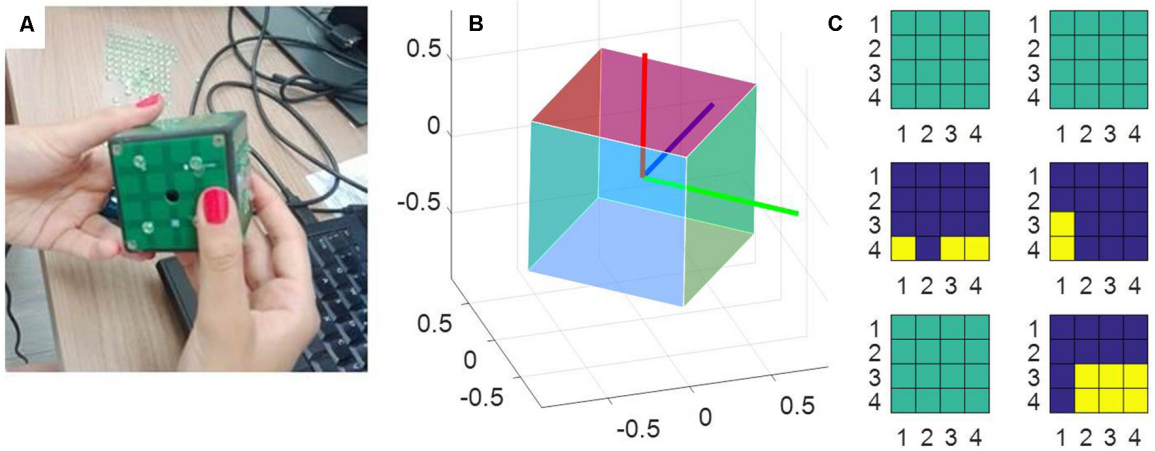

FIGURE 1 | The measurement tool. **(A)** A picture of the iCube with the raised pins positioned on its faces. **(B)** A representation of the cube orientation in space, plotted with MATLAB. **(C)** A snapshot of the activation of the tactile sensors on each of the six faces. Yellow indicates the cells currently touched, blue the cells currently not touched on the same face. Sea green indicates faces that in that instant are totally inactive (i.e., with no cell touched).

to 0.3 pF, so as to allow the device to sense contacts without the need to apply pressure. Orientation estimation was based on a Motion Processing Unit™ (MPU), a nine axes integrated device, combining a three axes MEMS gyro, a three axes MEMS accelerometer, a three axes MEMS magnetometer and Digital Motion Processor™ (DMP). The MPU combined information about acceleration, rotation and the gravitational field in a single flow of data. The information about touches and rotation from iCube were sent to a laptop computer through a serial protocol. The reception was performed through a radio module XBEE together with an integrated circuit developed by FTDI (Future Technology Devices International Ltd., Glasgow, UK) and occurred on average every 348 ms (±52 ms, SD). The communication was constituted of an exchange of properly formatted commands: starting byte, a byte with the address of the board to which the command had been sent, a byte defining the command, one or more optional bytes including the command parameters, the end byte. Through these messages, it was possible to assess the status of the tactile boards and the rotation of the device. These data were further analyzed in MATLAB to extract the pattern of touches, the amount of cube rotation and the speed of rotation (see "Data Analysis" section).

Protocol

Before experiment initiation the device iCube was prepared, connected in wireless mode to a laptop with MATLAB installed and the experimenter positioned on its faces a set of raised plastic gray pins (diameter: 0.5 cm, height: 0.2 cm). The distribution of pins on the cube faces was similar to that of a dice, with each face containing from 0 to 5 pins (see **Figure 2** for an example). There was however no limitation of the presence of two equal faces. The participant was comfortably seated in front of the table, where the cube was positioned on a support. Before the experiment subjects were invited to touch and explore the cube to allow for familiarization with the device. In particular, they were asked to try and count the number of pins on the surface of the cube, once with their eyes open and once with eyes closed. The familiarization lasted about 2 min.

In the experimental session participants were asked to explore the cube twice, with the task of understanding whether any change occurred in the pins allocation between the first and the second presentation. All trials, therefore, consisted of a first exploration (*pre*) followed by a second exploration (*post*). The two explorations could be performed either only haptically—with participants wearing a blindfold—or while also looking at the cube (see **Table 1**). Each of the two trial types was presented to the participants three times in randomized order, for a total of 12 explorations (i.e., six trials) for each subject. The experiment lasted about 25 min on average. At the beginning of each trial subjects received by the experimenter the instructions: e.g., "you will now explore the cube twice, both times with vision (or both times only haptically). Between the two explorations, the pins configuration on the cube might change or remain the same. Please explore the cube as long as you want and then lay the cube on the table and reply either 'same' or 'different.' " Then the experimenter handed the cube to the participant. The design was therefore similar to a "study-test" paradigm to assess memory and recall (e.g., Pensky et al., 2008), with the crucial feature of posing no time limit for any phase of the exploration. In the trials with vision, subjects were instructed to keep their eyes closed until they felt the cube touching their hands. In the haptic trial, they wore a blindfold during the whole time.

Between the two phases (*pre* and *post*) the cube could remain exactly the same, but rotated on the support, or could be changed (e.g., by removing or adding one pin to one of the faces or exchanging the pins of two different faces, see **Figure 2** for an example). These changes in orientation or pins configuration were rapidly operated by the experimenter, with an interval

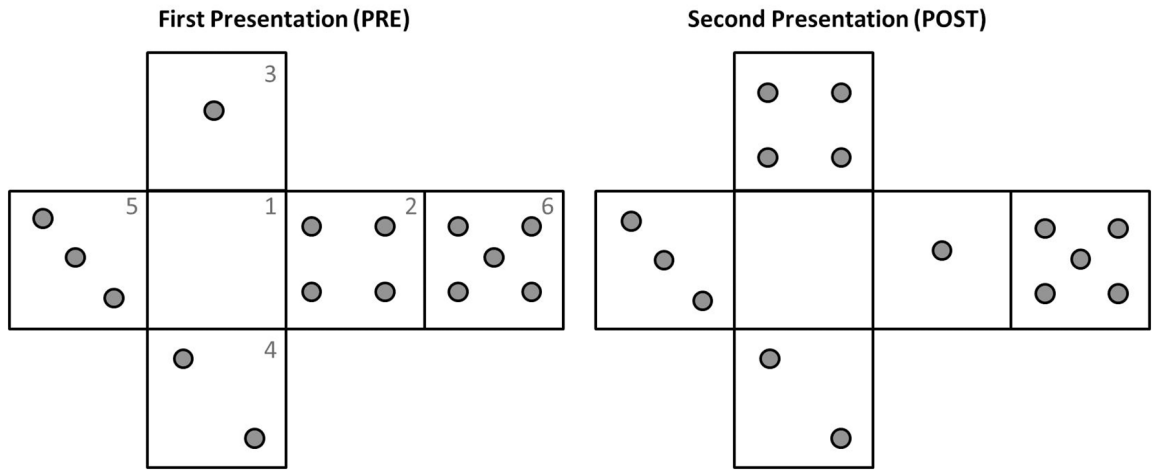

FIGURE 2 | Example of pins configuration during the two explorations of one trial. Note how the pins distributions in faces 3 and 2 have changed from *pre* to *post*. The numbers reported on the faces are indicated here just for illustrative purposes.

between explorations lasting on average less than a minute. In 2/3 of the trials, the correct answer was "Different," in 1/3 of the trials it was "Same." This uneven distribution was selected to minimize subjects' fatigue because pilot experimentation indicated that "same" trials were perceived as more difficult.

Data Analysis

The data about touches and rotations recorded by the iCube were analyzed in MATLAB as described in the following subsections.

Touches

From each of the six boards, representing the faces of the cube, the device reported a tactile map, i.e., a matrix of 4×4 elements of zeros and one, where one represents a touch. In the analysis we first considered the total number of touches occurred on all the six faces as a measure of tactile exploration. We also computed the exploration duration as the moment between the first and the last touch of the subject (manually cutting for each file the initial phase of recording, when the experimenter put the cube in the hands of the participant).

Rotation

The information about the orientation of the cube with respect to its starting position was provided in the form of a quaternion, which was then converted in MATLAB into a rotation matrix to compute instantaneous rotation. The instantaneous angular variation was computed by measuring the angle traversed over time by each of the three unitary axes orthogonal to the faces of the cube (see **Figure 1B**). In particular, given one *axis*:

$$\Delta\text{angle}_{axis}(\text{t}) \;=\; \arctan\left(\left|\frac{axis(t)\times axis(t-1)}{axis(t)\cdot axis(t-1)}\right|\right) * 180^{\circ}/\pi$$

TABLE 1 | Trials organization.

First presentation (*pre*)	Second presentation (*post*)	*CODE*
Haptic	Haptic	$H_1\_H_2$
Visuo-Haptic	Visuo-Haptic	$VH_1\_VH_2$

We integrated over time the rotations performed by the three axes, to get an estimation of the rotation impressed to the cube in all the possible different directions. To quantify the amount of rotation we considered the maximum value among cumulative sums of the rotations executed by the three axes. The instantaneous rotation speed was instead computed by dividing $\Delta\text{angle}_{\text{axis}}(\text{t})$ for the corresponding time interval and averaging the results across the three axes and across all the instants in a trial in which the cube was in motion (i.e., angular velocity >1°/s). This selection was done to assess actual velocity of rotation when the rotations were executed, without spuriously reducing the estimate with the analysis of the static phases.

Statistics

Statistical analysis has been performed on exploration duration, amount of rotation, rotation velocity and the number of touches, averaged among all trials of each condition for every subject. We checked for the presence of outliers, by evaluating whether any subject exceeded the average ± 2.5 standard deviations. This happened for two subjects, one in condition H_2 and the other in almost all conditions. We, therefore, eliminated these participants from the sample for all the subsequent analyses. To assess the difference in exploration due to its goal [memorize the structure (*pre*) vs. recall and compare (*post*)] we ran a repeated measures ANOVA, with TYPE (levels: *pre, post*) and MODALITY (levels: haptic, VH) as factors. A difference has been considered significant for $p < 0.05$.

RESULTS

To assess whether the exploration is planned differently when aimed at *memorizing* an object—study phase (*pre*)—than when it is used to *recall and compare* with a previously explored stimulus—matching phase (*post*)—we compared different properties of these two different exploratory phases, including features of the tactile exploration and characteristics of the

movements actively applied to the cube, both in presence and absence of vision.

Tactile Exploration

In **Figure 3**, top left panel, we show the exploration duration of the first and second explorations for the haptic (H) trials and the VH ones. On average, explorations take longer when performed in the haptic only modality (46.8 ± 5 s SE, Standard Error), than with the help of vision (20 ± 2 s SE) and the difference is significant (two-way repeated measures ANOVA, $F_{(1,14)} = 41.14$, $p < 0.001$). Moreover, in both modalities the first exploration, aimed at memorizing the cube configuration, lasts significantly longer than the second one, for recall and comparison (two-way repeated measures ANOVA, $F_{(1,14)} = 16.45$, $p = 0.0012$). This difference is more accentuated for the Haptic than for the VH trials, as confirmed by a significant interaction ($F_{(1,14)} = 6.3$, $p = 0.025$)[1]. This pattern of results is shown also by the individual data plotted in **Figure 3**, top right panel. Indeed, the majority of the symbols lie below the black dashed light, indicating a longer exploration in the memorizing phase than in the comparison one. The exact same pattern is replicated when assessing the total number of touches on the cube faces (**Figure 3**, bottom panels). Haptic exploration entails on average a larger number of touches than VH exploration (two-way repeated measures ANOVA, $F_{(1,14)} = 63.2$, $p < 0.001$) and this number decreases significantly between the first and the second phase of the trials (two-way repeated measures ANOVA, $F_{(1,14)} = 19.4$, $p < 0.001$). Again, the difference between the first and the second exploration is larger for the Haptic than for the VH trials (significant interaction, $F_{(1,14)} = 12.3$, $p = 0.003$).

To assess more in detail how haptic exploration changed as a function of the available sensory modality and of the task phase (memorization and recall) we estimated how long each of the six faces was touched on average in each of the haptic and VH trial, during the *pre* and the *post* phases (**Figure 4**). At first look, it emerges clearly that individual face exploration is shorter in the VH condition (bottom panels) than in the haptic one (top panels) and that in both modalities the main difference between *pre* and *post* is a general decrease of time. This pattern is similar for both "different" and "same" trials and also among the different faces. There is a tendency in most trials for faces 3 and 4 to be touched for a longer time. Since we are considering all the touches occurred during a trial, here are included also the contacts necessary for holding and rotating the cube and not only the ones purely aimed at exploring the surfaces. If we exclude from this computation all touches which lasted on the same cell for a consecutive period of more than 2 s (steady touches, most probably needed for support), the small temporal differences among faces disappear—in particular for the haptic trials (**Figure 5**). This suggests that on average participants tended to hold the cube from the back and frontal faces (face id: 3 and 4, respectively) for large parts of the trials while inspecting the other faces either visually or with the other hand as a function of the sensory condition. In summary, in terms of touches distribution, the exploration seems to follow a similar pattern during the encoding and the recall phases of the task, but with a much faster pace in the latter. From a qualitative comparison between behaviors in the "same" and "different" trials, no clear dissimilarities are visible. If participants had on average immediately stopped after finding the different face configuration in the "different" trials, a diverse pattern of time per face distribution would have been expected between these and the "same" ones, where a complete exploration was always necessary. The pattern instead looks remarkably similar, suggesting that in both types of trials, participants tended to analyze all faces in the *post*, before expressing their response. This observation is further confirmed by looking at the total time spent exploring in the *pre* and *post* phases separately

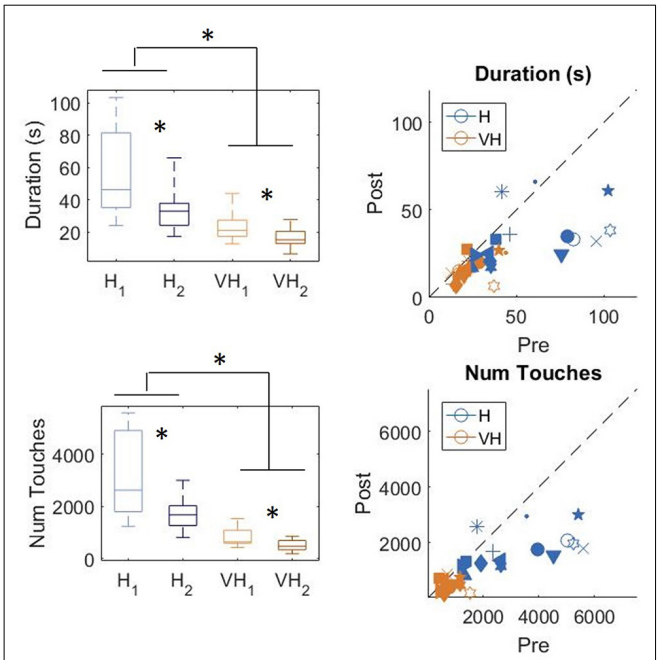

FIGURE 3 | Duration and number of touches. Left panels: distributions of duration (top) and number of touches (bottom) in the Haptic (H) and visuo-haptic (VH) modalities. The subscripts 1 and 2 indicate the first and second exploration of each trial, respectively. On each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points. Asterisks indicate significant difference. Right panel: plot of the individual subjects' average duration (top) and number of touches (bottom) in the second exploration (*post*) against the same variable in the corresponding first exploration (*pre*), color coded for modality. Different symbols represent different subjects. The dashed black line indicates identity. Elements below the line indicate subjects for which the values in the *post* were lower than in the *pre*.

[1]Since a Lilliefors test indicated that the Durations measured in the first visuo-haptic exploration (VH_1) were distributed non-normally ($p = 0.019$), we tested all differences related to Visuo-Haptic duration also with Wilcoxon signed rank tests, which confirmed the reported results. In particular, the first exploration lasts longer than the second one (paired *t*-test, $p = 0.003$ for Haptic, Wilcoxon signed rank $p = 0.001$ for Visuo-Haptic) and this difference is more accentuated for the Haptic than for the Visuo-Haptic trials (Wilcoxon signed rank test on the differences $H_2 - H_1$ vs. $VH_2 - VH_1$; $p = 0.018$). All differences are significant after Bonferroni correction.

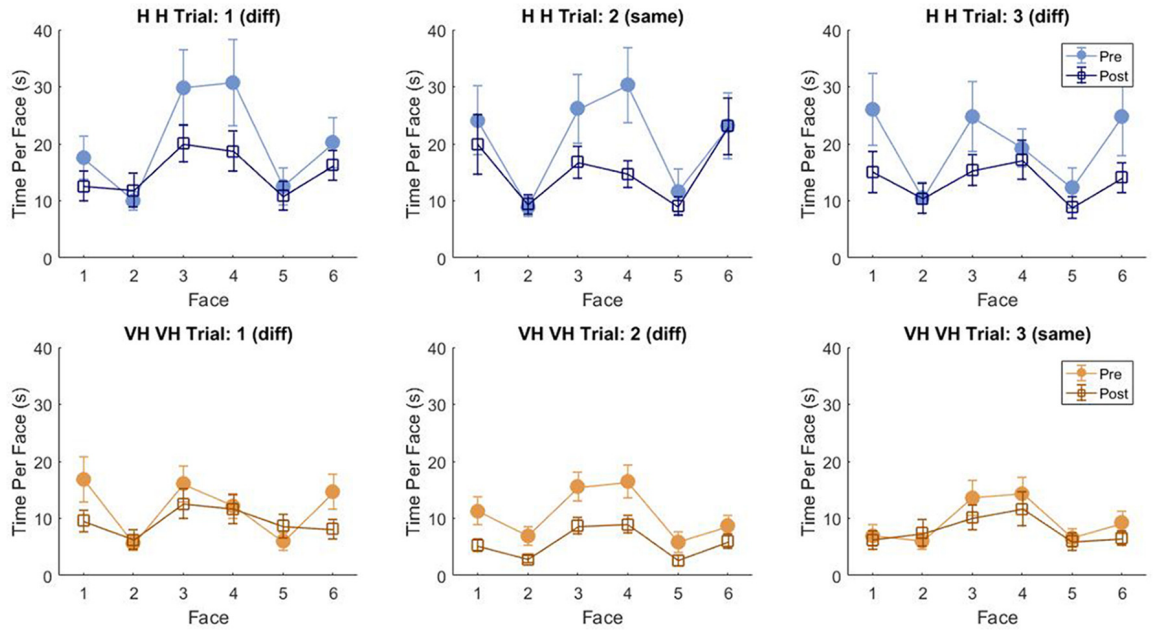

FIGURE 4 | Average touch duration per face. Average time in which each cube face has been touched at least on one cell in one frame during a trial. The three top panels refer to haptic explorations, the three bottom panels to VH exploration. Each panel represents a single trial, with different symbols being associated with *pre* and *post* phases. Error bars indicate standard errors of the mean.

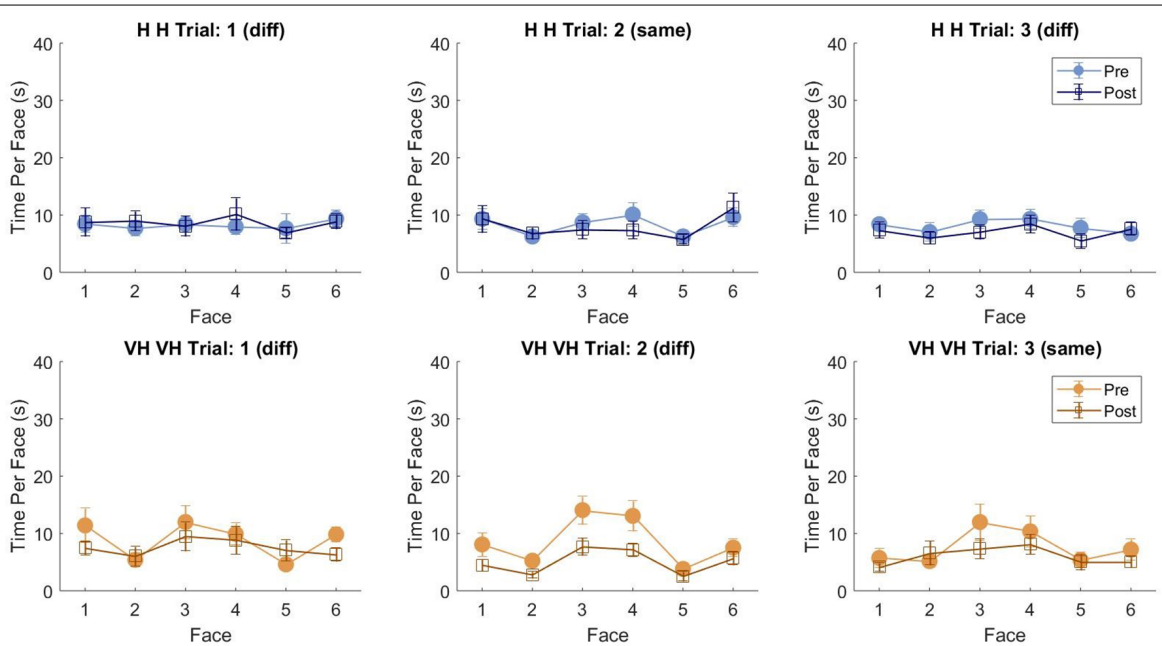

FIGURE 5 | Average touch duration per face (only short touches). Average time in which each cube face has been touched at least on one cell in one frame during a trial excluding all touches which lasted on the same cell for a consecutive period of more than 2 s (steady touches). Same graphical conventions as in **Figure 4**.

for each subject and for "same" and "different" trials (see **Figure 6**): in both typologies of trials there is a very similar decrease in exploration duration between the encoding and the matching phase.

Rotation

To gain a better insight in the modulation of the exploratory strategy as a function of the goal of the exploration, we analyzed how the cube was moved in the different conditions.

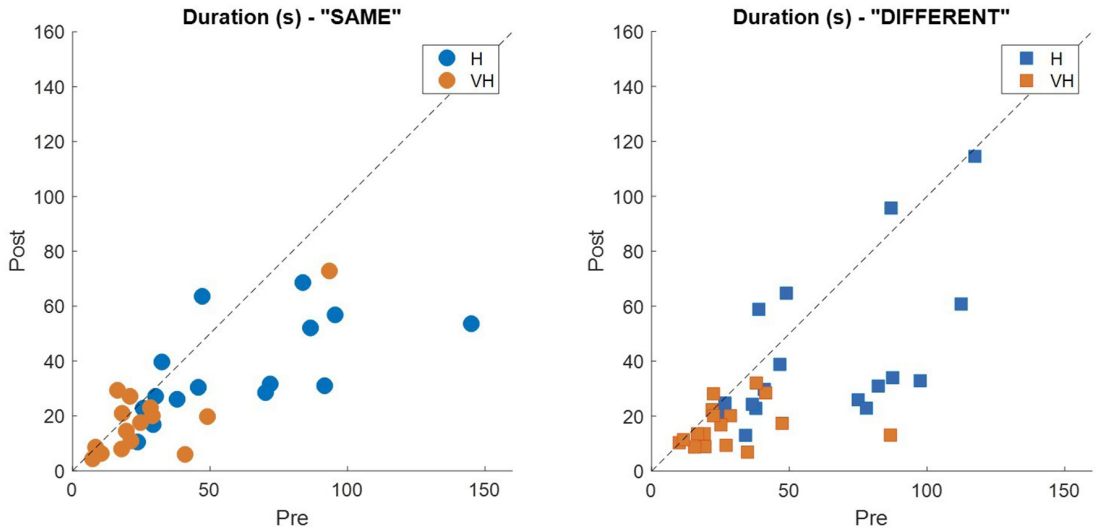

FIGURE 6 | Individual exploration duration. Each panel represents the average exploration duration during the *post* phase plotted against the corresponding duration in the *pre* phase for the "same" trials (left panel) and the "different" ones (right panel). Dashed lines indicate identity.

In **Figure 7**, top panels, the distributions of the total maximum amount of rotation is plotted on the left, while on the right individual subjects' values are presented. In the bottom panels, the corresponding graphs for velocity of rotation are shown. Participants rotated the cube significantly more in the VH condition than when exploring only haptically (two-way repeated measures ANOVA, $F_{(1,14)} = 17.3$, $p < 0.001$). The amount of rotation decreased significantly between the first phase of the trial and the second one (two-way repeated measures ANOVA, $F_{(1,14)} = 15.98$, $p = 0.0012$) and the decrease was similar between the two modalities (two-way repeated measures ANOVA, non-significant interaction $F_{(1,14)} = 0.36$, $p = 0.56$)[2]. The same pattern is visible in the individual data (top right panel).

Rotation velocity (bottom panels) was much faster in the VH condition than when exploration was performed only haptically (two-way repeated measures ANOVA, $F_{(1,14)} = 62.96$, $p < 0.001$). Moreover, it increased significantly during the second exploration (two-way repeated measures ANOVA, $F_{(1,14)} = 9.4$ $p = 0.008$), similarly in both modalities (non-significant interaction $F_{(1,14)} = 1.18$, $p = 0.29$).

Additionally, we computed for each temporal frame which cube face (or faces) were within a specific "cone of visibility." To do so we estimated the orientation of an hypothetical axis passing through the center of each face, orthogonal to it and outbound oriented, and we computed the angles formed by its' projections on the frontal and horizontal planes of the absolute frame of reference with respect to the ideal axis connecting the center of the cube starting position and the participant (at the same elevation with respect to the floor). If such angles were inferior or equal to ±45° and the direction of the axis was toward the participant, the face(s) were considered in the "cone of visibility." This choice was made by observing which faces were in direct view when participants held the cube in their hands. From this analysis, we could determine in which order the faces entered in the "cone" in different trials and whether there were sequences of orientations (or transitions) more frequent than others.

To do that we extracted a transition matrix: a 6 by 6 matrix in which each element corresponds to the number of times in which the transition has occurred between the face individuated by the row number and the face corresponding to the column number. For instance, the cell in row 2 and column 3 reports the number of times in which at first face 2 was in the cone of visibility and then face 3 entered the cone. An element in the diagonal instead would indicate for how many frames the same face was maintained consecutively in the cone of visibility.

For each subject we computed one matrix for *pre* and *post* for both the Haptic and the VH conditions, by summing the matrixes of all trials for each specific condition. We then normalized all cells dividing them by the maximum value of each matrix, leading to values ranging between 0 and 1, making each cell a proportion of the maximum amount of transitions occurred.

We also derived a single matrix for each condition, by summing over all the subjects the matrixes of all trials for each specific condition and normalizing the result. The derived matrixes are reported in **Figure 8**, panel A. To evaluate potential differences in proportion of transitions between *pre* and *post* sessions we additionally computed the difference between the two sessions for both the Haptic and the VH conditions (**Figure 8**, panel B).

[2]Since the rotations were not distributed normally in the two Haptic explorations (Lilliefors tests, $p = 0.034$ and $p = 0.037$, respectively), we replicated the analysis with non-parametric tests for all comparison related to Haptic Amount of Rotation. The decrease in amount of rotation in the Haptic modality did not reach significance (after Bonferroni correction: Wilcoxon signed rank for Haptic, $p = 0.035$), but the decrease was similar between the two modalities (Wilcoxon signed rank test on the differences H_2-H_1 vs. VH_2-VH_1: $p = 0.6787$).

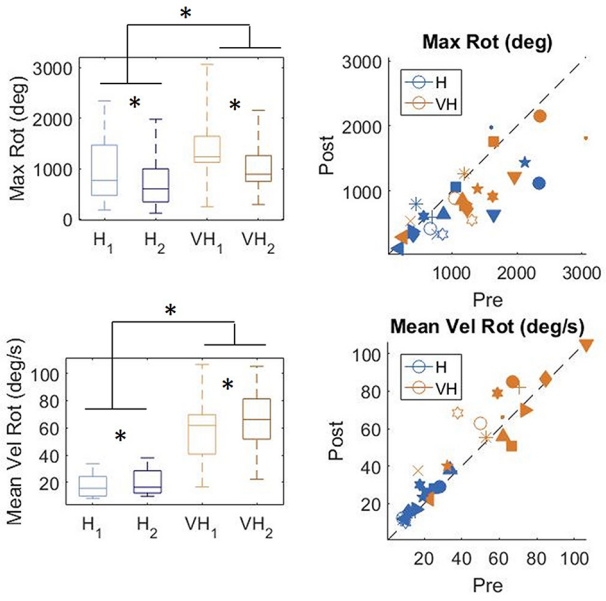

FIGURE 7 | Distribution of amount of cube rotation and average rotation velocity. Left panels: distributions; Right panels: individual data. *indicate significant difference. Same graphical conventions as in **Figure 3**.

In all graphs of panel A, it is clear that the face which remained most consecutively in the cone of visibility is face 4, which is actually the face which is handed toward the participant by the experimenter in most trials. Then, for all the faces there is a certain amount of time in which they are kept consecutively in a similar orientation toward the participant. The pattern is remarkably similar across all conditions, suggesting that even though the number of transitions might differ between different modalities, there is a tendency to maintain all the faces stable for a similar proportion of time during exploration.

The difference between transitions in the Haptic and in the VH condition is evident outside the diagonal: with vision available a lot more of transitions occur between different faces, and this phenomenon seems to increase further in the *post* session with respect to the *pre*. Conversely, in the Haptic condition, the proportion of transitions occurring between different faces is much smaller than the maintenance of a single face in the same orientation and seems to remain almost unchanged between the *pre* and the *post* conditions. The larger increase of transitions between different faces in the *post* VH condition outside the diagonal is significant ($X^2_{(1,N=60)} = 4.44$, $p = 0.035$) and can be visualized in the larger number of yellow off-diagonal cells in **Figure 8**, panel B, top graph, if compared with the corresponding Haptic matrix (bottom graph).

To verify this observation, we computed for each subject the difference in proportion of transitions between the *pre* and the *post* conditions, by subtracting the corresponding matrixes. We then counted the number of cells with positive values in the resulting difference matrix, considering only the 30 cells outside the diagonal. Positive values correspond to the transitions for which the proportion of occurrence increased from the *pre* to the *post* trials. In the VH conditions about 36% (±14%) of such transitions increase, whereas in the Haptic conditions only about 18% (±14%) of the transitions increase and this difference is significant (paired sample *t*-test $t_{(14)} = -3.6$, $p = 0.003$).

This might be interpreted suggesting that a difference in exploratory patterns occurs between *pre* and *post* sessions and that such change differs between Haptic and VH explorations. With vision, participants perform more rotations of the cube from one face the other corresponding to the large number of transitions (outside the diagonal). Moreover, this phenomenon tends to increase in the *post* session, where probably participants attempt to check the evaluations and maps they built in the *pre* session, by assessing relative positions of different configurations of pins, rapidly rotating the cube in all possible configurations and also exploiting orientations that give the possibility to glance at more faces at the same time (i.e., looking from a vertex, enabling the vision of two or even three faces at the same time). Conversely in the Haptic condition—especially in the *post* session—it seems that participants tend to select a reduced number of fixed rotations (e.g., switching from face 4 in front to face 6 and then vice versa) and to keep the resulting position, to be able to then explore the cube faces by moving the hands over them with a relatively stable spatial frame of reference.

DISCUSSION

This work shows for the first time that even in presence of a well-known object, humans change the way they explore it, when they manipulate it to encode what is on its faces, vs. when they manipulate it again to recall it and make a comparison with the first exploration. Not only the latter process becomes faster and involves fewer touches, but it also entails faster and different rotations. Hence, memorization and recall are not only processed by different brain areas (Rojas-Hortelano et al., 2014) but are supported by significantly different behavioral patterns, suggesting a tuning of the exploratory plans guided by the current action goal.

Interestingly, this marked difference in exploration between the two tasks is clearly present for both Haptic and VH exploration, though being more accentuated for the haptic-only condition. Hence, even when vision is available to guide manipulation, still the two explorations differ significantly. This suggests that memorization requires more effort independently of the modality with which exploration is performed and not only when it is limited to just manual analysis.

Crucially, our experimental design allowed unlimited time for both exploration and recall, leaving to participants the free decision of how (and how long) to explore the object in both phases. As a result, they could take advantage of the free time to thoroughly explore the cube in both presentations to facilitate the recognition. Indeed, replicating one-to-one the same exploratory strategy twice (both in *pre* and *post*) could *a priori* represent a reasonable method to simplify the recognition of similarities and differences among the two presentations. Conversely, most participants tailored their exploration to the different nature of the task (memorizing vs. recall) performing the two explorations differently, not only in terms of timing,

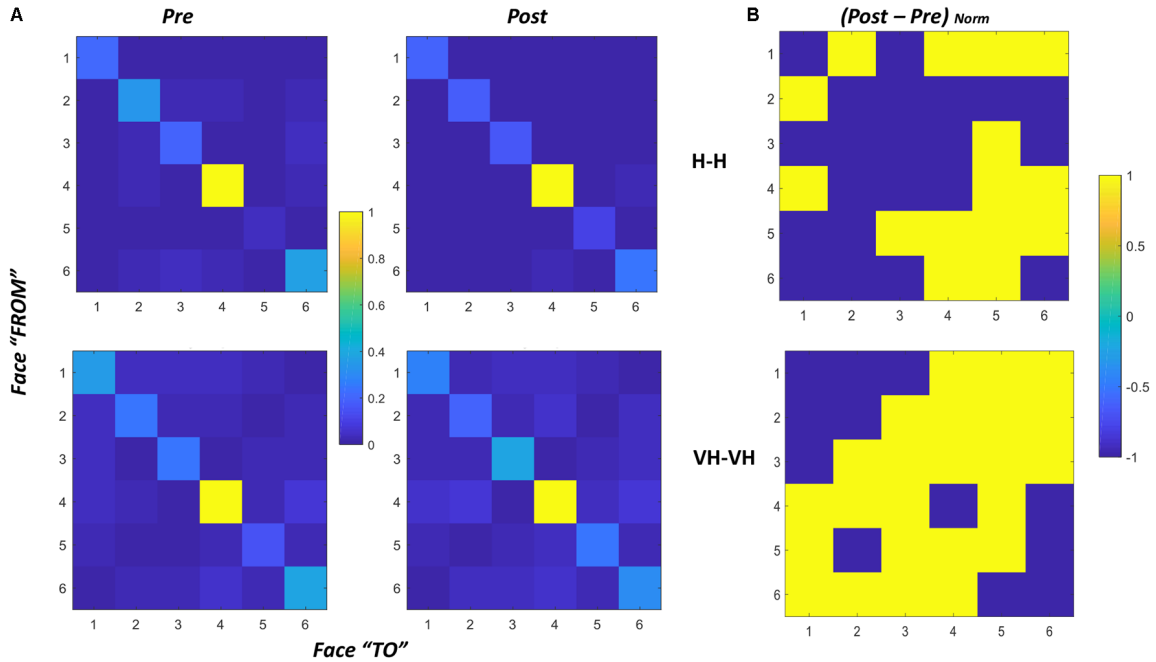

FIGURE 8 | Transition matrices. **(A)** Transition matrices for *pre* and *post* trials, for Haptic (top) and VH (bottom) conditions for all subjects. Each cell represents the proportion of transitions from one face (row id) to the other (column id) in the cone of visibility. The proportion is computed with respect to the maximum of the matrix. Cells on the diagonal reflect the proportion of time instants in which the same face was maintained in the cone. **(B)** Normalized difference between the *post* and *pre* matrixes for Haptic (top) and VH (bottom) conditions. Values have been set to 1 when positive, −1 when null or negative. As a result, yellow cells indicate transitions which proportionally increased from *pre* to *post*.

but also in the number of touches and the amount, type and speed of rotations. Interestingly, the reduction in exploration time was observed also in the "Same" trials, in which the cube was not modified between the two explorations. So, even when visiting the whole cube was necessary to provide an accurate response, the *post* exploration was performed differently from the *pre* one. In summary, uni- and bimodal exploratory strategies are modified as a function of the goal of the exploration, i.e., memorization or recall, even when the properties to be analyzed in the object and the sensory modalities available are kept constant.

Additionally, the results demonstrated that the availability of vision—together with haptics—substantially changes how the object is manipulated, leading to faster decisions, faster and larger rotations and a reduced number of touches. The better temporal performance observed in the VH condition hints to a higher efficiency of simultaneous visual and haptic exploration with respect to unimodal haptic exploration. This finding extends previous results obtained in classical geometric shapes recognition tasks (Hatwell et al., 2003). The reduction in the number of touches is consistent with the role of vision as a guide, providing a quick "preview" of the object properties, and limiting the instantiation of an extensive haptic exploration when the visual encoding is sufficient to give a response (Klatzky et al., 1993). It is worth noting that notwithstanding the reduction in the total number of touches in the VH condition, in our experiment participants still performed several touches, which might suggest that touch was used not only to support cube motion, but also to actively gather haptic information, in support to visual inspection. A relevant contribution of haptic exploration to the response, even in the presence of vision, might be due to the property of the stimulus to be assessed. Indeed, the analysis of the pins configuration can be interpreted as a form of texture discrimination rather than a pure geometrical task (as shape recognition) and haptic sensing is particularly efficient for texture perception (Jones and O'Neil, 1985).

The addition of vision had an opposite effect on how the object was touched and how it was rotated. Indeed, while the number of touches decreased, the amount of cube rotations and their speed significantly increased from the Haptic to the VH condition. This suggests a guidance of vision in the selection of the responses. While haptically it is possible to explore simultaneously different faces of the cube, as the front and the back, with fingers and thumbs respectively (Newell et al., 2001), with no need for a complete rotation of the object, vision requires that all faces are positioned so as to allow for a visual inspection, inducing subjects to perform larger rotations. These rotations can, however, be performed at a very fast pace, as they are mainly finalized to put each face in better view rather than being part of the strategy for tactual exploration. As a result, the VH recognition is significantly faster than the Haptic one, even if it involves larger rotations of the object to be analyzed.

The current experimental setting has some limitations, in that it provides only an object-centered description of the touches and rotations occurred, without allowing to understand exactly how visual analysis and haptic exploration are coordinated. Another factor that the current investigation did not address is how the bimanual coordination unfolds during the exploration. The discussion of the relative roles of the right and the left hand in dichaptic exploration is still controversial, with some evidence in favor of a higher sensitivity of the left hand—e.g., for curvature (Squeri et al., 2012) or for geometrical shape discrimination (Fagot et al., 1997), but depending on a variety of factors, as gender, type of shape, exploratory approach (Streri, 2003). The analysis described here clearly shows how also the presence of vision and the memorization goal are determining factors in the planning of the bimanual exploratory strategy adopted by participants.

Notwithstanding these limitations, the current study has demonstrated that exploratory strategies are not only tailored to the property to be extracted—as described in the traditional Exploratory Procedures classification—but are finely tuned to the current goal of the shape perception task (memorize/encode vs. or recall/match).

Shedding light on how different factors shape haptic exploration and how this is connected with efficient perception of object properties could help develop novel training protocols designed to help participants showing perceptual or memory deficits or to support learning during development. Multiple evidence suggests that haptic and VH exploration can facilitate letters and shapes understanding in young children (Bara et al., 2004; Kalenine et al., 2011). As a consequence, teachers and occupational therapy practitioners often engage children in multi-sensory experiences as part of teaching or treatment, respectively (Coté, 2013). A better understanding of how haptic exploration is tailored by sensory-motor and task constraints could help in appropriately designing these activities to maximize their impact. Moreover, a systematic modeling of the features of efficient exploration in healthy individual could also allow detecting the occurrence of abnormal exploratory behaviors that emerge during life—either due to developmental changes or to the set in of a disease. For instance, Mild Cognitive Impairment leads to significant deficits in haptic tasks (Grunwald et al., 2002), among the spectrum of deterioration of memory and perceptual-motor capabilities associated with this condition. In contrast, haptic memory has been shown to be very well preserved in healthy ageing (Sebastián et al., 2011), while being even more compromised in patients with dementia—e.g., Alzheimer disease (Ballesteros and Reales, 2004). A simple and non-invasive procedure providing information on the manipulation strategy adopted during haptic memory tasks could represent a valid addition to the assessment measures currently in place, supporting the quantitative evaluation of both perceptuo-motor skills and memory processes.

We also posit that the use of simple noninvasive tools as the sensorized object described here could be in future used to augment the automatic assessment of haptic exploration strategies, reducing the need of manual annotation of videos, to increase reproducibility of the measures, a currently crucial challenge in the context of haptic analysis (Jansen et al., 2013, 2015). Indeed, the automatic extraction of the temporal dynamics of the exerted touches could allow reconstructing on average the effector's motion features, together with the cube rotation. This information could in future be used to derive similarities and differences with those exploratory procedures which are principally defined by the kinematics of the exploring hand.

ETHICS STATEMENT

The research protocol was approved by the Regional Ethical Committee (Comitato Etico Regione Liguria—Sezione 1), all participants provided their written informed consent, received a compensation of 10 euro and followed the same experimental procedure.

AUTHOR CONTRIBUTIONS

All authors contributed to the design of the experiment. MA and FD cured the data collection. AS, MA and FD cured the data analysis. AS and GS contributed to the writing of the manuscript. All authors revised the manuscript.

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Resting-State Brain Network Dysfunctions Associated With Visuomotor Impairments in Autism Spectrum Disorder

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Background: Individuals with autism spectrum disorder (ASD) show elevated levels of motor variability that are associated with clinical outcomes. Cortical–cerebellar networks involved in visuomotor control have been implicated in postmortem and anatomical imaging studies of ASD. However, the extent to which these networks show intrinsic functional alterations in patients, and the relationship between intrinsic functional properties of cortical–cerebellar networks and visuomotor impairments in ASD have not yet been clarified.

Methods: We examined the amplitude of low-frequency fluctuation (ALFF) of cortical and cerebellar brain regions during resting-state functional MRI (rs-fMRI) in 23 individuals with ASD and 16 typically developing (TD) controls. Regions of interest (ROIs) with ALFF values significantly associated with motor variability were identified for for patients and controls respectively, and their functional connectivity (FC) to each other and to the rest of the brain was examined.

Results: For TD controls, greater ALFF in bilateral cerebellar crus I, left superior temporal gyrus, left inferior frontal gyrus, right supramarginal gyrus, and left angular gyrus each were associated with greater visuomotor variability. Greater ALFF in cerebellar lobule VIII was associated with less visuomotor variability. For individuals with ASD, greater ALFF in right calcarine cortex, right middle temporal gyrus (including MT/V5), left Heschl's gyrus, left post-central gyrus, right pre-central gyrus, and left precuneus was related to greater visuomotor variability. Greater ALFF in cerebellar vermis VI was associated with less visuomotor variability. Individuals with ASD and TD controls did not show differences in ALFF for any of these ROIs. Individuals with ASD showed greater posterior cerebellar connectivity with occipital and parietal cortices relative to TD controls, and reduced FC within cerebellum and between lateral cerebellum and pre-frontal and other regions of association cortex.

Conclusion: Together, these findings suggest that increased resting oscillations within visuomotor networks in ASD are associated with more severe deficits in controlling variability during precision visuomotor behavior. Differences between individuals with ASD and TD controls in the topography of networks showing relationships to visuomotor behavior suggest atypical patterns of cerebellar–cortical specialization and connectivity in ASD that underlies previously documented visuomotor deficits.

Keywords: autism spectrum disorder, resting-state functional MRI, visuomotor control, precision grip, cortical–cerebellar connectivity, amplitude of low-frequency fluctuations, functional connectivity

INTRODUCTION

Autism spectrum disorder (ASD) is characterized by difficulties in social interaction and communication, a restricted repertoire of interests and stereotypic behaviors (American Psychiatric Association, 2013). Sensorimotor deficits are common in ASD including reduced accuracy of ballistic and smooth pursuit eye movements (Takarae et al., 2007; Schmitt et al., 2014), gait abnormalities (Esposito and Ventola, 2008; Calhoun et al., 2011), macrographia (Fuentes et al., 2009), and atypical finger mannerisms (Anzulewicz et al., 2016). Reduced sensorimotor control interferes with multiple adaptive skills (Travers et al., 2016), and more severe motor deficits appear to be related to more severe social (Haswell et al., 2009; Landa et al., 2016), cognitive (Estes et al., 2015), and language impairments in ASD (Iverson, 2010).

Our group has documented that individuals with ASD show increased force variability relative to typically developing (TD) controls during visually guided precision gripping (Mosconi et al., 2015; Wang et al., 2015) and isometric index finger abduction (Wang et al., 2017). Precision force control is essential for manual dexterity, and increased grip force variability is associated with diminished capacity to execute manual motor tasks such as writing, buttoning clothes, and manipulating small or delicate items (Potter et al., 2009). Non-human primate and task-based functional magnetic resonance imaging (tb-fMRI) studies have delineated distinct cortical–cerebellar networks involved in reactively adjusting sustained precision motor behavior in response to visual information (Kelly and Strick, 2003; Vaillancourt et al., 2006; Coombes et al., 2010). Specifically, visual–spatial information is processed in calcarine cortex and relayed to posterior parietal cortex (Glickstein, 2000). Visual feedback information is translated directly to ventral and dorsal premotor cortex, and then to primary motor cortex in order to adjust outgoing motor commands. Additionally, parietal–ponto–cerebellar–thalamo–motor cortical pathways (Glickstein, 2000; D'Mello and Stoodley, 2015) integrate sensory feedback error information to refine motor outputs at the periphery. Within the cerebellum, anterior (I–V) and inferior (VIII–X) lobules are densely connected with somatomotor and brainstem circuits and are involved in basic sensorimotor behaviors (Nitschke et al., 2005; Stoodley and Schmahmann, 2009, 2010). In contrast, the more phylogenetically advanced lateral hemispheres (crus I–II) innervate pre-frontal and association cortices via dentate nuclei and thalamus (Ramnani, 2006). The extent to which these distinct cortical–cerebellar circuits are functionally affected and associated with visuomotor impairments in ASD is not yet known.

Consistent and growing evidence from neuroimaging studies suggests that ASD is characterized by abnormalities of distributed functional networks, rather than focal impairment. Task-based fMRI (tb-fMRI) studies have documented reduced activation of cortical–cerebellar networks accompanied by increased recruitment of supplementary motor area during simple sequential finger tapping (Mostofsky et al., 2009). Using diffusion tensor imaging, several studies have demonstrated reduced white matter microstructural integrity within fronto–parietal networks (Fitzgerald et al., 2018), cortical–basal ganglia networks (Barnea-Goraly et al., 2004; Shukla et al., 2010; Nair et al., 2015), brainstem (Hanaie et al., 2016), and both middle and superior peduncles of the cerebellum in ASD (Catani et al., 2008; Brito et al., 2009; Hanaie et al., 2013). These studies suggest that aberrant functional and structural connectivity of cortical and subcortical networks supporting sensorimotor control may contribute to sensorimotor impairments in ASD.

The examination of brain activity during resting-state fMRI (rs-fMRI) is a well-validated approach that allows characterization of intrinsic properties of regional- and network-level functional activation and connectivity (Biswal et al., 1995; Fox and Raichle, 2007). During rest, the brain displays spontaneous low-frequency (0.01–0.08 Hz) blood oxygen level-dependent (BOLD) fluctuations reflecting neural activity when goal-directed cognitive behavioral actions and external sensory inputs are minimized relative to active task conditions (Biswal et al., 1995; Zuo et al., 2010). Importantly, these low-frequency

Abbreviations: ASD, autism spectrum disorder; tb-fMRI, task-based functional MRI; rs-fMRI, resting-state functional MRI; BOLD, blood-oxygen-level dependent; FC, functional connectivity; ROI, region of interest; ALFF, amplitude of low-frequency fluctuations; FSIQ, full scale IQ; PIQ, performance IQ; VIQ, verbal IQ; RBS-R, repetitive behaviors scale-revised; SFG.R, right superior frontal gyrus; MFG.L, left middle frontal gyrus; IFG.L, left inferior frontal gyrus; PreCG.R, right pre-central gyrus; PoCG.L, left post-central gyrus; SPG.L, left superior parietal gyrus; SPG.R, right superior parietal gyrus; SMG.R, right supramarginal gyrus; ANG.L, left angular gyrus; PCUN.L, left precuneus; HES.L, left Heschl's gyrus; STG.L, left superior temporal gyrus; MT/V5. R, right middle temporal gyrus including visual area 5; SOG.R, right superior occipital gyrus; SOG.L, left superior occipital gyrus; MOG.L, left middle occipital gyrus; CAL.R, right calcarine cortex; DCG.L, left median cingulate gyrus; CBL.Vermis VI, cerebellar vermis VI; CBL.Crus I.L, left cerebellar crus I; CBL.Crus I.R, right cerebellar crus I; CBL.Crus II.L, left cerebellar crus II; CBL.Crus II.R, right cerebellar crus II; CBL.VIII.L, left cerebellar lobule VIII.

fluctuations during rest show high levels of temporal correlation with discrete proximal and distal brain regions that comprise specialized brain networks involved in cognition and behavior as determined by tract-tracing, histopathological, and tb-fMRI studies (Fox and Raichle, 2007; Fox et al., 2007; Ma et al., 2011). Moreover, coherent intrinsic BOLD fluctuations account for a majority of the BOLD–behavior relationship observed during tb-fMRI (Fox et al., 2006, 2007). For example, the resting amplitude of low-frequency fluctuations (ALFFs) and functional connectivity (FC) of frontal–parietal motor networks are strongly associated with the rates at which individuals are able to learn novel manual motor skills (Ma et al., 2011).

Few studies have characterized regional ALFF during rest in ASD, although several rs-fMRI studies have documented atypical FC in patients relative to controls (Just et al., 2004; Jones et al., 2010; Tyszka et al., 2014; Cerliani et al., 2015; Hull et al., 2017). A consistent observation of these studies includes reduced long-distance cortical FC in individuals with ASD, with effects most pronounced within sensorimotor, default mode, and visual perceptual networks (Assaf et al., 2010; Di Martino et al., 2014; Hahamy et al., 2015). Recent studies showed decreased cerebellar connectivity to somatomotor and visual cortices in ASD relative to controls that was related to more severe ASD symptoms (Khan et al., 2015; Cardon et al., 2017). These findings suggest that intrinsic functional communication between discrete regions of visual–motor brain networks may be selectively impaired in ASD and related to key clinical features of the disorder. It remains unclear whether intrinsic ALFF and FC of these networks are associated with visuomotor impairments in ASD.

The present study aimed to characterize intrinsic functional properties of distinct brain regions and networks associated with visually guided precision motor control in individuals with ASD and matched TD controls. ALFF and FC were quantified during rs-fMRI and compared with precision grip force variability during a visuomotor task previously studied in ASD (Mosconi et al., 2015; Wang et al., 2015). Based on prior findings showing increased involvement of "non-visuomotor" networks in ASD during simple motor tasks (Takarae et al., 2007; Mostofsky et al., 2009), we used a data-driven rather than a pre-defined region of interest (ROI) approach to identify cortical and subcortical brain regions associated with force variability. This approach has the advantage of identifying relevant ROIs that are outside primary sensorimotor networks but still associated with visuomotor behavior. FC between brain regions identified as significant in our ALFF–force variability analysis was then compared between groups and examined in relation to visuomotor behavior. Given prior findings that more severe motor abnormalities (Haswell et al., 2009; Estes et al., 2015; Landa et al., 2016) and FC alterations (Khan et al., 2015; Cardon et al., 2017) of visuomotor networks are associated with more severe ASD symptoms, we also examined the relationship between visuomotor network ALFF and FC with clinical ratings of social–communication abnormalities and restricted and repetitive behaviors in individuals with ASD.

METHODS

Participants

Twenty-three participants with ASD and 16 healthy controls completed a rs-fMRI scan. Of these participants, 10 individuals with ASD and 11 age-, gender-, and IQ-matched controls also completed a task of visually guided precision gripping during a separate tb-fMRI run (**Table 1**). Among the participants who completed the rs-fMRI run, 12 (ASD = 10, controls = 2) were not administered the tb-fMRI procedure, as the protocol was developed subsequent to the rs-fMRI study initiation. Six participants (ASD = 3, controls = 3) completed the rs- and tb-fMRI studies, but their data were not included due to technical or task compliance issues (e.g., intermittent relaxation of force during the task).

Individuals with ASD were recruited through community advertisements and local clinical programs. All participants with ASD met classification criteria for autism on the Autism Diagnostic Inventory-Revised (ADI-R) (Lord et al., 1994) and for autism or autism spectrum on the Autism Diagnostic Observation Schedule (ADOS) and were diagnosed with ASD according to DSM-5 criteria based on expert clinical opinion (American Psychiatric Association, 2013). IQ was assessed using the Wechsler Abbreviated Scales of Intelligence (Wechsler, 2011). No individuals with ASD had any known genetic syndrome associated with ASD (e.g., fragile X syndrome).

Control participants were recruited from the community and had a score of eight or lower on the Social Communication Questionnaire (SCQ) (Rutter et al., 2003). Control participants were excluded for current or past psychiatric or neurological disorders, family history of ASD in first- or second-degree relatives, or a history in first-degree relatives of a developmental or learning disorder, psychosis, or obsessive–compulsive disorder. No participants were taking medications known to affect motor performance at the time of testing, including antipsychotics, stimulants, or anticonvulsants (Reilly et al., 2008). No participant had a history of head injury, birth asphyxia, or non-febrile seizure. Study procedures were approved by the local Institutional Review Board. Adult participants provided both informed and written consent, and minors provided assent in addition to written consent from their legal guardian.

Visuomotor Data Acquisition

MRI scanning was completed on an Achieva 3-Tesla Philips system (Philips Medical Systems, Andover, MA). Each scanning session included a T1-weighted high-resolution structural scan (repetition time = 8.1 ms; echo time = 3.373 ms; flip angle = 12°; field of view = 256 × 204 × 160 mm^3; matrix = 256 × 204 × 160; 160 sagittal slices; voxel size = 1 mm^3; no gap). The T1 scan was performed prior to functional scans to facilitate functional data registration to standardized space.

Experimental procedures for the fMRI visuomotor test were similar to laboratory tests reported previously from our group (Mosconi et al., 2015; Wang et al., 2015). Prior to MR imaging, each participant's maximum voluntary contraction (MVC) was measured using a custom Bragg grating fiber optic force transducer (Model sm130; Neuroimaging Solutions, Gainesville,

TABLE 1 | Demographic and diagnostic characteristics [mean (SD)] for TD controls and individuals with ASD.

	Individuals who completed both tb-fMRI and rs-fMRI			Individuals who completed rs-fMRI		
	Controls ($n = 11$)	**ASD ($n = 10$)**	***p***	**Controls ($n = 16$)**	**ASD ($n = 23$)**	***p***
Age in years	22.82 (4.38)	21 (5.58)	0.414	23.31 (4.11)	19.09 (5.90)	0.018†
Range	*17–33 years*	*14–32 years*		*17–33 years*	*10–32 years*	
% Male[a]	90.9%	100%	1.000	93.8%	91.3%	1.000
FSIQ	120.70 (11.19)	109.90 (16.05)	0.098	121.07 (11.25)	101.78 (16.43)	0.000***
Range	*94–133*	*79–129*		*94–138*	*78–129*	
PIQ	118.80 (11.60)	108.80 (15.85)	0.125	118.40 (10.44)	104.00 (14.87)	0.002**
Range	*94–133*	*83–129*		*94–133*	*79–129*	
VIQ	118.00 (12.03)	109.10 (16.27)	0.181	118.87 (12.55)	99.39 (18.50)	0.001**
Range	*95–135*	*80–133*		*95–140*	*64–133*	
ADOS social		9.70 (3.89)			9.68 (3.68)	
Range		*4–16*			*4–16*	
ADOS RRB		2.70 (2.00)			2.68 (1.55)	
Range		*0–7*			*0–7*	
RBS-R total		23.14 (13.68)			26.85 (19.74)	
Range		*9–47*			*0–78*	

FSIQ, full scale IQ; PIQ, performance IQ; VIQ, verbal IQ; ADOS Social, ADOS social affect algorithm score; ADOS RRB, ADOS restricted and repetitive behaviors algorithm score; RBS-R total, repetitive behaviors scale-revised total score.
[a]Chi-square (χ^2) statistics using Fisher's exact test.
*†$p < 0.05$, *$p < 0.01$, **$p < 0.005$, ***$p < 0.001$.*

FL). The transducer was housed in a precision grip apparatus. Participants were instructed to hold the apparatus using the right thumb, middle, and index fingers (**Figure 1**; e.g., Neely et al., 2016). Participants then were instructed to press as hard as they could for three 5-s trials, and the average of their maximum force during these trials was used as their MVC.

During the scan, participants rested their hands at their sides and used their right hand to grip the force transducer without moving their arm while viewing visual feedback regarding their performance. A stationary TARGET bar (red/green) located in the middle of the screen was set to 60% of each individual's MVC. The TARGET bar turned from red to green at the beginning of each trial to cue participants to begin pressing the gripping device. Participants' motor performance was represented as a white FORCE bar that moved upward with increases in force output. Participants were instructed to press on the transducer so that the white FORCE bar reached and stayed at the same level as the stationary TARGET bar.

The visual angle of the FORCE bar was set to 0.623 as we have done previously (Mosconi et al., 2015). Participants completed five 24-s blocks in which they pressed on the transducer while receiving visual feedback. Each run began and ended with 24-s rest blocks, and each force block was separated by 24-s rest blocks (total scan time: 4:50). Only participants successfully completing at least three force trials were included in final analyses.

Resting-State fMRI Data Acquisition

During a 5-min rs-fMRI scan (240 brain volumes; 33 interleaved axial slices per volume; TR = 1,500 ms; echo time = 25 ms; flip angle = 60°; field of view = 220 × 114.2 × 220 mm^3; voxel size = 3.438 × 3.438 × 3.4 mm^3; 1-mm gap), participants were instructed to keep their eyes closed and refrain from any cognitive, language, or motor behavior as much as possible. Participants were queried regarding their ability to stay awake following each run; only runs in which participants reported staying awake were included in analyses.

Seed ROIs associated with visuomotor behavior were identified using the subset of participants who completed tb-fMRI (10 individuals with ASD and 11 matched controls). Clusters with significant correlations between ALFF and sustained force variability were defined separately for the ASD and TD groups. FC analysis was performed using the significant ROIs identified in these ALFF–force variability analyses as seed regions, but then analyzed across all participants who completed the rs-fMRI study as described below.

Visuomotor Data Processing and Analysis

Each force trace was low-pass filtered *via* a double-pass fourth-order Butterworth filter at a cutoff of 15 Hz in Matlab 2017a (MathWorks, Inc., Natick, MA). The first and last 3 s of each trial were excluded from the analyses to reduce variability related to the rates at which individuals initially increased their force level or relaxed their force at the end of trials. Thus, the middle 18 s of each sustained force trial was analyzed. To quantify individuals' motor variability, we examined the standard deviation of force during each trial for each individual. Mean force of each trial was also analyzed to ensure that participants understood and completed the task.

Rs-fMRI Data Processing

The rs-fMRI pre-processing was performed using the Data Processing Assistant for Resting-State fMRI 3.0 toolbox

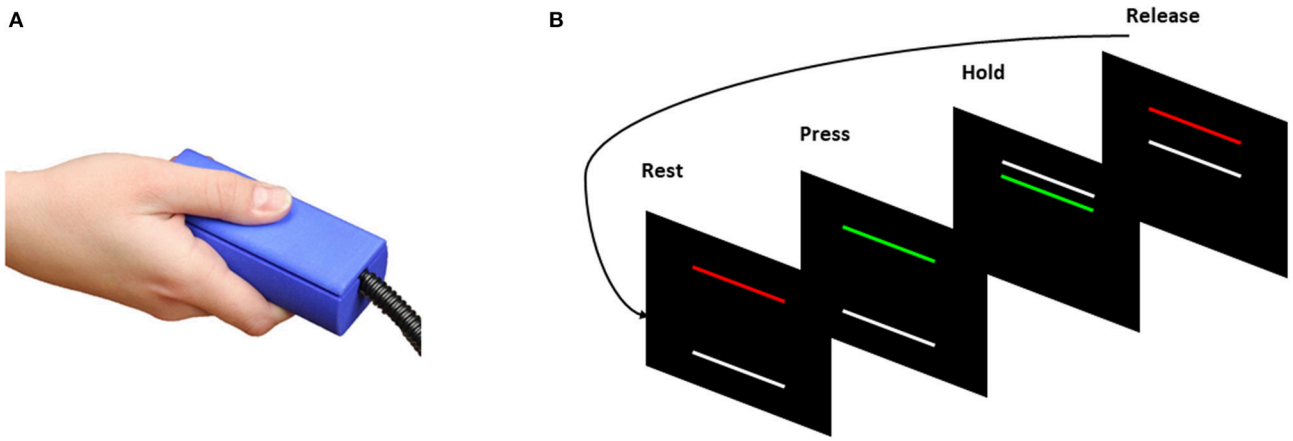

FIGURE 1 | (A) Individuals pressed against the Bragg grating fiber optic force transducer during a test of precision grip. **(B)** Visual information individuals received during task-based fMRI (tb-fMRI). Participants viewed two horizontal bars presented against a black background (Rest/Release). The TARGET bar (red/green) was stationary during each trial. The TARGET bar turned from red to green at the beginning of each trial to cue participants to begin pressing the force transducer. The white FORCE bar moved upward with increased force. The discrepancy between the TARGET and FORCE bars provided online visual feedback to the participants about their motor performance.

(DPARSF 3.0; http://rfmri.org/DPARSF) in MATLAB. The first 10 volumes of each run were discarded to reduce artifacts caused by magnetic instability (Zhang et al., 2012). Volumes were slice-time corrected and realigned to the middle slice. Rigid body realignment parameters were estimated for each individual, and data were excluded if individuals showed head motion $\geq$3 mm in the horizontal plane or over 3° in rotation. The rs-fMRI data of one individual with ASD were removed from the original dataset due to excessive head motion. There were no significant differences between individuals with ASD and controls for any of the six motion parameters (three translational and three rotational) (**Supplementary Table 1**).

Each individual's rs-fMRI data were registered to their own T1-weighted anatomical scan and spatially normalized to Montreal Neurological Institute (MNI) space using the unified segmentation–normalization algorithm in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Specifically, individuals' structural images were coregistered to the functional image after motion correction, and the transformed structural images were segmented into gray matter, white matter, and cerebrospinal fluid by using a unified segmentation algorithm. The motion corrected functional images were spatially normalized to MNI space and resampled to 3 × 3 × 3 mm voxels using the normalization parameters estimated during unified segmentation. We regressed out nuisance covariates including linear trends, friston 24 head motion parameters, white matter, and cerebrospinal fluid signal. Data were spatially smoothed using a 4-mm full width at half maximum (FWHM) Gaussian filter and bandpass-filtered at the range from 0.01 to 0.08 Hz to remove slow drift and high-frequency components (Biswal et al., 1995; Lowe et al., 1998). No global signal regression was performed to avoid introduction of spurious correlations of the rs-fMRI data (Gotts et al., 2013).

Amplitude of Low-Frequency Fluctuations and Functional Connectivity Analysis

ALFF analysis was conducted using the rs-fMRI data analysis toolkit v1.1 (http://www.restfmri.net/forum/rest_v11) in MATLAB. For each individual's rs-fMRI data, the filtered time series of each voxel was transformed to the frequency domain to obtain the power spectrum using a fast Fourier transformation (taper percent = 0, FFT length = shortest). The ALFF was derived from the averaged square root of the power spectrum across frequencies from 0.01 to 0.08 Hz (Zang et al., 2007). The ALFF of each voxel was then normalized by the averaged ALFF value of the whole brain.

Seed ROIs for FC analysis were identified using data from the subset of participants (10 individuals with ASD and 11 controls) who completed both rs-fMRI and tb-fMRI runs by determining clusters with significant correlations between ALFF and sustained force variability separately for each group. Monte Carlo simulation was used to correct for multiple comparisons (Ledberg et al., 1998). Based on AlphaSim calculations, clusters including $\geq$90 contiguous voxels showing significant correlations with $p < 0.05$ at voxel level were identified and are reported to maintain family-wise $p < 0.05$.

FC analysis was performed based on rs-fMRI of all participants using a seed-based voxel correlation approach (Hull et al., 2017). Three dimensional 6-mm-radius seeds were created based on selected ROIs using the PickAtlas toolbox in SPM8 (http://fmri.wfubmc.edu/software/PickAtlas). The center of the sphere for each identified voxel cluster was the coordinate with the greatest correlation between motor variability and ALFF value. To quantify whole-brain connectivity of identified seeds, individual time series for each ROI were extracted and correlated with each voxel in the brain to create a whole brain connectivity map. Each participant's correlation map was then converted to z-statistic maps using Fisher r-to-z transformations.

Statistical Analyses

One-way ANOVAs were conducted to compare groups on force variability. For imaging data, statistical analyses were conducted using rs-fMRI data analysis toolkit V1.8 (http://www.restfmri.net/forum/REST_V1.8) in MATLAB (Zang et al., 2007). ROIs identified as significantly associated with force variability in the ASD or control group were merged. Then, the maximal ALFF value within each ROI was extracted from each individual and compared between patients and controls using two-sample *t*-tests with the SPSS 22 software (Armonk, NY, USA). For voxel-based comparisons of FC correlation maps between groups, two-sample *t*-tests was used with REST_V1.8 software in Matlab. Age and sex were included as covariates for both statistical analyses. A default mask (dimension = 61 × 73 × 61) was applied for all statistical analyses. Additionally, for individuals with ASD, we examined the relationship between strength of FC for networks that showed significant between-group differences and IQ scores and clinical ratings of social–communication deficits (i.e., ADOS social communication algorithm score). Statistical thresholds for correlation analyses were set at $p < 0.05$ (two-tailed) after false discovery rate (FDR) correction. To inform hypotheses of future studies, uncorrected correlation *r* and *p*-values are also presented in **Supplementary Tables 3–7**.

RESULTS

Visuomotor Behavioral Measure

Individuals with ASD and TD controls showed similar MVCs ($t_{11.43} = 0.41$, $p = 0.69$; ASD: mean = 55.70 N, SD = 20.68 N; TD: mean = 52.82 N, SD = 8.01 N) and mean force ($t_{12.12} = 0.18$, $p = 0.86$; ASD: mean = 31.46 N, SD = 10.58 N; TD: mean = 30.81 N, SD = 4.67 N). The difference in sustained force variability for individuals with ASD and TD controls was not statistically significant ($t_{9.59} = 1.68$, $p = 0.13$; ASD: mean = 3.38 N, SD = 4.07 N; TD: mean = 1.19 N, SD = 0.77 N). While this group comparison was not significant as we have seen in our prior studies (Mosconi et al., 2015; Wang et al., 2015), the effect size was large (Cohen's $d = 0.75$).

Relationships Between ALFF and Sustained Force Variability

Seven ROIs in each group showed significant correlations between whole brain voxel-wise ALFF measures and sustained force variability (**Table 2** and **Figure 2**). ROIs that were positively correlated with force variability for TD controls after corrections for multiple comparisons included left inferior frontal gyrus, right supramarginal gyrus, left angular gyrus, left superior temporal gyrus, and bilateral cerebellar crus I. Left cerebellar lobule VIII ALFF was negatively correlated with force variability for controls.

For individuals with ASD, ALFF levels of the right precentral gyrus, left post-central gyrus, left precuneus, left Heschl's gyrus, right middle temporal gyrus (including MT/V5), and right calcarine cortex were positively correlated with force variability. ALFF levels in the cerebellar vermis VI were negatively correlated with increased force variability in ASD. After FDR correction, none of these 14 ROIs showed between group differences in ALFF (all corrected *p*'s < 0.05; **Supplementary Table 2**).

TABLE 2 | Montreal neurological institute (MNI) coordinates of selected seed regions of interest (ROIs) which showed significant correlations between amplitude of low frequency fluctuations (ALFF) and sustained force standard deviation during precision grip in TD controls and individuals with ASD.

ROIs	MNI coordinates			Number of voxels	Mean (SE)	Correlation coefficient *Z*
	X	Y	Z			
ROIs identified from controls						
Left inferior frontal gyrus	42	15	24	110	−0.13 (0.10)	0.94*
Right supramarginal gyrus	66	−27	39	103	−0.03 (0.06)	0.93*
Left angular gyrus	−54	−60	30	180	0.06 (0.07)	0.90*
Left superior temporal gyrus	−57	−18	3	31	−0.13 (0.10)	0.95*
Left cerebellar crus I	−45	−48	−33	190	0.04(0.13)	0.94*
Right cerebellar crus I	24	−78	−27	612	0.17 (0.17)	0.97*
Left cerebellar lobule VIII	21	−57	48	147	−0.08 (0.04)	−0.90*
ROIs identified from individuals with ASD						
Right precentral gyrus	42	−18	42	391	−0.08 (0.05)	0.94*
Left postcentral gyrus	−33	−24	45	113	0.01 (0.05)	0.93*
Left precuneus	−6	−36	57	241	−0.07 (0.06)	0.94*
Left Heschl's gyrus	−45	−15	9	369	−0.00 (0.08)	0.98*
Right middle temporal gyrus	60	−15	−9	250	−0.11 (0.11)	0.94*
Right calcarine cortex	15	−57	15	342	−0.25 (0.13)	0.94*
Cerebellar vermis VI	6	−69	−15	306	−0.05 (0.11)	−0.96*

Negative X values indicate locations in the left hemisphere.
All results are AlphaSim error corrected, $^{\dagger}p < 0.05$, $^{*}p < 0.01$, $^{**}p < 0.005$, $^{***}p < 0.001$.

Functional Connectivity in Individuals With ASD and TD Controls

Intra-cerebellar FC was reduced across multiple lobules in ASD (**Table 3** and **Figure 3**). Compared to TD controls, individuals with ASD showed decreased FC between right cerebellar crus II and seeds within left cerebellar lobule VIII, bilateral cerebellar crus I, and cerebellar vermis VI. Individuals with ASD also showed reduced FC between left cerebellar crus I and right cerebellar lobule IX, left cerebellar crus II, and cerebellar vermis VI.

Cerebellar FC with pre-frontal and temporal cortical targets was reduced in ASD relative to TD controls. Individuals with ASD showed decreased FC between right cerebellar crus II and both left Heschl's gyrus and left superior temporal gyrus. For left cerebellar crus I and cerebellar vermis VI, individuals with ASD showed lower FC than controls with left middle frontal gyrus and right superior frontal gyrus.

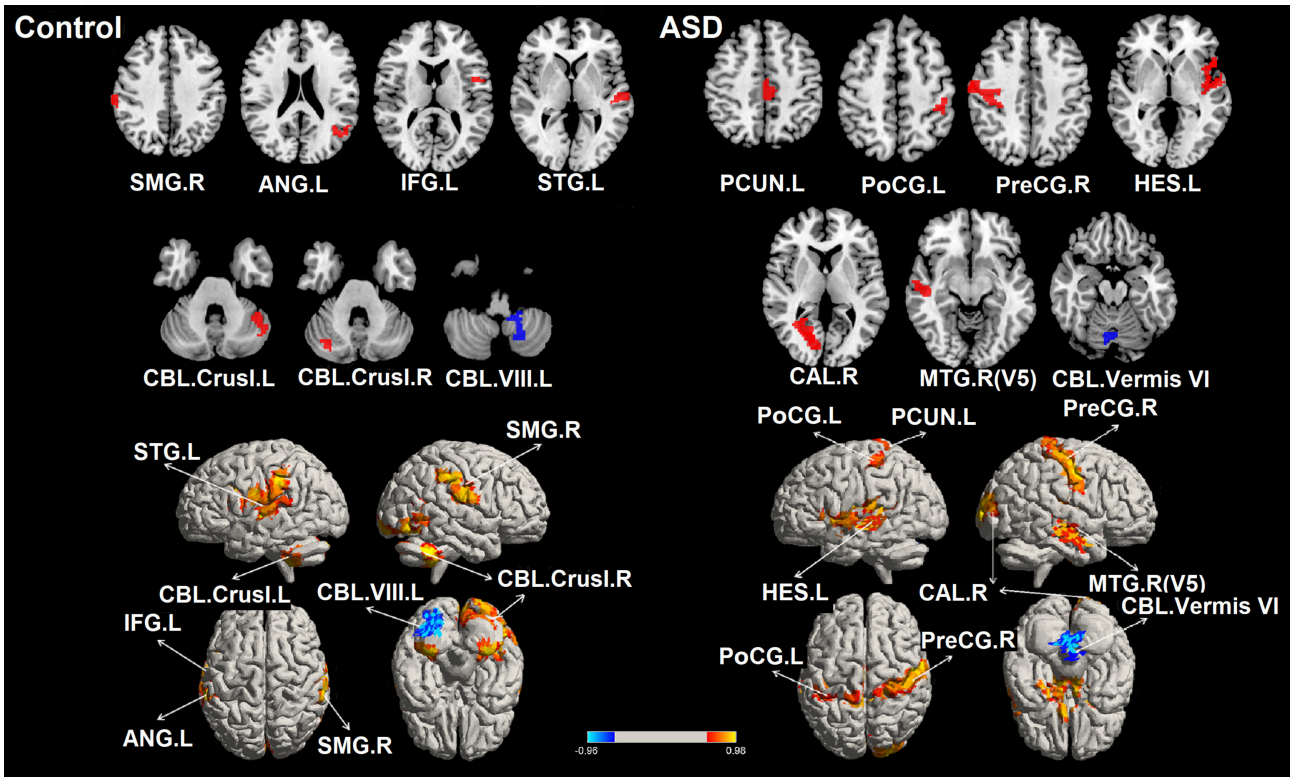

FIGURE 2 | Significant correlations between the whole brain amplitude of low-frequency fluctuations (ALFFs) and sustained force standard deviation in individuals with ASD and TD controls. The red areas depict voxels presenting positive correlations, whereas the blue areas depict voxels showing negative correlations with sustained force standard deviation. IFG.L, left inferior frontal gyrus; PreCG.R, right precentral gyrus; PoCG.L, left postcentral gyrus; SMG.R, right supramarginal gyrus; ANG.L, left angular gyrus; PCUN.L, left precuneus; HES.L, left Heschl's gyrus; STG.L, left superior temporal gyrus; MT/V5. R, right middle temporal gyrus including visual area 5; CAL.R, right calcarine cortex; CBL.Vermis VI, cerebellar vermis VI; CBL.Crus I. L, left cerebellar crus I; CBL.Crus I. R, right cerebellar crus I; CBL. VIII. L, left cerebellar lobule VIII.

Cerebellar FC with occipital and parietal cortical targets was primarily elevated in ASD relative to controls. Individuals with ASD showed increased FC between left cerebellar lobule VIII and bilateral superior occipital gyrus and left superior parietal gyrus. FC between right cerebellar crus I and left superior occipital gyrus was also increased in ASD relative to controls. Right cerebellar crus II FC with left precuneus was reduced in ASD compared to controls.

Demographic and Clinical Correlations

After FDR correction, no significant correlations were found between ALFF values of our 14 selected ROIs and IQ scores for healthy controls (**Supplementary Table 3**) or individuals with ASD (**Supplementary Table 4**). No ROI ALFF values were related to ASD clinical ratings for patients (**Supplementary Table 4**). No significant correlations were identified between each cortical–cerebellar FC and IQ scores (**Supplementary Table 5**) or ADOS social communication algorithm scores for individuals with ASD (**Supplementary Table 6**). Increased FC between left precuneus and right superior parietal gyrus was associated with less severe RBS-R rated repetitive behaviors in ASD. No significant correlations were identified between FC of cortical–cerebellar networks and sustained force variability for TD controls (**Supplementary Table 7**). Greater FC between left cerebellar crus I and right cerebellar lobule IX was associated with reduced sustained force variability in individuals with ASD (**Supplementary Table 7**).

DISCUSSION

In the present study, we identify multiple discrete cortical and cerebellar brain regions showing intrinsic functional oscillations that covary with precision visuomotor ability. The pattern of ROIs showing intrinsic functional properties associated with visuomotor variability were distinct for individuals with ASD and TD controls. Specifically, greater ALFF values of sensorimotor cortical and cerebellar brain regions were associated with greater force variability (i.e., less force precision) in ASD, whereas greater ALFF values in cortical and cerebellar brain regions that comprise higher-order association networks were related to greater force variability in TD controls. Further, we find that FC in patients was greater in cerebellar–occipital and cerebellar–parietal circuits associated with fundamental sensory and sensorimotor processes, whereas it was reduced

TABLE 3 | Between group comparison of functional connectivity (FC) between ROIs identified in ALFF analyses and whole-brain data (positive *T*-values represent greater FC in ASD vs. TD controls).

ROIs	Brain regions	MNI coordinates			Number of voxels	*T*-value
		X	Y	Z		
ROIs identified from TD controls						
Right supramarginal gyrus	Left middle cingulate gyrus	−9	−21	48	93	4.00***
Left angular gyrus	Right superior frontal gyrus	18	51	39	123	−5.05***
	Right superior occipital gyrus	27	−72	18	184	5.04***
Left superior temporal gyrus	Right cerebellar crus II	42	−78	−42	168	−4.20***
Left cerebellar crus I	Right superior frontal gyrus	27	48	42	92	−4.58***
	Left middle frontal gyrus	−27	42	33	110	−3.95***
	Left cerebellar crus II	−39	−66	−48	198	−4.92***
	Right cerebellar crus II	51	−63	−45	288	−4.96***
	Right cerebellar lobule IX	12	−48	−48	120	−3.79***
Right cerebellar crus I	Left superior occipital gyrus	−12	−84	18	175	4.65***
	Right cerebellar crus II	42	−81	−42	160	−4.08***
Left cerebellar lobule VIII	Left superior parietal gyrus	−21	−57	45	159	4.62***
	Left superior occipital gyrus	−21	69	30	141	4.47***
	Right superior occipital gyrus	27	−69	33	209	4.97***
	Right cerebellar crus II	42	−78	−42	137	−3.70**
ROIs identified from individuals with ASD						
Left precuneus	Right superior parietal gyrus	18	−63	48	143	4.36***
	Right cerebellar crus II	6	−84	−39	92	−3.67**
Left Heschl's gyrus	Left superior parietal gyrus	−24	−39	48	101	3.96***
	Right cerebellar crus II	21	−81	−57	105	−3.75**
Cerebellar vermis VI	Right superior frontal gyrus	24	57	15	187	−5.20***
	Left middle frontal gyrus	−27	45	36	138	−3.94***
	Left middle occipital gyrus	−24	−84	24	102	4.49***
	Left cerebellar crus I	−45	−57	−39	277	−4.93***
	Right cerebellar crus II	42	−81	−42	241	−4.42***

Negative X-values indicate locations in the left hemisphere.
*All results are AlphaSim corrected, †p < 0.05, *p < 0.01, **p < 0.005, ***p < 0.001.*

in cerebellar–frontal and cerebellar–temporal cortical circuits involved in more complex multisensory and cognitive processes. Overall, these findings suggest atypical specialization of brain networks associated with visuomotor behavior in ASD.

Intrinsic Cortical–Cerebellar Activity and Visuomotor Precision

ALFF power during rest reflects intrinsic oscillations of local circuits including changes in synaptic activity (Fox et al., 1988), neurotransmitter recycling (Magistretti and Pellerin, 1999), and synchronous neuronal firing (Logothetis, 2002; Scholvinck et al., 2010). Long-duration rs-fMRI studies of non-human primates have identified strong correlations between local field potentials and fluctuations in BOLD time series from nearby ROIs at both gamma and lower-frequency bands with consistent time lags of 6–8 s (Shmuel and Leopold, 2008), implying a causal relationship between spontaneous neuronal activity and local hemodynamics. Neuroimaging studies in humans have further demonstrated that intrinsic brain activity accounts for a significant proportion of variance (up to 74%; Fox et al., 2007) of tb-BOLD responses and motor behavioral outcomes (Fox et al., 2006, 2007). Intrinsic BOLD oscillations thus represent important signals interacting with task-related neuronal functions to support adaptation to task demands (Lv et al., 2018). Based on these observations, it has been postulated that the relative balance of intrinsic oscillations and task-specific regional activation is critical for guiding precise sensorimotor behaviors.

Our finding that force variability was significantly correlated with distinct cortical and cerebellar brain regions supports this hypothesis. Positive associations between intrinsic cortical activity and force variability suggest that resting activity of sensorimotor networks in ASD serves as "interference" during visuomotor behavior by either reducing signal-to-noise ratios of task-dependent cortical functions or attenuating the extent to which cortical circuits can dynamically adjust to support the onset and maintenance of behavior. Findings that ALFF levels of cerebellar regions (**Table 2**; i.e., left cerebellar lobule VIII in controls and cerebellar vermis VI in ASD) dedicated to sensorimotor processes were anti-correlated with force variability suggest that reduced intrinsic cerebellar activations are associated

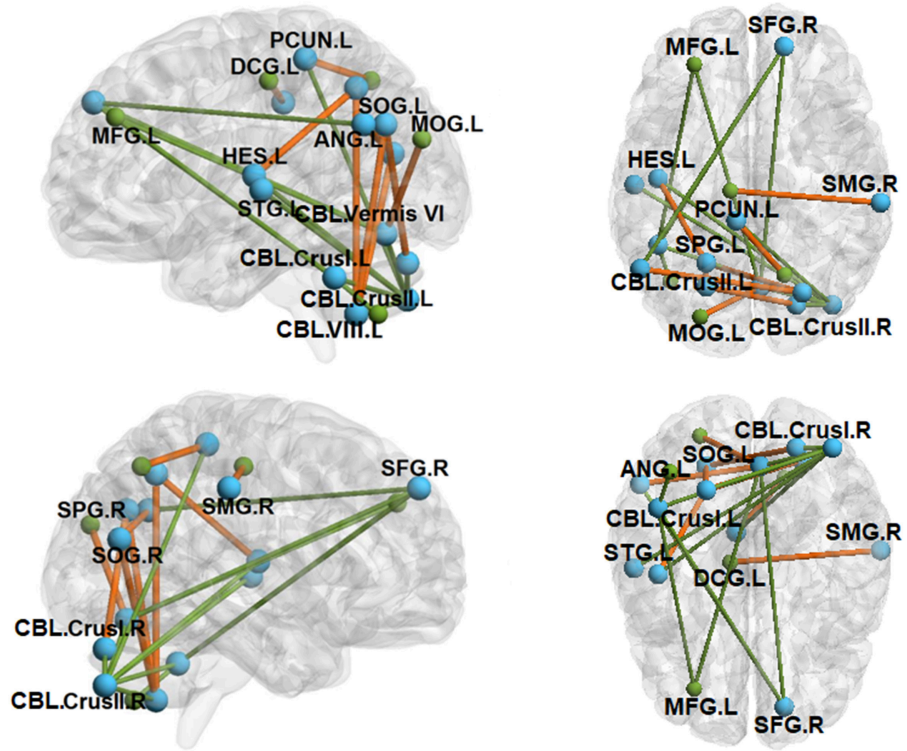

FIGURE 3 | Between-group functional connectivity (FC) maps (ASD vs. TD). Green lines depict FC reductions in individuals with ASD relative to TD controls, while yellow lines represent FC elevations in ASD relative to TD controls. SFG.R, right superior frontal gyrus; MFG.L, left middle frontal gyrus; SPG.L, left superior parietal gyrus; SPG.R, right superior parietal gyrus; SMG.R, right supramarginal gyrus; ANG.L, left angular gyrus; PCUN.L, left precuneus; HES.L, left Heschl's gyrus; STG.L, left superior temporal gyrus; SOG.L, left superior occipital gyrus; SOG.R, right superior occipital gyrus; MOG.L, left middle occipital gyrus; DCG.L, left median cingulate gyrus; CBL.Vermis VI, cerebellar vermis VI; CBL.Crus I.L, left cerebellar crus I; CBL.Crus I.R, right cerebellar crus I; CBL.Crus II.L, left cerebellar crus II; CBL.Crus II.R, right cerebellar crus II; CBL.VIII.L, left cerebellar lobule VIII. All clusters were AlphaSim corrected and statistically significant at $p < 0.05$.

with attenuation of inhibitory output that serves to adjust cortical output during continuous sensorimotor behaviors. Cerebellar cortical output *via* Purkinje cells provides an inhibitory drive on cortical targets that supports refinement of motor behavior in response to sensory feedback (Stein and Glickstein, 1992). Cerebellar lobule VIII and vermis VI each innervate sensory and motor cortices, including frontal eye fields, parietal eye fields, inferior and superior parietal lobules, primary sensory cortex (S1), and primary motor cortex (M1) to support control of precision visuomotor activities (Glickstein, 2000; Ramnani, 2006). Our findings suggesting that reduced inhibitory drive on sensory and motor cortices at rest relates to greater motor variability may reflect amplified task-related modulation of sensory and motor cortices during action contributing to greater output variability over time.

Our brain–behavior results implicate independent cortical and cerebellar regions in the control of visuomotor behavior among individuals with ASD and TD controls. Analyses of individuals with ASD showed positive associations between force variability and ALFF of right M1, left S1, left precuneus, right middle temporal gyrus including MT/V5, and striate cortex (V1). V1 and precuneus are primarily involved in processing initial visual inputs, whereas MT/V5 is dedicated to supporting processing of visual motion. Left S1 and right M1 both are actively involved in prehensile movements including finger tapping (Muller et al., 2001; Mostofsky et al., 2009), grasping (Cavina-Pratesi et al., 2010), and precision gripping (Ehrsson et al., 2000; Coombes et al., 2010). While S1 involvement in visuomotor tasks is typically right dominant, M1 activity is typically lateralized to the contralateral (left) hemisphere. Greater left S1 and ipsilateral (right) M1 activation are seen when visuomotor tasks are more difficult (Post et al., 2009), as in our test of precision force at 60% of individuals' maximum output. Additionally, individuals with ASD showed an inverse relationship between motor variability and intrinsic activity of cerebellar vermis VI, a region implicated in the guidance of precision visuomotor behaviors including saccadic and smooth pursuit eye movements (Takarae et al., 2007).

In contrast to individuals with ASD, brain–behavior associations for TD controls implicate regions outside of primary sensorimotor networks and include cortical and cerebellar association circuits. Specifically, left inferior frontal gyrus is involved in visuomotor gripping (Ehrsson et al., 2000) and learning of complex finger tapping sequences (Muller et al., 2002)

due to its reciprocal projections with principal sensorimotor areas of premotor cortex, frontal eye fields, and striatum (Husain, 1991). Right supramarginal gyrus supports motor programming through its connections to the paracentral lobule, supplementary motor area, premotor cortex, and insula (Hesse et al., 2006). Angular gyrus is dedicated to attentional processes supporting individuals' engagement during goal-directed behaviors (Arsalidou and Taylor, 2011). Cerebellar crus I activity during basic visuomotor tasks scales with the frequency of visual feedback, suggesting that it is critical to adapting ongoing motor behavior to complex sensory information (Vaillancourt et al., 2006). Important regional variations thus suggest that TD controls' visuomotor behavior may be disrupted by intrinsic cortical–cerebellar activities in association networks involved in more complex processes. In contrast, individuals with ASD appear more susceptible to intrinsic variations affecting primary sensorimotor networks, suggesting a greater reliance on more fundamental neural networks to support the refinement of basic visuomotor behavior.

ALFF frequencies reported here are within the range of delta oscillations (0–4 Hz) associated with force variability during slow isometric force production and neocortical "common drive" modulation of the skeletal motor neuron pool (De Luca et al., 1982a; Lodha and Christou, 2017). Our finding that greater ALFF power in sensorimotor networks is associated with elevated force variability in ASD but not in TD individuals suggests that patients show atypical common drive modulation of neuromuscular systems during rest. In the context of our previous EMG findings documenting reduced delta modulation and reduced linkage between modulation of the motor neuron pool at multiple frequency bands (i.e., delta, beta, and gamma) and force variability in ASD (Wang et al., 2017), our rs-fMRI findings implicate alterations in corticomuscular coherence that contribute to a reduced ability to precisely control force output in patients. Other studies also identified the effect of motor learning on beta and gamma band modulation within the context of individualized differences (Witte et al., 2007; Mendez-Balbuena et al., 2012). As precision visuomotor control is repetitively implicated in individuals with ASD at multiple target force levels (Mosconi et al., 2015; Wang et al., 2015), studies examining corticomuscular coherence at multiple frequency bands using EEG and EMG (Mendez-Balbuena et al., 2012) are warranted.

Intra-Cerebellar and Cortical–Cerebellar Functional Connectivity in ASD

Relative to TD controls, individuals with ASD showed reduced intrinsic FC between medial cerebellar lobules dedicated to sensorimotor processes (vermis VI, posterior VIII and IX) and lateral lobules involved in higher-order processes (crus I/II), suggesting reduced interactions of distinct functional circuits within the cerebellum (**Table 3** and **Figure 3**). These distinct cerebellar circuits are anatomically and functionally linked to separate cortical targets. Medial lobules innervate dorsomedial thalamic nuclei and motor and parietal cortices, whereas crus I/II are more ontogenetically and phylogenetically advanced and are most densely connected with pre-frontal and association cortices (Ramnani, 2006). During goal-directed activities, cerebellar crus I/II circuits are involved in integrating complex and multi-sensory information (Nitschke et al., 2005; Stoodley and Schmahmann, 2009; D'Mello and Stoodley, 2015). Reduced FC between crus I/II and more medial cerebellar circuits in ASD suggest deficits integrating multisensory information and utilizing higher-level inputs to guide sensorimotor behaviors. Our FC results are consistent with prior DTI studies that show decreased white matter microstructural integrity within the cerebellum and within fiber tracts connecting cerebellar cortex and dentate nucleus in ASD (Sivaswamy et al., 2010; Jeong et al., 2014; Crippa et al., 2016). The cerebellum has also been consistently implicated in histological and MRI studies of ASD that identified reductions in the number and size of Purkinje cells (Bauman, 1991; Courchesne, 1997; Whitney et al., 2008) and hypoplasia of lobules V–VII (Courchesne et al., 1988). Our findings indicate that cerebellar pathology and white matter microstructural variation may be associated with reduced communication between functionally distinct circuits in ASD.

We also documented that cerebellar FC with pre-frontal cortical targets, including right superior and left middle frontal gyri are reduced in ASD, while cerebellar FC with posterior parietal and occipital cortices is elevated (**Figure 3**). These findings are consistent with prior studies documenting reduced rs-FC between right cerebellar crus I/II and contralateral pre-frontal cortex and inferior/middle temporal gyrus (Khan et al., 2015), and between right crus I and contralateral superior frontal gyrus, middle frontal gyrus, thalamus, anterior cingulate gyrus, and parietal cortex in adolescents with ASD (Verly et al., 2014). Reduced tb-FC of the cerebellum and M1, supplementary motor area, and thalamus was also reported during sequential finger tapping in ASD (Mostofsky et al., 2009). Consistent with findings of reduced FC between cerebellum and cortex, DTI studies of individuals with ASD have documented atypical white matter microstructural integrity of the primary cortical input and output pathways of the cerebellum—the middle and superior peduncles (Catani et al., 2008; Brito et al., 2009; Hanaie et al., 2013). As cerebellar circuits integrate and relay error information to frontal, parietal, and temporal cortices (Glickstein, 2000; D'Mello and Stoodley, 2015), reduced cerebellar FC with the left middle frontal gyrus, right superior frontal gyrus, and contralateral superior temporal gyrus suggests that cerebellar disconnectivity may play a key role in a broad range of neurodevelopmental dysfunctions in ASD, including executive, language, and multisensory processes. Further, greater FC between the cerebellum and occipital and posterior parietal circuits suggests increased reliance on more basic sensory information for guiding behavior, as suggested previously in studies of motor learning (Haswell et al., 2009; Izawa et al., 2012).

Limitations and Future Directions

One limitation of the present study is the small sample of individuals who completed both tb- and rs-fMRI runs. However, given the large magnitudes of correlations between selected ROIs and sustained force variability (**Table 2**), our results appear to be robust. Additional analyses across larger samples will be important for determining how these brain–behavior

relationships vary across the broader ASD population. Future studies may also examine separate measures of intrinsic brain activity in relation to behavioral issues in ASD. For example, recent studies have quantified the dynamics of intrinsic brain activity using the variance of ALFF over time (Li et al., 2018; Liao et al., 2019). While we focused on mean ALFF across the time series based on the strong relationships previously demonstrated between mean ALFF and sensorimotor behavior (Ma et al., 2011), examination of temporal variability may provide key insights into neural mechanisms. Future studies should also examine the relationships between intrinsic activity of sensorimotor networks and behavior across both hands, as lateralized deficits of sensorimotor behavior and brain function have been identified in ASD (Kleinhans et al., 2008). As we have previously found that the severity of visuomotor deficits in ASD varies as a function of visual feedback gain and force level (Mosconi et al., 2015), determining the extent to which brain–behavior linkages vary across different levels of visual feedback and force load will also be important for future studies. Finally, analysis of FC of cerebellar–cortical systems in relation to DTI data quantifying white matter microstructural integrity alterations in these networks in ASD may provide important new insights into mechanisms associated with sensorimotor network dysfunctions and elevations in motor variability.

CONCLUSIONS

The current work demonstrates that intrinsic neural oscillations in sensorimotor cortical and cerebellar circuits are strongly associated with visuomotor precision in both individuals with ASD and TD controls. We also show important regional and network dissociations of the intrinsic functional anatomy of visuomotor control in ASD and TD. Our findings of reduced intracerebellar, cerebellar–frontal, and cerebellar–temporal FC in ASD suggest that previously documented pathologies of the cerebellum may interfere with multiple developmental functions involving both basic sensorimotor and higher-order association networks. As disruptions of basic sensorimotor processes involving cortical–cerebellar circuits are seen in ASD across the lifespan, focus on intrinsic functional properties of these networks may provide important insights into neurodevelopmental processes that interfere with both early emerging and more complex behaviors.

ETHICS STATEMENT

All study procedures were approved by the Institutional Review Boards at the UT Southwestern Medical Center and Children's Hospital of Dallas. Adult participants provided both informed and written consent, and minors provided assent in addition to informed and written consent from their legal guardian.

AUTHOR CONTRIBUTIONS

MM and JS are responsible for the conception and design of the research. ZW scored and analyzed the behavioral data. MM, JS, QG, and SL supervised YW to score and analyze the neuroimaging data. ZW and YW performed statistical analyses. ZW, YW, SL, QG, JS, and MM interpreted the experimental results. ZW and YW prepared figures and tables and drafted the manuscript. Each author edited the manuscript. All authors have approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnint.2019.00017/full#supplementary-material

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ORIGINAL RESEARCH
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Dizziness and Convergence Insufficiency in Children: Screening and Management

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Objective: In children screened for dizziness with vergence disorders, we tested short and long term efficacy of orthoptic vergence training (OVT) and instructions to reduce screen usage.

Methods: Prospective study: Of the 179 children referred for vertigo or dizziness (over 3 years) with ophthalmological disorder as the only problem after complete oto-neuro-vestibular testing, 69 presented vergence insufficiency, and 49 accepted to participate in this study. 109 healthy children served as controls. All subjects had classic orthoptic evaluation and video binocular movement recordings during various oculomotor tasks. Patients were evaluated before OVT (M0), 3 months after the end of OVT (M3) and 9 months after the end of OVT (M9). Statistics compared orthoptic and oculomotor parameters between patients and controls over time with one-way ANCOVA, and mixed models, controlling for age and gender.

Results: Patients reported vertigo that was usually rotatory, lasting <15 min, associated with or alternating with headache (50%). Their exposure to small video screens and TV was intensive (~3.6 h per day). At M0, all orthoptic and oculomotor parameters were statistically different in patients relative to controls ($p < 0.0001$) except for divergence. At M3, vertigo symptoms had disappeared in all of the patients, and all eye movement parameters improved significantly ($p < 0.0001$). At M9, this improvement remained stable or continued.

Conclusion: Vergence disorders (assessed by abnormal orthoptic and oculomotor parameters) can generate symptoms of dizziness in children. Orthoptic treatment and instruction to reduce screen usage has a significant and long term effect on vertigo symptoms as well as oculomotor performances. Dizzy children should be screened for vergence disorders.

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WHAT THIS STUDY ADDS

Dizziness in children can be associated exclusively with insufficient convergence. Orthoptic training and instructions to reduce screen exposure made dizziness symptoms disappear and improved all eye movement parameters for 6 months. Vergence disorders should be screened for in dizzy children.

Keywords: vertigo, pediatric, vergence disorders, saccades, orthoptic training, video screen usage

INTRODUCTION

It is well known that ophthalmological disorders can induce headaches but most general practitioners, and many specialists, still do not recognize them as a cause of dizziness. We first published evidence that dizziness in children could result from vergence insufficiency VI and dizziness improves when the ophthalmological disorder is treated by orthoptic training, correction of a refractive problem with glasses, or both (Anoh-Tanon et al., 2000). A recent report on a cohort of 1037 vertiginous children found 15% with an ophthalmological disorder as the only cause of dizziness (Wiener-Vacher et al., 2018).

Vertigo (false perceptions of movement of oneself or the environment) and dizziness (sensation of imbalance) may originate at various levels of the central nervous system, where multisensory inputs (including vestibular, somesthetic, proprioceptive, and visual inputs) are integrated (Brandt, 2003; Rine and Braswell, 2003; Braswell and Rine, 2006; Wiener-Vacher et al., 2012; Leigh and Zee, 2015). During rapid head movements, vestibular input triggers oculomotor responses to maintain stable gaze. When the head is fixed, visual inputs trigger a combination of several oculomotor responses: saccades (where gaze jumps from one target to another), smooth pursuit (where gaze follows a slowly moving target), optokinetic responses (where eyes follow the scrolling of the peripheral landscape during movement), and vergence (where both eyes converge on targets getting closer or further away). Dysfunction of these systems can lead to blurry vision, dizziness, vertigo, and headaches particularly after activities requiring intense attention, and convergence during long periods of time (such as reading, playing videogames or looking at mobile telephone screens). Vergence dysfunction can also impair learning activities such as writing or reading (Gaertner et al., 2013; Lions et al., 2013).

In our clinic specialized for diagnosing and treating dizzy children, we observed over the past 5 years an increase of the prevalence of oculomotor disorders as the only cause of the dizziness (10–15%) (Wiener-Vacher et al., 2018). We suggest that this increase of symptomatic vergence disorders may be due to the growing use of electronic devices with small video screens that are very demanding for convergence and diverse controlled saccades during long periods of time. Impacts of computer use on vision have been described by Rosenfield (2016) in adults as "Computer vision syndrome (a.k.a. digital eye strain)" including vergence disorders as well as vertigo. To our knowledge, there are no reports on the effects of video screen exposure on oculomotor disorders in children.

The effects of orthoptic training on oculomotor performance remain controversial. Some publications report that orthoptic training is effective for oculomotor problems such as VI (Van Leeuwen et al., 1999; Bucci et al., 2004; Alvarez et al., 2010; Von Noorden and Campos, 2012) but others claim that this effect is ephemeral (less than a month) (Rawstron et al., 2005). Nevertheless there is evidence that vergence training can modify saccades and vergence parameters in healthy adult subjects (Jainta et al., 2011).

This study shows that dizziness in children can be related to VI (particularly convergence) and occurs in patients with prolonged daily screen exposure. OVT and instructions to reduce screen exposure in these patients has a positive effect on dizziness symptoms as well as correcting oculomotor disorders. Furthermore it shows for the first time that these improvements persist for at least 6 months in these children.

Patient Group

Forty-nine children (25 boys, 24 girls, 9–13 years old) with VI participated in the study (from 179 children over 3 years diagnosed with ophthalmological problems of which 69 were VI as the only cause of their dizziness). The selection criteria were:

- No history of vestibular, neurological or psychiatric pathology.
- Normal clinical oto-neuro-vestibular examination as described below.
- Absence or minor refraction anomalies (within the range of −1 to +1) with cycloplegic refractometry.
- VI confirmed with ORTE.

Patients were tested before the OVT (M0), 3 months after OVT ended (M3) and 9 months after the end of OVT (M9).

Control Group

In order to establish normal reference values for the ORTE and the OCME, 109 healthy children (boy/girl ratio = 0.98, 6–17 years old) were recruited from hospital employees' and patients' families. All had normal clinical oto-neuro-vestibular evaluations and normal visual acuity.

For ethical reasons we couldn't test the effect of the OVT on healthy children since they had no complaints and no VI.

Abbreviations: CG, control group; M0, initial evaluation before OVT; M3, evaluation 3 months after M0 and the end of OVT; M9, evaluation 9 months after the end of OVT; NPC, near point of convergence; OCME, oculomotor evaluation; ORTE, orthoptic evaluation; OVT, orthoptic vergence training; VI, vergence insufficiency.

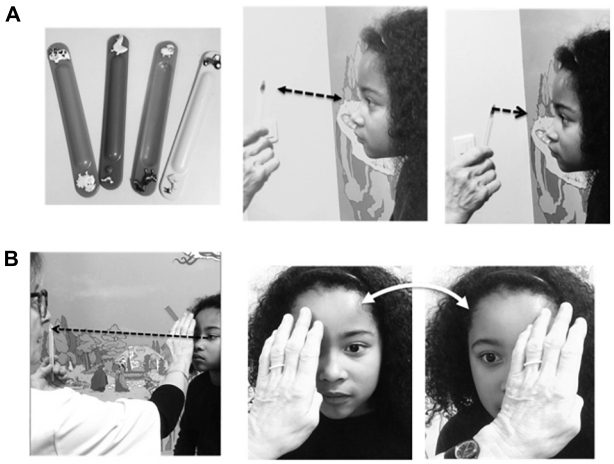

FIGURE 1 | Clinical screening for vergence insufficiency. This test includes 2 steps: **(A)** Looking for an abnormally long NPC during convergence (normal value ≤ 7 cm). (Left) Small targets were child-friendly stickers pasted onto tongue depressors. (Middle) The subject is instructed to fixate on the target as it was moved away from and toward the nose. (Right) As the target approaches the nose, the child must indicate when the target appears double and/or the clinician detects the distance, where smooth binocular eye movements cease and convergence breaks, with one eye ceasing fixation. This corresponds to the NPC. **(B)** Testing for misalignment of the eyes (heterophoria). (Right) Each eye is covered and uncovered by the clinician's hand while the child fixates on a far target (dashed line and arrow in photo on left). If the eyes remain stable there is no misalignment. If one eye moves when uncovered this indicates a heterophoria.

We evaluated the impact of OVT at M3 and M9 on patients by comparing their oculomotor parameters values to their values at M0 and to those of controls.

Data concerning the symptoms and time of screen exposure for both groups were obtained by asking children and parents.

Oto-Neuro-Vestibular Evaluation

In both groups, a clinical examination was performed including otologic assessment (otoscopy and acoumetry), neurological and vestibular testing (HIT head impulse test, VOR with videoscopy). Patients had a complete battery of tests to exclude those with any vestibular disorders. This included vestibular canal testing (bithermal caloric test, rotatory chair test, Video Head Impulse Test) and vestibular otolith testing (cervical vestibular evoked myogenic potentials) (Wiener-Vacher et al., 2012).

Visual Acuity Evaluation

Controls and patients were screened for normal visual acuity in each eye (≥20/20) for far and near viewing. The Parinaud test used for near vision and the Monoyer scale for far vision (Von Noorden and Campos, 2012). Normal refraction for patients was confirmed with cycloplegia between +1 and −1 (with a refractometer after applying drops of 0.5% cyclopentolate, *Skiacol*® to the eyes).

Initial ORTE

VI was clinically screened for in both groups by the physician via a simple screening test for weak or asymmetrical eye convergence movements and eye misalignment (see **Figure 1** and **Supplementary Videos S1, S2**). An optometrist assessed vergence performances with a classic test protocol including:

- Measure of near point of convergence (NPC): as distance between nose and target when the target moved toward the nose is first seen as double, or convergence is disrupted (Alvarez et al., 2010; Ajrezo et al., 2016).
- Eye covered-uncovered test: one eye is successively covered and uncovered while the subject gazes at targets at 5 m and 30 cm. If the eyes stay stable when uncovered this excludes a latent eye misalignment corrected by binocular fixation (heterophoria, i.e., latent deviation of a covered eye when the other is not covered); this is not a VI (Cooper, 2011).
- Measurement of the fusion amplitude for divergence and convergence using a Berens prism bar (Cooper, 2011) with targets at 5 m and 30 cm.
- Measurement of the stereoscopic depth discrimination using the TNO (Random dot test, Netherlands Organization for Applied Scientific Research).

The OVT prescribed for all patients included a total of 12 sessions occurring twice a week.

The main goal of OVT was to improve binocular vision for all eye movements at near and far distances. All orthoptists asked patients to perform the same exercises during each session: ocular saccades and pursuits at near distance as well as divergence and convergence at both far and near distances with several instruments: Berens prism bar, synoptophore, and stereograms.

The patients were encouraged to make efforts to increase vergence amplitudes and reach normal values, then to repeat all exercises to make the responses automatic and effortless (Cooper, 2011).

Patients and their families were also encouraged to reduce videoscreen exposure daily. However, it was not possible to reliably track adherence to this recommendation.

Oculomotor Evaluation

Eye movements were recorded from each eye independently with the Mobile EyeBrain Tracker (Mobile EBT®, SuriCog) at a recording frequency of 300 Hz and 0.25° precision. The program permits separate analyses of the different components of the eye movements (saccadic, convergent, and divergent).

Six conditions of combinations of saccades and vergence were studied: saccades alone with far or near vision (thus imposing constant vergence), divergence and convergence alone without saccades, combined saccades with convergence, and divergence (**Figure 2**).

Statistical Analyses

A one-way ANCOVA with control for age and gender was conducted at each evaluation date (M0, M3, and M9) to test for

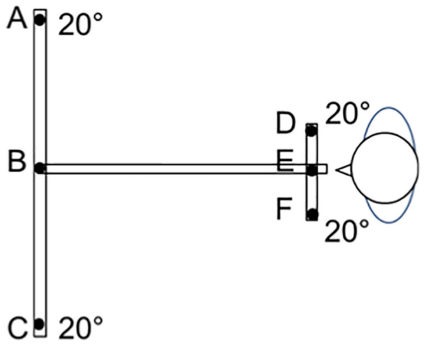

FIGURE 2 | The LED target set up (overhead view). LEDs were presented as targets on a board placed at the child's eye level. The subject was seated in a dark room, with the head stabilized via a headrest supporting the forehead and chin. Calibration was made during binocular viewing at the beginning of the session (Lions et al., 2013). The child was instructed to look at targets randomly presented at 20° from the midline either at 150 cm (a distance not requiring vergence) or at 20 cm (where convergence is continuously required). Eye movements between adjacent distal targets (between LEDs B and A or B and C) involve saccades alone with no convergence. Eye movements between near targets (E and F or E and D) involve saccades alone with constant convergence. Vergence movements (convergence and divergence alone) were performed for targets presented along the medial plane (convergence from B to E and divergence from E to B). Combined saccade+vergence movements are involved when the target change of distance and laterality from the midline (saccades+divergence between E and A, or E and C, and saccades+convergence B and D, or B and F).

a statistically significant difference in orthoptic and oculomotor parameters between individual patients and the CG.

Mixed models for longitudinal data (MMLD) adjusted for age and gender evaluated the effect of time after training on orthoptic and oculomotor parameters in the patient group (M0 vs. M3, M0 vs. M6, and M3 vs. M6).

For all multiple comparisons, p-values were corrected with a Holm adjustment. All statistical tests were two-tailed and $p < 0.05$ is considered statistically significant. All analyses were done with SAS software version 9.4. All p values less than 0.0001 are reported as $p < 0.0001$ rather than exact values.

RESULTS

Vertigo Symptoms (Figure 3)

Patients reported vertigo as a rotatory sensation in 80% of the cases (40/49) with a sensation of falling in 48% (24/49). The vertigo was usually brief, lasting less than 15 min but recurrent during the day (71%; 35/49) (ranging from once a day to almost continuous in one case).

Headache was reported in 86% (42/49) of the cases, associated with or alternating with vertigo. The pain was frontal in 62%, temporal in 33% or occipital in 16%, pulsatile (56%) or continuous (44%). In 40%, instability, nausea, photo- and phono-phobia, or abdominal pain were experienced during the crises.

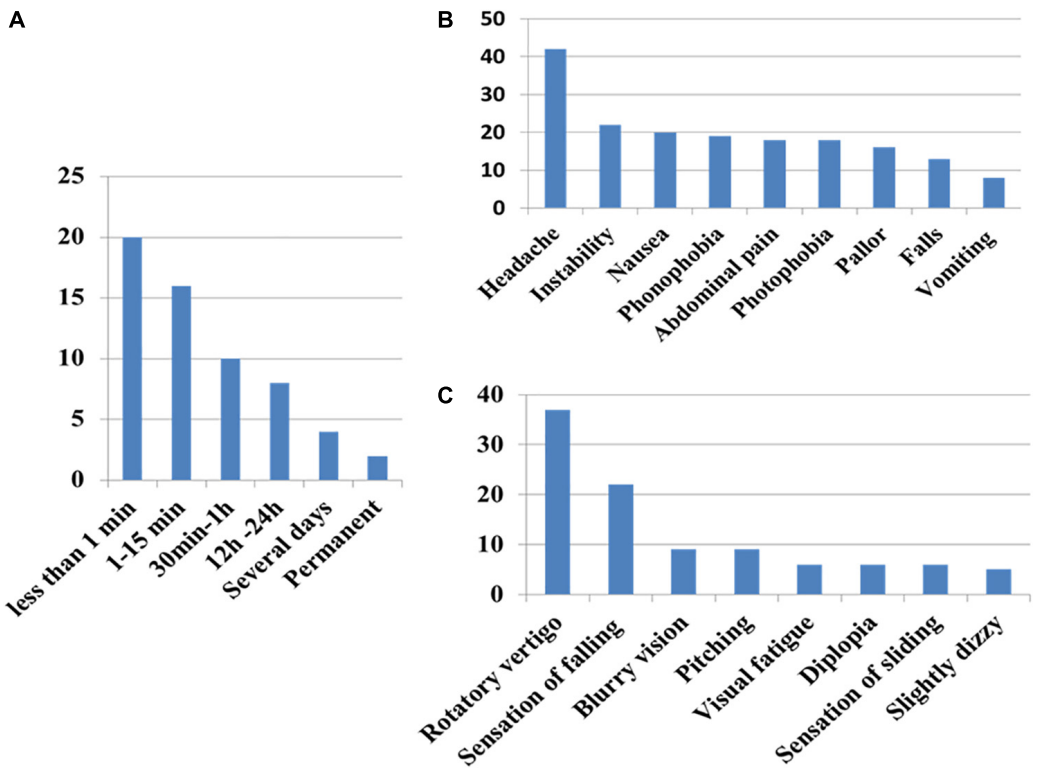

FIGURE 3 | Clinical patterns of symptoms of vergence insufficiency in the patient group **(A)** duration of the sensation **(B)** associated symptoms, and **(C)** sensation perceived. Note that sensation of rotation is never an intense continuous rotatory vertigo as observed in acute vestibular loss.

Triggering factors were usually stress and ocular fatigue. Children complained at the end of the day (56%) but also in the morning (63%). Symptoms were reported after reading (31%), at school (46%), during or after sport activities (49%), and after using small video screens (31%). The duration of the daily screen exposure (for small screens, mobile phone, video consoles, computer and television) was rather high in the patient group: the mean ± standard deviation was 5.0 ± 3.3 h per non-school days and 2.3 ± 1.7 h on school days. The weighted average was 3.65 h. The CG reported somewhat less exposure: mean 3.9 ± 2.1 h per non-school day and 1.6 ± 1.4 h on school days. The weighted average was 2.8 h. The difference between groups did not reach statistical significance, perhaps because of the high variability and the sample size (p = 0.054, after correction for age and gender).

Forty-five percentage of the patients reported previous episodes of headache without dizziness or vertigo. Patients' parents or siblings reported VI in 15% and migraine in 25% of cases.

M0: All Patients Had Abnormal Orthoptic and Oculomotor Performances Compared to Controls

At M0 patients had convergence, but not divergence, insufficiency: the NPC was significantly more distant (**Figure 4A** and **Supplementary Tables S2**, **S3**), amplitudes of convergence at far and near vision were significantly lower in patients than in CG (see **Figure 4B** and **Supplementary Tables S2**, **S3**). In contrast, no patients had divergence amplitudes significantly lower than the controls for near and far vision (**Figure 4C** and **Supplementary Tables S2**, **S3**).

Gains were greater (**Figure 5**) and latencies (**Figure 6**) were significantly longer in patients than controls (**Supplementary Tables S4**, **S5**) for all conditions tested. Velocities were significantly lower in patients than controls in all conditions (**Figure 7** and **Supplementary Tables S4**, **S5**).

M3: Orthoptic and Oculomotor Values Improve After OVT

At M3 the NPC of all patients were not significantly different from controls. Amplitudes of far and near convergence improved significantly and reached values higher than those of controls (**Figure 4A** and **Supplementary Tables S2**, **S3**).

For most of the oculomotor conditions, gain was not significantly different from control values (**Figure 5** and **Supplementary Tables S4**, **S5**) except for near saccades and combined saccades with convergence. This could be due to remaining weakness of convergence capabilities. Latencies decreased significantly for all conditions, reaching shorter latencies than controls for near and far saccades without vergence (**Figure 6** and **Supplementary Tables S4**, **S5**). Velocities of vergence alone or combined with saccades were not significantly different from control values (**Supplementary Tables S4**, **S5**) or values could be even higher than controls (see **Figure 7**). Velocities for saccades (far and near) and combined saccades with convergence and divergence remained significantly lower in patients than controls (**Supplementary Tables S4**, **S5**).

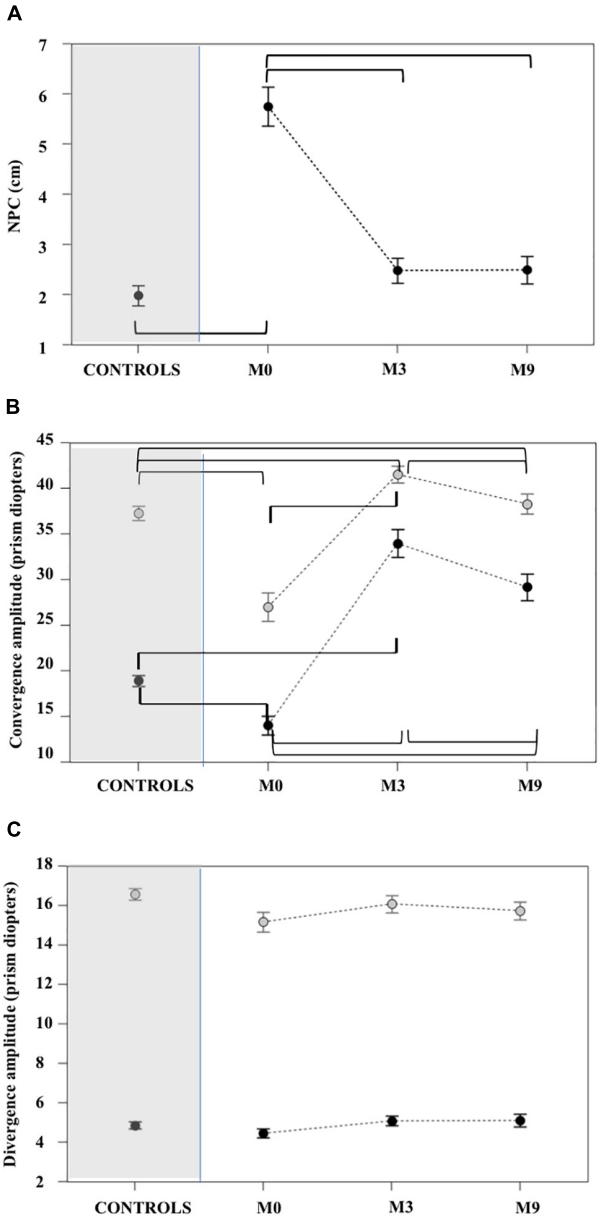

FIGURE 4 | Near point of convergence **(A)**, far and near convergence **(B)** and divergence **(C)** measured with ORTE (mean ± standard deviation). Brackets indicate statistically significant comparisons. At M3 the NPC values for the patient group **(A)** improved relative to M0 and were not significantly different from controls. Amplitudes of near (gray circles) and far (black circles) convergence **(B)** improved significantly in patients at M3 and M9 and these values were greater than controls. Divergence amplitudes **(C)** were not significantly different in patients relative to controls at M0, M3, and M9. For all statistical values see **Supplementary Tables S2**, **S3**.

M9: Improvement of Orthoptic and Oculomotor Parameters Persists in Patients

Orthoptic parameters in patients remained improved, without significant differences or better when compared to controls

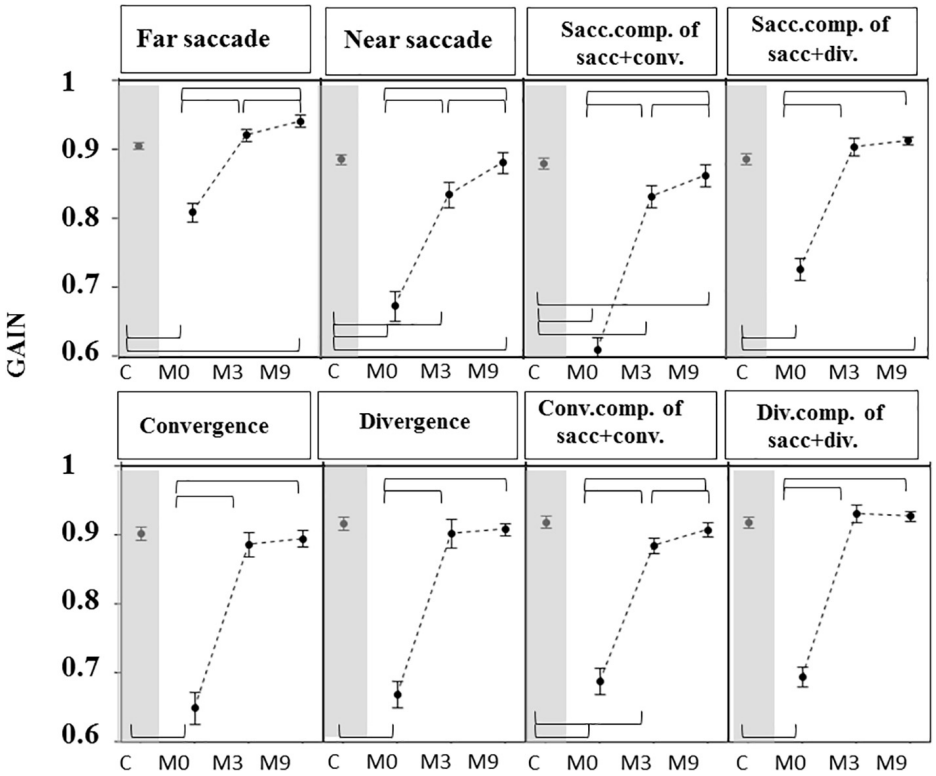

FIGURE 5 | Gain of eye movements measured in OCME for the six test conditions (mean ± standard deviation). For combined saccades+vergence, the saccadic and vergence components are shown individually and labeled: sacc. component, conv. component, and div. component. Statistically significant differences are indicated by braces. Gains at M0 were lower in patients than in controls (C) for all eight conditions. At M3, gains were not significantly different from control values for far saccades, convergence, divergence, and combined saccades with divergence but were still significantly different from controls for near saccades and for both components of saccades combined with convergence. For all statistical values see **Supplementary Tables S5**, **S6**.

at M9 (see **Figures 4A–C** and **Supplementary Tables S1**, **S2**). Oculomotor performances at M9 remained also normal (**Figures 5–7** and **Supplementary Tables S4**, **S5**).

Gains continued to improve significantly between M3 and M9 for saccades to near and far targets and for saccades+convergence (**Figure 5** and **Supplementary Tables S4**, **S5**). Latencies continued to decrease significantly between M3 and M9 for saccades to near targets, divergence and divergence+saccades (**Figure 6** and **Supplementary Tables S4**, **S5**). Velocities did not change significantly from M3 to M9 (**Figure 7** and **Supplementary Tables S4**, **S5**).

The analysis with mixed models show that improvement of the orthoptic and oculomotor parameters obtained with OVT was statistically significantly maintained over time (**Supplementary Tables S3**, **S6**). From M0 to M3 the improvement of all oculomotor parameters was statistically significant (**Supplementary Table S6**), except for velocities of saccade alone, combined saccades with divergence and convergence.

From M3 to M9, improvements were still statistically significant for some parameters: near and far saccade gains increased and latencies of near saccades decreased significantly (**Supplementary Tables S4**, **S5**). The gain of saccades + convergence increased (**Figure 4** and **Supplementary Tables S4**, **S5**). Latencies of saccades continued to decrease for divergence combined with saccades (see **Figure 5** and **Supplementary Tables S4**, **S5**).

DISCUSSION

Children complaining of vertigo or dizziness may have no vestibular pathology, but rather suffer from oculomotor problems including poor saccades and vergence disorders (Anoh-Tanon et al., 2000), in particular convergence insufficiency. Orthoptic treatment led to the disappearance of subjective symptoms of vertigo (Anoh-Tanon et al., 2000) and also improved static and dynamic oculomotor performance. Such improvement persisted and some parameters even progressed further from M3 to M9. At M0, VI patients' exposure to video screens was found to be intensive (∼3.6 h per day) and this was discouraged.

Poor Oculomotor Performance in Children With Vertigo

This is the first study recording saccades, vergence and combined saccade-vergence movements before and after orthoptic training in a group of VI children complaining of vertigo but with

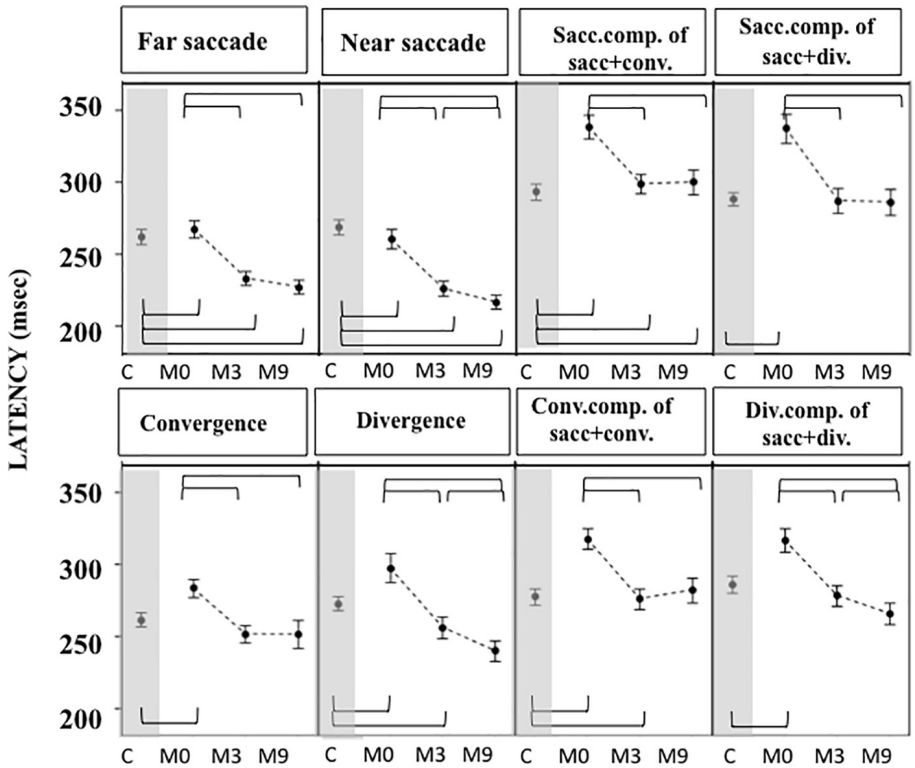

FIGURE 6 | Latencies measured in OCME in the six test conditions (mean and standard deviation). Only statistically significant differences were indicated: top row braces shows differences between M0 vs. M3, M0 vs. M9, and M3 vs. M9 and bottom row shows all differences with controls. Latencies at M0 were significantly longer in patients compared to controls for all conditions tested. At M3 latencies decreased significantly for all conditions after OVT, reaching shorter latencies than controls for near and far saccades. For all statistical values see **Supplementary Tables S5**, **S6**.

no vestibular pathology. Before training we measured poor oculomotor performance in these children and found that all made saccades with longer latencies, lower gain and lower velocities than controls. Note that the latency of eye movements includes the preparation time for performing vergence and/or saccadic movements, involving several processes: shift of attention to the visual target, disengagement of oculomotor fixation and computation of the upcoming movement parameters (Fischer, 1987; Seassau and Bucci, 2013). All of these processes are associated with activation of several cortical areas, particularly the parietal cortex and frontal lobe (Leigh and Zee, 2015). In patients with cortical lesions, latencies increase due to cortical dysfunction (Findlay and Walker, 1999). Thus, in the VI children with vertigo, we hypothesize the presence of a minor central dysfunction in the initiation and in the triggering of eye movements.

Our results confirm that the accuracies of saccades and vergence movements are severely impaired in VI children suffering dizziness (Riddell et al., 1990; Bucci et al., 2004; Gaertner et al., 2013). Such poor accuracy could be due to a poor visual localization of the target as a direct consequence of the VI, as suggested previously (Pierrot-Deseilligny et al., 1995; Bucci et al., 2004; Gaertner et al., 2013). Such an impairment in target localization could have an impact on the preparation and the execution of the eye movements, leading to longer latencies, more inaccurate and slower eye movements. These problems could delay learning processes involving saccades and vergence such as reading and writing. Thus screening for VI in children with learning disabilities is of interest.

Effect of Orthoptic Training

Our study shows that OVT in VI patients can suppress vertigo symptoms and improve saccades and vergence performances (decreasing latencies, improving eye movement precision, and velocity). OVT is widely recommended by clinicians for improving vergence capabilities (Von Noorden and Campos, 2012), but only a very few studies quantitatively showed the effect of OVT on eye movements. Vergence exercises were reported to change vergence movement dynamics in a small population of normal (Von Noorden and Campos, 2012) and VI children (Bucci et al., 2004). Alvarez et al. (2010) observed in adults with VI a correlation between orthoptic training, improvement of vergence dynamics and cerebral changes with fMRI (Alvarez et al., 2010, 2014). Subjects with VI showed a significantly lower activation of cerebellar vermis, frontal and parietal cortex with vergence movements compared to healthy adults with no VI, and this activity improved significantly after a total of 18 h of OVT (Alvarez et al., 2014). These findings suggest that

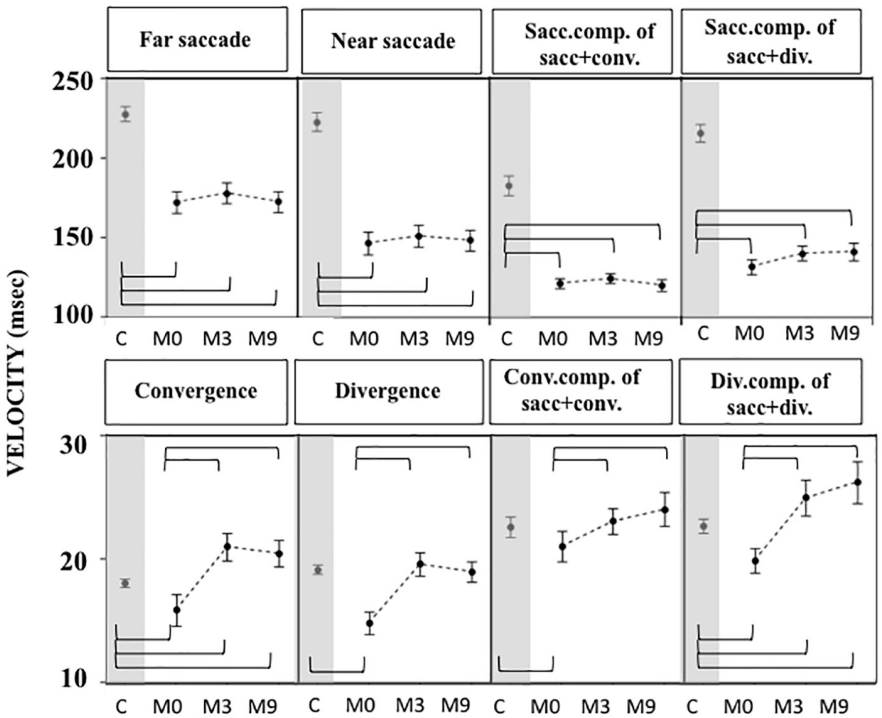

FIGURE 7 | Velocities of eye movements measured in OCME in the eight conditions in controls, and in patients at M0, M3, and M9 (mean and standard deviation). Only statistically significant differences were indicated: top row braces shows differences between M0 vs. M3, M0 vs. M9, and M3 vs. M9 and bottom row shows all differences with controls. Velocities at M0 were significantly lower in patients compared to controls in all conditions except for convergence+saccade, likely because of a compensatory increase of saccade velocity. After training the velocities were still significantly lower in patients than controls for far and near saccades, and for combined saccades with convergence and divergence. In contrast, velocities of vergence movements (alone or combined with saccades) in patients reached values not significantly different from or even higher than controls. For all statistical values see **Supplementary Tables S5**, **S6**.

OVT may act at a central level. The continued improvement in oculomotor performances we observed 6 months after the end of the OVT support the hypothesis of central changes. However, this does not exclude a peripheral effect of training on eye muscles.

Clinical Considerations

Quantitative recordings of eye movements together with clinical orthoptic tests proved useful here for detailed diagnosis and treatment follow-up of dizzy children with no vestibular or neurological disorder. OVT improved their VI and this persisted and even continued to improve after the end of the training.

It is thus important to promote screening of VI in vertiginous children, in particular those with neither vestibular nor neurological disorders, and if vergence disorder is found to prescribe ORTE.

In our pediatric balance evaluation center, vertigo due to vergence disorders is the second more frequent diagnosis after migraine; its prevalence increases every year (from 10% in 2014 to 15% in 2018) (Wiener-Vacher et al., 2018). The American Association of Pediatrics (Swing et al., 2010) recommended limiting children's exposure to video screens for many reasons. Our results show that symptomatic cases of VI tend to be associated with longer exposure to small video screens than controls. Today many children are exposed to small video screens for long periods of time. The increasing prevalence of symptomatic VI that we observed could be explained by this lifestyle. For health care and prevention, children and particularly those who suffer VI should be advised to reduce video screen exposure. Note, however, that video screen exposure was on average 2.8 h per day year around for the CG while it was 3.6 h per day for the dizzy patients with VI. Thus despite their intensive screen exposure, controls were not dizzy and that would support the hypothesis of a pre-existing latent or minor vergence disorders that were aggravated by the intense exposure in patients.

CONCLUSION

Pediatric patients with vertigo and dizziness associated with convergence insufficiency benefit from orthoptic training with a significant improvement of the vertigo symptoms as well as oculomotor performances. These effects persist after the end of OVT and even continue to progress for some parameters. Vergence disorders should be screened for in all dizzy children with normal neurological and vestibular examinations, and then followed by treatment and instructions for reduced video screen exposure.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

This investigation adhered to the Declaration of Helsinki principles and was approved by our local Human Experimentation Committee (10801-AOR09078, AFSSAPS B100388-40). Written consent was obtained from the children's parents after careful review of the experimental procedures with them.

AUTHOR CONTRIBUTIONS

SW-V and MPB conceived and designed the study, drafted the initial manuscript, reviewed the manuscript for intellectual content, and finalized the submitted version. SIW performed a statistical analysis, reviewed the manuscript for intellectual content, revised the manuscript, and finalized the submitted version. RO and LA designed the data collection instruments, collected the data, and carried out the initial analyses. CA, DM, and PB developed the data base and carried out the statistical analyses. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnint.2019.00025/full#supplementary-material

TABLE S1 | Orthoptic parameters for control and patient groups at M0, M3, and M9.

TABLE S2 | Mean ANCOVA differences between patient and control groups and 95% confidence interval for orthoptic parameters (multiple comparisons controlling for age and gender). Significant values are highlighted in gray.

TABLE S3 | The effect of orthoptic training over time on orthoptic parameters with mixed models for longitudinal data (mean differences with minimum-maximum of the 95% confidence interval). Same format as **Supplementary Table S2**.

TABLE S4 | Mean values of oculomotor parameters (with standard deviations) over all conditions of the saccade/vergence for controls and patient groups at M0, M3, and M9.

TABLE S5 | Mean ANCOVA differences mean with minimum-maximum of the 95% confidence intervals in oculomotor parameters between patient and control groups (Multiple comparisons controlling for age and gender). Statistically significant differences are highlighted in gray.

TABLE S6 | The effect of orthoptic training over time on oculomotor parameters with mixed models for longitudinal data (mean differences with minimum-maximum of the 95% confidence interval). Statistically significant differences are highlighted in gray.

VIDEO S1 | Screening for weak or asymmetrical vergence eye movements and misalignment.

VIDEO S2 | Left eye weak convergence and without eye misalignment.

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frontiers in Integrative Neuroscience

ORIGINAL RESEARCH
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Modeling Interval Timing by Recurrent Neural Nets

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The purpose of this study was to take a new approach in showing how the central nervous system might encode time at the supra-second level using recurrent neural nets (RNNs). This approach utilizes units with a delayed feedback, whose feedback weight determines the temporal properties of specific neurons in the network architecture. When these feedback neurons are coupled, they form a multilayered dynamical system that can be used to model temporal responses to steps of input in multidimensional systems. The timing network was implemented using separate recurrent "Go" and "No-Go" neural processing units to process an individual stimulus indicating the time of reward availability. Outputs from these distinct units on each time step are converted to a pulse reflecting a weighted sum of the separate Go and No-Go signals. This output pulse then drives an integrator unit, whose feedback weight and input weights shape the pulse distribution. This system was used to model empirical data from rodents performing in an instrumental "peak interval timing" task for two stimuli, Tone and Flash. For each of these stimuli, reward availability was signaled after different times from stimulus onset during training. Rodent performance was assessed on non-rewarded trials, following training, with each stimulus tested individually and simultaneously in a stimulus compound. The associated weights in the Go/No-Go network were trained using experimental data showing the mean distribution of bar press rates across an 80 s period in which a tone stimulus signaled reward after 5 s and a flash stimulus after 30 s from stimulus onset. Different Go/No-Go systems were used for each stimulus, but the weighted output of each fed into a final recurrent integrator unit, whose weights were unmodifiable. The recurrent neural net (RNN) model was implemented using Matlab and Matlab's machine learning tools were utilized to train the network using the data from non-rewarded trials. The neural net output accurately fit the temporal distribution of tone and flash-initiated bar press data. Furthermore, a "Temporal Averaging" effect was also obtained when the flash and tone stimuli were combined. These results indicated that the system combining tone and flash responses were not superposed as in a linear system, but that there was a non-linearity, which interacted between tone and flash. In order to achieve an accurate fit to the empirical averaging data it was necessary to implement non-linear "saliency functions" that limited the output signal of each stimulus to the final integrator when the other was co-present. The model suggests that the

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central nervous system encodes timing generation as a dynamical system whose timing properties are embedded in the connection weights of the system. In this way, event timing is coded similar to the way other sensory-motor systems, such as the vestibulo-ocular and optokinetic systems, which combine sensory inputs from the vestibular and visual systems to generate the temporal aspects of compensatory eye movements.

Keywords: temporal coding, perception of time, interval timing, temporal averaging, peak procedure

INTRODUCTION

The temporal encoding of significant events in neural structures has become an important consideration in a wide range of adaptive behaviors. The importance of time estimation in humans was first described by François (1927), but publicized by Hoagland (1933, 1935), when he noticed that his wife counted from 1 to 60 at an estimated 1 count/sec differently when her temperature changed as a result of influenza (Hoagland, 1935). Other studies on interval timing showed that a visual stimulus associated with a standard duration could be identified amongst other intervals that were given to subjects (Wearden et al., 1997). This work showed that this estimation of time intervals could be scaled for standard deviation as the interval was increased according to Weber's law (Wearden et al., 1997).

Following Pavlov's work on conditioning (Pavlov, 1927) and subsequent work on operant conditioning (Skinner, 1938, 1951), there has been growing interest in studying how animals learn to time the arrival of key events, such as reward (Staddon and Higa, 1999; Staddon, 2005). In a typical experiment, for instance, a stimulus might be presented and in its presence food reward is made available for the first response that occurs after t seconds have elapsed. At issue is how the animal encodes the time at which food becomes available and then distributes its behavior accordingly.

Research on interval timing has generated a variety of different theoretical models, the most popular of which is based on an internal "pacemaker-accumulator theory" (Treisman, 1963; Church, 1978) also known as scalar expectancy theory (SET) (Gibbon, 1977). According to this "clock model" (Gibbon, 1977; Gibbon et al., 1984), the onset of a signal closes a switch that gates pulses to an accumulator until a reinforcement signal ends the accumulation of pulses that are stored in a reference memory. This accumulation of the number of stored pulses establishes a distribution of values related to the reinforced duration. On subsequent trials the signal causes retrieval of a value from reference memory, and responding is based on a discrepancy rule and a decision threshold. The difference between the current accumulated time (working memory) and the reference memory value is constantly updated, and responding is predicted to occur when the ratio of that difference to the reference memory value falls below some decision threshold. As trial time elapses, the relative difference decreases and the probability of responding increases. However, if reinforcement is omitted and the trial signal remains beyond the expected time of reinforcement, the relative discrepancy grows and responding decreases again. This pattern of responding is typically observed in empirical studies when averaging responses across many individual trials in a task known as the "peak procedure" (Catania, 1970; Roberts, 1981).

Several other authors have objected to the pacemaker-clock approach to interval timing and have proposed alternative frameworks (Killeen and Fetterman, 1988; Grossberg and Schmajuk, 1989; Church and Broadbent, 1990; Machado, 1997; Staddon and Higa, 1999; Staddon, 2002, 2005; Matell and Meck, 2004; Oprisan and Buhusi, 2011; Buhusi and Oprisan, 2013). Several of these alternative approaches rest on the notion that as time passes from the onset of a stimulus, processing initiated by that stimulus undergoes a series of discriminable states and that the dominantly active state at the moment of reward becomes strengthened. In this way, learned behavior can be said to be "timed." This notion was, perhaps, first noted by Pavlov (1927, p. 104) in his attempt to explain the phenomenon of "inhibition of delay":

> "*nerve cells which are being excited pass through a series of successive physiological changes. In accordance with this it is obvious that if a definite unconditioned reflex is repeatedly evoked coincidently with any one particular physiological state of the cerebral cells, it is this definite state and no other that acquires a definite conditioned significance.*"

The idea expressed by Pavlov has been formalized in a variety of ways. For example, Staddon and Higa (1999) introduced a "multiple time scale model" of habituation as the basis of interval timing, Grossberg and Schmajuk (1989) introduced a "spectral timing" approach, and Killeen and Fetterman (1988) and Machado (1997) assumed a series of stimulus-initiated behavioral states as the basis of temporal control. In any given conditioning trial, once that particular state associated with reward is re-entered then responding will arise.

A third class of theories has also been developed to explain interval timing. Church and Broadbent (1990) introduced a connectionist model and Matell and Meck (2004) and Oprisan and Buhusi (2011); also (Buhusi and Oprisan, 2013) extended this to a neural network equivalent – the "striatal beat theory." The basic notion is that the brain contains multiple oscillators, i.e., neurons that fire with different periodicities, and time can be encoded as the unique oscillator firing pattern present at the moment of reward. Subsequently, when that firing pattern is approximated on a given conditioning trial, responding becomes more likely through a pattern matching decision process.

Another approach, using large interconnected neurons have also been applied to modeling certain fundamental aspects of interval timing behavior. One key feature of the timing system is that as the interval to be estimated increases, the variance of

responding around that estimate also increases in accordance with Weber's law. This is known as the "scalar timing" principle (Gibbon, 1977, 1992; Gibbon et al., 1984). The approach of using "large population clock neurons" that give rise to a diffusion equation whose drift rate is learned within these interconnected neurons was utilized to model scalar expectancy in peak timing experiments (Simen et al., 2011, 2013; Luzardo et al., 2017; Hardy and Buonomano, 2018). Different intervals are timed, in this model, by different proportions of neural populations that generate timing pulses engaged by the stimulus, with higher proportions effectively increasing the diffusion drift rate such that a response is triggered sooner.

One empirical phenomenon that has been especially challenging for all approaches is how multiple stimuli are combined to generate timing behavior. Matell and his colleagues have noted that when rodents are trained in a peak procedure with two separate stimuli indicating different intervals to time, responding to the stimulus compound reflects an averaging of the two intervals rather than memories for each trained interval (Swanton et al., 2009; Swanton and Matell, 2011; Matell and Henning, 2013; Matell and Kurti, 2014; Delamater and Nicolas, 2015; De Corte and Matell, 2016a,b). This result is problematic because most theories anticipate behavior to be controlled by each of the two intervals trained separately. For instance, first consider SET's assumptions. When trained with different stimuli, reference memory should include one distribution of trained intervals appropriate for stimulus A and a second distribution for stimulus B. When a stimulus compound is presented, stimulus AB, one interval from each of the two reference memory distributions should be retrieved and responding should emerge whenever a working memory representation of elapsed time is close to each of those two intervals. In other words, there is no mechanism for responses to compound stimuli to reflect the average of the two intervals built into SET. Similarly, if timing were related to a series of discriminable states initiated by presentation of the stimulus, then, once again, whenever the system approached those two dominant states trained individually, responses should be maximal at each of those two times rather than at some intermediate time. The multiple oscillator and striatal beat theories would have similar difficulty because each reinforced activation pattern should govern responding, in a manner analogous to SET.

In this paper, we explore the use of a simple RNN model to predict interval timing. Our approach differs from others in several ways. First, we do not assume that a clock system is engaged to generate a steady stream of pulses. Rather, our RNN has a dynamic response to an input stimulus, which is modeled by a step function in Tone and Flash. The response is determined by input weights and recurrent feedback weights that are learned by a reinforcement signal at a specific time from stimulus onset. The weights of the RNN are stored in memory (i.e., in the network itself) but can be updated (learned) with repeated exposure to signal and reinforcement. Second, as developed in greater detail below, we assume that different recurrent processes adopt distinct "Go" and "No-Go" behavioral functions that summate within a "timing circuit" and this, ultimately, feeds into a final recurrent integrator output stage that governs the system's response. Finally, we make the important additional assumption that when multiple stimuli are presented together (as in a temporal averaging study), interactions among the stimuli take place such that the effective "salience" of each stimulus is impacted by the other. On the basis of the temporal dynamics of the recurrent processes that make up the network, we show that temporally organized behaviors can be trained with empirical data obtained from rodents performing in a peak procedure. Thus, the final recurrent process with its learned weights generates a distribution of output that matches the response patterns of the animal. Furthermore, after training different recurrent systems with different intervals of reinforcement and different stimuli (such as a tone and flash), we show that dynamic interactions between these two systems can predict temporal averaging. This idea suggests that the encoding of interval timing is embedded in the connection weights of a relatively small RNN, without the need for a fixed internal clock (Staddon, 2005). In addition, by considering the recurrent interactions within and between neural processing units, other aspects of dynamic temporal control may be shown to emerge in empirically meaningful ways.

MATERIALS AND METHODS

Experimental Data

Procedures

Subjects

Male and female Long–Evans rats ($n = 8$ of each) bred at Brooklyn College (from Charles River Labs descent) were housed in a colony room on a 14:10 light:dark schedule, and throughout the experiment were maintained at 85% of their free feeding weights (ranging from 223 to 263 g for females, and from 348 to 377 g for males). All procedures on these animals were approved by the IACUC of Brooklyn College, and were in compliance with NIH guidelines as identified in the *Guide for the Care and Use of Laboratory Animals, 8th Ed.*

Preliminary training

The rats first learned to retrieve food pellets (45 mg TestDiet 5TUM, 45 mg Bio-Serv #50021) from a recessed "food magazine" (3.0 cm × 3.6 cm × 2.0 cm, length × width × depth) located on the front wall of a rectangular shaped conditioning chamber (BRS Foringer RC series, measuring 30.5 cm × 24.0 cm × 25.0 cm, length × width × height). These chambers were housed inside separate sound- and light-resistant shells (Med Associates, ENV-022V). During each of 2 days, the rats were placed in the conditioning chambers for 2, 20-min sessions. In each session, one of the two pellet types was delivered to the food magazine 20 times at random, with the order counterbalanced. A response lever (4.0 cm in width) was located 3.0 cm to the left of the magazine and 8.0 cm above the chamber floor (that consisted of steel rods spaced 2.0 cm apart). On the next day the rats learned to press this response lever to obtain food reward, until 30 rewards of each type were earned.

Peak procedure

Over the next 40 days the rats were trained on the "peak procedure." In each training session, the rats learned to obtain

reward for the first lever press response occurring after 5 s from the onset of an 80-s tone stimulus (Med Associate sonalert, 2900 Hz and 6 dB above background level of 74 dB provided by a ventilation fan), and 30 s from the onset of an 80-s flashing light stimulus. These stimulus-interval assignments were not counterbalanced because prior research has shown that temporal averaging effects are more likely to occur with the present assignments (Swanton and Matell, 2011; Matell and Henning, 2013; Matell and Kurti, 2014). Two 28 volt light bulbs were used for this purpose and these were located in the top of the rear wall of the chamber opposite the food magazine, behind a translucent plastic sheet used to protect the bulbs and diffuse the light. The lights flashed at a frequency of 2/s with equal on-off pulses. The chamber was dark otherwise. In each training session, there were 16 conditioning trials with the tone stimulus and 16 with the flash stimulus. The inter-trial interval (i.e., time from offset of one stimulus to onset of the next) was 40 s in each of the first two sessions, 80 s in the next two sessions, and 120 s in each session thereafter. The 40 training sessions were arranged in "blocks" of four training sessions. In each of the first two blocks of training, reinforcement was made available for the first lever press response occurring after the critical time interval on a predetermined 75% of the tone and flash trials. In the next three training blocks, reinforcement was made available on 25% of the tone and flash trials. Thereafter, reinforcement was made available on 25% of the tone trials and 75% of the flash trials (in order to maintain comparable peak levels of responding on each trial type). Importantly, the non-reinforced tone and flash trials were regarded as "probe" trials in which lever press responding was assessed in 1-s intervals, starting 20 s prior to stimulus onset and extending for the entire 80 s of stimulus presentation. In each conditioning session the order of these reinforced and non-reinforced probe trials was randomly determined.

Temporal averaging assessment

The same procedures continued in the 11th block of training as in the 10th block. However, four additional non-reinforced "probe" trials occurred in which the tone and flash stimuli were presented as a simultaneous stimulus compound. Responding on each of these non-reinforced probe trials with tone, flash, and the tone + flash compound constituted the main data of interest.

RESULTS

Experimental Results

Mean lever-presses per second on non-reinforced tone and flash probe trials were recorded in 1-s time bins for the 20-s period preceding stimulus onset and for the entire 80-s stimulus period. The data were averaged across trials and days for an individual subject and across rats for each 4-session block of training. The response functions on both Tone (**Figures 1A–E**) and Flash (**Figures 1F–J**) trials were progressively shaped over the course of training, with responding peaking increasingly closer to the anticipated reward times as training proceeded. There were three noteworthy differences between tone and flash responses by the end of training. First, in the presence of the Tone, the response rapidly rose and peaked close to the anticipated time of food availability (5 s) and declined rapidly thereafter (**Figure 1E**). In the presence of the Flash stimulus, the peak response occurred close to the anticipated time of food availability (30 s) but it rose to that peak value more slowly than for Tone, before declining gradually thereafter and more slowly than for Tone. Second, these differential patterns of responding emerged more quickly over training to the Tone stimulus than the Flash (Compare **Figures 1A–E** with **Figures 1F–J**). Third, while the responses in both stimuli rose more rapidly than they declined, the overall variability in responding (e.g., the width of the response distributions) was larger for the flash than for the tone stimulus, a fact that is likely due to the length of the time interval to be estimated. It is also noteworthy that by the end of training, responding to the Tone stimulus decreased to below-baseline (i.e., pre stimulus) levels. This suggests that the stimulus actively inhibited lever press responding late in the Tone stimulus, periods in which food was never presented but were clearly differentiated from the early periods in which food was frequently available. Such behavior was not apparent to the Flash stimulus.

Lever press responses during Block 11 included non-reinforced probe trials with the Tone, Flash, and Tone + Flash stimuli. For the purposes of analysis, the data were normalized in each 1-s time bin by expressing response rate as a proportion of maximal response rate. The maximal response rates on each trial type averaged across animals did not appreciably differ (mean maximal response rates on Tone, Flash, and Tone + Flash trials, respectively, were 1.00, 0.96, and 0.98 responses/s). The normalization was done for each of the three (3) cues separately and the slightly different rates reflect the differing peak times across animals. However, when peak rates were computed for each individual animal, the maximal response rate on tone trials (mean = 1.10 responses/sec) was significantly less than on flash trials (mean = 1.28 responses/sec) and on compound trials (mean = 1.23) trials [$F(2,28) = 4.84$, $p < 0.05$]. The model simulations were compared to the response rates averaged across animals. Once again, differential responses were observed to the Tone and Flash stimuli, when tested individually, with earlier and less variable responses to Tone (**Figure 2A**) than to Flash (**Figure 2B**). Importantly, responses to the Tone + Flash compound stimulus reflected an "averaging" of the two individual functions with a phase delay with reference to Tone alone and phase advance with reference to Flash alone. Notably, it did not result in peaks occurring at 5 and 30 s. This indicates that there is an integrative dynamic process that combines the responses to the stimuli rather than a simple summation of the two individual response functions.

To explore these data quantitatively we first evaluated the response functions on these three trial types using the curve-fitting method of Matell and Kurti (2014). The normalized lever press data on Tone, Flash and Compound trials for each animal were fit with a dual asymmetric sigmoid function, using the curve fitting package in Matlab, Cambridge, MA, United States. The time at which the function reached a peak (Peak Time) was then

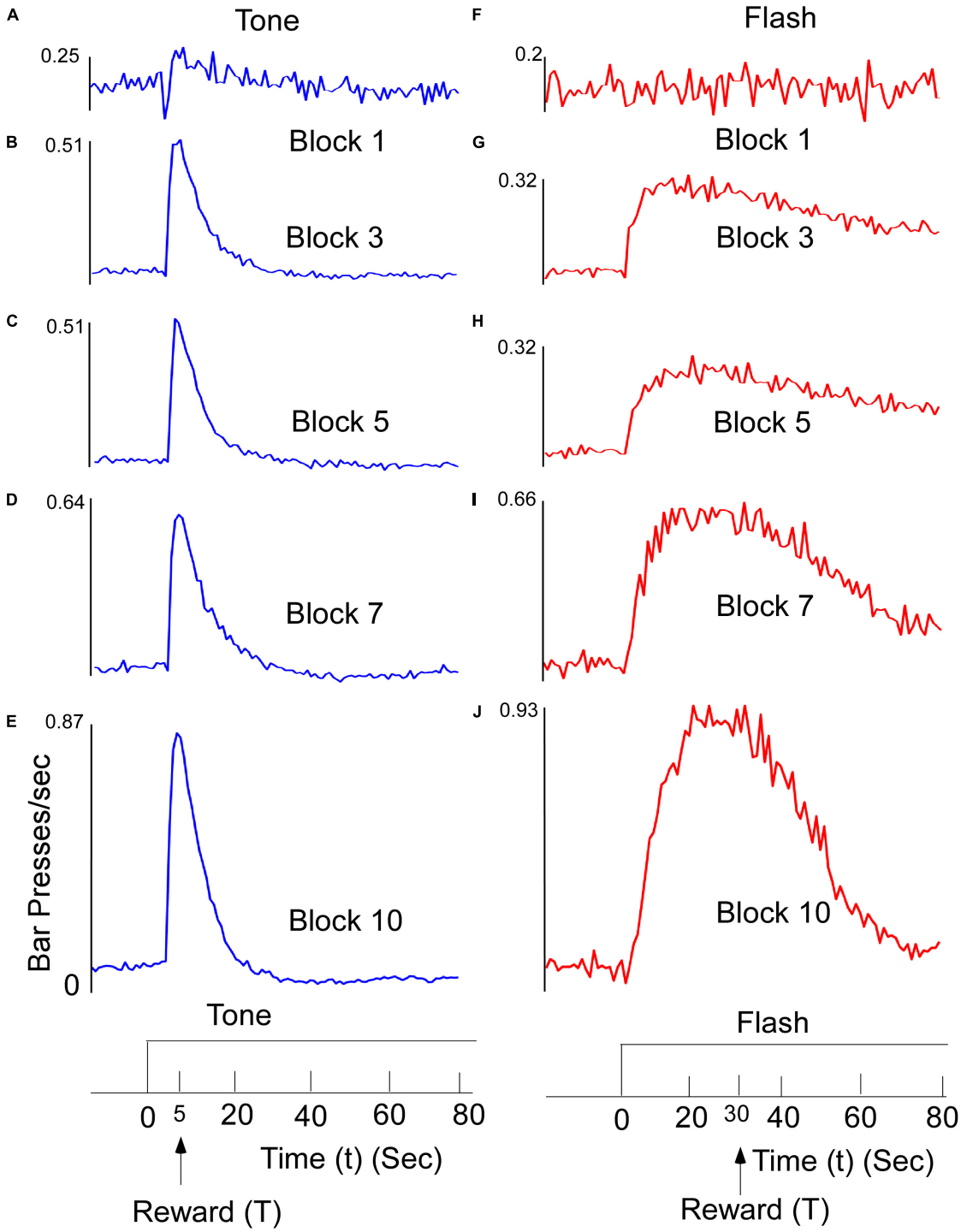

FIGURE 1 | Learned reward timing on tone **(A–E)** trials. Animals were trained to press a Bar to receive a food reward at T = 5 s following onset of a 60-s Tone stimulus. Animals received the reward for the first lever press after this time. Interspersed throughout training, rats were also tested on non-reinforced "probe" trials to determine how responding was temporally distributed within the trial. Bar Press frequency was computed by counting the number of Bar Presses within a 1 s window. The peak Bar Press/sec was computed and increased rapidly and then declined. The width of the function describing the Bar press frequency decreased as a function of training, but the time to reach the peak stayed relatively constant. The data are presented across representative 4-session Blocks of training. **(F–J)** Learned timing for Flash input. Animals received the food reward for the first lever press occurring after T = 30 s. The time to reach peak Bar Press frequency was longer, but as the animals learned, the width of the function decreased.

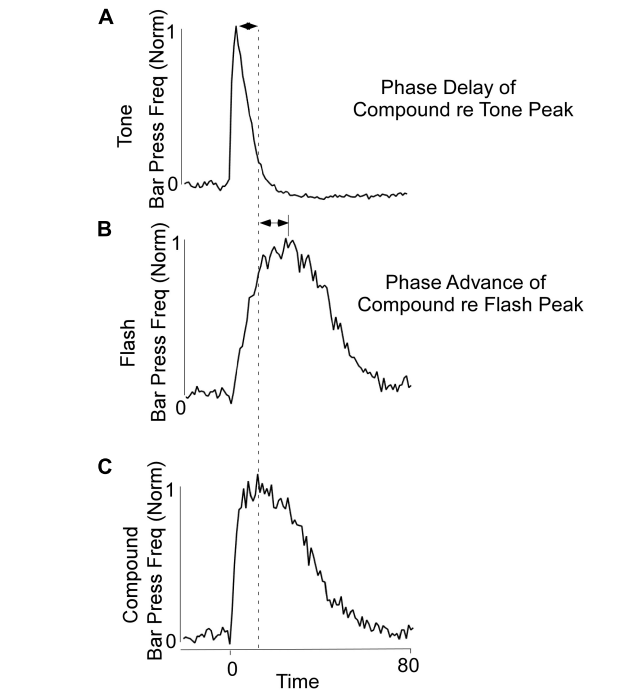

FIGURE 2 | Average Bar Press frequency on non-reinforced probe trials for Tone **(A)**, Flash **(B)**, and Combined Tone + Flash **(C)** trials in Block 11 of training. There was a phase delay of the Combined Tone + Flash relative to Tone alone trials and a Phase advance of the Combined Tone + Flash relative to Flash alone trials. Thus, the Combined Tone + Flash stimulus generated a response function whose peak and variance were between those observed on Tone and Flash trials. Whereas the peak of the Combined Tone + Flash function was somewhat closer to Tone alone, its width was closer to that seen to Flash alone.

determined for the 16 animals for Tone, Flash and Compound stimuli. The width of the function was determined as the time between values that were reduced by 50% from the peak value of the function (Width) (Matell and Kurti, 2014). These values as well as their averages over all animals are shown in **Table 1**. The data from one animal was not included in this analysis because response rates on Flash and Compound trials did not display a clear peak function.

First, we observed significant differences in peak times on these three trial types [means (±SEM) = 4.7 (0.18), 26.1 (0.70), and 16.2 (0.64) for Tone, Flash, and Compound, respectively], $F(2,28) = 366.45$, $p < 0.000001$. *Post hoc* tests using the method of Rodger (1974) confirmed that peak times on each trial type differed from one another with the peak times for Flash > Compound > Tone, and with the peak time for Compound approximating the arithmetic average of the peak times for Tone and Flash.

Second, we computed the coefficients of variation (CV, width/peak time) for each animal for Tone, Flash and Compound trials in order to determine if responding was scalar invariant (Gibbon, 1977; Church et al., 1994). The mean CVs significantly differed for Tone, Flash, and Compound trials

TABLE 1 | Each rat had 10 blocks of training.

Rat #	Peak time (PT)	Width 50% (W-50)	Ratio PT/W-50
Flash			
1	21.75	50.19	0.4332
2	25.14	38.73	0.6489
3	22.21	37.78	0.5877
4	27.98	35.94	0.7784
5	N/A	N/A	N/A
6	26.34	50.34	0.5231
7	30.68	37.70	0.8137
8	25.69	43.35	0.5925
9	26.26	34.27	0.7660
10	28.86	44.08	0.6545
11	30.99	43.33	0.7152
12	25.00	43.56	0.5738
13	24.06	36.58	0.6575
14	27.21	32.14	0.8466
15	25.78	45.97	0.5607
16	24.22	34.99	0.6920
Avg.	26.14	40.60	0.6563
Tone			
1	5.93	10.38	0.5711
2	6.22	12.70	0.4893
3	4.17	6.87	0.6066
4	4.02	6.56	0.6126
5	N/A	N/A	N/A
6	5.04	8.33	0.6046
7	5.03	7.90	0.6363
8	4.67	9.83	0.4746
9	4.60	7.37	0.6240
10	4.44	14.85	0.2988
11	3.68	10.80	0.3406
12	4.06	7.53	0.5384
13	4.30	7.80	0.5510
14	4.51	8.39	0.5370
15	4.99	7.22	0.6905
16	5.24	10.40	0.5036
Avg.	4.72	9.13	0.5225
Compound			
1	12.76	45.64	0.2795
2	19.06	34.81	0.5474
3	14.8	35.54	0.4164
4	14.41	34.57	0.4168
5	N/A	N/A	N/A
6	13.99	33.94	0.4121
7	12.49	28.17	0.4432
8	19.12	39.51	0.4839
9	17.56	36.42	0.4821
10	17.49	37.93	0.4610
11	16.14	36.64	0.4404
12	15.09	39.16	0.3853
13	15.77	33.54	0.4700

(Continued)

TABLE 1 | Continued

Rat #	Peak time (PT)	Width 50% (W-50)	Ratio PT/W-50
14	19.39	27.90	0.6947
15	20.23	38.36	0.5272
16	14.91	36.32	0.4104
Avg.	16.21	35.90	0.4580

The Compound Probe sessions were contained in Block 11 and comprised non-reinforced presentations of flash, tone, and compound trials. The peak Time (PT) and Width between 50% of peak times (W-50) was obtained using the method of curve fitting used by Matell and Kurti (2014). The ratio of the peak time to the Width (W-50) using the method of Matell and Kurti (2014) could then be used as a measure of scalar invariance.

[means, respectively, = 1.95 (± 0.13), 1.57 (± 0.70), 2.26 (± 0.12), $F(2,28) = 10.38$, $p < 0.0005$], and *post hoc* tests confirmed that the three trial types differed from one another and were ordered as follows: Compound > Tone > Flash. This indicates that although the widths of the response functions increased with the interval to be timed, they did not do so proportionally with peak time (**Table 1**).

Neural Net Modeling

Bar press distributions for flash and tone inputs were conceptually modeled by using a neural net that has dynamic properties, whose dynamics, i.e., speed of response, are associated with specific nodes that have recurrent feedback loops (**Figure 3**). The input layer comprises separate sensory units, which code auditory and visual stimuli, denoted by Tone and Flash inputs. Output from these units enter the "Timing" component of the network, which consists of two recurrent units for each sensory input. These recurrent units are conceptualized as executing "Go" and "No-Go" behavioral functions. Outputs from these Go/No-Go units feed into a "Sum Operator" unit for the Tone and Flash inputs when presented individually (**Figure 3**). The summation of the Go/No-Go units implement a second order dynamical system with short and long time constants whose outputs oppose each other. A second order system has been useful in modeling the semicircular canal dynamics of the vestibulo-ocular reflex, which then activates a central velocity storage integrator (Raphan et al., 1979). These early modeling approaches motivated the development of the RNN presented in this study. A parsimonious feature of this approach is that there is a minimal number of neural processing units, each behaving as an integrator with a different time constant, can be used to model the response to constant tone, flash and compound stimuli. Approaches using large populations of neurons have also been used to model interval timing (Hardy and Buonomano, 2018). An approach that uses a single integrator driven by a bistable input layer consisting of a population of units generating a stochastic ramp and trigger (SRT) has been shown to generate a drift-diffusion model that simulates scalar invariance in interval timing (Simen et al., 2011). We found that we could better fit the compound test data in this study by using a piecewise linear activation function in the Sum Operator unit that had a linear part that saturated with too much or too little input. It was also required to implement a saliency operator, which was implemented by cross coupling between the two stimuli such that their effective "saliency" is diminished when presented together (see Saliency Operator Description below). All of the weights connecting nodes up to this point in the network were assumed to be modifiable, so they could learn the timing characteristics associated with the data. Output from the Tone and Flash summation units then feed into a final recurrent integrator unit that uses a linear activation function [f(x) = x]. Output from this integrator unit serves as the basis of the model's performance. We now consider the details of the RNN and how learning was implemented to simulate the data across these two modalities.

Recurrent Neural Network Model for Generating Interval Timing

The model was implemented using Matlab's Neural Net ToolBox, and learning of the weights was obtained using Matlab's machine learning libraries. The simulated data were generated from the model as output activation vectors corresponding to the 20 pre-stimulus and 80 stimulus time steps for each stimulus (Tone, Flash), and the mean lever press response data (normalized to maximal response rates, see **Figure 2**) for a particular stimulus were used to train the weights to minimize the mean square error of the comparison.

We first consider how a single recurrent loop implements timing (**Figure 4A**) and sheds light on the solution to the overall timing problem by combining multiples of these recurrent loops to implement a dynamical system. A single recurrent neural processing unit can be described analytically by a feedback loop, which is delayed by a single time step, represented by z^{-1}, and a feedback weight, w_1 (**Figure 4B**). This has been described in the system theory literature as an *integrator* (Zadeh and Desoer, 1963). The concept of an integrator has been utilized in modeling a wide range of phenomena related to the saccadic system (Raphan and Cohen, 1981; Robinson, 1981; Raphan, 1998), denoted by the velocity-position integrator, which has played an important role in transforming the velocity commands generated centrally to the position commands that the eye muscles receive to hold the eyes (Robinson, 1981; Raphan, 1998; Seung et al., 2000). Additional integrators have been identified in the vestibulo-ocular reflex (Raphan et al., 1979; Raphan and Cohen, 1996, 2002), vestibulo-sympathetic system (Raphan et al., 2016), and locomotion system (Cho et al., 2006, 2010; Osaki et al., 2008). In some instances, our definition of "integrator" has also been referred to as a "leaky" or "impure" integrator (see also Seung et al., 2000; Simen et al., 2011), whereas a feedback weight of 1, is referred to simply as an integrator (Zadeh and Desoer, 1963; Seung et al., 2000).

The integrator can be defined by a difference equation, which naturally lends itself to implementation as a RNN. The state, x(n), denotes the current state of the integrator and x(n + 1) is the next state after 1 time step, which is updated by a dynamic process.

The integrator can be represented analytically as

$$x(n+1) = W_1 x(n) + W_0 U(n) \quad (1)$$

where x(0) is the initial value of the state and there is a linear activation function (**Figure 4C**). The weight, w_1, is the recurrent feedback weight, which determines the rate at which the output

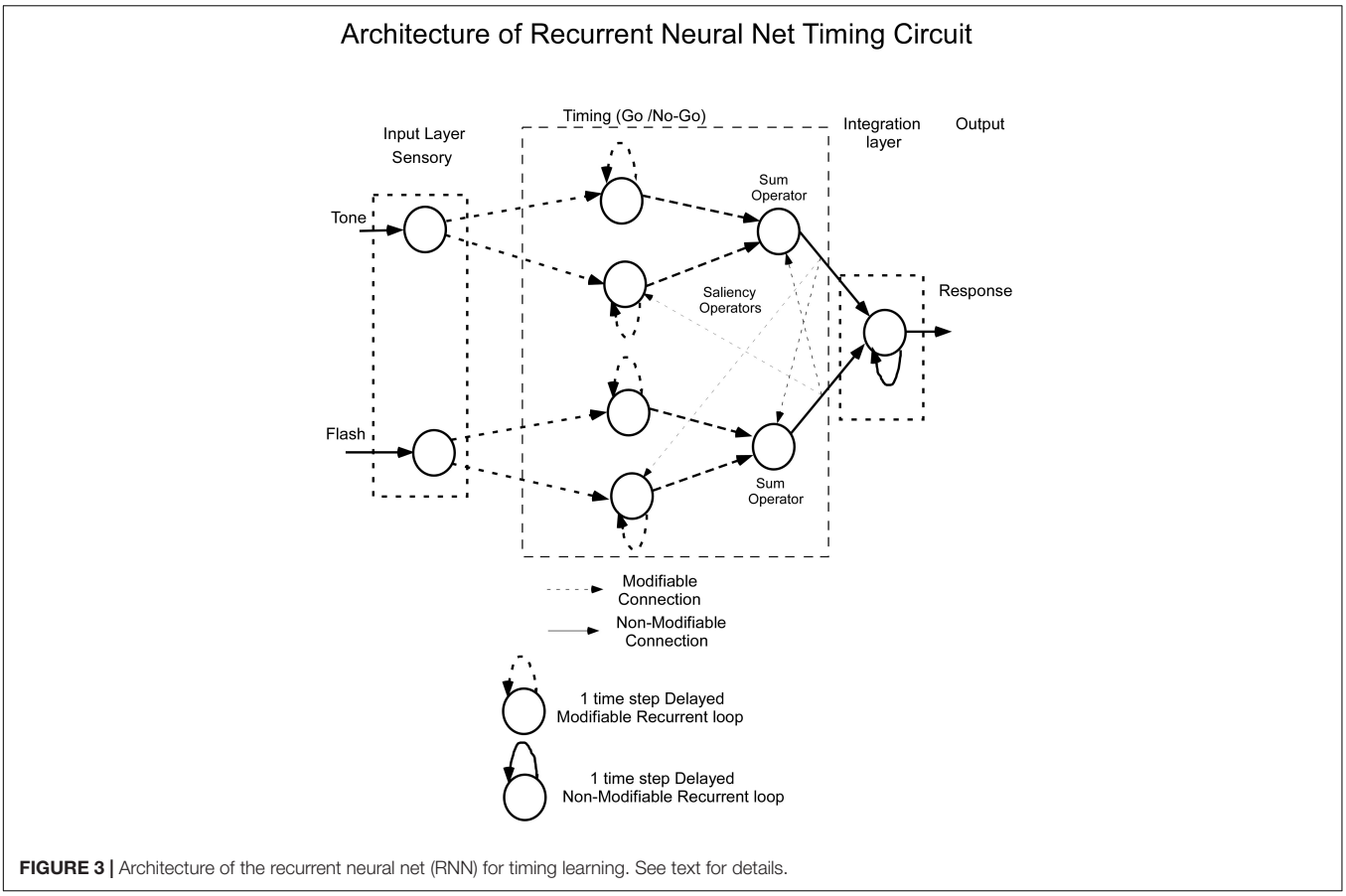

FIGURE 3 | Architecture of the recurrent neural net (RNN) for timing learning. See text for details.

rises to a steady state level. The feed-forward gain (W_0), i.e., the weight connecting an input unit to the integrator unit, determines the asymptotic level to which the output rises in response to a step input, U(n). When the feed-forward gain, W_0, is set to W_0 = 1- W_1, the asymptotic output level is equal to the input unit's activation level, which depends on an activation function that is linear (**Figure 4D**). The rate at which that asymptote is achieved depends upon w_1. In response to a step input (**Figure 4D**), this unit's behavior, for a Matlab simulation, changes over time differently for three different feedback weight values (where w_1 = 0.9, 0.8, 0.5, **Figure 4D**), illustrating its timing capability from a long time constant to a short time constant. Notice (1) that in all three cases, the asymptotic output level matches the input level, and (2) that the closer the feedback weight, w_1, is to 1.0, the longer is the rise time to reach the steady state value.

When implementing neural nets, non-linear activation functions, such as squashed S functions are generally used to increase the flexibility of the learning (Winston, 1993). In the Matlab toolbox, this squashed S activation function is the tanh(•). The integrator can now be represented mathematically by the following equation:

$$x(n + 1) = \tanh[w_1(x(n)) + w_0u(n)] \quad (2)$$

where the output of the integrator is squashed before it is fed back. It is implemented in Matlab as shown in **Figure 4E**. The response to steps of input follow the slow, medium, and fast rise although the weights are now different because of the squashed S function that generates the next state x(n) (**Figure 4F**). Because the input and recurrent weights are not aligned, the asymptotes that the responses rise to are different.

The Matlab implementation of the RNN timing model for Tone and Flash stimulus inputs is a combination of integrators as shown in **Figure 5**. The delays in the feedback loops for recurrent units are denoted by having a 1 time step associated feedback path. This meant that each recurrent unit stimulated itself through weights, w_3 – w_4, w_9 – w_{10}, and w_{14}.

It should be noted that the activation functions at each layer were different and suited for the intended purpose of the model. The first layer learned the weights from the data and a tanh function was used as the activation function for units at this layer. In the second layer (summator), the activation function was dependent upon whether one or two stimulus inputs were presented to the network. If a single stimulus was presented the activation function was linear, but if both stimuli were presented we used separate piecewise linear functions whose slopes, saturation and cutoffs differed for the two stimuli. As described in more detail below (Saliency Implementation), this was done in order to implement the idea that concurrently presented stimuli could interfere with one another's processing, i.e., their "salience."

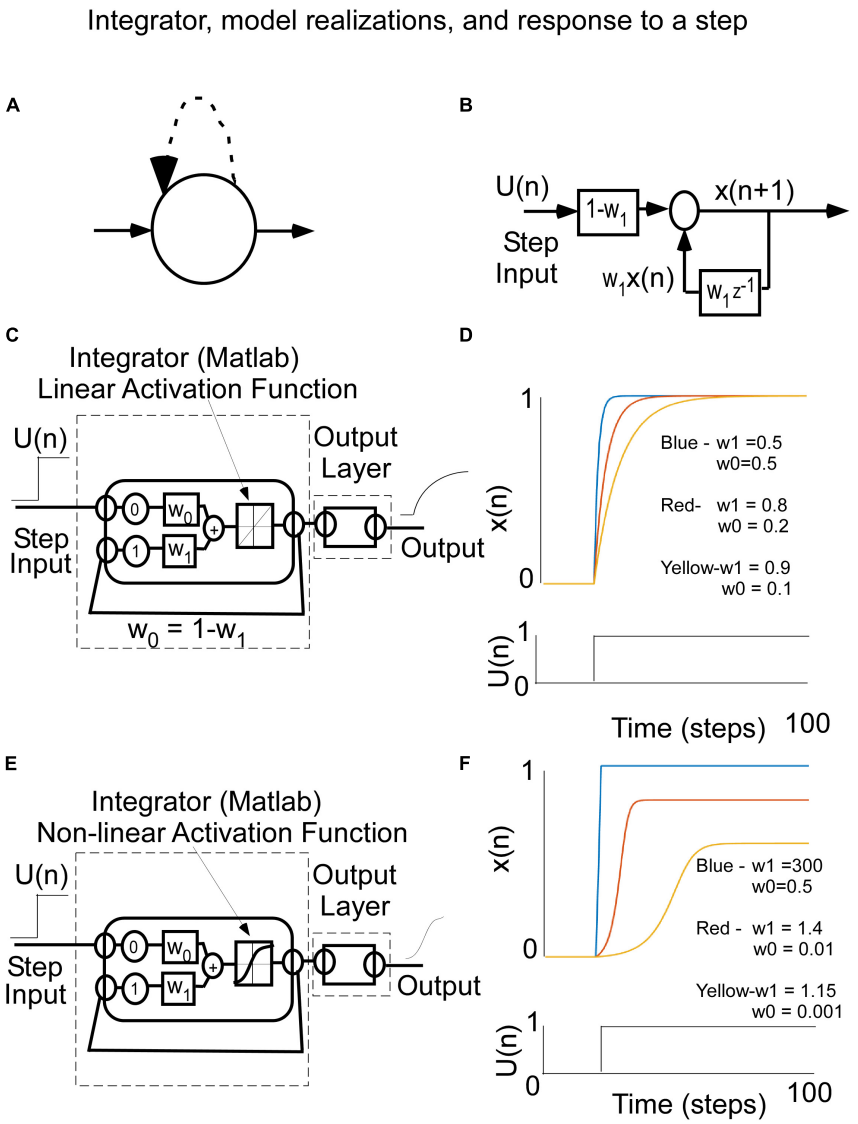

FIGURE 4 | (A) Recurrent Node representation for a simple integrator. **(B)** Control theoretic input–output representation of an integrator The input in U(n) and the output is the state of the integrator. The w_1 is the feedback (recurrent) weight and z^{-1} represents a one time step delay. The feedforward weight is W_0. When $W_0 = 1\text{-}W_1$, the state x(n) rises to a value 1 with a rise time that is that is related to W_1. **(C)** Matlab Implementation of the integrator with a linear activation function. **(D)** Response of the integrator to a step of input for different weights, w_0 and w_1. Note that the closer the weight w_1, which is the recurrent feedback weight, gets to 1, the longer the rise time to its steady state value. **(E)** Matlab implementation of the integrator with a squashing activation function [tanh(·)]. **(F)** This leads to a wider range of weights that produce different types of rise behavior. The larger the recurrent weight, the faster the response is to the steady state.

The final integrator was implemented with a linear activation function as its weight was fixed throughout the learning process. While the model explained the dominant features of the timing data, the saliency functions were necessary to more accurately fit the data for combined tone and flash compound tests (see Description and Equations implemented for Saliency, below).

Model Equations Without Saliency

As explained above, the model is a dynamical system, which can be described as an interconnection of integrators.

For Tone input, the state update equations of the Go and No-Go units can be given as:

$$T_{Go}(n+1) = \tanh[W_3(T_{Go}(n)) + W_1(U_{Tone}(n))] \quad (3)$$

$$T_{NoGo}(n+1) = \tanh[W_4(T_{NoGo}(n)) + W_2(U_{Tone}(n))] \quad (4)$$

The initial state, T_{Go} (0) and T_{NoGo} (0) was assumed to be zero during the first 20 time steps, which was pre-stimulus state and the response was assumed to be due to the stimulus.

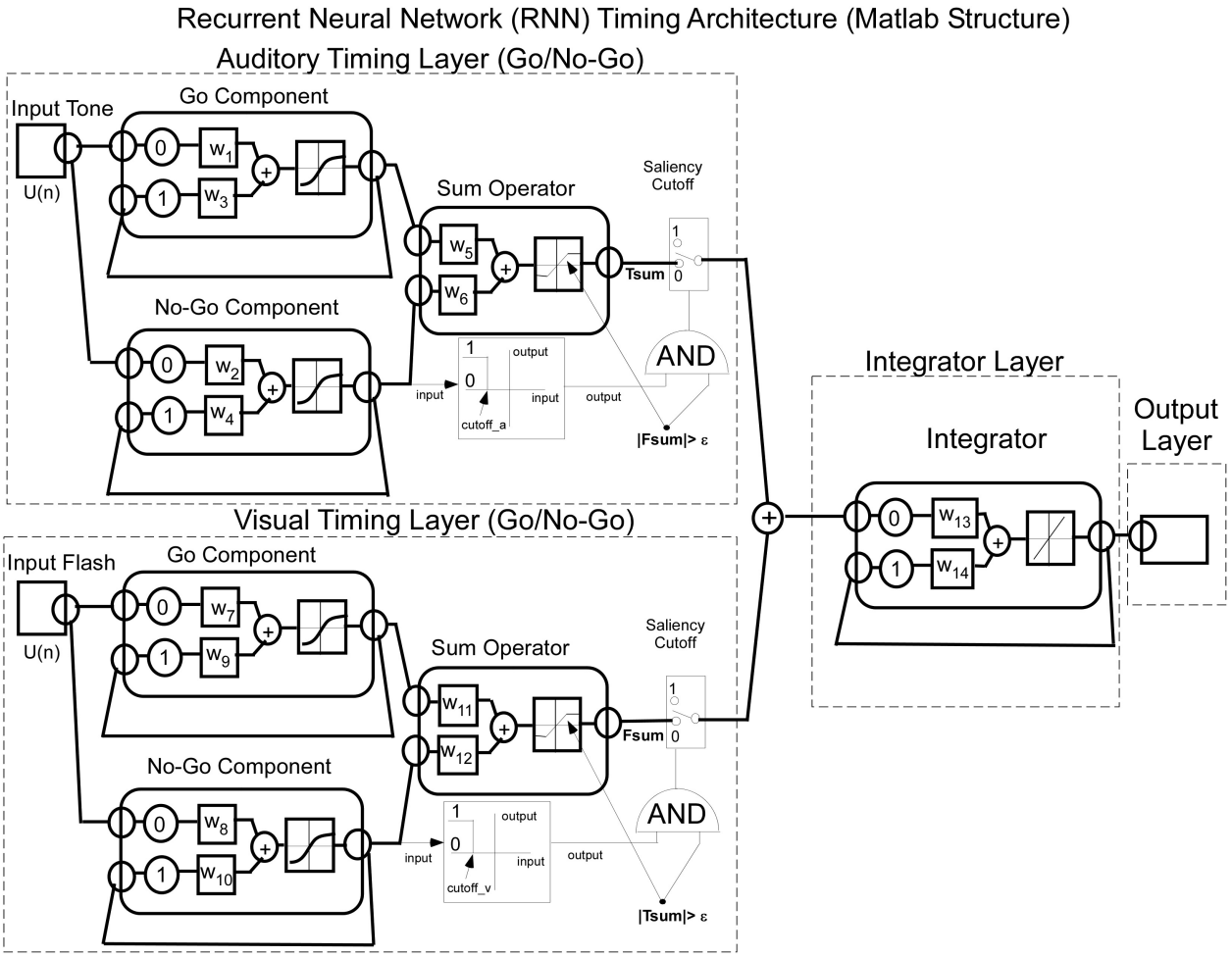

FIGURE 5 | Matlab implementation of the RNN used to model interval timing. Note that each of the Tone and Flash stimulus inputs converge onto two distinct "timing" nodes (Go and No-Go) that adopt opposing fast and slow integrator behavioral functions with squashing activation function behavior. Output from these two timing nodes are then summed and are operated on by a saliency operator, which then adds their outputs and drives a fixed weight integrator.

For Flash input, the state update equations of the Go and No-Go units can be given as:

$$F_{Go}(n+1) = \tanh[W_9(F_{Go}(n)) + W_7(U_{Flash}(n))] \quad (5)$$

$$F_{NoGo}(n+1) = \tanh[W_{10}(F_{NoGo}(n)) + W_8(U_{Flash}(n))] \quad (6)$$

For both Tone and Flash inputs, the Go and No-Go units feed into a Sum Operator (**Figure 5**).

When Tone and Flash responses are induced separately (no saliency), the Sum Operators have linear activation function and the update equations are given by:

$$T_{Sum}(n) = W_5 T_{Go}(n) + W_6 T_{NoGo}(n) \quad (7)$$

$$F_{Sum}(n) = W_{11} F_{Go}(n) + W_{12} F_{NoGo}(n) \quad (8)$$

Where n is the present time step and n + 1 is the next time step.

The summation is just used as input to the final Integrator whose equation is given by:

$$x(n+1) = W_{14} x(n) + W_{13}[T_{Sum}(n) + F_{Sum}(n)] \quad (9)$$

Saliency Operator Equations

In this model, saliency is defined as the conditions that must be met when two (or more) stimuli are present for one stimulus to modify the transmission of the other stimulus. The saliency operation that we considered was based on the examination of the compound response as compared to the response predicted without saliency. One aspect of the compound response based on our data (**Figure 2**) was that it did not have an overshoot as was predicted by the model without saliency (see section Machine Learning for Combined Tone + Flash Trials below). This required the presence of a saturation effect to limit the overshoot.

Thus, the saliency operation in this model for Flash interfering with Tone processing is the saturating of the summed Tone Go/No-Go signal when the Flash signal is present. This is represented by a modification of the activation function in the Sum Operator for Tone by incorporating a saturation and a modification of the slope that produces T_{Sum} (**Figure 5**).

The implementation of saliency had bilateral effects, where not only does the Flash stimulus interfere with Tone's processing, but the No-Go component of the Tone stimulus, can also affect Tones's combined Go/No-Go processing.

With the saliency operator defined as above, the outputs of the Sum Operator for Tone when Flash is present are given by:

If

$$W_5T_{Go}(n) + W_6T_{NoGo}(n) \geq 0 \quad (10)$$

Then,

$$T_{Sum}(n) = \text{Min}[T_{Sat}(W_5T_{Go}(n) + W_6T_{NoGo}(n)), T_{Sat}] \quad (11)$$

Similarly,

If

$$W_5T_{Go}(n) + W_6T_{NoGo}(n) < 0 \quad (12)$$

Then,

$$T_{Sum}(n) = \text{Max}[T_{Sat}(W_5T_{Go}(n) + W_6T_{NoGo}(n)), -T_{Sat}] \quad (13)$$

That is, if the weighted summation, $T_{Sum}(n)$, is less than zero, then the output of the summator is the maximum (Max) of the negative of Tone saturation ($-T_{Sat}$) and the weighted summation scaled by T_{Sat}. Thus, while negative signals are implemented, but in reality, both Tone and Flash are always positive.

Similar equations of saliency were implemented for Flash processing when being interfered with by Tone:

If

$$W_{11}F_{Go}(n) + W_{12}F_{NoGo}(n) \geq 0 \quad (14)$$

Then,

$$F_{Sum}(n) = \text{Min}[F_{Sat}(W_{11}F_{Go}(n) + W_{12}F_{NoGo}(n)), F_{Sat}] \quad (15)$$

and,

If

$$W_{11}F_{Go}(n) + W_{12}F_{NoGo}(n) < 0 \quad (16)$$

Then,

$$F_{Sum}(n) = \text{Max}[F_{Sat}(W_{11}F_{Go}(n) + W_{12}F_{NoGo}(n)), -F_{Sat}] \quad (17)$$

Where T_{Sat} and F_{Sat} are the saturating values and slopes for the Sum Operator's activation functions for Tone and Flash, respectively.

Because the decline in the compound response function was somewhat faster than that for Flash, it indicated that if there was a slight presence of the Tone Sum after a long time, i.e.,:

If

$$|T_{Sum}| > \varepsilon \quad (18)$$

Where ε is a value close to zero, then the Flash processing can still be modified by the activation of an AND gate together with the Flash No-Go component being less than **cutoff_v**, which outputs a 1 and the Flash is cutoff from activating the final integrator, i.e., the saliency cutoff switch is in the 1 position, and final integrator is just left to discharge without input from Flash processing.

Similarly, there is a modification of the process that produces T_{Sum} (**Figure 5**). If there is a presence of the Flash sum signal, i.e.,:

If

$$|F_{Sum}| > \varepsilon \quad (19)$$

and the Tone No-Go component is less than **cutoff_a**, then the output of its AND gate is 1 and the Tone processing is cutoff, i.e., the saliency cutoff switch is in the 1 position. The final integrator is similarly just left to discharge without input from Tone processing.

These components of saliency insure that there can be no activation of the final integrator with low level interference activations. These saliency features were simulated and compared to the compound data after learning Tone and Flash separately (see below).

Machine Learning Methodology

To determine weights that would make the model fit the data we used the distribution of lever press responses across 100 time steps (20 pre stimulus, 80 during stimulus) to train the network to generate a 100 time step output activation vector that approximated the training vector. The weights that minimized the mean square error between the behavioral data and network output vectors were learned by utilizing the Levenberg–Marquardt algorithm from the Matlab Neural Net Toolbox to train the network. The training basically implements a back-propagation algorithm through time (BPTT) (Hagan et al., 1996; Haykin, 2004). This algorithm unfolds the RNN into a multilayered feed-forward network where each time epoch constitutes another layer (see **Appendix A** for a simplified description of the unfolding mechanism). Once the RNN is unfolded, multilayered back propagation with the Levenberg–Marquardt refinement can be used to identify the weights (see **Table 2**). As described above, saliency functions were also implemented between the Flash and Tone summation units, to better approximate the network's performance to the Tone + Flash compound empirical data (see **Table 2** for saliency constants).

Machine Learning for Tone Trials

The system was first trained by keeping the final integrator and flash component weights fixed while also maintaining the flash input at zero. The data came from the non-reinforced probe trials with Tone in Block 11 of training (**Figure 2A**). Once these weights were learned, we inspected the output activation levels of the Go and No-Go recurrent units for each of the 100 steps on a simulated Tone trial. The Tone input generated a rapid rise in activation of the "Go" unit and a slower decrease in activation of the "No-Go" unit. Thus, the two counteracted one another over time (**Figures 6A,B**). The summated response (measured by the summation unit's output activation level) was a pulse (**Figure 6C**), which was then used to drive the

TABLE 2 | The model weights W_1–W_6 were learned from the final average Tone Response data.

Model weights		Descriptor	Saliency weights	
W_1	0.2342	Tone Go Input weight	cutoff_a	−0.8632
W_2	−0.1774	Tone No-Go Input weight		
W_3	57.3471	Tone Go recurrent weight	cutoff_v	−0.2
W_4	1.3228	Tone No-Go recurrent weight		
W_5	9.7741	Tone Go Sum weight	Auditory Sum Operator Threshold	1.6
W_6	11.3814	Tone No-Go Sum weight		
W_7	0.00081236	Flash Go Input weight	Visual Sum Operator Threshold	0.85
W_8	−0.000521	Flash No-Go Input weight		
W_9	865.0088	Flash Go recurrent weight	Time to reach cutoff_a	27 time steps
W_{10}	1.1229	Flash No-Go recurrent weight		
W_{11}	0.9659	Flash Go Sum weight	Time to reach cutoff_ v	54 time steps
W_{12}	1.6959	Flash No-Go Sum weight		
W_{13}	0.1	Final Integrator Input Weight		
W_{14}	0.9	Final Integrator recurrent weight		

The model weights W_7 – W_{12} were learned from the final average Flash Response data. The final integrator weights, W_{13} and W_{14} were chosen to have a reasonably long time constant that can be used to integrate the Tone and Flash Timing information from the previous layers. The Saliency weights were chosen to improve fits of the model to the data after the learning took place.

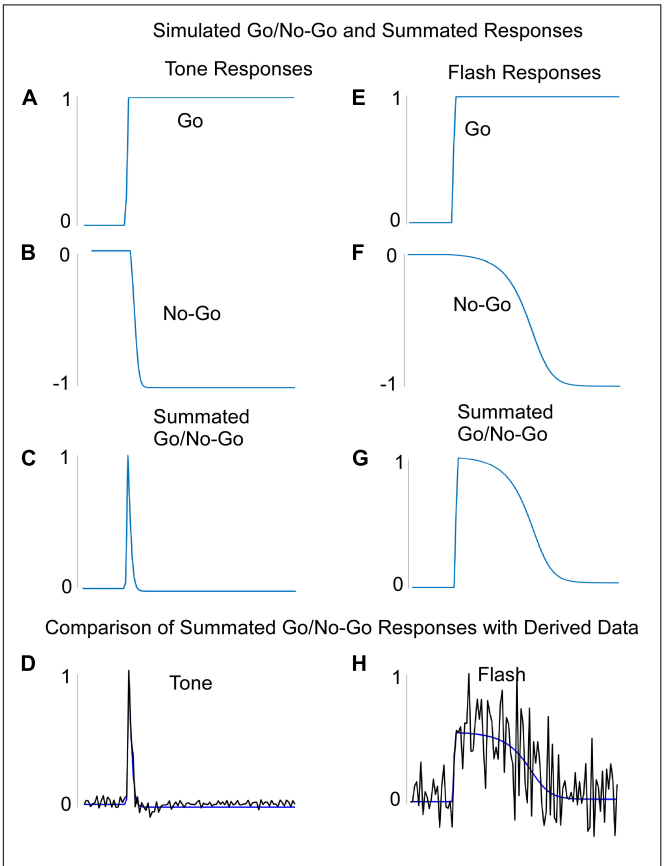

FIGURE 6 | (A–D) The learned Go **(A)**, No-Go **(B)**, and summated Go/No-Go responses to a Tone input. The Summated Go/No-Go response compares favorably with the derived data for this response. **(E–H)** The learned Go **(E)**, No-Go **(F)**, and summated Go/No-Go **(G)** responses to a Flash input. The Summated Go/No-Go response compares favorably with the derived data for this response **(H)**. Note that the "derived" data was based on the values needed to produce the actual rodent empirical data when these were fed into the integrator unit.

final integrator. When this summated function (**Figure 6C**) was applied to the integrator, the simulated tone response fit the data with great fidelity (**Figures 6D**, **7A**). Because the flash input was zero, the flash component did not impact the response of the system to a pure tone.

Machine Learning for Flash Trials

The network was similarly trained to respond to the Flash stimulus, but the Tone weights were kept constant as found during Tone learning. The final integrator weights were kept from learning as well and they were the same used for training Tone. The Go and No-Go units to a Flash input, respectively, also displayed a fast rise and slower fall in activation (**Figures 6E,F**). Notably, the No-Go unit decreased its output at a slower rate than on simulated Tone trials (**Figures 6B,F**). The Flash summation unit, which summed over its separate Go and No-Go units, showed a similar pulse to that of the Tone summation unit, but weights that were learned delayed the phase and increased the variance over time as compared to Tone (**Figures 6C,G**). When this widened pulse was applied to the final integrator whose weights were the same as for Tone input, the simulation fit the Flash data almost perfectly (**Figures 6H**, **7B**). These simulations indicate that the Tone and Flash timing data could be generated separately by having different timing subsystems for the two stimuli that, nonetheless, combine at the final integration level.

Machine Learning for Combined Tone + Flash Trials

After training the Tone and Flash separately (**Figures 7A,B**), the model was then tested to determine whether it would predict the combined response to Tone and Flash inputs. Without altering any of the weights and without incorporating any saliency functions, the model predicted a shift in phase when the inputs were combined (**Figure 7C**). It also predicted a rapid rise in responding to the compound stimulus, close to that seen to Tone

(**Figure 7A**), as well as a slow decline in responding closely aligned to the Flash alone (**Figure 7B**). However, it did not accurately predict the shapes on these simulated compound trials. The peak was close to that of Tone (Compare **Figure 7C** with **Figure 7A**), and simulated responding to the compound stimulus overshot the empirical response function (**Figure 7C**). This discrepancy between model output and empirical data prompted us to consider a role that saliency might play in governing system performance. When we introduced the "saliency functions" for tone and flash components of the network described above, the empirical and simulated data fit with a much-reduced error (**Figure 7D**).

Because these parameters were non-linear functions, they could not be learned and the various saliency parameters of the effects of tone on flash and flash on tone were adjusted in a trial and error way. We did so in order to reduce the mean squared error between the empirical behavioral function on compound trials and the model's response function on compound trials once parameters for Tone and Flash were learned.

Thus, the best predictor of the fit to the average compound data occurred when Tone was saturated by the interference of Flash, causing a rise in response close to that of tone and a saturation and cutoff of Flash and Tone to produce the decline in the compound response function. Tone interfered with the Flash internal signal at a time when the Flash No-Go signal was sufficiently negative (i.e., had surpassed a certain threshold). Conversely, the internal summed Go and No-Go Tone signal was cutoff when the Tone No-Go unit was sufficiently negative and surpassed a different threshold. Because the Flash signal rose to the threshold slower than the Tone, there was a non-symmetric non-linear interaction that governed the dynamic responses. The principle of Saliency that we have established is that while Tone and Flash can effectively interfere with the other, they are governed by different saturation and cutoff functions. The saturation level is determined by the presence of the other signal and the cutoff is determined by the No-Go component of the same signal. Both the saturation and cutoff are present only when both signals are processed simultaneously; otherwise, these

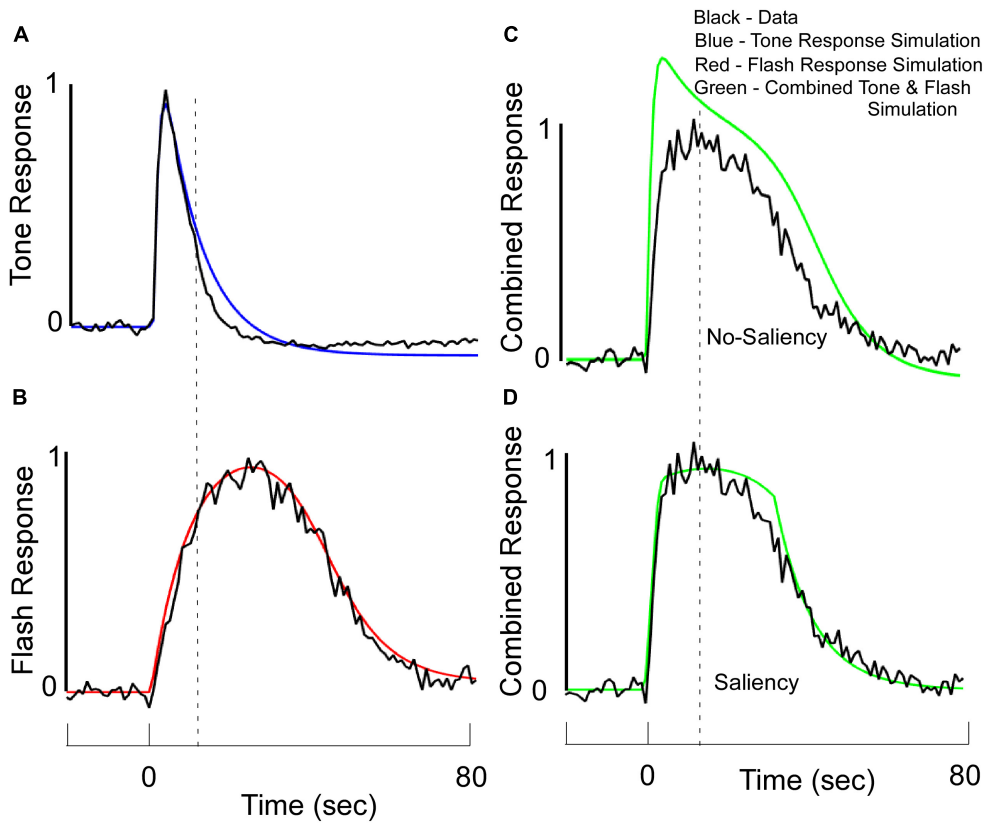

FIGURE 7 | Average Normalized Response Functions observed on Tone **(A)**, Flash **(B)**, and Combined Tone + Flash probe trials in Block 11 **(C,D)** overlayed with the RNN model's simulated data. Note, these simulated data reflect output values from the large fixed weight integrator. The model was trained to fit the empirical data for the Tone only and Flash only trials. **(C)** The fits to the data when there was no saliency and the Tone and Flash responses were linearly combined. **(D)** A stronger fit to the combined Tone + Flash data was obtained when we assumed that the simultaneous presentation of both stimuli diminished the "salience" of one stimulus on the other, but the learned weights in the network were not changed. These data support the idea that salience interactions among stimuli may be required to understand temporal averaging effects. See text for more details.

units adopt a simple linear activation function (as was used on trials with one or the other stimulus present).

DISCUSSION

This study showed that a simple RNN, one that has local feedback, could simulate rodent data from a peak interval timing task. In particular, we demonstrated that the model could be trained to produce the different response functions we obtained with rats performing in a peak timing task when one stimulus (Tone) indicated reward availability after 5 s and a second stimulus (Flash) after 30 s. Further, we showed that the model approximated the behavior of the animals when probed with a compound stimulus (Tone + Flash). Specifically, both the rats and the model responded to the stimulus compound by "averaging" the two independent temporal estimates to reward such that maximal responding occurred at an interval intermediate between the two signaled by the individual stimuli (Swanton et al., 2009; Swanton and Matell, 2011; Matell and Henning, 2013; Matell and Kurti, 2014; Delamater and Nicolas, 2015; De Corte and Matell, 2016a,b).

The RNN neural network architecture presented in this study is unique in that it models interval timing using a small number of neurons to encapsulate the dynamic properties of interval timing. A feature of the model is that it converts a sensory step input into an appropriately timed pulse. This could be related to the observation that single cell neural firings in the dorsal striatum display temporal firing properties that match interval timing behavior (Matell et al., 2003). Furthermore, our model suggests that there should exist neurons that code the separate Go and No-Go components of the timing signal. This is in contrast to other RNN models that seek to derive properties of scalar timing directly from large populations of interconnected neurons (Simen et al., 2011; Luzardo et al., 2017; Hardy and Buonomano, 2018). Despite its simplicity, our model has some common conceptual features with other models interval timing. For example, SET (Church et al., 1994), the multiple-time-scale memory model of timing (Staddon and Higa, 1999; Staddon, 2005), spectral timing (Grossberg and Schmajuk, 1989; Buhusi and Oprisan, 2013), behavioral theories of timing (Killeen and Fetterman, 1988; Machado, 1997) and diffusion drift theories (Simen et al., 2011; Luzardo et al., 2017) all assume that integration or summation over time is an important component of temporal encoding. SET assumes that there is a pacemaker clock whose pulses are counted (i.e., integrated) over time, with response decisions obeying particular decision rules (Gibbon and Church, 1990). In this way, SET can be thought of as a dynamical system, perhaps operating at a more computational, rather than a mechanistic, level of analysis. Church and Broadbent (1990) suggested one way to implement SET's key ideas within the framework of a feedforward connectionist network. Our approach differs from this and related models (Oprisan and Buhusi, 2011; Buhusi and Oprisan, 2013) by emphasizing the feedback components within a RNN. Multiple time scale, spectral timing, and behavioral theories of timing can be construed as assuming that the reinforced time is encoded as one component within a series of cascaded integrators, and that what is learned is the reinforced strengths, or values, of these different components. In particular, the component occurring maximally at the time of reward accrues maximal strength (also Pavlov, 1927). The RNN proposed in this study also utilizes the concept of an integrator, but we implement it as an individual node with a recurrent feedback loop whose weight determines its "time constant," i.e., its rate of growth and decay. In this way, maximal responding arises when the summation unit reaches its peak, but, importantly, this is accomplished in a system that neither requires a pacemaker-clock system nor a cascade of separate temporally discriminable states. A main distinction between our RNN approach and those of many other approaches is that temporal memory is encoded in the connection weights of the RNN and the recurrent loop weights.

The apparent simplicity of the RNN proposed here is noteworthy. Each input stimulus converges to two separate recurrent units that, themselves, converge on a single summation unit that, ultimately, feeds into a single response integrator unit. Thus, each stimulus is assumed to be part of a 4-unit dynamical "timing" system that feeds into a response output integrator unit (see **Figure 5**). One important discovery with this network was the observation that when training it to learn a particular response function (e.g., to Tone, or to Flash), the first two recurrent units always adopted opposing behavioral functions. We label one of these the "Go" function and the other the "No-Go" function. In essence, the RNN learns by developing a tendency to respond (Go) and a separate tendency to turn off the response (No-Go).

Through the combination of these two behavioral tendencies, the network learns to appropriately time its output. In an additional analysis we used the data from each block of training (**Figure 1**) to train the network and we inspected the status of the Go and No-Go units. We observed that the RNN rapidly acquired the tendency to respond through a strong activation of the Go unit, but only more slowly developed the opposing tendency to turn off the response through increasing suppression of the No-Go unit. In other words, just like the animals, the RNN rapidly learned to respond, but only with additional training learned to withhold responding at inappropriate times. SET, and other approaches, interprets the loss of responding at extended times in terms of an increasing dissimilarity between a representation of elapsed time to the remembered reinforced time. Here, we assume that it reflects the combined influences of opposing learned response tendencies that appear to be acquired at different rates. It remains to be determined whether including more than two recurrent units at this stage of the RNN would impact any of the model's predictions.

Another important issue regarding the RNN concerns how stochastic decision rules for responding can be incorporated. Our RNN model generates a deterministic output, which would have to be converted to a stochastic signal that could vary from trial to trial (or even within trial) (Church et al., 1994). This aspect of the model remains to be developed.

We earlier suggested that while many different types of models of interval timing can explain key aspects of interval timing data, all of those theoretical approaches generally have difficulty with the averaging phenomenon (Swanton et al., 2009; Swanton and Matell, 2011; Matell and Henning, 2013; Matell and Kurti, 2014;

Delamater and Nicolas, 2015; De Corte and Matell, 2016a,b). We replicated that empirical effect here, but went on to show how it might arise from the RNN framework. In particular, we showed that the response functions to the Tone + Flash compound produce a response timing function whose peak and variance was between those seen to Tone and Flash when given separately. In order to better fit these data, we assumed that the activation functions at the stage of inputs to the final response integrator unit needed to be modified by what we refer to as non-linear "saliency functions." In particular, we assumed that when the Tone and Flash stimuli were compounded, they mutually interfered with one another's processing (though not necessarily to the same degree). In this model, saliency is defined as the conditions that must be met when two (or more) stimulus are present for one stimulus to interfere with or block the transmission of the other. There were two aspects of the saliency operation that we considered. A primary saliency operation in this model is the saturating of the summed Go/No-Go signal when another signal is present. Thus, one signal such as Flash transmission can limit the transmission of the Tone response. Another saliency operation is the blockage of signal transmission at a certain point, which is represented by the switch. For Tone transmission, for example, it is in the 0 position for NO blockage and in the 1 position when there IS blockage. In this model, the switch is in the 1 position (blockage) when there is another signal (Flash) present AND the No-Go of the Tone response is negatively greater than some cutoff. This is the purpose of the AND gate and switch. Together, this type of saliency is capable of fitting the nuances of the compound data, once the weights for Tone and Flash have been learned separately. While this may not be a unique methodology for implementing saliency, it does suggest that saliency may play an important role in refining the response to compound stimuli once weights have been learned for responding to stimuli separately. It opens the possibility of more in depth studies of saliency and its role in implementing event timing.

The specific rules for determining salience disruptions, more generally, have not been elucidated. Other research has shown that mutual interference can occur when two stimuli are equivalently salient, but that asymmetrical disruptions occur when one stimulus is stronger (e.g., Mackintosh, 1976). Relatedly, Matell and Kurti (2014) showed that temporal averaging varied as a function of the differential reward probabilities and stimulus modalities of the early and late time signals. Our suggestion is that these effects reflect differential salience disruptions on compound trials when stimuli are differentially salient to begin with or when different reinforcement probabilities are used. This mutual disruption of signal processing could be an important consideration when interpreting temporal averaging studies.

A key aspect of any theory of interval timing should address its scalar timing property (Gibbon, 1977; Gibbon et al., 1984; Wearden et al., 1997) as it has long been recognized as a fundamental issue in timing (Gibbon, 1977). We utilized standard methods (taken from Matell and Kurti, 2014) to compute the peak times and widths of our average Tone, Flash and Compound data to determine whether there was scaling across these trial types. We defined the CV as the width of the behavioral function/it peak time for Tone, Flash, and Compound trials and observed that they were not constant and, therefore, not in accordance with scalar invariance. It is not clear why our data did not obey the scalar timing principle, but it may be related to the specific short and long intervals used (5 s, 30 s).

The RNN model proposed in this study is based on the contribution of combinations of integrators to timing performance (Staddon and Higa, 1999; Staddon, 2002, 2005). Our starting point was to ask, first, if different behavioral timing functions could be modeled using the RNN approach. We show that the network weights are trainable so that trained weights can simulate data from tone trials whose peak occurs at 5 s as well as from flash trials whose peak occurs at 30 s and fits our data almost exactly. For example, a constant tone or constant flash input can be trained individually to respond to a certain rise and fall time. In the RNN model, the constant tone input generated a fast rising Go response and a slower rising No-Go response in the negative direction at the first processing layer. When these two responses were summated, a pulse was generated. Because the time constants of these components were trained using the empirical data, the pulse contains information about time of reward (the peak value). When this pulse is processed through an activation function, it scales and shapes the pulse that can be input to a final integrator, with a long rise time, whose output can be used by higher "cognitive" centers to decide whether or not to respond by bar pressing. We also showed that this final integrator can then be used to integrate compound stimuli whose timing is different from its individual components. What is perhaps intriguing about this model's predictions is that we utilized the method of Matell and Kurti (2014) to compute the peak times, widths, and CVs for Tone, Flash and Compound data to determine whether there was scaling across these trial types. Although scalar invariance was not observed empirically, our model predicted these response functions with great fidelity. We suspect that different configurations of weights (within the networks "weight space") may, indeed, produce response outputs that conform to scalar invariance. This was not our focus here, but the present framework does present the possibility in future developments that the model could differentiate when and under what conditions scalar invariance may or may not be present.

More generally, the kinds of dynamic properties of systems that combine integrators are ubiquitous across various sensorimotor systems. The vestibulo-ocular and optokinetic reflexes are governed by combinations of feedback control mechanisms that have equivalent dynamical responses as the RNN proposed in this study (Raphan et al., 1977, 1979; Fanelli et al., 1990). The concept of an integrator is at the core of the model of the vestibulo-ocular reflex (VOR) (Raphan et al., 1979; Raphan and Cohen, 1996, 2002). For example, when the head is rotated with a step in velocity, eighth nerve afferents respond in a pulsatile manner as a second order system with a rising time constants of 0.003 s and an opposing falling time constant of 4–5 s (Fernandez and Goldberg, 1971; Goldberg and Fernandes, 1975; Wilson and Melvill-Jones, 1979; Raphan et al., 1996; Dai et al., 1999). This has been modeled with control systems using integrators with feedback similar to the RNN models proposed in this study (Raphan et al., 1996; Dai et al., 1999). The feedback mechanisms at this level comes from the viscosity of the endolymph fluid in the canal and the elasticity of the

cupula, in which is embedded the hair cells that drive the eighth nerve afferents that code the movement of the head (Wilson and Melvill-Jones, 1979). This model is similar to the summation of the Go and No-Go responses presented here.

An important contribution regarding VOR processing, is the presence of another integrator at the level of the vestibular nuclei in the brainstem that lengthens the time constant at the periphery to a longer time constant of about 12 s, seen in medial vestibular nuclei neurons (Waespe and Henn, 1977a,b, 1978) and in eye velocity responses (Raphan et al., 1979). This central integrator known as the velocity storage integrator is also accessed by the optokinetic system (Cohen et al., 1977), which then activates another integrator known as the velocity-position integrator (Skavenski and Robinson, 1973; Skavenski et al., 1981; Raphan and Cohen, 2002). The central feedback mechanisms are not local as they are in the mechanical feedback that occurs within semicircular canals. Rather, they appear to be more global and with projections across the commissure and back (Galiana et al., 1984; Wearne et al., 1997). These mechanisms were modeled using control theoretic concepts, which have the same architectural structure as the RNN proposed here, although there has been some work to model this integrator using neural nets (Anastasio, 1991). In addition, similar feedback control has been used to model locomotion reflexes (Raphan et al., 2001; Osaki et al., 2007, 2008) and an RNN with feedback has been used to model vestibulo-autonomic interactions (Raphan et al., 2016) to predict vaso-vagal responses as well as vaso-vagal syncope (Yakushin et al., 2016). This suggests that the central nervous system utilizes integrators to implement sensory motor transformations whose weights can be learned to adapt the behavior. It was of interest that the transformations that were utilized to model interval timing behavior were structured in the same manner as that for the VOR.

The RNN model proposed here has not addressed the important problem of identifying a trial-by-trial learning mechanism from the timing of the reward. Rather, we used the animals' asymptotic empirical data to identify a set of weights that result in appropriate network output on simulated Tone and Flash trials, and then we used this to predict network performance on stimulus compound trials. The reward timing is therefore embedded in these empirical responses, which are mapped to the RNN weights. The RNN developed separate Go and No-Go functional units, and we adopted particular saliency functions (based on stimulus cross-coupling and No-Go threshold mechanisms) in order to account for the temporal averaging effect. Therefore, we have provided a 'proof of concept' that the RNN framework can be usefully applied to model interval timing data and showed that temporal averaging effects may arise from that network. More specifically, we have shown that weights of the RNN can be found so that the model is capable of faithfully reflecting the empirical data arising when one stimulus signals a 5 s and another a 30 s reward time. But in order to show this we used a somewhat arbitrary learning algorithm (found in MatLab's toolbox) in conjunction with "teaching" signals provided by the animals' actual response functions. This approach shows that, in principle, the RNN is capable of producing a set of weights between nodes that could give rise to scalar timing and temporal averaging effects. But to show that the RNN could learn this in a more realistic way requires specification of the learning mechanisms whereby separate timing functions can be learned in a food-reinforced learning situation. One approach is that provided by "reinforcement learning" (Sutton and Barto, 2018), which could be applied to dynamical systems learning. However, we are not aware of its application to RNNs, and this would need to be developed further. Regardless of the details of how this might be accomplished, however, we have identified a new model structure that could be extremely important for our understanding of how the central nervous system encodes interval timing.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

All procedures used in the experiment with the rats were approved by the IACUC of Brooklyn College, and were in compliance with NIH guidelines as identified in the Guide for the Care and Use of Laboratory Animals, 8th Ed.

AUTHOR CONTRIBUTIONS

TR contributed to the overall conceptual framework for the study, and responsible for developing the model, and the organization and writing of the manuscript. ED contributed to the development of the implementation of the model in Matlab, writing the programs for comparison of model output with the data and identifying the weights to simulate the data, and writing of the manuscript. AD contributed to the experimental data, which was used as the database in the training of the model to determine the weights to simulate the data, writing of the manuscript, and conceptual development of the model.

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APPENDIX A

A recurrent neural net can be thought of as a feedforward network, in which the recurrent network is unfolded in time (**Figure A1**). If this network were to be trained over a 100 time steps, as is for the experiments modeled in this paper, we could unfold the network to create one 100 layers – one for each time step. The Back Propagation Through Time (BPTT) algorithm then represents a method for computing the gradients over these layers and this fixed network produces the same final result for the computed weights as the recurrent neural net with the same weights.

There are some problems with this type of learning, however. If a sigmoid transfer function is used, then if the output of the network is near the saturation point for any time point, the resulting gradient could be quite small and could impact the convergence. Another problem in training dynamic networks is the shape of the error surface. It has been shown that the error surfaces of recurrent networks can have spurious valleys that are not related to the dynamic system that is being approximated. The underlying cause of these valleys is the fact that recurrent networks have the potential for instabilities. However, it is possible, for a particular input sequence, that the network output can be small for a particular value greater than one in magnitude, or for certain combinations of values. Finally, it is sometimes difficult to get adequate training data for dynamic networks. This is because the inputs to some layers will come from tapped delay lines. This means that the elements of the input vector cannot be selected independently, since the time sequence from which they are sampled is generally correlated in time. Unlike static networks, in which the network response depends only on the input to the network at the current time, dynamic network responses depend on the history of the input sequence. The data used to train the network must be representative of all situations for which the network will be used, both in terms of the ranges for each input, but also in terms of the variation of the inputs over time.

Static multilayer networks can be used to approximate functions. Dynamic networks can be used to approximate dynamic systems. A function maps from one vector space (the domain) to another vector space (the range). A dynamic system maps from one set of time sequences (the input sequences) to another set of time sequences (the output sequences). For example, the network of **Figure A1** is a dynamic system. It maps from input sequences to output sequences. The BPTT algorithm starts from the last time point and works backward in time. In addition to the gradient, versions of BPTT can be used to compute Jacobian matrices, as are needed in the Levenberg–Marquardt algorithm. Once the gradients or Jacobians are computed, many standard optimization algorithms can be used to train the networks. The BPTT algorithm, however, usually requires more memory storage and for large networks, the memory requirements would be unmanageable. Despite the shortcomings of the BPTT algorithm, the recurrent neural network that we used has a small number of interconnected units and therefore, the BPTT algorithm using the Levenberg–Marquardt algorithm in the Matlab neural Net Package worked quite well in converging to weights that fit the data and gave insight into the internal workings of the timing generator.

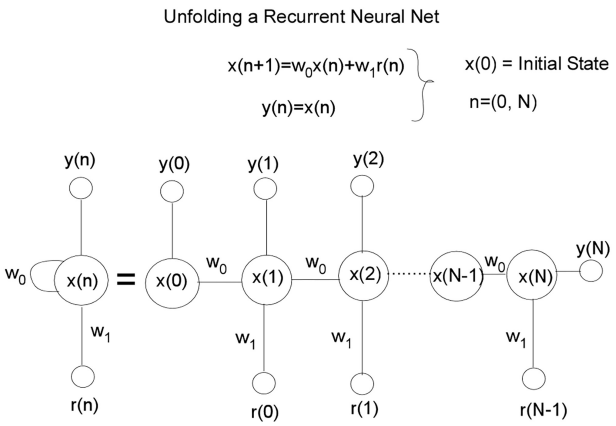

FIGURE A1 | The unfolding of a one dimensional recurrent neural net over time. The Back propagation through time (BPTT) algorithm can be used to identify the weights.

frontiers in Integrative Neuroscience

REVIEW
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Pre-emptive Intervention for Autism Spectrum Disorder: Theoretical Foundations and Clinical Translation

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Autism spectrum disorders (ASD) are an emergent public health problem, placing significant burden upon the individual, family and health system. ASD are polygenetic spectrum disorders of neural connectome development, in which one or more feedback loops amplify small genetic, structural, or functional variations in the very early development of motor and sensory-motor pathways. These perturbations trigger a 'butterfly effect' of unpredictable cascades of structural and functional imbalances in the global neuronal workspace, resulting in atypical behaviors, social communication, and cognition long-term. The first 100 days post-term are critically neuroplastic and comprise an injury-sensitive developmental window, characterized by a neural biomarker, the persistence of the cortical subplate, and a behavioral biomarker, the crying diathesis. By the time potential diagnostic signs are identified, from 6 months of age, ASD neuropathy is already entrenched. The International Society for Autism Research Special Interest Group has called for pre-emptive intervention, based upon rigorous theoretical frames, and real world translation and evaluation. This paper responds to that call. It synthesizes heterogenous evidence concerning ASD etiologies from both psychosocial and biological research literatures with complexity science and evolutionary biology, to propose a theoretical framework for pre-emptive intervention. This paper hypothesizes that environmental factors resulting from a mismatch between environment of evolutionary adaptedness and culture initiate or perpetuate early motor and sensory-motor lesions, triggering a butterfly effect of multi-directional cascades of atypical developmental in the complex adaptive system of the parent and ASD-susceptible infant. Chronic sympathetic nervous system/hypothalamic-pituitary-adrenal axis hyperarousal and disrupted parent-infant biobehavioral synchrony are the key biologic and behavioral mechanisms perpetuating these atypical developmental cascades. A clinical translation of this evidence is proposed, for application antenatally and in the first 6 months of life, as pre-emptive intervention for ASD.

Keywords: autism (ASD), pre-emptive, intervention, environmental factors, complex adaptive systems

Abbreviations: ASD, Autism spectrum disorder/s; FWB, First wave behaviorism/first wave behavioral; PNS, Parasympathetic nervous system; NDC, Neuroprotective Developmental Care; SNS, Sympathetic nervous system; SNS-HPA, Sympathetic nervous system and hypothalamic-pituitary-adrenal axis.

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BACKGROUND

What Is Autism?

Autism spectrum disorders (ASD) are a group of complex and heterogenous neurodevelopmental conditions, with life-long presentations in behavior, communication, cognition, and mental health. They are defined behaviorally, by early onset of persistent difficulties in social communication and interaction, sensory atypicalities, and repetitive, restricted interests and activities causing impairment in daily life. Due to the multiple and interdependently variable traits that pertain to autism, Autism-Related Disorders has been recently proposed as a more accurate clinical descriptor (Greenspan, 2018). ASD presents differently in males and females, in a ratio of 4:1. Traits of autism are continuously distributed in the general population and overlap with clinical phenotypes.

Autism spectrum disorders is diagnosed in one in 68, or 0.7%, of Australians. Prevalence has increased across all age groups between 2009 and 2015 in Australia, most markedly in the 5–14 years age group, consistent with international trends. This rapid increase is attributed to increased awareness and diagnosis. Although signs may emerge much earlier, most children are not diagnosed until at least 2 or 3 years of age, and the mean age of diagnosis in Australia is 4.5 years (Synergies Economic Consulting, 2011).

The total direct and indirect cost of ASD annually was estimated at $5.8 billion in Australia in 2010, with the high cost of treating ASD and its associated co-morbidities subject to intense media scrutiny during the initial roll out of the National Disability Insurance Scheme (Synergies Economic Consulting, 2011). The cost to the health system over the life-span for each individual diagnosed with ASD has been estimated at $2.4 million in the United States, or £1.5 million in the United Kingdom, higher than for asthma or diabetes (Buescher et al., 2014).

Etiology

Autism spectrum disorder are polygenic syndromes, associated with 102 genes in a 2018 genetic sequencing study, and also with *de novo* mutations (De Rubeis and Buxbaum, 2015; Satterstrom et al., 2018). However, any individual's complex genetic susceptibility is impacted by a myriad of environmental factors in intra-uterine and early life, which alter epigenomic regulation and phenotype expression (Tordjman et al., 2014; Keating, 2016; Mandy and Lai, 2016; Matas et al., 2016; Ismail et al., 2017; Barker et al., 2018).

Autism spectrum disorder is best conceptualized, applying a dynamic systems or complexity science framework, as a spectrum disorder of connectome development, that is, of the neural wiring of the brain, in which one or more feedback loops amplify small variations in very development. Initial lesions trigger a 'butterfly effect' of unpredictable cascades of structural and functional imbalances in the global neuronal workspace, which dynamically interact with and impact upon the infant's social and non-social environmental experiences, amplifying feedback loops and affecting behaviors, cognition, and social communication long-term (Fields and Glazebrook, 2017).

The initial lesions which cause feedback loop disruptions may be structural, either genomic or due to injury; or functional, for example, from changes in the monoaminergic system. Variable phenotypic expressions emerge out of the compensations of the child's neural networks in response to very early lesions, as myriad feedback loops in the complex adaptive system of the global neuronal workspace compensate for deficiencies and maintain the best possible functional stability. This may occur at the expense of, or with unusual development of and compensation by, higher order cognitive functions like memory, attention and executive functions. The heterogeneity of ASD reflects the multiple different disturbances that can occur along any one of multiple pathways. Dysregulation in any one neural or physiological pathway causes a cascade of events culminating in a cluster of symptoms (Fields and Glazebrook, 2017).

At the cellular level, feedback loop imbalances sculpt neuron morphology and synaptogenesis and alter synaptic transmissions by excitatory or inhibitory neurons. By the time a child is diagnosed with ASD, neuroanatomic patterns of excessive short-range connections and weakened long-range connections have emerged in vulnerable parts of the brain, including in parts of the prefrontal cortex associated with attention, social interaction, emotions, and executive control, and in a decreased density of axons below limbic cortices such as the anterior cingulate cortex (Zikopoulos and Barbas, 2010, 2013; Garcia-Cabezas et al., 2018; Zikopoulos et al., 2018; Trutzer et al., 2019).

Comorbidity of ASD with other medical disorders, including neurodevelopmental, psychiatric, and physical disorders, is common in both children and adults, further supporting the hypothesis that impaired neural and physiological developmental cascades occur in response to very early structural or functional neural lesions. Resultant neural and physiological morbidities then interact with co-occurring behavioral morbidities (Tye et al., 2019).

High-Risk Siblings

An infant who has an older sibling diagnosed with ASD is referred to in this paper as a high-risk sibling. An estimated 1 in 5 high-risk siblings will develop ASD (Ozonoff et al., 2011). High-risk siblings who do not develop ASD are nevertheless at risk of suboptimal developmental outcomes (Landa et al., 2012). High-risk siblings who develop ASD comprise only a small proportion of children diagnosed with ASD, and it is unclear to what extent findings from high-risk sibling studies can be generalized to ASD children from simplex families. When 244 two-year-olds diagnosed with ASD were compared, those with an older ASD sibling showed improved cognitive abilities relative to those from simplex families, perhaps because multiplex parents modify their parenting in response to their previous ASD experience (Dissanayake et al., 2019).

Autism Prodrome

Research in recent years has focused on identification of an autism prodrome, with the aim of developing effective screening

and early intervention. Signs that may be diagnostic of ASD, including repetitive behaviors, delayed motor and language skills, reduced gesturing, and impaired eye gaze patterns, emerge from 12 months of age (Bradshaw et al., 2015; Pierce et al., 2016; Petinou and Minaidou, 2017; Shen and Piven, 2017; Whitehouse, 2017; French and Kennedy, 2018; Green and Garg, 2018; Vivanti et al., 2018). From 6 months of age, high-risk siblings are more likely to show reduced attentiveness to parent, reduced affective signaling, poorer co-ordination of communication, and less use of gestures (Wan et al., 2018). These and other soft signs of neurodevelopmental vulnerability have been identified from 6 months of age in children who are later diagnosed with ASD, and may be evident even earlier (Bolton et al., 2012).

However, it's not possible to distinguish infants with behavioral signs of neurodevelopmental vulnerability who will be later diagnosed with ASD, from those with behavioral signs of neurodevelopmental vulnerability who will develop other neurodevelopmental disorders (Levit-Binnun and Golland, 2012). Because of the extreme heterogeneity of ASD, the non-specific nature of early signs of neurodevelopmental vulnerability, and the significant risks associated with early diagnosis, the search for clinically useful biomarkers of ASD in the first 6 months of life may not prove realistic (Goldani et al., 2014).

Pre-emptive and Early Intervention

This paper defines very early life as the first 100 days post-term, and early life as the first 12 months post-term, distinguishing these periods from intra-uterine life. Early intervention is defined here as an intervention for infants showing signs of neurodevelopmental vulnerability or ASD that is implemented between 6 to 18 months of age, and pre-emptive intervention as an intervention for infants less than 6 months of age, whether or not they are showing soft signs of neurodevelopmental vulnerability.

The International Society for Autism Research Calls for Rigorous Theoretical Framing and Real World Methodologies

In a 2019 discussion of gaps in ASD research, Bailey laments that:

> *. . . Genetic studies continue to search for risk variants of small effect in complex groups, biological research proceeds in its attempt to find unitary underlying commonalities in members of the ASD category, and early detection research continues to search in vain for markers with clinically useful specificities and sensitivities.*

In this context, Dawson calls for "new and paradigm-shifting interventions and research methodologies which embrace heterogeneity," and Charman highlights the need to "[transfer] new knowledge about risk factors, biomarkers, and behavioral signs into practical applications" for health professionals (Amaral et al., 2019).

In an era of big data, theoretical framing is key to ongoing knowledge development and cost-effective, sustainable innovation (Campbell et al., 2007; Fox et al., 2014; Ioannadis, 2014). Between 2015 and 2017, the International Society for Autism Research sponsored a Special Interest Group on *Implementing and Evaluating Community-based Early Intervention*. The Special Interest Group calls for:

- Carefully developed theoretical frames to guide early intervention development, evaluation, and implementation;
- An understanding of factors that make interventions more likely to be adopted and implemented in the real world;
- Rapid progression from treatment theory to community-based effectiveness trials, with community-partnered participatory research;
- An iterative approach consistent with the principles of real world research, with multidirectional knowledge exchange occurring between different phases of the research (Vivanti et al., 2018).

Green and Garg observe that large amounts of public and private money are currently being invested internationally in interventions for ASD which lack rigorous theoretical frames or an evidence base, and call for integration of psychosocial and biological intervention models, in order to eludicate potential synergies between promising psychological and biological intervention mechanisms in ASD (Green and Garg, 2018). Green asks that:

> *. . . prodromal intervention should not be tied to legacy theories of the past [but] be responsive to emerging results from basic science to get a more profound understanding of early developmental process and how best to intervene (Green, 2019).*

Here, I aim to respond to these calls, as a primary care medical practitioner who specializes both clinically and as a researcher in the care of parents with infants. This paper integrates existing evidence across multiple disciplines and etiological models, in order to explicate the theoretical frames which underlie a proposed interdisciplinary pre-emptive intervention for ASD, applied from the antenatal period and throughout the first 6 months of life.

The International Society for Autism Research Special Interest Group also calls for isolation and evaluation of 'active ingredients' of ASD interventions (Vivanti et al., 2018). But from a complexity science perspective, epigenomic regulation of heterogenous ASD genetic susceptibility is impacted by a myriad of interacting and co-evolving environmental variables (Tordjman et al., 2014; Keating, 2016; Matas et al., 2016; Mandy and Lai, 2016; Ismail et al., 2017; Barker et al., 2018). A reductive approach which aims to identify a small number of active ingredients is, applying complexity science, predicted to have limited real world application. This is because any ingredient or mix of specific selected ingredients (that is, simplified, linear interventions) risk unintended outcomes when applied to complex adaptive systems (Campbell et al., 2007; Braithwaite et al., 2018). This may be one reason why guidelines and interventions designed in university or tertiary settings often lack relevance in community settings (Steel et al., 2014).

A complexity or whole systems approach demands development of a comprehensive and integrated pre-emptive

intervention, translated from a rigorous theoretical frame, to address the many environmental factors which co-evolve together and interact with the epigenome, to protect against or mitigate expression of ASD genetic susceptibility. That is, holistic pre-emptive intervention is applied flexibly in order to stabilize as many of the myriad feedback loops within the complex adaptive system of each unique infant's global neuronal workspace and within the complex adaptive system of the infant's unique family as early as possible, in order to have the greatest chance of success (Marin, 2016; Green, 2019).

Leading primary care researchers have developed innovative methodologies suitable for the evaluation of complex interventions, since community-based healthcare is typically confronted by complex problems requiring early, complex, cost effective, preventive interventions (Plsek and Greenhalgh, 2001; Campbell et al., 2007; Greenhalgh et al., 2015; Braithwaite et al., 2018).

Why Pre-emptive Intervention?

To date, early intervention studies for ASD have been conducted after 6 months of age, either with high-risk siblings or with infants showing signs of neurodevelopmental vulnerability. They derive from applied behavior analysis and social communication models of ASD etiology, applying parent-mediated enrichment of the social interactive environment, and demonstrate preliminary positive outcomes on parent-infant interactions. In particular, evaluation of these early interventions suggest that undemanding synchronous parent behavior, which notices and responds to the child's focus without suggesting a new action, is associated with stronger joint attention and language skills throughout childhood (Steiner, 2013; Rogers et al., 2014; Baker et al., 2015; Baranek et al., 2015; Bradshaw et al., 2015; Green, 2017; Petinou and Minaidou, 2017; French and Kennedy, 2018; Green and Garg, 2018; Wan et al., 2018; Whitehouse et al., 2019).

However, functional neuroimaging of high-risk siblings who later develop ASD demonstrates altered cortico-cortical connectivity from 6 months of age (Lewis et al., 2017; French and Kennedy, 2018). Regional MRI volumes across the whole brain in 4–6 month old high-risk siblings show larger cerebellar and subcortical volumes than in low risk infants, linked to more repetitive behaviors at 36 months (Pote et al., 2019). These findings suggest that initial changes in the global neuronal workspace occur either prenatally or in very early life. By the time an infant is 6 months old, the effects of very early structural or functional lesions in the global neural workspace are already entrenched, and developmental cascades of multi-directional perturbations in motor, sensory, and social interactions are underway. That is, early interventions evaluated to date have been applied to an already fundamentally altered neural landscape.

Studies of pre-emptive intervention for ASD, as defined in this paper, have not yet appeared in the scientific literature. This paper integrates current understandings of neural network developmental dynamics to support the hypothesis that ASD course alteration is possible, beginning with antenatal anticipatory guidance, followed by intervention in the first months of life.

THE FIRST 100 DAYS POST-TERM: WINDOW OF CRITICALLY INJURY-SENSITIVE NEUROPLASTICITY

Neural and Behavioral Biomarkers

Neural network development activity peaks between the last three months *in utero* and the first three months post-birth, spanning from the beginning of cortical subplate diminution until the subplate disappears as permanent circuitries in the primary motor, somatosensory, and visual cortex take over. Until then, sensory-motor information continues to be relayed through subplate neurons, which sculpt permanent cortical templates. Cortical subplate neurons are known to be selectively sensitive to injury, and very early sensory-motor defects due to subplate neuronal injury have been implicated in neurodevelopmental disorders, including ASD (Kanold and Luhmann, 2010; Wess et al., 2017; Hadders-Algra, 2018a).

From 10 weeks post-conception, the brainstem and spinal cord central pattern generator initiates general movements. These disappear by three to 5 months post-birth, as fidgety movements and goal directed, affect-driven movements of the limbs emerge and the cortical subplate disappears. Hadders-Algra notes that the emergence, blooming, and eventual disappearance of general movements coincides temporally with the emergence, dominance, and disappearance of subplate synaptic activity. She hypothesizes that general movement complexity and variability are modulated by the cortical subplate and mediated by subplate motor efferents. In her model, abnormal general movements result from lesions in either the subplate or in subplate motor efferent connections within the periventricular white matter (De Graaf-Peters and Hadders-Algra, 2006; Hadders-Algra, 2007, 2017, 2018a).

Significantly, at the time of birth and in the middle of the period of peak neural network development, noradrenergic alpha 2 receptors and glutamatergic *N*-methyl-D-aspartate receptors are temporarily overexpressed, with high serotonergic innervation and dopaminergic turnover. Hadders-Algra hypothesizes that monoaminergic exuberance from birth until three months of age is associated with increased neural excitability, including of motoneurons. She speculates that monoaminergic exuberance offers evolutionary advantage, and is expressed in the writhing character of general movements. This over-expression resolves as the permanent cortical circuitries take over from the subplate (De Graaf-Peters and Hadders-Algra, 2006; Hadders-Algra, 2007, 2017, 2018a).

Behaviorally, persistence of the cortical subplate and increased monoaminergic excitability correspond post-birth with the infant crying diathesis. The crying diathesis can be viewed, then, as a behavioral biomarker, and the cortical subplate as a neural biomarker, of the critically injury-sensitive neuroplasticity of an infant's first 100 days of life.

Stress Response Settings

Neural templates for emotional and stress regulation are laid down in the cortical subplate and cortex, and in subcortical structures such as the basal ganglia and the amygdala, during the

developmentally critical first 100 days post-birth. These templates form the basis for secure psychological attachment and mental health life-long. As originally proposed by Greenspan in the Affect Diathesis Model, this paper hypothesizes that chronic sympathetic nervous system and hypothalamic-pituitary-adrenal axis (SNS-HPA) hyperarousal has monoaminergic impacts on the brainstem and cortical subplate during the critical window of monoaminergic excitability and injury-sensitive neuroplasticity, and is a key physiological mechanism disrupting capacity for attention on developmental tasks, mediating atypical developmental cascades (Schore, 2001; Greenspan, 2002; Lydon et al., 2016; Marin, 2016; Mandy and Lai, 2016; Ismail et al., 2017; Patel et al., 2018).

Although genetic susceptibility to long-term HPA axis dysregulation varies, painful perceptual experiences in very early life, including fear or chronic stress (experienced physiologically as chronic SNS-HPA hyperarousal), risk lifetime changes in SNS-HPA axis settings and dopaminergic, serotonergic, or noradrenergic circuitries. The HPA axis does not return to its 'unstressed' state, normal patterns of cortisol release fail to emerge, and behaviorally, infants show difficulty moving out of hyper-aroused or hypo-aroused states.

Stress also increases the permeability of the blood brain barrier, allowing penetration of pro-inflammatory cytokines. The immune dysregulation associated with chronic stress and increased allostatic load has widespread effect on physiological systems underlying developmental processes and mental and physical health, including on growth, metabolism, immunity, and cognition (Marin, 2016; Barker et al., 2018; Koss and Gunnar, 2018).

Executive functions and the development of sustained intention, including joint attention, are particularly sensitive to stress. In 12 months old neurotypical infants, those with high autonomic reactivity to stressors show short attention durations, and those with lower autonomic reactivity show longer attention durations (Wass et al., 2018). In older children diagnosed with ASD, stress triggers worsened signs and symptoms, impairing the capacity to achieve developmental tasks, which further upregulates the stress response, perpetuating multi-directional developmental cascades. This paper proposes that the same chronic SNS-HPA hyperarousal known to impact negatively on capacity to perform developmental tasks in the older child similarly impedes an infant's capacity to perform the vital motor and sensory-motor developmental tasks of very early life.

Chronic SNS-HPA Hyperarousal in Very Early Life Is Modifiable by Environmental Factors

The infant crying diathesis is characterized by high levels of cry initiation, which are stable across cultures. In high income countries, the crying diathesis is characterized by durations of cry that average about 2 h a day for the first 6 weeks, decreasing to 72 min a day at 12 weeks, and has resolved, for most, by about 16 weeks post-birth (Wolke et al., 2017). Twenty percent of families report excessive crying, of over 3 h a day, for at least 3 days a week, and many more report cry-fuss problems (Wake et al., 2006). But importantly, infant crying is modifiable according to socioculturally determined environmental factors. Cultures more likely to breastfeed, offer physical contact, and provide cued care have the same high frequency of cry initiation in this period, but at least halved cry durations (St James-Roberts et al., 2006; Wolke et al., 2017).

This paper proposes pre-emptive intervention for ASD which integrates the neurobiological model of cry-fuss problems (**Table 1**). In the complex adaptive system of parent and infant, problems of crying and fussing, feeds, and sleep often interact and co-evolve, and may be referred to as 'regulatory problems,' or 'dysregulation.' The neurobiological model of cry-fuss problems conceptualizes problem crying as a behavioral biomarker of chronic SNS-HPA hyperarousal, that is, of stress in very early life (Douglas et al., 2011; Douglas and Hill, 2013a). Crying and fussing, or chronic SNS-HPA hyperarousal, emerge when the parent-infant complex adaptive system is unable to stabilize multiple feedback-loop disruptions arising from interacting and co-evolving environmental factors (Douglas et al., 2011).

The neurobiological model of cry-fuss behaviors identifies three key environmental causes of chronic SNS-HPA hyperarousal in the first 100 days post-term:

(1) Suboptimal environmental stimulation
(2) First wave behavioral (FWB) approaches to infant sleep
(3) Unidentified and unmanaged feeding problems, either
    (a) Poor satiety
        (i) due to compromised milk transfer in breastfeeding, typically due to suboptimal fit and hold ('latch and positioning'), and resultant low supply
        (ii) due to feed-spacing in either breast or formula-fed infants
    (b) Functional lactose overload in breastfed infants
    (c) Conditioned SNS-HPA hyperarousal with feeds, most often due to positional instability at the breast, or coercive practices during either breast or bottle feeds (Douglas and Hill, 2013a).

In the neurobiological model of cry-fuss problems, these disruptive environmental factors interact unpredictably in the context of monoaminergic excitability in the first 100 days of life and predispose to:

(1) High levels of SNS-HPA arousal, which temporarily trigger more SNS-HPA arousal, in a positive feedback loop of inconsolable crying (correlating with monoaminergic excitability);
(2) Development of conditioned SNS-HPA hyperarousal in response to particular triggers e.g., conditioned SNS-HPA hyperarousal with breastfeeding or bottle-feeding;
(3) Temporary re-setting of the baby's 'stress thermostat' in response to chronically high levels of SNS-HPA arousal (also known as 'difficulty shifting arousal states'), which resolves at the end of the crying period;
(4) Permanent re-calibration of the 'stress thermostat' in biologically susceptible infants (that is, permanent alteration of neural connectivity), due to:
    (a) Alteration of the infant's stress response settings, and/or
    (b) Cascade effects of altered parent-infant interactions;

TABLE 1 | Current explanatory models for cry-fuss problems.

Theoretical model	Key management strategies	Summary of evidence
Medical condition		Not supported by evidence (Douglas, 2013; Bergmann et al., 2014; Gieruszczak-Bialek et al., 2015; O'Shea et al., 2017; Gordon et al., 2018)
(1) 'Reflux' or GORD	(1) Anti-secretory medications	
(2) Allergy	(2) Maternal elimination diet	
(3) Tongue-tie or upper lip-tie (in absence of classic tongue-tie)	(3) Frenotomy	
(4) Lactose intolerance	(4) Lactose-free formula	
Normal developmental phase (Zeifman and St James-Roberts, 2017)	Support carer coping. Reassure crying will pass Entrain infant biology with first wave behavioral (FWB) strategies.	Ignores evidence that crying durations are modifiable by infant care practices (Wolke et al., 2017). High level evidence shows that FWB strategies do not decrease night waking (Bryanton et al., 2013; Douglas and Hill, 2013b; Kempler et al., 2016; NHMRC, 2017)
'A mysterious disorder of the microbiota-gut-brain axis' (Partty and Kalliomaki, 2017; Rhoads et al., 2018; Zeevenhooven et al., 2018)	Probiotics	Probiotics may decrease crying in breastfed infants (placebo response 66%) but studies do not control for the breastfeeding problem of functional lactose overload and do not take into account complex bidirectional nature of gut-brain axis (multiple confounders). Gut dysbiosis is a confounder, not a cause (Fatheree et al., 2017; Sung et al., 2017).
Neurobiological (Douglas and Hill, 2013a)	Neuroprotective Developmental Care, which Integrates lactation and sleep science, neuroscience, brain- gut-microbiota science, evolutionary biology, applied functional contextualism.	Preliminary studies positive (Douglas et al., 2013; Ball et al., 2018) Requires further research.

(5) Maternal chronic SNS-HPA hyperarousal in response to infant crying, feed, and sleep problems, which increases the risk of:

   (a) Postnatal anxiety and/or depression
   (b) Avoidance behaviors (Douglas and Hill, 2013a).

Chronic SNS-HPA Hyperarousal in Very Early Life Permanently Alters Stress Response Settings in Susceptible Infants

Multiple studies link infant regulatory problems in the domains of feed, cry-fuss behaviors and sleep with impaired developmental and behavioral outcomes in later childhood. This association is strongest if there is more than one regulatory problem, or if the regulatory problem persists beyond 4–5 months of age (DeGangi et al., 2000; Desantis et al., 2004; Von Kries et al., 2006; Schmid and Wolke, 2014; Winsper and Wolke, 2014; Santos et al., 2015; Laetitia et al., 2016; Sidor et al., 2017; Williams et al., 2017; Bilgin et al., 2018; Breeman et al., 2018; Cook et al., 2019). Regulatory problems in infancy have also been linked with avoidant adult personality traits (Bauml et al., 2018). Multiple persistent regulatory problems are associated with attention problems in later childhood, and trajectories of attention problems from childhood to adulthood (Bilgin et al., 2018). A comparative study which claims to show no link between regulatory problems in the first 3 months of life and behavior problems at 2–3 years of age retrospectively excludes infants whose crying was found to persist at 6 months of age (Bell et al., 2018). Yet it is not possible to predict which infants with cry-fuss problems in very early life will have persistent crying at 5 or 6 months of age, and intervention should be offered promptly to all parents reporting problem crying.

More 'cued care' at term predicts fewer regulatory problems at three months, but after the first highly neuroplastic 100 days, patterns of chronic SNS-HPA hyperarousal may become entrenched in susceptible infants, since cued care beyond this age does not result in less regulatory problems. Biological vulnerabilities such as preterm birth provide more significant prediction of long-term regulatory problems (Bilgin and Wolke, 2017). But upregulated infant behavior, or crying and fussing, also predisposes to negative affect and mood problems in parents (Vik et al., 2009; Cook et al., 2017), which disrupt the parent's capacity to engage and lengthen reciprocity chains. This bi-directional disruption to parent-infant biobehavioral synchrony adds to allostatic load in infants.

These findings corroborate the neurobiological model of infant crying, which argues that while most families are resilient, and suffer no long-term effects of excessive infant crying, cry-fuss problems are a behavioral biomarker of infant stress and disrupted parent–infant biobehavioral synchrony due to modifiable environmental factors, and affect the infant's capacity to attend to developmental tasks. In a small but important subset of genetically susceptible infants or psychosocially vulnerable families, cry-fuss problems are then associated with long-term SNS-HPA axis disruption and developmental impairment (DeGangi et al., 2000; Desantis et al., 2004; Von Kries et al., 2006; Schmid and Wolke, 2014; Winsper and Wolke, 2014; Santos et al., 2015; Laetitia et al., 2016; Sidor et al., 2017; Williams et al., 2017; Bilgin et al., 2018; Breeman et al., 2018; Cook et al., 2019).

Primacy of Motor Lesions in Development of ASD

Motor, sensory, cognitive, and social communication development are not independent phenomena occurring in sequential order or in discrete neuroanatomic locations, but are complex functions which dynamically co-evolve within the global neuronal workspace of the complex adaptive systems of

the developing fetus and infant (Whyatt and Craig, 2013; Estes et al., 2015; Fields and Glazebrook, 2017).

Up to 60% of children with ASD have motor deficits, including impaired postural control, impaired motor planning and sequencing, which affects gesture planning and imitation, and low motor tone (Paquet et al., 2016; Paquet et al., 2019). Prospective or feedforward mechanisms of motor timing, also referred to as sensory-motor intentionality, are typically disrupted in ASD, as is perceptual awareness of others' intentions conveyed in body movement or eye gaze. Torres et al. argue that motor disruption is a core feature of ASD, requiring assessment methods suitable for inclusion in clinical diagnostic criteria (Torres et al., 2013).

This paper draws on the hypotheses concerning the primacy of either *in utero*, intra-partum, or very early life motor lesions in ASD etiology, as detailed in ground-breaking syntheses by Torres et al. (2013), Trevarthen and Delafield-Butt (2013), Delafield-Butt and Trevarthan (2017), Dadalko and Travers (2018), and Delafield-Butt et al. (2018). These researchers propose that a structural (genetic or injury) or functional (monoaminergic) motor lesion is sustained in the brainstem systems or cortical subplate in the critically neuroplastic window *in utero*, intra-partum, or in very early life, which impairs prospective, affect-driven movement. Cascades of imbalance between local and global connectivity emerge, resulting in atypical neurological, psychological, and behavioral development. Chronic SNS-HPS hyperarousal and disrupted parent-infant biobehavioral synchrony are key physiological and behavioral mechanisms which either predispose to very early motor lesions, or perpetuate the effects of very early motor lesions.

Infants require complex and unpredictable postural variability from birth in order to optimize postural control strategies; they also require rich and active movement experiences in order to learn to perceive visual and tactile stimuli (Hadders-Algra, 2010; Dusing, 2016; Hadders-Algra, 2017; Hadders-Algra, 2018b). Reduced movement complexity and variability, that is, reduced movement repertoire, associated with decreased affect-driven prospective movement from birth, impairs sensory feedback and parent response, limiting capacity to process sensory information (Delafield-Butt et al., 2018). In the phase of secondary variability of general movements, these infants have further difficulty selecting an appropriately adapted strategy from of their repertoire, due to limited variability (Hadders-Algra, 2010; Dadalko and Travers, 2018; Delafield-Butt et al., 2018; Hadders-Algra, 2018b). Shafer et al. (2017) propose that motor stereotypy is a downstream manifestation of low motor complexity. The reduced exploratory motor drive evident in many ASD children is hypothesized to begin with these deficits in primary and secondary variability of movement, which cascade to motor coordination difficulties and impaired complex motor sequencing (Denisova and Zhao, 2017) (**Box 1**).

The social communication developmental pathway depends throughout early life on motor competency and capacity for motor synchrony with another, including mutual gaze, joint attention and shared positive affect, attention disengagement, gesture, touch, and language learning (Yu and Smith, 2017). For example, from birth multiple sequential motor and sensory-motor patterns lead to joint attention. By three months of age, social gaze is the primary modality of coordinated interactions, occurring 30–50% of the time in low risk infants. But attention to eyes declines between 2 and 6 months in infants who later develop ASD (Jones and Klin, 2013), and high-risk siblings perform lower on visual attention tasks at 2 and 3 months of age compared to low-risk infants (Bradshaw et al., 2019).

Eye contact, joint attention, and touch synchrony are driven from birth by the motor-emotional system for the enjoyment of shared experience (Greenspan, 2002; Trevarthen and Delafield-Butt, 2013). Early motor and coordination deficits result in atypical control of eye movements, delays in development of gestures such as pointing, and associated impairment of joint attention (Landa et al., 2013). In neurotypical infants, touch synchrony, the coordination of affectionate touch with episodes of shared gaze, increases significantly from 3 to 9 months with the development of fine-motor skills. In this time, episodes of shared gaze decrease to about a third of the time, while shared attention to objects increases dramatically. This emphasizes the dynamic relationship between downstream development in the motor domain, which allows infants to crawl, grasp, and manipulate objects, and development of social competencies. 'Sticky' attention to objects is one of the earliest biomarkers of ASD, evident from 7 to 14 months. Difficulty disengaging attention interferes with social orienting and impairs social communication skills.

Speech comprehension relies on multi-sensory integration, predominantly auditory, enhanced by concomitant visual information; speech capacity relies on motor competence (McCleery et al., 2013; Akhtar et al., 2016; Stevenson et al., 2018).

The relationship between motor development in infancy and later cognition correlates neurally with the involvement of extensive cortico-subcortical networks and structures such as the dorsolateral prefrontal cortex and the neocerebellum in both motor and cognitive functions (Heineman et al., 2018).

Up to 87% of individuals with ASD demonstrate atypical sensory processing, often categorized as hyper-sensitive, hypo-sensitive, or sensory-seeking behaviors. From 6 months of age, signs of sensory processing abnormality are predictive of social communication deficits and repetitive behaviors in childhood, and ASD diagnosis (Robertson and Baron-Cohen, 2017).

Synchronous multisensory experiences, including proprioceptive, haptic, visual, and auditory, enhance neural connectivity and sensory-motor processing (Werchan et al., 2018). This paper hypothesizes that impoverishment of environmental stimulation, both social and non-social, chronic SNS-HPA hyperarousal, and disrupted parent-infant biobehavioral synchrony, result in trajectories of compensatory sensory hyper- or hypo-sensitivity and sensory seeking behaviors. In the same way that short-sightedness is a neuronal consequence of prolonged exposure to interior environments with suboptimal opportunities for long-distance focus, this paper proposes that the three variations of sensory processing difficulty are compensations within the global neuronal workspace for suboptimal motor and sensory-motor enrichment in very early life. Compensatory trajectories vary according to the

BOX 1 | Selected studies corroborating the hypothesis that very early life motor lesions initiate the atypical developmental trajectories of ASD. See Torres et al. (2013), Trevarthen and Delafield-Butt (2013), Delafield-Butt and Trevarthan (2017), Dadalko and Travers (2018), Delafield-Butt et al. (2018) for comprehensive reviews. A retrospective 2008 study of videos of 20 children diagnosed with ASD demonstrated impaired complexity and variability of general movements and absent or abnormal fidgety movements in infancy (Phagava et al., 2008). A 2013 prospective, longitudinal study of 235 low and high-risk infants aged 6–36 months demonstrated developmental differences from 6 months of age, and poorer fine motor skills were evident in the high-risk infants by 14 months of age (Landa et al., 2013; Bradshaw et al., 2018). In a 2014 study of 158 high-risk siblings and low risk infants, repetitive and stereotyped motor behavior was observed as early as 12 months of age in high-risk siblings who are were diagnosed with ASD at 24 months of age (Elison et al., 2014). A 2017 comparative study of 71 low and high-risk infants showed that high-risk siblings who later develop learning delays have less variety of general movements in the first 8 weeks post-birth, and less variety of movements in response to language, suggesting less flexible sensory-motor systems (Denisova and Zhao, 2017). A 2018 study comparing 86 high-risk siblings with 113 low risk infants showed that high-risk siblings who walked independently at 12 months of age had superior social-communication skills relative to high-risk siblings who weren't walking. A 2019 prospective study of 437 high-risk siblings and 188 low risk infants were assessed at 6 and 36 months. High-risk siblings have poorer fine motor skills and delayed motor development at 6 months, though these findings are not predictive of later diagnosis of ASD (Iverson et al., 2019).

environmental experience, predispositions, and feedback loops activated within that individual infant.

PARENT–INFANT BIOBEHAVIORAL SYNCHRONY OPTIMIZES RECIPROCITY CHAINS AND PROTECTS STRESS RESPONSE SETTINGS

Biobehavioral Synchrony Integrates Psychosocial and Biological Models of Parent–Infant Interaction

Feldman's theory of parent-infant biobehavioral synchrony integrates psychosocial and biological models of parent-infant co-regulation, in order to describe interactions which optimize secure psychological attachment and other long-term developmental outcomes (Feldman, 2007, 2016). This paper uses the term biobehavioral synchrony to refer to reciprocal motor and multi-sensory parent–infant exchanges, integrating behavioral observation and parent-reported psychosocial experience with neural and physiological correlates. Biobehavioral synchrony significantly expands upon the concept of synchrony used in social communication models of ASD etiology (Green et al., 2015). Biobehavioral synchrony stabilizes the myriad physiological, neural, and behavioral feedback loops that operate within the complex adaptive system of parent and infant.

To give one example, biobehavioral synchrony between a parent and newborn occurs during contact with a carer's body and skin. This early proximity, known as skin-to-skin contact, comprises rich and complex sensory-motor stimulation for the infant, and supports both positive parent affect and early infant experience of secure psychological attachment. Skin-to-skin contact improves glucose and oxygen levels, downregulates the infant's autonomic nervous system, including heart and respiratory rates, and improves breastfeeding outcomes due to sensory-motor activation of mammalian reflexes (Moore et al., 2016).

This paper offers an interdisciplinary synthesis of heterogenous studies investigating infant dysregulation, stress, and parent-infant biobehavioral synchrony. Infant dysregulation may be caused by, and may cause, disrupted parent–infant biobehavioral synchrony. Both parent and infant stress may disrupt parent–infant biobehavioral synchrony, and each may also result from disrupted parent–infant biobehavioral synchrony. In this way, disruptions to parent–infant biobehavioral synchrony in very early life may trigger a butterfly effect of multi-directional cascades of impaired cognitive, social, emotional and self-regulatory skills in ASD susceptible children.

Cued Care

In very early life, an infant signals or cues his or her biological needs, e.g., for milk, or for richer sensory-motor experience, through affect-driven motor behaviors and autonomic cues. Motor behaviors include spinal extension or writhing spinal movements, postural changes, grimaces, and non-speech-like vocalizations, including groans and cries. Autonomic cues include reddened face, increased respiratory rate, and tremors. An infant increases intensity of signaling along a spectrum of SNS-HPA arousal, from mild agitation to screaming.

'Cued care' has also been known as 'responsive care' or 'attunement,' and refers to parenting behaviors which respond to infant cues. Cued care is the foundational behavioral mechanism underlying parent-infant biobehavioral synchrony (Shonkoff and Phillips, 2000; Mansfield and Cordova, 2007; Fogel et al., 2008; Swain et al., 2013; Matas et al., 2016). The care-giver aims to downregulate the infant's SNS-HPA axis and meet the infant's needs for safety, nutrition, and loving human-mediated sensory-motor nourishment, by providing eye-contact, touch, motor interaction, and sounds, including speech and song, and also by providing non-human sensory-motor nourishment, such as opportunities for experience of the complex non-human natural environment. Milk and sensory-motor enrichment upregulate the parasympathetic nervous system (PNS), which downregulates the SNS. Downregulation of the SNS-HPA axis facilitates learning and joint attention, as well as cognitive and social processes (Greenspan, 2002).

Extinction of cues may occur when the infant does not receive adequate responses to his or her communications, a process elucidated in the operant conditioning principles of the first wave of the school of behaviorism (FWB), which has been widely applied in infant-care from the mid-twentieth century. The rate at which infant cues are extinguished may also depend on an infant's underlying genetic or biological susceptibility.

Behaviorally hypo-aroused infants offer minimal cues and are at particular risk of cue extinction. That is, they may

quickly respond to SNS-HPA hyperarousal with 'learned helplessness.' Minimal cues result in less parental engagement, shortened reciprocity chains, and less opportunities for joint attention, reinforcing the extinction of cues. For these behaviorally hypo-aroused infants, cued care from birth protects against cascades of disrupted development (Greenspan, 2002).

Reciprocity Chains

Reciprocity chains refer to back-and-forth exchanges which incorporate infant responses to parent-initiated communication as well as parental responses to infant-initiated cues. Behavioral reciprocity chains build on cued care, resulting in parent–infant biobehavioral synchrony. Cues from the infant, including affect-driven movements toward the parent, and sensory-motor and speech responses from the parent, including co-ordination of gaze, downregulate and organize the infant's autonomic, motor, and attentive states. Reciprocity chains also facilitate lactation and downregulate parental stress responses, and are driven by the powerful evolutionary drive for mutual enjoyment.

Between the age of 2–3 months, specific combinations of interactive behaviors become more frequent and turn into constellations of behaviors. From a complexity perspective, these are 'attractor states,' recurring patterns that shape neural networks. In the second half of the first year of life, sensory-motor reciprocity chains are increasingly organized, symbolic, and complex, evolving into protophones and verbal communications, although the capacity to perceive and respond to complex non-verbal sensory-motor communication remains foundationally important for social communication throughout life.

Parent–infant biobehavioral synchrony, or enjoyment of increasingly long and complex sensory-motor reciprocity chains, is necessary from birth in order to protect cognitive, language, and social communication development (Britto et al., 2017). This paper proposes that very early life motor or sensory-motor lesions disrupt biobehavioral synchrony, either resulting in, caused by, or perpetuated by chronic SNS-HPA hyperarousal. The resultant shortened, less complex reciprocity chains trigger cascades of atypical sensory processing, motor skills, language, and cognition typical of ASD.

For example, at 4 months of age, the optimal interactive structure comprises a predominance of 'infant-leads-parent-follows,' but this is less likely to be found with high-risk siblings, even though maternal responsivity, scaffolding, and linguistic input are the same. By 6 months of age, high-risk siblings demonstrate attenuated social attention and fewer speech-like vocalizations. Although some parents may respond in the second half of the first year of life to minimal infant cuing with disengagement and decreased gaze, many parents of high-risk siblings become directive, in an effort to impose attentional shift and scaffold communicative behaviors. Unfortunately, either pattern may perpetuate a cascade of impaired social development in susceptible infants. 'Undemanding synchrony,' a pattern of noticing and responding to the infant's cues without attempting to control, is the optimal parent response (Green et al., 2017).

Chronic SNS-HPA Hyperarousal in Very Early Life Triggers Atypical Developmental Trajectories in ASD Susceptible Infants

The Avon Longitudinal Study of Parents and Children, a prospective longitudinal cohort study of the offspring of 14,541 pregnant women, demonstrates that high levels of irritable or unsettled infant behavior in very early life predicts autistic traits at 6 months and 2 years of age (Bolton et al., 2012). This association is corroborated by other longitudinal and retrospective studies (**Box 2**).

Motor development, as this paper argues, is primary for healthy development of sensory, social, and cognitive skills, and is facilitated by rich sensory-motor experience, including irregular postural variation. However, the benefits of rich sensory-motor experience occur when the newborn is in a quiet, alert state and able to integrate sensory feedback from primary and secondary generalized movements and other environmental experience (Hadders-Algra, 2018b). This paper hypothesizes that chronic SNS-HPA hyperarousal in very early life, most commonly associated with unsettled infant behavior, but sometimes with hypoaroused behaviors, as discussed below, disrupts capacity to maintain necessary attention on sensory feedback to achieve developmentally appropriate motor and sensory-motor learning tasks, including auditory and visual tasks, joint attention, and motor planning and sequencing (Bilgin et al., 2018; Breeman et al., 2018). These disruptions further perpetuate chronic SNS-HPA axis hyperarousal and add to allostatic load as the child continues to struggle with ongoing developmental tasks, until ASD is diagnosed.

The hypothesis that chronic SNS-HPA hyperarousal disrupts the capacity to attend and develop motor skills is corroborated by the Danish National Cohort study. Although this cohort showed no link between transient 'infantile colic' and developmental coordination disorder in later childhood, infants with persistent crying were at increased risk of developmental coordination disorder (Milidou et al., 2015). A prospective study of 37 infants with colic found transient developmental lags in both fine and gross motor skills at 6 months. They were less responsive and less able to maintain optimal functioning for months after resolution of the crying, though these subtle impacts resolved by 12–18 months of age (Sloman et al., 1990).

Corroborating the hypothesis that chronic SNS-HPA hyperarousal plays a key role in perpetuating disrupted feedback loops in ASD etiology, 4-month-old infants later diagnosed with ASD show altered respiratory sinus arrhythmia response to a social stressor compared to non-autistic infants. Similarly, toddlers later diagnosed with ASD have less variable respiratory sinus arrhythmia at 18 months of age (Sheinkopf et al., 2019). ASD adolescents report high levels of anger-focused rumination (Patel et al., 2018).

Hypoaroused Behavior

Although many children diagnosed with ASD are retrospectively reported to have had regulatory problems in the domains of

BOX 2 | Studies demonstrating links between unsettled behavior in very early life and ASD.
In a 2013 audit of 208 preschool children with ASD, 50% of whom had intellectual disability, child health records demonstrated significantly more consultations for crying, feeding or sleeping problems regulatory problems in the first 18 months of life for children who were later diagnosed with ASD (Barnevik et al., 2013).
A 2017 retrospective study investigating 80 monozygotic twin pairs and 46 dizoygotic twin pairs with autistic traits and ASD found that early medical events, such as low birth weight or prenatal valproate exposure, and poor sleep, feeding problems, frequent vomiting, and crying in the first year of life, were associated with an increased risk of autistic traits and ASD. Cumulative load of risk factors correlated with severity of ASD. The authors concluded that non-shared environmental stressors, or allostatic load, resulted in epigenomic expression of autistic traits and ASD in individuals with genetic vulnerability (Willfors et al., 2017).
A 2018 analysis of the Danish National Birth Cohort showed that parents of the 973 children later diagnosed with ASD reported the same rates of periods of crying for over half an hour in the first 6 months of life as non-ASD children. However, when infants who had excessive crying in both groups were compared, those later diagnosed with ASD had cried longer per day and more days per week in the first 6 months than their excessively crying controls. Mothers who reported difficulty caring for their infant when interviewed at 6 months and 18 months post-birth were more likely to have a child later diagnosed with ASD (Lemcke et al., 2018).
In a 2018 retrospective comparative study of 200 children, parent-reported persistent infant crying, and excessive crying with long duration, that is, higher allostatic load, was associated with later diagnosis of ASD (Bag et al., 2018).
In a 2019 longitudinal sample of 282 high-risk siblings compared with 114 low risk infants, assessed at 6, 12, and 24 months of age, 6-month-old high-risk siblings later diagnosed with ASD exhibited poorer parent-reported regulatory capacity and less surgency compared to high-risk siblings and low risk infants not later diagnosed with ASD. Approach behaviors such as smiling and vocal reactivity were less. At 12 months, these temperament differences persisted, with high-risk siblings who were later diagnosed with ASD demonstrating decreased surgency or approach, impaired regulatory capacity, and increased negativity compared with typically developing infants (Paterson et al., 2019).

feeds, sleep and cry-fuss problems in very early life, other children diagnosed with ASD are retrospectively characterized as 'easy babies,' whose behavioral regulation difficulties emerged in toddlerhood or later.

The Affect Diathesis model proposes that these behaviorally hypoaroused ASD-susceptible infants also experience chronic SNS-HPA hyperarousal, which is subjected to PNS override (Greenspan, 2002). In behaviorally hypoaroused babies, affect-driven sensory-motor cues are partially extinguished, either due to biological propensity or early 'learned helplessness' or both, resulting in less parental engagement and shortened reciprocity chains. These infants may be particularly vulnerable to the developmental impacts of suboptimal environmental stimulation and disrupted parent–infant biobehavioral synchrony (Middlemiss et al., 2012).

Atypical Crying Acoustics

One month old infants later diagnosed with ASD are reported by parents to have louder, more distressed, less typical cries, as if in pain (English et al., 2018). At 6 months of age, parents report that high-risk siblings produce cries with higher pitch and greater variability than low risk infants, as if in pain. High-risk siblings later diagnosed with ASD produced the widest frequency range and most poorly phonated cries. Parents report difficulty understanding the causes of crying episodes in the early life of infants later diagnosed with ASD (Esposito et al., 2017). Paradoxically, by 12 months of age, high-risk infants and infants later diagnosed with ASD have shorter cry durations, with higher frequency and decreased number of pauses compared to low-risk or neurotypical toddlers (Unwin et al., 2017). This paper proposes that environmental factors, that is, one or more problems of feeds, sleep and unmet needs for richer sensory-motor and social stimulation, may induce cries at the extreme end of the spectrum of distress in very early life, triggering aversive or avoidant responses in the care-giver, and finally, extinction of cries, associated with cascades of atypical development (Bag et al., 2018). In very early life, infant crying is commonly inappropriately attributed to gut pain (Bergmann et al., 2014; Gieruszczak-Bialek et al., 2015; Gordon et al., 2018).

Suboptimal Sensory-Motor Stimulation

The neurobiological model of infant cry-fuss problems argues that infants in a low sensory environment cry because of unmet needs for sensory-motor enrichment, in a biological bid to optimize development of neural circuitries during a window of critically sensitive neuroplasticity and monoaminergic excitability (Douglas and Hill, 2013a). The human infant's biological requirement for rich postural variability and rich and complex sensory-motor reciprocity chains for normal motor development has been discussed previously. Interestingly, a study of infant macaques found that rich social environments in the first week of the macaques' lives improved gaze-following and social skills at 7 months of age (Simpson et al., 2016).

Regulatory difficulties in very early life are typically interpreted, through a linear causative theoretical frame, as an early sign of sensory processing deficit in infants who later develop ASD. By 12 months, when neural changes are entrenched, sensory processing and regulatory reactivity are known to predict parent-reported executive function deficits in children later diagnosed with ASD (Robertson and Baron-Cohen, 2017; Stephens et al., 2018). But this paper applies the neurobiological model of cry-fuss problems to argue that infant dysregulation in very early life is less likely to be a sign of pre-existing sensory processing problems, and more likely to be a behavioral biomarker of chronic SNS-HPA hyperarousal resulting from environmental factors, including suboptimal sensory-motor stimulation, which predispose to sensory processing problems.

Feeding Dysregulation

Children diagnosed with ASD are 5 times more likely to have a feeding problem than neurotypical children (Peverill et al., 2019). In very early life, 'feeding problems' refer to fussing at the breast or bottle, and breast or bottle refusal. Infant feeding problems often result in a cascade of worsening parental anxiety, controlling parental feeding practices, and conditioned infant withdrawal or distress (Estrem et al., 2016). The latter is referred to in the neurobiological model of cry-fuss problems as 'conditioned SNS-HPA hyperarousal' with feeds, and may result in temporary re-setting of the HPA axis in the critically

sensitive neural networks of very early life (Douglas and Hill, 2013a). Feeding-related signs such as refluxing or back-arching and fussing with the breast or bottle are often inappropriately medicalized, and underlying causes, such as positional instability with breastfeeds or conditioned SNS-HPA hyperarousal with breast or bottle feeds, remain untreated (Douglas, 2013). Feeding problems may escalate into a developmental trajectory of dysregulated feeding behavior into childhood (Winsper and Wolke, 2014). In the older child, the term 'feeding problems' encompasses a range of concerns, including food selectivity, problematic mealtime behavior, and oromotor challenges, and are most commonly the downstream effect of early life feeding problems. Children with feeding problems may withdraw from or reject their mother's touch; their mothers touch them less; and shared eye contact is reduced (Estrem et al., 2016).

Feeding problems in children diagnosed with ASD decrease from time of diagnosis to clinically insignificance by school age, the same pattern followed by feeding problems in neurotypical children. However, ASD children with higher levels of anxiety or mood difficulties and externalizing behavioral challenges are most at risk of a chronic course of feeding problems, with bi-directional negative effects on family interactions, emphasizing the role of chronic SNS-HPA hyperarousal in perpetuation of atypical developmental trajectories (Peverill et al., 2019).

Sleep Dysregulation

There are strong links between sleep disruptions in the first months of life and impaired cognitive, sleep and behavioral outcomes at school-age (Williams et al., 2016, 2017). However, a prospective birth cohort of 5151 children in the Netherlands found that reports of sleep problems co-occur with autistic traits in early childhood beginning from 18 months of age, when parental reports of sleep problems are resolving for neurotypical infants (Verhoeff et al., 2018).

Children with ASD show more frequent 'bedtime resistance,' sleep anxiety, and longer durations of night waking than children with other development disabilities (Cohen et al., 2014; Valicenti-McDermott et al., 2019). A study of 1201 children with ASD demonstrates links between aggressive behavior and sleep disruption, and proposes that this link is mediated by chronic SNS-HPA hyperarousal, noting that children with ASD experience a great deal of stress resulting from impairments in their ability to communicate with others or to understand the world around them (Shui et al., 2018). Sleep difficulties worsen behavior and cognition problems for the ASD child, and impact negatively on his or her family's communications.

This paper proposes that sleep problems in children with ASD are a consequence, first, of elevated levels of anxiety or chronic SNS-HPA hyperarousal, and, second, disrupted circadian rhythms, both worsened by the widespread application of 'sleep training' or FWB sleep strategies for children with ASD, which paradoxically disrupt the circadian clock.

Adults with ASD have dampened cortisol secretion and subjective somatic physiological arousal, associated with increased sleep onset latency, poorer sleep efficiency, and increased waking after sleep onset. This corroborates the hypothesis that dysregulation of the SNS-HPA is a key mechanism underlying phenotypic expression of ASD (Baker et al., 2019).

Chronic SNS-HPA Hyperarousal Affects the Gut Microbiome and Metabolic and Immune Settings in ASD

The gut is a major microbial–host interface, and a dominant immune organ. Immune function, HPA axis regulation, and the microbiota-gut-brain axis interact and co-evolve in the mother-infant complex adaptive system, and are each affected by stress. The infant gut microbiome in very early life has life-long effect on the settings of metabolism, immune, endocrine, and gut health. Gut dysbiosis is associated with impaired gut barrier integrity, inflammation and autoimmune disease (Cenit et al., 2017; Warner, 2018).

Mothers of children with ASD are twice as likely to report at least one gastrointestinal symptom in their child between 6 and 36 months. High-risk siblings have a greater prevalence of gastrointestinal symptoms. Young children diagnosed with ASD demonstrate significant gut dysbiosis (Coretti et al., 2018). Chronic constipation is the most common gastrointestinal problem in children with ASD. Many children with ASD have functional gastrointestinal tract disorders relating to selective eating, medications, and differences in sensory processing, which are associated with increased anxiety (Tye et al., 2019). Gastrointestinal problems may worsen developmental cascades of child behavior problems and disrupted family interactions. Studies show a strong correlation between gastrointestinal dysfunction and autism severity, across all domains including speech, social and behavioral (Ding et al., 2017). Early clinical trials suggest that by targeting the gut ecosystem, both ASD and gastrointestinal tract symptoms can be impacted, suggesting potential shared mechanisms (Tye et al., 2019).

However, this paper proposes that widespread inappropriate medicalization of infant behavioral cues, usually as signs of gut problems, results in a failure to identify and manage the underlying environmental factors which precipitate chronic SNS-HPA hyperarousal in very early life. Inappropriate medicalization of upregulated behavior and the failure to identify and manage underlying clinical problems have deleterious effects on gut health long-term. Instead of applying a linear and causative disease model which assumes that gut dysbiosis causes excessive infant crying, the neurobiological model of cry-fuss problems proposes that chronic SNS-HPA hyperarousal and feeding problems, and also the widely prescribed proton pump inhibitors, interact in the complex adaptive system of the mother and infant, out of which gut dysbiosis emerges (Douglas P.S. and Hill P.S., 2011; Douglas and Hill, 2013a; Castellani et al., 2017; Rhoads et al., 2018). This paper proposes that these and other environmental factors disrupt parent-infant biobehavioral synchrony, precipitating multiple trajectories of atypical development in ASD susceptible infants.

Chronic inflammation is emerging as the critical pathophysiological feature of mental disorders generally, associated with dysregulation of normal microglial synaptic pruning (Cenit et al., 2017). Siniscalco hypothesizes that pro-inflammatory processes

and immune alterations in very early life are etiological events for autism (Siniscalco, 2015) (**Box 3**). ASD has been linked with:

- Increased pro-inflammatory cytokines in the cerebro-spinal fluid
- Acquired mitochondrial dysfunction, an early sign of neurodegeneration
- Decreased antioxidants in urine
- Higher plasma GABA levels
- Amino acid and neuropeptide disruptions
- Increased autoimmune antibodies targeting central nervous system proteins.

Chronic SNS-HPA hyperarousal releases cortisol, which alters intestinal motility, gut epithelial permeability, and induces changes in gut microbial composition. This paper argues that the pro-inflammatory state of children with ASD arises from chronic SNS-HPA hyperarousal, or stress and anxiety, with long-term multi-directional impacts upon gut health, behavior and immune trajectories (De Palma et al., 2014; Gottfried et al., 2015; Siniscalco, 2015; Ding et al., 2017; Carter, 2019).

Excessive infant crying, or chronic SNS-HPA hyperarousal, is a pro-inflammatory state, impacting on gut microbiome and permeability, with long-term effects on metabolic, immune and mental health in susceptible infants (Partty et al., 2017; Rhoads et al., 2018). The Affect Diathesis model theorizes that behaviorally hypo-aroused infants also experience chronic SNS-HPA hyperarousal, with PNS override, which would be expected to similarly predispose to pro-inflammatory states (Greenspan, 2002). Anxiety disorder is common in adults with ASD, and symptoms of anxiety cause substantial functional impairment in ASD adults more broadly (Tye et al., 2019). This paper proposes that chronic anxiety and gastrointestinal problems in children and adults diagnosed with ASD are a downstream effect of chronic SNS-HPA axis dysregulation that begins in very early life.

Dysregulated Parent Mood Increases the Risk of ASD in Susceptible Children

A meta-analysis of nine observational studies shows increased ASD risk in children exposed to parental affective, depressive, and bipolar disorders (Ayano et al., 2019). Previous findings of links between maternal antenatal antidepressant use and ASD are attributed to the confounder of maternal mood disorder (Sujan et al., 2019). But there is, in particular, an increased risk of ASD in children of mothers who experience affective and depressive disorders, and this can be attributed to the developmental impacts of disrupted parent–infant biobehavioral synchrony in susceptible infants.

Depressed mothers with a 6-month-old baby show lower baseline vagal tone, linked with poorer emotional regulation and sensitivity to stress; less vagal brake, linked with poorer emotional regulation; less joint attention; and less initiation of interaction including touch and vocalization with the infant. Depressed mothers are less likely to touch the child, take significantly longer to respond to changes in their infant's behavior, and have less capacity to repair interactive errors. A cycle of mutual disengagement occurs, as the infant develops flat affect and makes fewer bids for joint gaze and response. Matched states are more likely to be negative, in distress or anger. Difficulties in mother-infant biobehavioral synchrony are detected not only in cases of full-blown clinical depression but among mothers with chronic sub-clinical symptomatology (Tietz et al., 2014). An anxious mother may direct very active communications toward her baby, which are not attuned to the infant's cues. The resultant shortened, less complex reciprocity chains between both highly anxious and depressed mothers and their infants in very early life predict the child's emotional dysregulation in later life (Feldman, 2007).

BIOLOGY-CULTURE MISMATCH IN VERY EARLY LIFE GENERATES ENVIRONMENTAL FACTORS WHICH INCREASE THE RISK OF ASD IN SUSCEPTIBLE INFANTS

A susceptible infant experiences biology-culture mismatch as adversity. Although the human infant is highly adaptive across a wide variety of culturally determined infantcare practices, a significant gap between cultural practices and evolutionary expectation may result in chronic SNS-HPA hyperarousal in very early life, in addition to other risks (Barr, 1990, 1999; Hofer, 2002; Douglas, 2005; McKenna et al., 2007; Ball, 2008;

BOX 3 | Evidence demonstrating that ASD is a pro-inflammatory state.

Many autism susceptibility genes are localized in the immune system and related to immune or infection pathways (Carter, 2019), and ASD is associated with an increased prevalence of specific immune-related conditions (Tye et al., 2019). Endoplasmic reticulum stress, oxidative stress, and apoptosis have been proposed as molecular mechanisms underlying autism (Dong et al., 2018). The oxidative and integrated stress responses are upregulated in the autism brain and may contribute to myelination problems (Carter, 2019). Increased autoantibodies directed toward central nervous system proteins have been observed in children with ASD, which may signal heightened inflammatory processes or an autoimmune component that could decrease the integrity of the mucosal barrier (Tye et al., 2019). Cytokines are proteins produced by neurons that regulate immune responses including hematopoiesis, inflammation, immune cell proliferation and differentiation.

Pro-inflammatory cytokines are found in higher levels in children with more severe ASD symptoms compared to ASD children with milder symptom presentation (Tye et al., 2019). A recent meta-analysis of cytokine levels in unmedicated individuals with ASD, mostly children, confirmed an abnormal cytokine profile, characterized by elevations in proinflammatory cytokines and reduced levels of anti-inflammatory cytokines (Masi et al., 2014).

ASD is associated with altered expression of genes associated with blood-brain barrier integrity, increased neuroinflammation, and possibly impaired gut barrier integrity. Immunological abnormalities affect both the gastrointestinal system and microglial cells of the brain and CNS. Monocytes, the precursors for macrophages, dendritic and microglial cells, show significant pro-inflammatory dysfunctions in ASD children. Individuals with ASD have upregulated inflammatory cytokines which induce blood brain barrier disruption. Altered blood brain barrier permeability directly influences neural plasticity, connectivity and function, and is hypothesized to underlie impairments in social interaction, communication behavior (Siniscalco, 2015).

Gettler and McKenna, 2011; Koss and Gunnar, 2018; Renz-Poster and De Bock, 2018). This paper proposes that the initial motor and sensory-motor neural lesions of ASD are more likely in susceptible children when environmental factors are mismatched with biological expectations during the critically neuroplastic, injury-sensitive first 100 days post-birth, resulting in chronic SNS-HPA hyperarousal which either triggers or perpetuates multi-directional cascades of atypical development.

In very early life, the infant evolved, in the *Homo sapien's* environment of evolutionary adaptedness, to expect:

(1) Rich environmental stimulation, including
   (a) Prolonged physical contact with older children and adults, including co-sleeping and diverse and frequent social sensory-motor enrichment
   (b) High levels of postural variability
   (c) Multi-centric social interactions
   (d) Complex non-social environmental stimulation, e.g., outdoors.
(2) Affect-driven, increasingly long, sensory-motor reciprocity chains with caring older children and adults. Mutual positive affect between adults and infants, or enjoyment and delight, has ensured *Homo sapiens*' evolutionary survival.
(3) Human milk transferred directly from the lactating breast to the infant gut, optimizing the gut microbiome, and metabolic, endocrine, and immune protection. Frequent and flexible breastfeeding facilitates increasingly long and complex motor and sensory-motor reciprocity chains.

Currently, in very early life, parents receive a great deal of conflicting advice from health professionals concerning breastfeeding, infant sensory needs, unsettled infant behavior and parent-infant sleep, and consult with multiple providers (McCallum et al., 2011; Schmied et al., 2011). There are serious gaps in health professional training across disciplines in management of breastfeeding and infant behavior problems (Renfrew et al., 2012; Rimer and Hiscock, 2014; Kirby et al., 2015; Gavine et al., 2017); widespread inappropriate medicalization of infant behavior, risking worsened outcomes; and substantial evidence that popularly applied approaches to breastfeeding and infant regulatory problems do not help parents and babies, or make breastfeeding, crying and sleep problems worse (Douglas, 2013; Svensson et al., 2013; Bergmann et al., 2014; Gieruszczak-Bialek et al., 2015; Kempler et al., 2016; Thompson et al., 2016; Woods et al., 2016; NHMRC, 2017; O'Shea et al., 2017; Gordon et al., 2018).

That is, the gap between biological expectation and socioculturally determined advice concerning infantcare is profound in contemporary societies, placing susceptible infants at neurodevelopmental risk, and increasing the risk of maternal postnatal anxiety and depression (Stein et al., 2014; Dias and Figueiredo, 2015; Tsivos et al., 2015). Infant cry-fuss, feeding, and sleep problems are often highly stressful for parents, and predispose to maternal postnatal depression (Dorheim et al., 2009; Vik et al., 2009; Dias and Figueiredo, 2015).

This paper hypothesizes that three key environmental factors emerge from biology-culture mismatch, and interact in the complex adaptive system of the parent and infant, increasing the risk of ASD in susceptible infants: suboptimal environmental stimulation, disruption of parent-infant biobehavioral synchrony, and gut dysbiosis or feeding problems.

Suboptimal Environmental Stimulation

In Australia and in many countries today, parents continue to be advised that sleep training or FWB approaches are necessary for optimal developmental outcomes and good sleep habits (**Table 3**). Infant sleep training emerged in the 1950s and 1960s when the first wave of the school of behaviorism (FWB) in psychology was applied to infantcare. Yet high level evidence demonstrates no decreased night waking or reliably improved maternal mood scores as a result of FWB interventions, and no improvement in developmental outcomes (Price et al., 2012; Bryanton et al., 2013; Douglas and Hill, 2013b; Mindell and Lee, 2015; Price et al., 2015; Kempler et al., 2016; NHMRC, 2017; Pennestri et al., 2018).

This paper proposes that application of FWB approaches to infant sleep in very early life impacts negatively on neurodevelopmental outcomes in ASD-susceptible infants, because FWB approaches decrease environmental stimulation in four ways:

(1) Parents are advised to teach the infant 'good sleep habits' by having the infant sleep in a cot or on an immobile surface, often in a quiet dim room with deliberately minimized visual stimulation, iteratively throughout the day. This regular recourse to a low sensory interior environment impoverishes sensory-motor experience, both social and non-social, and fails to offer susceptible infants adequate task demands for optimal development of skills in interpretation of sensory information, adaptation of movements in response to external stimuli, and organization of postural control.
(2) Stimulation is problematized. Parents are advised to avoid social and non-social 'overstimulation.'
(3) Inadequate sensory-motor stimulation results in SNS-HPA arousal (crying and fussing), which parents are advised to interpret as 'tired signs' or 'overstimulation,' triggering more attempts to put the baby to sleep.
(4) Patterns of inadequate environmental enrichment may result in chronic infant SNS-HPA hyperarousal (Douglas and Hill, 2013a; Whittingham and Douglas, 2014; Ball et al., 2018).

Disruption of Parent–Infant Biobehavioral Synchrony

Our environment of evolutionary adaptedness offered infants rich opportunities for increasingly long and complex motor and sensory-motor reciprocity chains across the two dominant sites of parent–infant transaction in very early life, feeds and sleep. Patterns of cued care are associated in cross-cultural studies with downregulation of both infant and parents' SNS-HPA axes (Hamilton, 1981; Barr et al., 1991; St James-Roberts et al., 2006; Wolke et al., 2017). Parent–infant biobehavioral synchrony is disrupted by problems of crying and fussing, breastfeeding, and sleep.

TABLE 2 | Cry-fuss problems: popular sociocultural and clinical advice which disrupts parent–infant biobehavioral synchrony compared with Neuroprotective Developmental Care strategies which promote parent–infant biobehavioral synchrony.

Infant cues	Most likely cause	Popular diagnoses or advice	Neuroprotective Developmental Care strategy
Crying	Poor satiety	Normalize infant distress	Identify and manage underlying breastfeeding problems
Crying	Suboptimal sensory-motor stimulation	Teach to self-settle in cot in order to develop autonomous sleep; apply graduated extinction	Educate parents about infant's biological need for rich sensory-motor nourishment including physical contact
Grizzling and crying	Suboptimal sensory-motor stimulation	Overtired or overstimulated; place in low sensory environment; avoid eye-contact and interaction; teach to self-settle in cot	Use two tools, satiety with milk or satiety of sensory nourishment, to downregulate infant
Back-arching	Sign of protest	Oral ties, oesophagitis, reflux, wind pain or gas, colic, allergy	Use two tools of satiety with milk and satiety of sensory nourishment to downregulate infant; educate parents re appropriate spinal support for infants
Writhing, grunting, grizzling when lying in cot	Suboptimal sensory-motor stimulation	Oral ties, oesophagitis, reflux, wind pain or gas, colic, allergy	Offer sensory nourishment; educate re dialing up of SNS also activates gut

Inappropriate Medicalisation of Infant Cry-Fuss Behaviors

The neurobiological model of cry-fuss problems proposes that chronic SNS-HPA hyperarousal, manifesting as crying and fussing in otherwise well infants, emerges out of a mismatch between environment of evolutionary adaptedness and various socioculturally determined environmental factors in contemporary life.

But overdiagnosis and overtreatment, including in children, is a growing global concern, and occurs commonly in very early life, with deleterious effects (Coon et al., 2014; Brownlee et al., 2017). Parents are often taught that their infant's cry results from physical pain, e.g., from reflux, aerophagia-induced reflux, food allergies or intolerances, lactose intolerance, or from oral connective tissue restrictions (Bergmann et al., 2014; Gieruszczak-Bialek et al., 2015; O'Shea et al., 2017; Gordon et al., 2018; Kapoor et al., 2018). The resultant inappropriate medicalization risks unintended outcomes and perpetuates disruption to parent-infant biobehavioral synchrony (**Table 2**).

First Wave Behavioral Interventions for Parent–Infant Sleep

In response to emerging neuroscience and psychological attachment research, FWB approaches have adopted a discourse which emphasizes the importance of cued care for secure attachment and optimal mental health outcomes. Yet paradoxically, FWB sleep advice continues to actively disrupt cued care and biobehavioral synchrony, by advising parents to behave in directive and non-contingent ways, iteratively, day and night (**Table 3**). More extreme FWB strategies, such as minimizing eye contact and interaction at sleep-time throughout the days and nights, iteratively present a 'still face' to the infant, which is known to result in greater efforts by the infant to engage at first, before he or she withdraws from cueing (Bertin, 2006). Standard FWB approaches advise parents to not respond to SNS-HPA arousal, or to delay responses, or to respond but not as they believe the baby intends. Parents are advised to iteratively override the powerful biological cues of sleepiness after feeds by applying behaviors such as burping, holding upright, or wrapping, at the same time as they are instructed to achieve prescribed nap frequencies and durations.

FWB approaches may exacerbate parental anxiety, which predisposes to poor sleep efficiency and postnatal depression (Blunden et al., 2016; Etherton et al., 2016). The parent trying to enforce sleep because she believes it necessary for her baby's healthy development is likely to feel anxious and distressed when the baby 'resists sleep' multiple times a day; the infant 'resisting sleep' (that is, whose sleep pressure is not yet high enough for easy sleep) is repeatedly subject to the biological stress of a low sensory environment, resulting in chronic SNS-HPA hyperarousal and increased allostatic load. This cycle may place behaviorally hypo-aroused infants at particular risk.

The link between sleep problems in very early life and behavioral and sleep problems in later childhood may be, paradoxically, mediated by the widespread sociocultural and clinical application of sleep training or FWB strategies, resulting in cascades of both parent and infant chronic SNS-HPA hyperarousal (Simard et al., 2017; Williams et al., 2017; Ball et al., 2018). FWB approaches also disrupt the dyadic synchrony of the circadian clock, by promoting long blocks of sleep during the day (Thomas et al., 2014).

Unidentified and Unmanaged Breastfeeding and Unsettled Infant Behavior Problems Increase Risk of Maternal Postnatal Depression

Cry-fuss problems, poor maternal sleep efficiency, and breastfeeding problems are key modifiable risk factors for post-natal depression (Vik et al., 2009; Stein et al., 2014; Dias and Figueiredo, 2015; Tsivos et al., 2015; Werner et al., 2015). Each of these may arise from, or be exacerbated by, a mismatch

TABLE 3 | Sleep problems: popular sociocultural and clinical advice which disrupts parent–infant biobehavioral synchrony.

First wave behavioral belief	First wave behavioral strategy	Disruption to parent–baby biobehavioral synchrony
Teaching self-settling improves infant sleep		
	Don't let baby fall asleep with breastfeed or bottle-feed	Overrides powerful biological cue of sleepiness
	Put baby down in cot drowsy but awake, even if baby grizzles and cries for a time	Ignores infant cue; interprets infant cue as 'resisting sleep'; baby may be crying due to suboptimal sensory-motor nourishment
	Create sleep associations with cot, white noise, swaddling, music, low sensory environment	Sleep is under stimulus-control of sleep pressure, not 'associations'; baby develops negative associations with sleep place and rituals, interpreted as 'resisting sleep'
	Sleep in quiet dark room during day	Worsened night-waking after 2–3 weeks, due to disruption of circadian clock
Feed-play-sleep cycles make life more manageable for parents		
	Don't let baby fall asleep with breastfeed or bottle-feed	Overrides powerful biological cue of sleepiness
	Put baby down in cot drowsy but awake	Baby cries due to suboptimal sensory-motor nourishment, interpreted as 'resisting sleep'
	Space out feeds	Baby cries due to hunger; Undermines breastfeeding success
Baby needs a lot of sleep for optimal brain development		
	Sleep breeds sleep	Worsened night-waking after 2–3 weeks, due to disruption of circadian clock
	Achieve 'second sleep cycle' during day-time naps	Worsened night-waking after 2–3 weeks, due to disruption of circadian clock
	Sleep routines with estimates of time awake and ideal duration of sleep	Baby is expected to spend longer asleep than actually needs, disrupting the biological sleep regulators
Mustn't let baby get over-tired		
	Prescribed list of 'tired cues'	Disempowers parents by undermining confidence in their capacity to experiment and learn what they baby is cueing
	Put baby down at first 'tired cue'	Promotes disruption of the biological sleep regulators, due to disruption of the circadian clock and inadequate sleep pressure
	Put baby to bed early at night (6–7 pm)	Promotes disruption of the biological sleep regulators, due to disruption of the circadian clock and inadequate sleep pressure
Mustn't let baby get overstimulated		
	Avoid leaving house or engaging in play or social activity in lead up to sleep times	Baby may be cuing for richer sensory-motor nourishment, not tiredness
Baby who grizzles and cries is 'resisting' sleep		Baby may be cuing for richer sensory-motor nourishment, not tiredness

between popular sociocultural and clinical approaches, and infant biology. Health professionals report inadequate training in management of breastfeeding and unsettled infant behavior problems, and often recommend approaches which have been shown not to help, or may even worsen these problems, with associated deleterious effects on parent–infant biobehavioral synchrony (Price et al., 2012; Bryanton et al., 2013; Douglas and Hill, 2013b; Rimer and Hiscock, 2014; Mindell and Lee, 2015; Price et al., 2015; Blunden et al., 2016; Etherton et al., 2016; Kempler et al., 2016; Thompson et al., 2016; Gavine et al., 2017; NHMRC, 2017; Pennestri et al., 2018).

There is widespread recognition of the importance of prevention of, or early detection and treatment of, perinatal anxiety and depression, the most common mental health condition post-birth. Winnicott proposed that a state of maternal preoccupation or heightened sensitivity develops toward the end of pregnancy and lasts throughout the postpartum period. From an evolutionary perspective, this heightened state supports a woman's ability to anticipate her infant's needs and to respond to her infant's unique cues, and may include anxious, hypervigilant and intrusive thoughts about the infant. In contemporary contexts, often characterized by minimal social support, the same heightened state increases her risk of postnatal anxiety and depression if infant behavior problems emerge.

Gut Dysbiosis and Feeding Problems

In the *Homo sapiens* environment of evolutionary adaptedness, the complex behavior of breastfeeding is the dominant site of parent-infant motor and sensory-motor interaction in very early life. The infant gut is part of the enteromammary immune system, an extension of the maternal gastrointestinal and immune systems facilitated by breastfeeding. Breastfeeding offers powerful protection of the gut microbiome, with positive impact upon immune and metabolic health.

Successful breastfeeding is linked with increased amounts of cued care (Brown and Lee, 2013), which leads to longer and more complex reciprocity chains. Early breastfeeding is driven by infant stepping, suck and swallow movements (secondary variable movements) and complex sensory-motor interactions, offering rich and diverse environmental stimulation and opportunities for affect-driven sensory-motor reciprocity chains. This may explain associations between breastfeeding and higher IQ (Belfort, 2017). Cued care also improves breastfeeding duration (Brown and Arnott, 2014). Breastfeeding difficulties, and lactation-related breast pain predispose women to postnatal depression, with an associated cascade of related crying behaviors and disruption of mother–infant biobehavioral synchrony (Howard et al., 2006; Brown et al., 2015; Dias and Figueiredo, 2015).

Breastfeeding, either exclusively or with some formula supplementation, is associated with decreased risk of ASD (Schultz et al., 2006; Boucher et al., 2017; Tseng et al., 2017; Bittker and Bell, 2018; Manohar et al., 2018). A Spanish multicenter birth-cohort study of 1,346 children showed that longer duration of breastfeeding protected against autistic traits (Boucher et al., 2017). The Danish National Birth cohort study of 76,322 mothers showed that breastfeeding for less than 6 months was associated with an increased risk of diagnosis of ASD (Lemcke et al., 2018). High-risk siblings are more likely to be weaned earlier. Weaning after 6 months and exclusive breast milk are also associated with protection against gastrointestinal tract symptoms, in particular constipation and abdominal distress, in high-risk siblings (Penn et al., 2016).

This paper proposes that unidentified clinical breastfeeding problems such as positional instability or conditioned dialing up at the breast, may cause chronic SNS-HPA hyperarousal, premature weaning, and related gut dysbiosis (**Table 4**). Unfortunately, popular clinical approaches often don't help, or may even worsen breastfeeding problems (Svensson et al., 2013; Thompson et al., 2016; Woods et al., 2016; O'Shea et al., 2017). Developmental trajectories of feeding problems and disrupted parent–infant biobehavioral synchrony may result (Douglas and Hill, 2013a).

TARGETING KEY ENVIRONMENTAL FACTORS FOR PRE-EMPTIVE INTERVENTION

A prospective cohort study of 85,176 children in Norway shows that folic acid supplementation, taken by the mother from 4 weeks prior to 8 weeks after the first day of her last menstrual period, is associated with a lower risk of ASD. For this reason, folate supplementation from pre-conception is recommended as an initial step in pre-emptive intervention (Suren et al., 2013).

Pre-emptive intervention for ASD then aims to promote infant neuro-resilience by preventing, or re-stabilizing, disrupted feedback loops emerging from small motor and sensory-motor structural or functional lesions in very early life. Drawing on the preceding review of heterogeneous interdisciplinary research literature, the following will require optimization:

(1) Environmental enrichment, social and non-social (that is, 'rich motor and sensory-motor nourishment')
(2) Parent–infant biobehavioral synchrony, which is promoted by
    (a) Cued care for downregulation of the SNS-HPA axis
    (b) Multi-lateral strategies for management of cry-fuss problems
    (c) Avoidance of inappropriate medicalization of infant behavior
    (d) Healthy regulation of parent-infant sleep
    (e) Increasingly long and complex, multi-sensory, affect-driven reciprocity chains, often infant-initiated
    (f) Optimal parent mental health, which requires
        (i) Effective intervention for the key modifiable risk factors of breastfeeding, sleep and cry-fuss problems
        (ii) Strategies for managing difficult thoughts and feelings
(3) Gut microbiota and mitigation of pro-inflammatory states, promoted by
    (a) Optimizing breastfeeding
    (b) Teaching paced bottle-feeding
    (c) Avoiding inappropriate medical diagnoses.

Over the past 20 years, robust peer-reviewed theoretical frames have been developed for a program known as Neuroprotective Developmental Care (NDC or 'the Possums programs'), which targets these environmental factors as anticipatory guidance in the antenatal period and as clinical and educational support throughout the first 6–12 months of life. NDC has applied an integrative, primary care generalist's lens to heterogenous evidence from the fields of neuroscience, evolutionary biology, developmental psychology, and the medical, lactation and sleep sciences (Douglas P. and Hill P., 2011; Douglas P.S. and Hill P.S., 2011; Whittingham and Douglas, 2014; Douglas and Keogh, 2017; Douglas and Geddes, 2018). NDC is a novel, community-based, parent-mediated, early life clinical and educational intervention. The term 'Neuroprotective Family Centered Developmental Care' has been previously applied to Heideleise Als pioneering work in the hospital setting with prematurely born infants, demonstrating improved development outcomes in this population (Als, 2009; Altimier and Phillips, 2016).

NDC has been delivered clinically in the Australian primary care setting since 2011 and is also available as online programs and for interdisciplinary health professional education.

Environmental Enrichment

This paper accepts the primacy of motor lesions *in utero*, intra-partum, or in very early life in ASD etiology. Pre-emptive intervention therefore advocates adult-mediated motor and sensory-motor environmental enrichment post-birth, both socially and non-socially, if early motor deficits are to be

TABLE 4 | Breastfeeding problems: popular sociocultural and clinical advice which disrupts parent-infant biobehavioral synchrony compared with Neuroprotective Developmental Care strategies which promote parent–infant biobehavioral synchrony.

Breastfeeding: infant cue	Most likely cause	Popular diagnoses or advice	Neuroprotective Developmental Care strategy
Difficulty coming onto the breast	Positional instability, breast tissue drag, landing pad encroachment	Oral ties, oesophagitis, reflux, wind pain or gas, colic, allergy	Optimize fit and hold to optimize positional stability (gestalt breastfeeding)
Back-arching and pulling off at the breast	Positional instability, breast tissue drag, landing pad encroachment	Oral ties, oesophagitis, reflux, wind pain or gas, colic, allergy	Optimize fit and hold to optimize positional stability (gestalt breastfeeding)
Dialing up at the breast	Positional instability, breast tissue drag, landing pad encroachment	Oral ties, oesophagitis, reflux, wind pain or gas, colic, allergy	Optimize fit and hold to optimize positional stability (gestalt breastfeeding)
Dialing up whenever approaches breast or during breastfeeding ('oral aversion')	Conditioned hyperarousal (dialing up) of SNS, often secondary to positional instability but persisting once fit and hold are corrected	Oral ties, oesophagitis, reflux, wind pain or gas, colic, allergy	Comprehensive intervention for conditioned hyperarousal of SNS
Marathon feeds or excessively frequent feeds	Poor milk transfer	Oral ties, oesophagitis, reflux, wind pain or gas, colic, allergy	Optimize fit and hold to optimize milk transfer (gestalt breastfeeding)
Falls asleep at the end of a breastfeed	Normal biological process (↑ parasympathetic nervous system response, ↑ oxytocin, ↑ cholecystokin)	Allows bad habits or sleep associations to develop	Parents educated about healthy function of the biological sleep regulators

prevented or early disruption of feedback loops stabilized. Patterns of rich postural variability, with general movements of the infant's limbs and trunk occurring against the adult's changing bodily configurations (rather than in the context of long periods on an immobile cot or mattress), and patterns of rich multi-centric social interactions, that is, physically interactive play and enjoyment with older children and adults, are required to optimize sensory-motor feedback (Lickliter, 2011).

The NDC sensory domain is unique amongst infant-care programs in de-problematizing sensory stimulation, and offers parents multiple practical strategies for environmental enrichment, also referred to as healthy sensory-motor nourishment. Physical contact with the infant is encouraged where this is sensibly possible, which helps address the infant's biological need for complex and continuing postural variation. NDC supports the primary carer to leave the low sensory environment of the Western interior daily for social contact, activities, and tasks, including frequent walks, as carers focuses on creating a rich, full and meaningful life, in large part outside the home, and to have the infant sleep day or night in the vicinity of the carer.

Optimizing Parent–Infant Biobehavioral Synchrony

The first step to optimal parent–infant biobehavioral synchrony is the elimination of clinical disruptors, which allows attention to the small communications of the infant to emerge. The second step is to encourage attention to the present moment and prioritize playful enjoyment of the baby.

Multi-Domain Management of Cry-Fuss Problems

NDC applies a systematic 5-domain approach to management of cry-fuss problems in parents, developed from the neurobiological model of cry-fuss problems (Douglas and Hill, 2013a). Each of the domains (feeds, sleep, environmental enrichment, infant gut and health considerations, and parental mood) integrate multiple evidence-based strategies. Preliminary evaluation of the 5-domain approach shows halved crying and fussing durations (Douglas et al., 2013). NDC applies a 'dialing up' and 'dialing down' metaphor, referring to up-regulation and down-regulation of the SNS-HPA, to describe parents' co-regulatory efforts as they respond to their infant's cues (Douglas and Hill, 2013a).

Healthy Regulation of the Infant's Circadian Clock and Sleep–Wake Homeostat

The NDC sleep domain comprises the Possums Sleep Program, which enriches environmental stimulation, both social and non-social, by offering the only existing evidence-based alternative to FWB approaches for parent-infant sleep (Whittingham and Douglas, 2014; Ball et al., 2018; Douglas, 2018). Preliminary evaluation confirms the Possums Sleep Program has high levels of acceptability to parents and improves their quality of life (Ball et al., 2018). Parents are educated about maintenance of healthy function of their baby's sleep regulators, and offered evidence-based information which minimizes anxiety about sleep. This includes:

(1) Educating parents about normal sleep variability and normal night-waking trajectories
(2) Differentiating between excessive night waking and normal night waking
(3) Identifying circadian clock disruption or satiety problems that result in unnecessary disruption of parent-baby sleep,
(4) Removing disruptors to sleep efficiency for parents and baby
(5) Education about how to optimize the function of the two biological sleep regulators, the circadian clock and the sleep–wake homeostat.

Strategies for Managing Difficult Thoughts and Feelings

NDC aims to change parents' relationship with difficult thoughts and feelings in order to optimize enjoyment of the baby, which results in increasingly long and complex reciprocity chains of interaction. Traditional Cognitive Behavioral Therapy, which has been extensively investigated perinatally and is demonstrated as effective, promotes disputing irrational thoughts, which in the perinatal context may paradoxically worsen the biological predisposition to maternal rumination, precipitating worsened anxiety and depression (Monteiro et al., 2018). For this reason, NDC integrates applied functional contextualism, popularly known as Acceptance and Commitment Therapy (ACT), a third wave behaviorism. ACT has a burgeoning evidence-base for depression and anxiety, overtaking traditional Cognitive Behavioral Therapy on some indicators, with preliminary positive findings for parenting (Blackledge and Hayes, 2006; Ruiz, 2010; Whittingham et al., 2014).

ACT avoids unnecessary pathologizing of mental health challenges. It helps parents clarify values, and targets rumination, which predisposes mothers to perinatal anxiety and depression and which has been thought to mediate impaired infant development outcomes (DeJong et al., 2016). ACT offers a range of strategies for cognitive defusion and expansion of attention, in order to help parents manage difficult thoughts and feelings. ACT teaches behavioral activation skills, in the service of values, demonstrated to be effective with postnatal anxiety and depression (Lavender et al., 2016; Marchesi et al., 2016), and mindfulness, or anchoring in the present moment, demonstrated to assist in anxiety and depression generally (Gotink et al., 2015). ACT offers acceptance-focused processes and self-compassion, also demonstrated to help prevent postpartum depression and anxiety (Monteiro et al., 2018). A study of 139 parents of children with ASD showed that those who demonstrated more self-compassion experienced less stress and improved quality of life (Bohadana et al., 2019).

ACT is vitally concerned with context, and is well-suited for integration into health professionals' consultations for common infantcare problems post-birth. ACT strategies are integrated into the Possums 5-domain approach to cry-fuss problems, the Possums Sleep Program, and gestalt breastfeeding (Whittingham and Douglas, 2016; Douglas and Keogh, 2017). Within these programs, women with moderate to severe mental illness are appropriately diagnosed, treated pharmaceutically as needed, and referred for ongoing psychological support.

'Growing Joy in Early Life'

Unlike early intervention programs derived from social communication models, NDC does not instruct parents to avoid intrusiveness or directiveness, or offer suggestions about the meaning of infant cues and how to respond, which may inadvertently communicate assumptions of parental incompetence and may also increase parental anxiety. Similarly, NDC also does not employ tools such as the Neonatal Observation Scale to teach parents about their infant's behavioral repertoire and communication competence in the first six months of life (Nugent, 2013). Instead, NDC educates parents about the benefits of reciprocity chains, and encourages enjoyment of the baby. NDC proposes that parental competence and evolutionary drive for enjoyment of the baby will emerge in families once disruptive sociocultural and clinical advice are removed, underlying clinical problems are identified and repaired, and the importance of satisfying and socially engaged days outside the home explained.

NDC confidence in parental competence is corroborated by a study of 864 parent–newborn pairs observed spending time together as the baby lay close to the parent. Parents were given minimal instructions, but asked to interact with the baby comfortably, as they saw fit. Most of the 480 full-term newborns showed subtle affect-driven initiation of arm movements toward the parents as they interacted, though this was somewhat reduced in the prematurely born infants, and all parents engaged in quiet and supportive interaction without being intrusive (Delafield-Butt et al., 2018).

Optimizing Breastfeeding and Enjoyment of Feeds

Optimizing gut health in susceptible infants requires not only avoidance of unnecessary medicalization of unsettled infant behavior, but importantly, the support of breastfeeding success, with its known gut and immune protections.

A systematic review of ultrasound studies of breastfeeding mother-baby pairs forms the biomechanical basis for a new 'gestalt' model of clinical breastfeeding support, out of which the NDC gestalt breastfeeding approach is developed (Douglas and Geddes, 2018). Gestalt breastfeeding builds on the pioneering advance of baby-led breastfeeding, which emphasizes cued care by activating and responding to the baby's mammalian reflexes, but points to the evidence showing that this is not enough to achieve pain-free effective milk transfer for many women (Svensson et al., 2013; Woods et al., 2016). The gestalt approach educates about the biomechanics of effective breastfeeding. Impaired fit and hold and the resultant infant cues of fussing at the breast, difficulty coming onto the breast, back-arching, maternal nipple pain, marathon feeds, excessively frequent feeds, excessive night waking, and poor weight gain, are addressed applying gestalt methods (Douglas and Keogh, 2017; Douglas and Geddes, 2018).

Formula, which offers the human infant unphysiological doses of cow's milk protein, lacks a myriad immune factors, micro-nutrients, and live bacteria, predisposing to short- and long-term gut dysbiosis and increased risk of immune system alterations. DNA methylation, a key mechanism of epigenomic regulation, is highly responsive to diet. Nutrients and bioactive compounds are known to alter the expression of genes at the transcriptional level and result in long-term phenotypic changes (Barker et al., 2018).

However, women feed babies in complex contemporary sociocultural contexts, facing multiple disruptors, pain, and conflicting clinical advice. NDC is strictly non-judgmental concerning mode of infant feeding, and teaches paced bottle-feeding in order to optimize feeding-related reciprocity

TABLE 5 | Bottle-feeding problems: popular sociocultural and clinical advice which disrupts parent–infant biobehavioral synchrony compared with Neuroprotective Developmental Care strategies which promote parent–infant biobehavioral synchrony.

Bottle-feeding: infant cue	Most likely cause	Popular diagnoses or advice	Neuroprotective Developmental Care strategy
Back-arching and fussing	Positional instability	Oral ties, oesophagitis, reflux, wind pain or gas, colic, allergy	Paced bottle-feeding
Back-arching and fussing	Does not want more milk; pressure on feeds due to spacing	Oral ties, oesophagitis, reflux, wind pain or gas, colic, allergy	Paced bottle-feeding
Back-arching and fussing	Conditioned hyperarousal of SNS	Oral ties, oesophagitis, reflux, wind pain or gas, colic, allergy	Paced bottle-feeding, health professional support to build enjoyable feeding associations

chains in formula or expressed-breastmilk-fed infants. NDC also identifies and manages conditioned dialing up with the bottle, which is commonly inappropriately medicalized (**Table 5**).

CONCLUSION

ASDs are complex polygenic syndromes displaying highly variable phenotypic expressions and co-morbidities. Myriad environmental factors are known to modulate epigenomic regulation of ASD phenotypic expression. By 6 months of age, when the first signs of atypical connectivity emerge in neuroimaging studies, neuropathy may be entrenched. This paper responds to a recent call from the International Society for Autism Research for the development of theoretical foundations for, and clinical translation of, pre-emptive intervention for ASD.

The first 100 days of human life are characterized by injury-sensitive neuroplasticity, correlating with in the neural biomarker of cortical subplate persistence and the behavioral biomarker of the crying diathesis. Environmental factors in these critically injury-sensitive first 100 days may either initiate or perpetuate structural or functional lesions in global neuronal workspace of susceptible infants. This paper builds on ground-breaking etiological models concerning the primacy of motor lesions in ASD etiology, to propose that chronic SNS-HPA hyperarousal and disrupted parent-infant biobehavioral synchrony are key mechanisms facilitating perpetuation of the atypical developmental trajectories of ASD, triggered by very early motor and sensory-motor lesions.

Existing clinical guidelines for protection of infant growth and development in very early life, including support for feeds and sleep, are confused and conflicting, exacerbating parental anxiety. From an evolutionary perspective, popular clinical and cultural advice often promotes biology-culture mismatch, resulting in chronic SNS-HPA hyperarousal and disrupted parent–infant biobehavioral synchrony. Health professionals are not adequately trained in the identification and management of common clinical problems arising in the domains of feeds, sleep, cry-fuss problems and parental mood, which disrupt parent–infant biobehavioral synchrony. This health system context places ASD susceptible

BOX 4 | When to apply NDC as pre-emptive intervention.
Families with the following risk factors for ASD should be offered NDC pre-emptive intervention as soon as possible, beginning with anticipatory antenatal education and throughout the first 6 months of life:

- Child diagnosed with ASD
- Parent diagnosed with ASD.

Further potential indicators for NDC pre-emptive intervention are perinatal factors associated with later diagnosis of ASD, including:

- Antenatal depression
- Pre-eclampsia
- Premature birth
- Caesarian section under general anesthesia
- Infant hypoxic-ischemic encephalopathy (Chien and Lin, 2015; Getahun et al., 2017).

The following factors that have been associated with infant neurodevelopmental vulnerability also warrant NDC as pre-emptive intervention:

- Unsettled infant behavior
- Atypical crying acoustics
- Breastfeeding or feeding difficulties
- Maternal postnatal depression
- Plagiocephaly (marker of increased risk of transient motor and developmental delay, suggesting need for enriched sensory-motor experience)
- Other signs of developmental vulnerability (Martiniuk et al., 2017; Renz-Poster and De Bock, 2018)

If efficacy of NDC is demonstrated in infants at risk, a second phase would evaluate NDC as a health system response, replacing current conflicting and confusing advice from multiple health professionals in order to offer consistent, evidence-based care which optimizes neuroprotection for all parents and infants from the antenatal period onward, universally available through usual maternal and child health services.

infants, and infants susceptible to other neurodevelopmental disorders, at risk.

Any pre-emptive intervention for ASD that aims to apply a focused selection of 'active ingredients' risks exacerbating parental anxiety, because parents already receive conflicting and confusing advice concerning common infant-care problems. Contact with these providers will continue. This paper argues that a comprehensive, holistic, community-based approach to the care of at-risk siblings is necessary to support parents who must inevitably navigate the health system, and proposes an Australian program known as NDC.

Research funding for evaluation of primary care initiatives remains scarce and is diminishing. Evaluation of NDC as pre-emptive intervention for ASD (**Box 4**), using real world methodologies suitable for complex community-based interventions, is required.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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Conflict of Interest: PD is the medical director of a small primary care charity, Possums Education, which sells Neuroprotective Developmental Care programs online for parents, and also as education workshops for health professionals www.possumsonline.com. All revenue raised is returned into the development of education and research.

frontiers in Integrative Neuroscience

ORIGINAL RESEARCH
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How Much Instructional Time Is Necessary? Mid-intervention Results of Fundamental Movement Skills Training Within ABA Early Intervention Centers

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Background: The purpose of this study was to explore the question of the minimal amount of instructional time needed to still be effective by assessing the efficacy at mid-intervention of an early fundamental movement skill (FMS) intervention for preschoolers with Autism Spectrum Disorder (ASD).

Method: Fourteen preschoolers participated in this randomized controlled trial daily over 10 weeks (10 h total at mid-intervention). A two-factor mixed MANOVA tested the significance of group*time interactions for two dependent variables: object control and locomotor raw scores on the Test of Gross Motor Development—III.

Results: Group*time interactions approached significance with large effect sizes on the vector of both dependent variables and in a univariate fashion on object control scores, but not locomotor scores.

Conclusions: These findings hold relevance for physical educators working with young children with ASD, indicating that 10 h of FMS instruction, at least in this form, is not adequate to improve FMS.

Keywords: autism spectrum disorder, motor skills, visual supports, applied behavior analysis, intervention

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INTRODUCTION

Although not part of the diagnostic criteria for Autism Spectrum Disorder (ASD), children with ASD show frequent motor delays (Staples and Reid, 2010) that begin early in life and become more significant with age (Lloyd et al., 2013). It appears that motor skills in this population also relate to developmental areas beyond the physical (MacDonald et al., 2014), emphasizing the importance of fundamental movment skill (FMS) training and adapted physical education (APE) services for children with ASD. This is increasingly relevant for young children, given the strong evidence that early intervention is effective for children with ASD (Reichow et al., 2012).

Early FMS interventions for those with ASD show promising results in research settings. A 12-week FMS intervention implemented by APE researchers improved object manipulation and overall motor scores for nine 4-year-olds with ASD (Bremer et al., 2015). Some individual FMS improved from an intervention orchestrated by APE researchers and a special education teacher within an early intervention classroom with five children aged 3–7 showing ASD-like characteristics

(Bremer and Lloyd, 2016). Finally, the locomotor and ball skills of 11 children aged 4–6 (nine controls) improved in a summer-camp motor skill intervention, again implemented by APE researchers (Ketcheson et al., 2017).

FMS interventions for children with ASD show promising results; however, no matter the setting, competing curricular demands will always be present. It is therefore necessary to know how much direct FMS instructional time is required, in order to justify lesson time. In the 2018, Patricia Austin Award Presentation, this question was addressed as part of a meta-analysis (Case, 2018). The findings showed a substantial publication bias, wherein most published interventions showed a significant treatment effect, and very few used less than 12 instructional hours. The issue, it appears, is underreporting of FMS interventions with null results. Thus, the question of minimal instructional time needed is still unanswered.

Early Intensive Behavioral Intervention (EIBI) centers, frequently based on Applied Behavior Analysis (ABA) techniques, have gained attention in recent times. These centers are generally specified for children with severe ASD who qualify for intensive behavioral treatment before entry into kindergarten. There are typically no APE services offered in this environment. The EIBI environment has not been used for early FMS intervention. These centers typically use individualized therapy plans and a small staff to student ratio, thus they hold promise as a delivery platform for early FMS intervention services.

The purpose of this study was to explore this question of minimally effective FMS instruction time within an ecologically valid environment, the EIBI clinic. Baseline data and mid-intervention outcomes are presented here from a 20-week FMS randomized controlled trial. Post-intervention and follow-up results will be published separately upon their collection and analysis.

MATERIALS AND METHODS

Participants

Fourteen children were recruited from two campuses of an ABA EIBI clinic and randomized within each campus to form a control ($n = 6$) and intervention group ($n = 8$; **Table 1**).

Procedure

All procedures were approved by an ethical board before data collection began and all participants' caregivers gave informed consent. Caregivers supplied descriptive characteristics on a questionnaire. Autism Severity (Autism Diagnostic Observation Scales–2 calibrated severity score; ADOS-2) was measured by ABA staff and reported at baseline. Anthropometrics and FMS (Test of Gross Motor Development—III—TGMD—III) were assessed prior to any baseline and following 10 weeks (mid-intervention) of intervention. For anthropometrics, height without shoes was measured to the nearest 2 cm (Seca Stadiometer) and weight in light clothing was measured to the nearest 0.1 kg (standing scale). Body mass index (BMI) percentile was calculated according to Centers for Disease Control growth curves.

Intervention

Direct FMS instruction sessions lasted 15 min each and occurred 4 days per week for 10 weeks. Each session consisted of discrete trial training in one of the 13 FMS (run, gallop, skip, hop, jump, slide, two-handed strike of a stationary ball, one-handed strike of a self-bounced ball, one handed dribble, kick, two-handed catch, overhand throw, and underhand throw) for one individual child. Trials were implemented by the EIBI behavior technician already working with the child. One research staff was present to answer questions and collect video. Each trial consisted of viewing a tablet-displayed video of the FMS, a picture task card, and an abbreviated verbal direction (Breslin and Rudisill, 2011). Following this stimulus, the participant completed one trial of the skill, with the behavior technician implementing a most-to-least physical prompting hierarchy and providing immediate differential reinforcement (reinforcement following attempts, and more potent reinforcement following correct attempts). An additional 5 min per day, 4 days per week for 10 weeks, the entire intervention group at each campus played rotating active social games without direct instruction in FMS. The control group continued therapy as usual and were simply tested on FMS twice with an interval of 10 weeks.

Measures

TGMD-III

The present study used picture task cards (Breslin and Rudisill, 2011), short standardized instructions (Breslin and Rudisill, 2011), and administration provided by a single live model (Allen et al., 2017). Video-recorded assessments were scored by individuals blinded to group (control or intervention) and time (baseline or mid-intervention), and whom had achieved 90% reliability (Rintala et al., 2017) using videos and scores disseminated by the assessment authors.

Statistical Analysis

To check equivalence of groups, a MANOVA was conducted to compare the control and intervention groups' baseline TGMD—III and ADOS-2 scores. A two-factor mixed MANOVA (each subject tested twice in time but belonged to only one group) tested the significance of group*time interactions for TGMD–III raw scores in object control and locomotor subtests from baseline to mid-intervention between the two groups. This allowed tests of the multivariate interaction effects as well as the univariate interaction effects for each dependent variable. The assumptions of linearity of relationships among the dependent variables, multivariate normality, and homogeneity of variance-covariance matrices between groups were tested before conducting the mixed MANOVA, even though sample sizes were not more than 1.5 times different (Leech et al., 2014). Matrix scatterplots and correlation matrices showed linearity but not multicollinearity ($r < 0.80$; Vatecheva et al., 2016). Before beginning any parametric tests, normality was assessed using the Shapiro–Wilk test for normality; the dependent variables did not violate the assumption of normality. Box's M test was conducted to check the homogeneity assumption, and no significant differences were found between the covariance matrices; Wilk's Lambda was an appropriate test to use. A significant interaction

TABLE 1 | Comparison of control and intervention group TGMD—III raw scores from baseline to mid-intervention by repeated measures MANOVA, and baseline descriptive information.

	Control (n = 6)		Intervention (n = 8)		Total (n = 14)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
TGMD—III						
Pre-intervention Locomotor	5.000	4.195	4.750	4.683	4.857	4.312
Pre-intervention Ball skills	5.167	3.764	5.625	4.984	5.429	4.345
Pre-intervention Total	10.170	7.574	10.380	8.700	10.290	7.927
Mid-intervention Locomotor	5.800	4.760	8.500	9.040	7.462	7.557
Mid-intervention Ball skills	6.600	3.580	12.250	7.940	10.077	7.017
Mid-intervention Total	12.400	7.700	20.750	16.450	45.000	17.539
Descriptive information						
Height (cm)	104.700	7.562	105.260	6.536	105.050	6.390
Weight (kg)	17.167	4.574	18.400	2.662	17.938	3.230
BMI percentile	70.000	39.509	68.875	29.902	69.308	32.284
ADOS-2 CSS	7.500	1.975	8.286	2.059	7.923	1.977
Gender	3F; 3M		1F; 7M		4F; 10M	
Age (months)	53.833	7.167	53.875	7.019	53.857	6.803
Annual Household Income	1< $24,000		1< $24,999		2< $24,999	
	2 $50,000–$75,000		1 $25,000–$49,999		1 $25,000–$49,999	
	3 missing		1 $50,000–$74,999		3 $50,000–$74,999	
			1> $75,000		1> $75,000	
			4 missing		7 missing	
Race	2 White (33.3%)		5 White (62.5%)		7 White (50%)	
	3 AA (50.0%)		2 AA (25.0%)		5 AA (35.7%)	
	1 Asian (16.7%)		1 Asian (12.5%)		2 Asian (14.3%)	

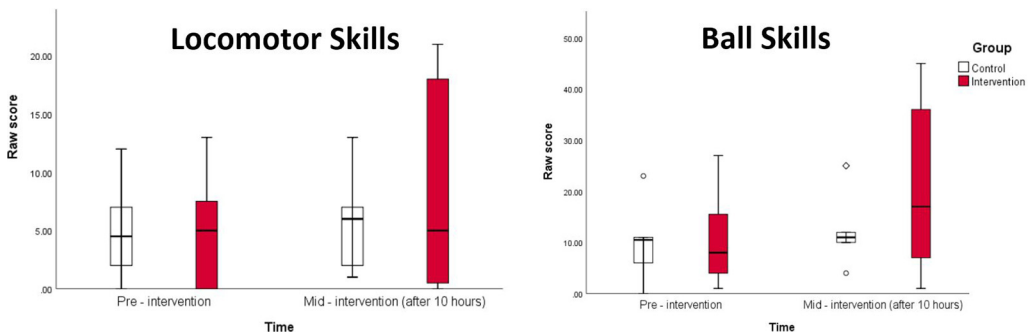

FIGURE 1 | Box-and-whisker plots of total, locomotor, and ball skill raw scores by interventional group at baseline and mid-intervention. Higher scores indicate better motor performance.

term would indicate that intervention group TGMD—III scores had improved by mid-intervention, compared to the control. Box-and-whisker plots visually represent these findings and Partial η^2 were calculated to represent effect sizes. All statistical procedures were carried out in SPSS version 25 (Nie et al., 1970) with a pre-determined alpha of 0.05.

RESULTS

No significant differences arose between groups at baseline, indicating successful randomization (see **Table 1**). The two-factor mixed MANOVA showed an insignificant interaction between time and group for the multivariate vector of the two dependent variables ($F_{(2,10)}$ = 2.436; p = 0.137; ES = 0.328). For univariate interaction effects, ball skills ($F_{(1,11)}$ = 4.640; p = 0.054; ES = 0.297) showed a large but insignificant effect size. The interaction term for locomotor skills ($F_{(1,11)}$ = 1.232; p = 0.291; ES = 0.101) was not significant nor approaching significance, and showed a medium effect size. **Table 1** and **Figure 1** detail these findings.

DISCUSSION

Here, 10 h of direct instruction did not alter FMS in this sample. A study in a similar population used 160 h of intervention, and saw improvements in all subtests on the TGMD—II by 40 h (Ketcheson et al., 2017). Another used 27 h of intervention and saw improvements in most children's catch, roll and strike, run, gallop, jump, and kick; however, the study's size did not allow for group statistics (Bremer and Lloyd, 2016). Finally, another study saw improvements in object control, but not locomotor or total motor scores after 12 h of intervention (Bremer et al., 2015). Of these reports, the current study reports the lowest dosage, yielding trending results for ball skills and total, but

not locomotor scores. Thus, this study adds knowledge that the minimal instruction time for this population lies somewhere above 10 h.

It should be acknowledged that the minimal instructional time question is addressed here independent of pedagogy, which is of utmost importance. FMS interventions to date have employed many pedagogies and drawn upon many different curricula. This consideration is not trivial, and this area should continue to be fervently investigated. However, the paucity of research and practical demands in this area necessitates a simplified discussion first: how much instructional time is necessary to make a difference in FMS, regardless of instructional methods?

The time*group interactions in this study show large effect sizes for ball skills and total scores that were not statistically significant, but approached significance. The interaction for locomotor scores did not approach significance and was of small effect size, suggesting a differential influence of the intervention. Ball skills may require less instruction because, unlike locomotor skills, their equipment communicates the purpose of the movement. This "purpose" information may provide an advantage for young learners with high severity ASD, of which this sample is largely comprised. Interestingly, a similar study previously found a similar differential influence upon locomotor vs. ball skills (Bremer et al., 2015). APE teachers may want to consider this when planning instructional time between these domains; locomotor skill improvement may be slower.

This is the first early FMS intervention to our knowledge that was designed and implemented in the EIBI setting. The implementors in this case were not educated in APE techniques, nor were they intimately familiar with the correct form of the FMS being taught. It is therefore likely that trained APE teachers might be more effective and therefore require less time to influence the FMS of similar participants. In addition, no positive data is presented, instead the current study is an analysis of mid-point results of a larger intervention from which data is forthcoming. Because of this, the current study does not include information on whether the children would have ever learned the motor skills if given enough instructional time. A further limitation is that only two subsets of motor skills were tested due to practical constraints of implementing a study within a functioning clinical center. In fact, one of these subsets (locomotor skills) is already known to be difficult to improve in children with ASD (Bremer et al., 2015). If other components of motor performance could have been tested, results could have been richer. The results approach significance with large effect sizes already at the mid-point, even with these implementors. The results of this study are not necessarily generalizable to every preschooler with ASD, as this sample was comprised of children with relatively severe ASD. Overall, this study adds to a growing body of literature examining methods for impacting the motor development of children with ASD.

IMPLICATIONS

Ten hours of direct FMS instruction (at mid-intervention) was not enough for the improvement of FMS in preschoolers with ASD. This amount and type of treatment approaches sufficiency for alteration of ball skills, but not locomotor skills.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Michigan State University Human Research Protection Program ethics board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

IF-K and JH conceived the study design. IF-K collected data, analyzed the data, and wrote the manuscript. JH collected data and reviewed the manuscript.

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frontiers in Integrative Neuroscience

REVIEW
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What Has Neuroimaging Taught Us on the Neurobiology of Yoga? A Review

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Yoga is becoming increasingly popular worldwide, with several implicated physical and mental benefits. Here we provide a comprehensive and critical review of the research generated from the existing neuroimaging literature in studies of yoga practitioners. We reviewed 34 international peer-reviewed neuroimaging studies of yoga using magnetic resonance imaging (MRI), positron emission tomography (PET), or single-photon emission computed tomography (SPECT): 11 morphological and 26 functional studies, including three studies that were classified as both morphological and functional. Consistent findings include increased gray matter volume in the insula and hippocampus, increased activation of prefrontal cortical regions, and functional connectivity changes mainly within the default mode network. There is quite some variability in the neuroimaging findings that partially reflects different yoga styles and approaches, as well as sample size limitations. Direct comparator groups such as physical activity are scarcely used so far. Finally, hypotheses on the underlying neurobiology derived from the imaging findings are discussed in the light of the potential beneficial effects of yoga.

Keywords: yoga, neuroimaging, PET, SPECT, MRI, neurobiology, connectivity

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INTRODUCTION

The term yoga is derived from a Sanskrit word *yuj* and means "union" or a "method of spiritual union." Yoga originated in India and is a behavioral practice that unites physical and mental training aimed at improving health and promoting personal transformation, with the ultimate goal of attaining *samadhi* (pure consciousness). According to *Patanjali*'s *Sutras*, yoga encompasses the following eight aspects (limbs): *yamas* (ethical guidelines, abstinence from immoral behavior), *niyamas* (self-discipline), *asana* (physical postures), *pranayama* (breath control), *pratyahara* (sensory withdrawal), *dharana* (concentration), *dhyana* (meditation), and *samadhi* (pure consciousness) (Villemure et al., 2014, 2015; Birdee et al., 2016; Eyre et al., 2016; Khalsa et al., 2016). Over the years, dozens of variations in yoga philosophy and styles have emerged. Some yoga styles are structured as a physical workout, while others put an emphasis on meditation. Meditation is a way of focusing and slowing down the stream of thoughts in the mind (Brewer et al., 2011). *Dhyana*,

Sanskrit for meditation, is one of the eight limbs of yoga according to *Patanjali's Sutras* and stresses the fact that yoga and meditation are interrelated. Several meditation techniques originate from yoga and are intrinsically connected with yoga. In addition, to train the mind to focus and prepare for meditation, physical movements of the body and breathing exercises are used and together from the triad of yoga trainings (Birdee et al., 2016).

Whereas yoga has been practiced in the East for thousands of years, it is now rapidly gaining popularity and interest in the Western world (Ivtzan and Jegatheeswaran, 2015). Data from a recent national health interview survey showed that 14.3% of the United States adults have done yoga in the past 12 months (Clarke et al., 2018). Furthermore, yoga practitioners are more likely female, younger, non-Hispanic white, college educated, higher earners, and of better health status (Cramer et al., 2016). The goal for many healthy individuals is to achieve fitness and flexibility, to reduce daily stress and improve energy as part of a healthy, active lifestyle (Barnes et al., 2008; Ivtzan and Jegatheeswaran, 2015; Cramer et al., 2016). Several yoga styles are practiced in western society, and most encompass the abovementioned triad of physical postures, breath control exercises, and meditation, although in many centers the primary focus is put on the *asana* (physical postures) of yoga (Ivtzan and Jegatheeswaran, 2015). There are also different intentions to practice yoga that may vary from purely physical to more spiritual. A recent study showed that the motivations to practice yoga are dynamic. With continued practice, there is a shift in intentions that are set by practitioners from more physical to spiritual development (Ivtzan and Jegatheeswaran, 2015).

On the other hand, yoga is also regularly practiced by people with physical and mental health problems. Cramer et al. (2016) performed an analysis on the cross-sectional data from the 2012 National Health Interview Survey. In this survey, most yoga practitioners reported positive outcomes resulting from their yoga practice, predominantly citing reduced stress, improved overall health, improved emotional well-being, improved sleep, and increased sense of control over their health. In addition to these subjective self-reported benefits, there is a growing body of clinical research studies on beneficial medical and psychological effects of yoga. Physiological effects of yoga include a decreased heart rate and blood pressure, and increased muscle strength (Hagins et al., 2014; Vardar Yağlı et al., 2015; Chu et al., 2016). Furthermore, improvement of depressive, anxious, and stressful states and relief in pain conditions has been demonstrated in several studies (Woolery et al., 2004; Cramer et al., 2013; Danucalov et al., 2013; Riley and Park, 2015; Wieland et al., 2017). Yoga may thus also offer a complementary auxiliary approach in various central nervous system (CNS) disorders such as depression, anxiety, posttraumatic stress disorder (PTSD) but also in schizophrenia and cognitive decline (Vancampfort et al., 2012; Cramer et al., 2013; Riley and Park, 2015; Brenes et al., 2018). Mostly, these effects are measured with self-reported questionnaires before, during, and after a yoga intervention and such an approach may be prone to bias and subjectivity. Non-invasive and easily accessible (bio)markers, for example blood pressure, medication use, and heart rate (variability), have been used as measurements of interest but remain aspecific.

There is growing interest in elucidating the neurobiological underpinnings of yoga. There are different methods to non-invasively investigate the neurobiology of the human brain including magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), electroencephalography (EEG), magnetoencephalography (MEG), transcranial direct-current stimulation (TDCS), transcranial magnetic stimulation (TMS), and blood biomarker analysis. For this review, our focus is on neuroimaging studies using MR-based techniques, PET, and/or SPECT, since these non-invasive modalities allow to to investigate the brain's structure and function *in vivo* and network functional effects *in vivo* with high spatial resolution (MRI) and sensitivity (PET).

The main purpose of the present review is to provide an overview of the existing structural and functional neuroimaging studies of yoga in a critical perspective, by examining the magnitude and the consistency of reported cerebral effects. We will address inconsistencies, highlight the gaps in our current knowledge, and discuss how better study designs and imaging probes may be able to address these.

In vivo BRAIN IMAGING TECHNIQUES

MRI, PET, and SPECT have been the medical neuroimaging techniques of choice to investigate the effects of yoga on the brain.

MRI refers to a family of imaging techniques that use strong magnetic fields, field gradients, and radio waves to excite hydrogen atoms in tissues and read out signals, depending on the properties of these tissues (Hendee and Morgan, 1984). The main advantages of MRI are its (sub)millimeter spatial resolution, non-invasiveness, and ability to discriminate tissues using their physical and biochemical properties. On the other hand, a relatively low sensitivity is a main disadvantage (Catana et al., 2013). MRI can be used to assess both cerebral structural and functional effects. Structural MRI (sMRI) provides anatomical information, delineating different tissues and brain structures, and can be used to assess volumetric changes or differences between populations. To assess and investigate possible changes in the white matter (WM) of the brain, diffusion tensor imaging (DTI) is commonly used (Sasson et al., 2010). DTI is sensitive to the diffusion characteristics of hydrogen. When diffusion is not restricted, hydrogen diffuses the same amount in all directions; the diffusion is called "isotropic." In contrast, when hydrogen diffusion is restricted and dominated by one direction, the diffusion is called "anisotropic." In WM, diffusion tends to be anisotropic due to the axon walls and myelin sheaths surrounding the axon, which is picked up in DTI, which is thus well-suited to investigate WM tract integrity. In addition to sMRI, functional MRI (fMRI) measures can be acquired, including resting-state or task fMRI, functional connectivity fMRI, magnetic resonance spectroscopy (MRS), and arterial spin labeling (ASL). *In vivo* MRS is a non-invasive specialized technique associated with MRI, where the differences in resonant frequency of various chemical compounds are measured and observed, in order to identify the MRS spectrum. This MRS spectrum reflects the biochemical

composition of a region of interest (or whole brain by voxel-based measurements), and each metabolite is identified by its unique position (Henning, 2018): GABA, glutamate + glutamine (Glx), reflecting major inhibitory and excitatory neurotransmitters, glycerophosphocholine + phosphocholine (Cho), a marker of cell membrane syntheses and breakdown, and N-acetyl aspartate and N-acetylaspartyl-glutamate (NAA/NAAG), a marker of neuronal and axonal viability and density (Verma et al., 2016). In resting-state or task fMRI, regional brain activity is detected based on blood oxygenation level-dependent (BOLD) changes in brain tissue that change with neuronal activity status (Chen and Glover, 2015). Correlation patterns of the activated regions can be identified with a functional connectivity analysis. Several networks have been robustly identified, including the default mode network (DMN), which is active in the absence of an attention-required task (Fox and Greicius, 2010), and the central executive (frontal) network (Trotta et al., 2018). Indirect neuronal functioning can be measured using ASL measuring absolute cerebral perfusion. In this technique, incoming arterial blood is magnetically labeled and the signal difference between labeled and unlabeled control images is proportional to regional perfusion (Haller et al., 2016).

PET is a molecular functional nuclear medicine imaging technique with very high (nanomolar) sensitivity. PET imaging makes use of a radioactively labeled radioligand administered in tracer quantities. Radiotracers consisting of a positron-emitting radioistope are bound to a compound that binds to target molecules such as neuroreceptors, reuptake transporters, or intracellular and extracellular proteins. Positron emission is located through measurement of coincident annihilation gamma rays detected by scintillator or solid-state PET-scan detectors (Sossi, 2018), and a quantitative physiological measure can be obtained such as receptor density or glucose metabolization rate. Although spatial resolution is less than that of sMRI, current equipment allows 3–4 mm spatial resolution (Vandenberghe et al., 2016).

Similarly, SPECT imaging is also used to visualize molecular and functional brain processes. In contrast to PET, the tracers are labeled with a single-photon emitting radioisotope. SPECT imaging provides quantitative images of the radiotracer distribution using a rotating scintillator gamma camera. In functional brain studies, SPECT has mainly been used to study regional brain perfusion (Goffin and van Laere, 2016).

REVIEW METHOD AND SEARCH STRATEGY

Articles were identified using MEDLINE[1] until the end of 2019, limiting the search to yoga and neuroimaging studies, using the following keywords: "yoga AND (magnetic resonance OR positron emission tomography OR single photon emission computed tomography OR MR OR PET OR SPECT or functional connectivity)." The obtained list of articles was afterward refined and screened for inclusion of at least one of the following words in the abstract: "magnetic resonance OR MR," "functional magnetic resonance imaging OR fMRI," "positron emission tomography OR PET," "single photon emission computed tomography OR SPECT," or "brain" AND "yoga" within the title or abstract (see below the study inclusion and exclusion criteria). Of the remaining results, all the abstracts were verified to describe neuroimaging methods in the investigation of cerebral effects of yoga.

We considered all studies using structural and functional neuroimaging to investigate different forms of yoga styles that met the following inclusion criteria: (i) articles that were available in English, German, Dutch, or French; (ii) articles that used neuroimaging techniques; and (iii) articles published in peer-reviewed scientific journals.

Studies were excluded in case of (i) individual case reports; (ii) effects of one-time practitioners; (iii) review articles; (iv) using neurophysiological techniques other than MRI, PET, or SPECT (e.g., EEG mapping, MEG, etc.).

RESULTS

Study Selection and Classification

A total of 93 studies were identified and reviewed. About two-thirds (59 of 93) of all studies did not meet the inclusion criteria, mainly because these studies did not focus on yoga itself or did not investigate neuronal effects, ending with a final set of 34 studies.

The included studies used different styles of yoga, which are briefly outlined in the Supplementary Appendix: Ashtanga, Iyengar, Vinyasa, Kripalu, Kundalini, Nidra, Sahaja, Sivananda, and Hatha yoga. The selected 34 articles were straightforward classified as "*structural/morphological neuroimaging studies*," which assessed structural brain changes by sMRI (n = 11, **Table 1**), or "*functional neuroimaging studies*," which assessed brain function, activation studies, or molecular targets (n = 26 studies, **Table 2**). The latter comprised MRS, task-based fMRI, resting-state fMRI (rsfMRI) with connectivity analysis, ASL, PET, and SPECT. Three of the 34 included articles used a combination of structural and functional imaging and were included in both sections. Six pairs of articles conducted different analyses in the same subjects and were included in both the structural and functional study sections.

Structural/Morphological Neuroimaging Studies on Yoga

All structural studies carried out in yoga practitioners between 2009 and 2019 performed voxel-based morphometry (VBM) analysis. VBM is a technique to quantitatively assess gray matter (GM) volume in predefined regions of interest (ROIs) or concentration (density) differences throughout the brain on a voxel-by-voxel or ROI basis (Hutton et al., 2009). Additionally, a voxel-based cortical thickness (VBCT) analysis can be performed, where cortical thickness is assessed by calculating the distance between the resulting WM-GM surface and GM-cerebral spinal fluid surface data (Lüsebrink et al., 2013).

[1] http://www.ncbi.nlm.nih.gov/pubmed/

TABLE 1 | Morphological brain imaging studies on yoga.

Imaging technique	Effect	Design	Yoga style	Number (♀), age (mean ± SD), yrs	Control group Control condition	Study
MRI-VBM	WB GMD	CSS	Hatha Yoga meditation (HYM) (MY)	HYM: 7 (6), 36.4 ± 11.9 CON: 7 (6), 35.5 ± 7.1	Non-meditators vs. yoga	Froeliger et al., 2012b
MRI-VBM MRI-VBCT MRI-DTI	WB GMD, GMT, ROI WM	CSS	Unspecified yoga (3Y)	YOG: 14 (9), 37.0 ± 6.6 CON: 14 (9), 36.7 ± 7.3	Non-meditators vs. yoga	Villemure et al., 2014
MRI-VBM	WB GMD	CSS	Unspecified yoga (3Y)	YOG: 14 (9) 37.0 ± 6.6 CON: 14 (9) 36.7 ± 7.3	Non-meditators vs. yoga	Villemure et al., 2015
MRI-VBM	WB GMD	CSS	Sahaja Yoga meditation (SYM)(YM)	SYM: 23 (17), 46.5 ± 11.4 CON: 23 (17), 46.9 ± 10.9	Non-meditators vs. yoga	Hernández et al., 2016
MRI-VBM	WB GMD	LS	Sahaja yoga meditation (SYM)(MY)	SYM: 12 (2): 21.6 ± 2.0 CON: 30 (12): 22.2 ± 1.3	SYM training vs. control group (waiting period)	Dodich et al., 2019
MRI-VBCT	WB GMT	CSS	Hatha Yoga (3Y)	YOG: 21 (21), 66.2 ± 4.5 CON: 21 (21), 67.9 ± 4.6	Non-meditators vs. yoga	Afonso et al., 2017
MRI-VBM	ROI GMV	CSS LS	Meditation and yoga practices (MY/3Y)	YOG: 289 (211), 61.9 ± 6.8 CON: 3453 (1830), 64.3 ± 7.7	Controls vs. practitioners of yoga, meditation and breathing exercises	Gotink et al., 2018
MRI-VBM	ROI GMD	LS	Yoga (3Y)	YOG: 7 (3), 69–81*	Before vs. after yoga training	Hariprasad et al., 2013
MRI-VBM	ROI GMD	CSS	Hatha, iyengar and kundalini yoga (3Y)	YOG: 13 (12), 35.8 ± 15.4 CON: 13 (12), 35.7 ± 14.6	Controls vs. yoga practitioners	Gothe et al., 2018
MRI-VBM	ROI GMD	LS	MBSR program (including yoga) (MY/3Y)	YOG: 27 (16), 35.2 ± 6.7	Before vs. after MBSR training	Hölzel et al., 2009
MRI-VBM	ROI GMV	LS	Combination of meditation and Kundalini yoga (MY/3Y)	YOG: 14 (6), 67.1 ± 9.5 CON: 11 (6), 67.8 ± 9.7	MET vs. yoga in MCI patients	Yang et al., 2016

*Imaging Technique: DTI, diffusion tensor imaging; MRI, magnetic resonance imaging; VBM, voxel-based morphometry; VBCT, voxel-based cortical thickness. Effect: GMD, gray matter density; GMV, gray matter volume; GMT, gray matter thickness; GM, gray matter; ROI, region of interest; WB, whole-brain; WM, white matter. Design: CSS, cross-sectional study; LS, longitudinal study. Yoga styles: 3Y, triad of yoga (including physical postures, breathing exercises, and meditation); HYM, Hatha Yoga meditation; MBSR, mindfulness-based stress reduction; MY, meditation yoga; SYM, Sahaja Yoga meditation. Number: SD, standard deviation. Control group Control condition: MCI, mild cognitive impairment; MET, memory enhancement training. *No information on mean age and SD.*

Several studies investigated morphological effects conducting a whole-brain analysis without *a priori* restriction to particular ROIs. Froeliger et al. (2012b) found higher GM density in a small group of experienced Hatha yoga meditation practitioners (n = 7) compared to a sex-, age-, and education-matched control group (n = 7). Particularly, higher GM density was found in the medial frontal gyrus, superior frontal gyrus, precentral gyrus, (para)hippocampal gyrus, insula, superior temporal gyrus, occipital gyrus, and cerebellum (Froeliger et al., 2012b). Similarly, greater GM density in cortical regions were also identified by Villemure et al. (2014), conducted in a group of experienced yoga practitioners (n = 14) vs. physically active controls (n = 14; matched in terms of sex, age, body mass index, handedness, education, and exercise level outside of yoga). These regions comprised cingulate gyrus, superior frontal gyrus, inferior parietal lobule, and insula. In addition, VBCT showed three brain regions with significantly increased GM thickness in yoga participants, including cingulate cortex, insular cortex, and primary somatosensory cortex, in agreement with the VBM analysis (Villemure et al., 2014). In a follow-up study in the same subjects, the authors also explored whether a yoga practice had a neuroprotective effect by comparing age-related GM decline (Villemure et al., 2015). They found a significant negative correlation between age and whole-brain GM density for controls, consistent with known atrophy effects of healthy aging, but this decline was not present in yoga practitioners. Furthermore, the years of yoga experience and hours of weekly yoga practice were positively correlated with regional GM density (Villemure et al., 2015). In experienced Sahaja yoga meditation participants (n = 23) compared to controls (n = 23), significantly larger GM density was found in the insula, ventromedial orbitofrontal cortex, and medial inferior temporal gyrus (Hernández et al., 2016). In subjects practicing the same yoga style, Dodich et al. (2019) investigated the modulation of a short-term yoga meditation training (four 1-hour sessions per week over four consecutive weeks), by randomly assigning 42 healthy meditation-naïve adults to either a control group (wait-list; n = 30) or a yoga group (n = 12). Compared to the controls,

TABLE 2 | Functional brain imaging studies on yoga.

Imaging technique	Effect	Design	Yoga style	Number (♀), age (mean ± SD), yrs	Control group Control condition	Study
A. Cerebral perfusion and glucose metabolism						
^{18}F-FDG PET	Glucose metabolism	CSS	Yoga meditation (YM)	YOG: 8 (2), 32, 21–39*	Wakeful condition in the same subjects	Herzog et al., 1991
$H_2{}^{15}O$ PET	CBF	CSS	Yoga Nidra (YM)	YOG: 9 (3), 23–41*	Control states in the same subjects	Lou et al., 1999
^{99m}Tc-ECD SPECT	CBF	LS	Iyengar yoga (3Y)	YOG: 4 (2), 45.0	Pre-program baseline scan	Cohen et al., 2009
^{99m}Tc-HMPAO SPECT	CBF	CSS	Kundalini chanting (YM)	YOG: 11 (5), 35.4 ± 13.5	BL in same subjects	Khalsa et al., 2009
fMRI ASL	CBF	CSS	Kundalini meditation (YM)	YOG: 10 (4), 53.7*	BL in same subjects	Wang et al., 2011
B. Neural activation						
fMRI	Neural activation	CSS	Kundalini, Acem tradition (MY)	YOG: 8 (5), 34.6 ± 9.7	BL in same subjects	Engström et al., 2010
fMRI	Neural activation	CSS	OM chanting (MY)	YOG: 12 (3), 28 ± 6	Production of "ssss. . ."	Kalyani et al., 2011
fMRI	Neural activation	CSS	Sahaja yoga meditation (MY)	YOG: 19 (11), 46.6 ± 9.5	Attention on breathing	Hernández et al., 2015
fMRI	Neural activation	CSS	*Patanljali* yoga: Yoga meditation (MY)	YOG: 4 (0), mid 60s*	Relaxation (control condition) vs. meditation	Mishra et al., 2017
fMRI	Neuronal fluctuations	LS	Hatha Yoga (3Y)	CON: 12 (12), 16–60* YOG: 23 (23), 16–60* AE: 23 (23), 16–60*	Patients with schizophrenia: control wait-list vs. yoga vs. aerobic exercises (AE)	Lin et al., 2017
fMRI	Neural activation	CSS	Undefined	YOG: 19 (16), 35.9 ± 11.5 CON: 12 (6), 32.9 ± 9.1	Recreational athletes vs. yoga practitioners (YP)	Wadden et al., 2018
fMRI	Neural activation	CSS	Hatha meditation (MY)	YOG: 7 (6), 36.4 ± 11.9 CON: 7 (6), 35.5 ± 7.1	Meditation-naive vs. controls	Froeliger et al., 2012c
fMRI	Neural activation	CSS	Hatha, iyengar, and kundalini (MY/3Y)	YOG: 13 (12), 35.8 ± 15.4 CON: 13 (12), 35.7 ± 14.6	Controls vs. yoga practitioners	Gothe et al., 2018
C. Functional connectivity						
fMRI	Functional connectivity	CSS	Hatha meditation (MY)	YOG: 7 (6), 36.4 ± 11.9 CON: 7 (6), 35.5 ± 7.1	Meditation-naive vs. controls	Froeliger et al., 2012a
fMRI	Functional connectivity	CSS	Sahaja yoga meditation (SYM)(YM)	SYM: 23 (17), 46.5 ± 11.4 CON: 23 (17), 46.9 ± 10.9	Meditation state vs. resting state	Hernández et al., 2018
fMRI	Functional connectivity	LS	MBSR program (including yoga) (MY/3Y)	MBSR: 18 (10): 37.5 ± 9.1 RR: 16 (9): 39.9 ± 10.3	MBSR vs. relaxation response RR training	Sevinc et al., 2018
fMRI	Functional connectivity	CSS	Hatha yoga (3Y)	CON: 20 (20), 68.2 ± 4.6 YOG: 20 (20), 66.5 ± 4.5	Elderly yoga practitioners vs. healthy yoga-naïve controls	Santaella et al., 2019
fMRI	Functional connectivity	LS	Meditation/Kundalini yoga (MY/3Y)	YOG: 14 (6), 67.1 ± 9.5 MET: 11 (6), 67.8 ± 9.7	MET vs. yoga in MCI patients	Eyre et al., 2016
fMRI	Functional connectivity	LS	Sahaja yoga meditation (SYM)(MY)	SYM: 12 (2): 21.6 ± 2.0 CON: 30 (12): 22.2 ± 1.3	SYM training vs. control group (waiting period)	Dodich et al., 2019
fMRI	Functional connectivity	CSS	Kripalu yoga, Vipassana meditation (MY/3Y)	YOG: 16 (11), 49.4 ± 7.8 MED: 16 (10), 54.1 ± 8.1 CON: 15 (9), 52.9 ± 9.8	Meditation vs. yoga vs. controls	Gard et al., 2014

(Continued)

TABLE 2 | Continued

Imaging technique	Effect	Design	Yoga style	Number (♀), age (mean ± SD), yrs	Control group Control condition	Study
D. Brain metabolites and neurotransmitters						
^{11}C-raclopride PET	Dopamine release	CSS	Yoga Nidra (MY)	YOG: 8 (0), 31–50*	Wakeful condition in same subjects	Kjaer et al., 2002
MRI-MRS	GABA	CSS	Yoga (3Y)	YOG: 8 (7), 25.8 ± 5.2 CON: 11 (5), 26.6 ± 7.6	Reading exercise	Streeter et al., 2007
MRI-MRS	GABA	LS	Iyengar yoga (3Y)	YOG: 19 (11), 23.9 ± 3.0 CON: 15 (11) 25.6 ± 4.9	Walking group	Streeter et al., 2010
MRI-MRS	NAA and MI	CSS	Yoga postures and breathing exercises (3Y)	YOG: 34 (?), 35–65* CON: 34 (?), 35–65*	Type 2 diabetes patients: Yoga + standard care vs. standard care	Nagothu et al., 2015
MRI-MRS	NAA and MI	CSS	Yoga postures and breathing exercises (3Y)	YOG: 5 (?), 35–55* CON: 5 (?), 35–55*	Type 2 diabetes patients: Yoga + standard care vs. standard care	Santhakumari et al., 2016
MRI-MRS	Metabolites	LS	Meditation/Kundalini yoga (MY/3Y)	YOG: 14 (6), 67.1 ± 9.5 CON: 11 (6), 67.8 ± 9.7	MET vs. yoga in MCI patients	Yang et al., 2016

*Imaging technique: ASL, arterial spin labeling; FDG, fluorodeoxyglucose; (f)MRI, (functional) magnetic resonance imaging; MRS, magnetic resonance spectroscopy; PET, positron emission tomography; SPECT, single-photon emission computed tomography. Effect: CBF, cerebral blood flow; GABA, γ-aminobutyric acid; MI, myoinositol; NAA, N-acetyl aspartate. Design: CSS, cross-sectional study; LS, longitudinal study. Yoga styles: 3Y, triad of yoga (including physical postures, breathing exercises and meditation); MBSR, mindfulness-based stress reduction; MY, meditation yoga. Number: SD, standard deviation. Control group/control condition: MET, memory enhancement training. *No information on mean age and SD.*

the yoga group showed increased GM density in the inferior frontal gyrus (pars orbitalis), correlated with general well-being after yoga training. Finally, in another study, Afonso et al. (2017) found increased cortical thickness in the left prefrontal area in 21 healthy elderly female experienced (practicing at least two times a week for a minimum of eight years) Hatha yoga practitioners compared to 21 age-, education-, and physical activity-matched controls.

Based on defined *a priori* hypotheses on behavioral effects of yoga such as reduction of anxiety and increased attention and their functional anatomical relationship, other studies analyzed structural MR data focusing on effects within specific ROIs, including the hippocampus, amygdala, or dorsal anterior cingulate cortex. Gotink et al. (2018) investigated the effect of meditation and yoga on the amygdala (as a central relay structure in emotional memory, fear, and anxiety processing) and hippocampus. In this large population-based study, including 3742 participants, of whom 289 practiced at least one hour of meditation or yoga per week for at least one year, a decrease in GM volume was observed in the left hippocampus and right amygdala (Gotink et al., 2018). It is worth mentioning that no distinction was made between yoga and meditation practice in this study, therefore obscuring the exact origin of the observed effects. The same study also described an additional analysis in a subsample of 218 subjects that had undergone a previous MR scan five years earlier and have been practicing yoga or meditation for five years or longer. In this longitudinal study, only an interaction between years of yoga/meditation experience and decreased volume of the amygdala was observed, without differences in the hippocampus (Gotink et al., 2018). Similar results were found in a small yoga intervention study (including physical postures, breath control exercises, and meditation) using VBM, performed in healthy elderly subjects (n = 7), without a control group (Hariprasad et al., 2013). GM density changes in the hippocampus as *a priori* ROI were examined after the yoga intervention of six months. The hippocampus is known to be affected by GM loss with aging, and previously elevations in serum brain-derived neurotrophic factor (BDNF) had been demonstrated in this region. Increased GM density in the hippocampus post-yoga intervention was found compared to baseline (Hariprasad et al., 2013). Based on the former results, Gothe et al. (2018) explored differences in GM density of the hippocampus specifically in 13 experienced yoga practitioners and 13 age- and sex-matched controls. The experienced yoga group contained Hatha, Kundalini, and Iyengar yoga practitioners. GM density was higher in the left hippocampus in the yoga group compared to controls (Gothe et al., 2018). Other structural imaging research focused on both the hippocampus and amygdala in order to investigate the effects of an 8-week mindfulness-based stress reduction (MBSR) intervention in subjects with elevated stress (n = 26), consisting of weekly group meetings and daily home mindfulness practices, including sitting meditation and yoga (Hölzel et al., 2009). Differences in the perceived stress scale were related to changes in GM density within the right amygdala (Hölzel et al., 2009). Finally, in a particular study, aiming at investigating neuroanatomical and neurochemical plasticity following either memory training or a yoga intervention in mild cognitive impairment (MCI) patients, Yang et al. (2016) conducted a longitudinal MR study to measure GM volume within the bilateral hippocampus and dorsal anterior

cingulate over 12 weeks. In total, 25 subjects were included and randomized to either the yoga group ($n = 14$) or the control group [memory enhancement training group ($n = 11$)]. An interaction between group and time was found: only the MCI patients who followed memory training sessions showed a trend toward increased volume in the dorsal anterior cingulate cortex after training (Yang et al., 2016).

Only one study has investigated the effects of yoga on WM integrity. In this study, Villemure et al. (2014) performed an ROI-based DTI analysis of the insular cortex in an experienced yoga cohort ($n = 14$) compared to controls ($n = 14$). They found higher fractional anisotropy (FA) in WM adjacent to the left insula compared to controls. Probabilistic tractography was used to track the WM pathway passing through this area, showing increased connectivity of the anterior and posterior insular regions. According to the authors, this might represent increased intrainsular connectivity in the yoga subjects (Villemure et al., 2014).

We have pooled the study specifics of the morphological studies in **Table 1**. Furthermore, **Figure 1A** shows a map of the different brain regions where morphological effects of yoga have been reported in the included studies. The insular cortex and hippocampus were the most frequently described regions with morphological thickening.

Functional Neuroimaging Studies on Yoga

Twenty-six studies have addressed the functional effects of yoga with neuroimaging, of which 21 studies used MR-based techniques, three studies used PET (including assessments of glucose metabolism—with ^{18}F-fluorodeoxyglucose (^{18}F-FDG), cerebral blood flow or perfusion—with ^{15}O-H_2O, and dopaminergic functioning—with ^{11}C-raclopride), and the two remaining studies used SPECT perfusion. We have grouped these studies in four coherent domains: effects on (mainly) resting-state function (perfusion and glucose metabolism), brain activation studies, network function (connectivity analyses), and molecular changes (MRS and neurotransmitters).

Effects of Yoga on Cerebral Perfusion and Glucose Metabolism

Changes in neuronal resting-state function in yoga subjects have been investigated in one glucose-metabolism PET study, one perfusion PET study, two perfusion SPECT studies, and one perfusion ASL MR study (**Table 2A** and **Figure 1B**).

Herzog et al. (1991) performed historically the first PET imaging study in yoga subjects, aiming to find possible alterations of cerebral glucose metabolism. A group of eight experienced healthy yoga meditation volunteers were scanned with ^{18}F-FDG PET in a 2-day protocol, during a normal wakeful resting control state and a yoga meditative relaxation state. Absolute regional cerebral metabolic rates of glucose (rCMRGlc) were obtained with dynamic ^{18}F-FDG PET imaging and arterial blood sampling, but no significant differences were found. Several significant regional differences of rCMRGlc ratios were found with increased glucose metabolism in the prefrontal to occipital ROIs, prefrontal to occipitotemporal ROIs, and superior frontal to superior parietal ROI ratio. Furthermore, during the state of yoga meditative relaxation, a smaller intersubject coefficient of variation was observed (Herzog et al., 1991).

In the first cerebral perfusion study, Lou et al. (1999) examined the neural regions subserving Nidra yoga meditation in nine experienced subjects using ^{15}O-H_2O PET. Subjects were injected eight times with ^{15}O-H_2O: two during an normal resting state, two during a resting state with auditory stimulation, and four during meditation, which was induced and maintained by auditory stimulation. While the global cerebral blood flow (CBF) remained unchanged throughout the experiment, a regional altered CBF pattern of meditation was observed according to the meditative content. Additionally, differential regional CBF activity upon meditation was found in regions thought to support an executive attentional network, such as the orbital and dorsolateral prefrontal gyrus, anterior cingulate gyrus, temporal gyrus, pons, and cerebellum (Lou et al., 1999). With the use of ^{99m}Tc-ECD (ethylcysteine dimer or bicisate) SPECT, perfusion was also examined in a small longitudinal, interventional study where participants ($n = 4$) underwent a 12-week Iyengar yoga training program (Cohen et al., 2009). Each participant received both a pre-program baseline and meditation scan and a post-program baseline and meditation scan. A significant decrease between the mean CBF ratios in the pre- and post-program baseline scans was detected in the following ROIs: amygdala, dorsal medial frontal cortex, and precentral and postcentral gyrus. Significant differences in pre- and post-program percentage change were observed in the medial frontal gyrus, prefrontal cortex (PFC), precentral gyrus, postcentral gyrus, and inferior and superior frontal gyrus (Cohen et al., 2009). ^{99m}Tc-HMPAO (hexamethylpropylene amine oxime or exametazime) SPECT was performed to examine perfusion changes during a Kundalini yoga chanting meditation in 11 experienced meditation participants (Khalsa et al., 2009). This study showed decreased rCBF during meditation compared to the control condition in the middle occipital, superior parietal, inferior temporal, and medial frontal gyrus. In contrast, during the meditation an activation was found in the posterior cingulate gyrus and left superior temporal gyrus (Khalsa et al., 2009). Finally, Wang et al. (2011) used ASL to investigate perfusion differences between baseline and two yoga/meditation states in Kundalini meditators ($n = 10$). In this study, the different meditation states (a "*focused-based*" practice and a "*breath-based*" practice) were compared to a pre-meditation and post-meditation baseline state. During the focused-based meditation task, increased CBF was observed in the medial frontal gyrus and caudate nucleus, while decreased CBF was detected in the inferior and superior occipital gyrus and inferior parietal lobule compared to the pre-meditation baseline control condition. Several cortical and limbic brain structures showed significant increases in CBF during the breath-based meditation task compared to the pre-meditation control state, including insula, amygdala, hippocampus, parahippocampus, and superior temporal gyrus, indicating different CBF patterns between the two meditation states. Moreover, strong positive correlations were observed between reported depth of meditation and increased CBF responses in the insula, inferior frontal

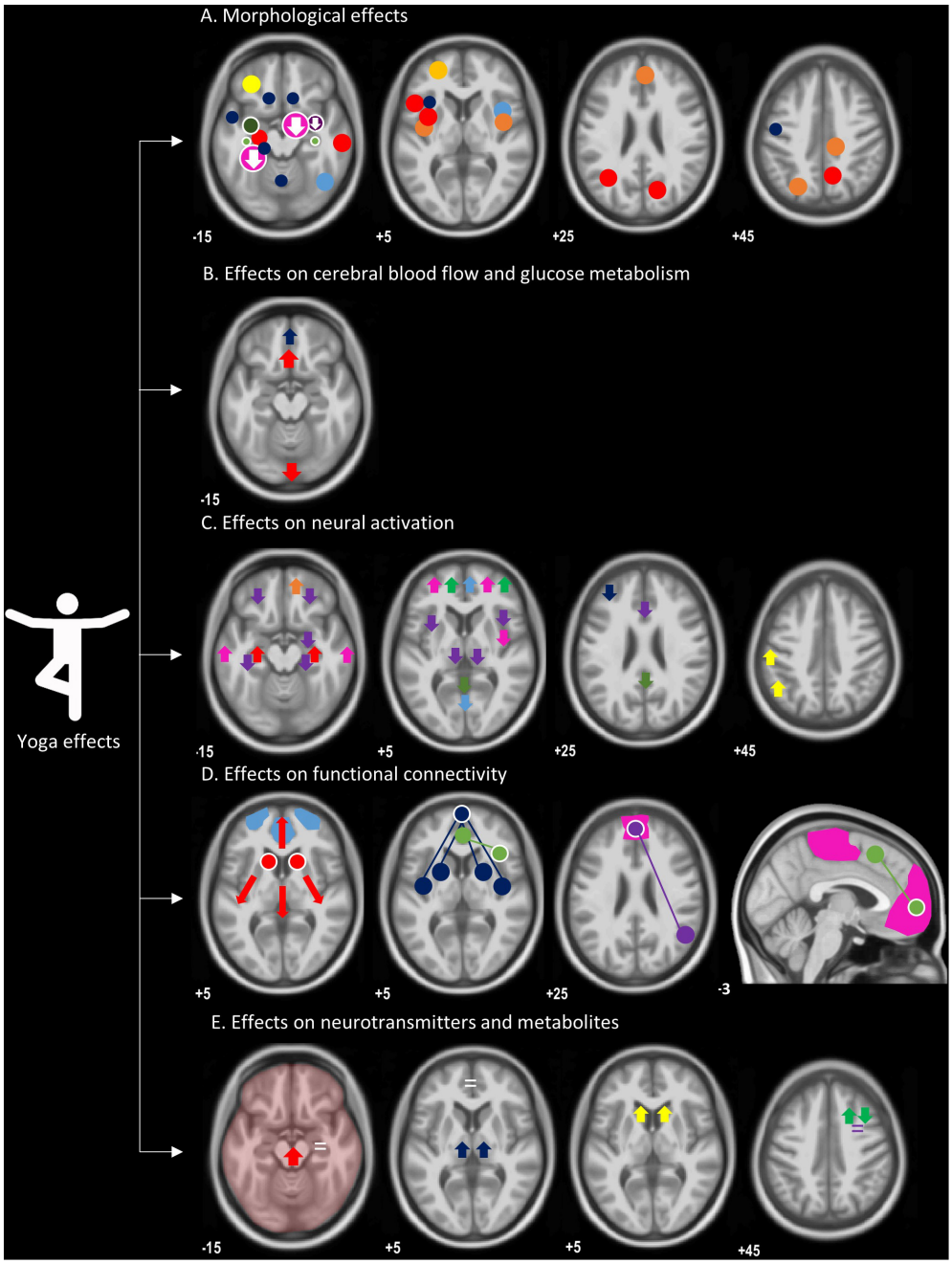

FIGURE 1 | An overview of morphological and functional effects of yoga, overlaid on a T1-weighted image. **(A)** Morphological effects: circles indicate regions with increased gray matter volume or density; regions with a decrease are represented by an arrow pointing down. Both whole-brain and ROI analyses are included, represented by circles without or with a white border, respectively. Size of the circles are a representation of group sizes: *light blue* (Hernández et al., 2016), *dark blue* (Froeliger et al., 2012b), *orange* (Villemure et al., 2014), *red* (Villemure et al., 2015), *yellow* (Dodich et al., 2019), *dark yellow* (Afonso et al., 2017), *pink* (Gotink et al., 2018), *dark green* (Gothe et al., 2018), *light green* (Hariprasad et al., 2013), *purple* (Hölzel et al., 2009), not in this figure (Yang et al., 2016). **(B)** Neuronal resting-state function: *red* (Herzog et al., 1991), *blue* (Khalsa et al., 2009), not in this figure (Lou et al., 1999; Cohen et al., 2009; Wang et al., 2011). **(C)** Neural activation: *red* (Engström et al., 2010), *purple* (Kalyani et al., 2011), *green* (Froeliger et al., 2012c), *pink* (Hernández et al., 2015), *orange* (Mishra et al., 2017), *yellow* (Wadden et al., 2018), *dark blue* (Gothe et al., 2018), and *light blue* (Lin et al., 2015). **(D)** Functional connectivity, seed regions (circles with a white border) connected (full lines) with brain regions (circles), representing higher functional connectivity: *red* (Gard et al., 2015); higher degree centrality for caudate nuclei, *blue* (Hernández et al., 2018), *green* (Sevinc et al., 2018), *pink* (Eyre et al., 2016), *purple* (Santaella et al., 2019), *light blue* represents a network with a change in power spectrum (Dodich et al., 2019), not in this figure (Froeliger et al., 2012a). **(E)** Neurotransmitters and metabolites: *yellow*, increased dopamine signaling (Kjaer et al., 2002); *red*, increased GABA without exact location (Streeter et al., 2007); *dark blue* (GABA levels) (Streeter et al., 2010); *green*, increased N-acetyl-aspartate (NAA) and decrease myoinositol (MI) (Nagothu et al., 2015); *purple*, no change in MI or NAA (Santhakumari et al., 2016); *white*, no change in metabolite levels in hippocampus and dorsal anterior cingulate cortex (Yang et al., 2016).

cortex, and temporal pole during the second meditation task, which had positive associations with subjective ratings of both connectedness and depth of meditation as well as a negative association with perceived stress (Wang et al., 2011).

Effects of Yoga on Neural Activation

Several fMRI studies investigated brain activations or fluctuations in yoga practitioners (**Table 2B** and **Figure 1C**).

Four studies acquired fMRI data during yoga meditation or breathing exercises. Firstly, in a cross-sectional fMRI study performed on eight Kundalini yoga meditation subjects (with less than two years of meditation practice), Engström et al. (2010) investigated whether moderately experienced yoga subjects would display activation in specific brain regions including the hippocampal formation and PFC with an on–off design (meditate vs. word condition). Engström et al. (2010) found activation in different regions when the subjects were meditating using a mantra, compared to pronouncing neutral words (control condition). The most significant activation during the meditation task was found in the bilateral hippocampus/parahippocampal formation. Other areas with significant neuronal activation were found in the middle cingulate cortex and the precentral cortex (Engström et al., 2010). Secondly, changes in activity in the hippocampal formation were also found in a somewhat atypical yoga study where the hemodynamic correlates of "*OM*" chanting were investigated. Healthy participants (n = 12, only four of these had formal training in yoga including meditation) were trained in chanting "*OM*" and were scanned during this activation condition (Kalyani et al., 2011). The continuous production of "*ssss*. . ." was used as control condition. *A priori* ROIs included the limbic brain regions, orbitofrontal cortex, anterior cingulate cortex, and thalami, based on the expected deactivation of these regions due to the vagal effect of the "OM" chanting. Significant deactivation was observed during "*OM*" chanting in the amygdala, anterior cingulate gyrus, hippocampus, insula, orbitofrontal cortex, parahippocampal gyrus, and thalamus (Kalyani et al., 2011). Thirdly, Hernández et al. (2015) investigated increased brain activation during Sahaja yoga meditation. In the control condition, meditators focused their attention on breathing, breathing movements, not worrying, and being relaxed, which is known as mindfulness meditation. After exploring the neural activity pattern with fMRI during yoga meditation (n = 19), they found an increased neural activity compared to the control condition in the medial frontal gyrus, anterior cingulate gyrus, and inferior frontal gyrus, insula, superior temporal gyrus, and medial temporal gyrus. The extent of activation relative to the control condition appeared to diminish progressively with later and deeper meditation stages. The authors suggested that this may be due to more focused activation. Additionally, the authors reported that the activation in the temporal lobes may be due to the presence of music during the meditation condition, in contrast to its absence during the control relaxation condition (Hernández et al., 2015). Fourthly, in parallel with the findings of Hernández et al. (2015), increased activation in the right prefrontal regions during a meditation phase (auditory fixation and visual fixation) was observed in four experienced *Patanjali* yoga practitioners. The authors did not report any statistics, detailed demographics, or scan acquisition parameters (Mishra et al., 2017).

Only one study examined neuronal activity during resting state in a population of females diagnosed with schizophrenia spectrum, comparing a yoga intervention (n = 23), with aerobic exercises (n = 23) and a control wait-list (n = 12) group (Lin et al., 2017). Local spontaneous neuronal fluctuations at rest, measured by the amplitude of low-frequency fluctuations (ALFF), have previously shown alterations in patients with schizophrenia (Hoptman et al., 2010). Although no results were reported regarding the differences at baseline between the groups, the yoga group exhibited significantly different ALFF in the precuneus and visual cortex compared to the control group and aerobic exercises group. Furthermore, the yoga intervention induced a significant decrease in ALFF in the precuneus (Lin et al., 2017).

Finally, neuronal activity during an emotional evoking or working memory task in experienced yoga practitioners were investigated by three different groups. More specifically, Wadden et al. (2018) investigated the effects on emotional regulation in experienced yoga subjects (n = 19, yoga styles unknown) compared to recreational athletes (n = 12, group-sports) using emotionally arousing visual stimuli. A higher activation during the emotion-evoking phase was found in the superior parietal lobule, postcentral gyrus, and anterior supramarginal gyrus of yoga subjects. These areas have been associated with attentional awareness and reduced egocentric bias. However, recreational athletes exhibited higher activation during the emotion-evoking phase in the inferior frontal gyrus and lateral occipital cortex, regions linked with cognitive reappraisal during emotional regulation (Wadden et al., 2018). Similarly, the neurocognitive correlates of emotion interference on cognition were examined in an fMRI study performed by Froeliger et al. (2012a). For this study, yoga practitioners (n = 7) and an age-, sex-, and years of education-matched control group (n = 7) had to perform an event-related affective viewing trial and Stroop trial during the fMRI session, preceded by a negative or neutral emotional distractor. During the viewing trials, greater activity was observed in the dorsolateral PFC. In contrast, for the Stroop trial, greater activation was observed in the superior frontal gyrus due to the group effect, where the control group showed larger activation responses. An interaction effect of the Stroop trial was seen in the ventrolateral PFC. Froeliger et al. (2012a) suggested that yoga meditation practitioners may selectively recruit dissociable frontal executive-dependent strategies in response to emotionally salient information. Only one study investigated the effects of a long-term yoga practice (consisting of meditation, physical postures, and breathing exercises) on working memory, performing a Sternberg working memory task during fMRI (Gothe et al., 2018). Twenty-six subjects whereof 13 experienced yoga practitioners and 13 age- and sex-matched controls were included in this study. Although no significant difference was found in terms of reaction time and performance accuracy between both groups, the fMRI results showed less activation in the dorsolateral PFC in yoga practitioners during the encoding phase of the Sternberg task. This result may point toward increased efficiency by experienced yoga practitioners while performing the task, which is in line with behavioral studies

suggesting the positive influence of yoga on working memory performance (Gothe et al., 2018).

Effects of Yoga on Functional Connectivity

Besides differences in brain activation, fMRI has also been used to investigate the potential effect of yoga on functional connectivity in the brain (**Table 2C** and **Figure 1D**). Methods used to investigate functional connectivity in the brain include ROI-to-ROI analyses, seed-to-voxel analyses, independent component analyses, and network-based statistics. Within the included studies, the following resting-state networks (RSNs) were investigated: DMN, dorsal attention network (DAN), executive control network (ECN), salience network, language network, and superior parietal network. For the seed basis analyses, most of the seeds were placed in the DMN, including the medial prefrontal cortex (mPFC) and precuneus/posterior cingulate cortex (PCC).

Froeliger et al. (2012a) investigated the effects of Hatha yoga meditation and meditation-state on four RSNs (DMN, DAN, ECN, and salience network) in a ROI-to-ROI analysis, hypothesizing that yoga meditation would be associated with greater functional connectivity between multiple RSNs. Firstly, higher interregional correlation values for the DAN in yoga practitioners (n = 7) compared to the controls (n = 7) were found. Secondly, interregional connectivity within nodes of the DAN was significantly higher for the yoga group. Within the yoga group, greater functional connectivity was observed during the meditative state between DAN and DMN nodes and between the anterior prefrontal node of the salience network and multiple DAN nodes, compared to the resting state. In contrast, during resting state, greater functional connectivity was found between the DAN and dorso mPFC (node of the ECN) and right insula (node of the salience network), compared to the meditative state (Froeliger et al., 2012a). In a seed-to-voxel fMRI study, Hernández et al. (2016) performed a functional connectivity analysis in the same subjects as their VBM study. They found a positive correlation between subjective perception of the depth of mental silence and GM density in mPFC. This region was subsequently used as a seed region for the seed-to-voxel functional connectivity analyses (Hernández et al., 2018). During the meditation state, functional connectivity between mPFC (seed/ROI) and anterior insula/putamen bilaterally increased significantly. In contrast, functional connectivity was significantly decreased between the mPFC and thalamus/parahippocampal gyrus throughout both the meditation and resting state (Hernández et al., 2018). Similarly, Sevinc et al. (2018) also used the mPFC as seed region to evaluate the common and dissociable neural correlates of an MBSR (n = 18) intervention (including yoga poses) and a relaxation response (RR; n = 16) program in a longitudinal setting. For this study, two *a priori* seeds were used: the mPFC (associated with focused attention) and the anterior insula (for its role in somatosensory awareness). Both interventions revealed functional coupling between mPFC (seed) and supplementary motor areas during meditation compared to rest. However, the MBSR program was uniquely associated increased functional connectivity between the anterior insula (seed) and the pregenual anterior cingulate cortex during body scan meditation compared with rest (Sevinc et al., 2018). Santaella et al. (2019) used the same seed (mPFC as main anterior seed of the DMN) to address neuronal connectivity in an healthy elderly female population (n = 40; same subjects as in Afonso et al., 2017). They found greater resting-state connectivity between the mPFC and the angular gyrus in the yoga group (n = 20), compared to paired yoga-naïve controls (n = 20) (Santaella et al., 2019).

In total, two studies used an independent component analysis (ICA) approach to investigate functional connectivity within and between identified networks. In an interventional longitudinal fMRI study, Eyre et al. (2016) investigated the correlation between functional connectivity and performance on memory tests, before and after yoga training (n = 14) or MET (n = 14) in patients with MCI. Subjects were randomly assigned to both groups. Analysis was performed on four networks identified by the ICA that are relevant in the research regarding long-term memory, including DMN, posterior DMN, language network, and superior parietal network. Results showed that improvement on the verbal memory performance was significantly positively correlated with greater connectivity within the DMN. Additionally, a significant negative correlation between changes in functional connectivity and changes in long-term visuospatial memory performance was observed in a cluster within the superior parietal network in both groups (Eyre et al., 2016). Applying an ICA as well, in addition to the VBM analysis, Dodich et al. (2019) also performed rsfMRI in the same subjects. A significant interaction effect (group × time) was observed in the frontal sector of the fronto-parietal network. Within this network, after Sahaja yoga training, decreased power was displayed at ultra-low frequencies and increased power in low-middle frequencies. This change in the power spectrum in the fronto-parietal network was correlated with well-being scores (Dodich et al., 2019).

Finally, in an attempt to disentangle differences between yoga and meditation as such, Gard et al. (2014) investigated functional connectivity in three different groups: yoga practitioners (n = 16), meditation practitioners (n = 16), and a control group (n = 15). Using Network-Based Statistics (NBS), which detects clusters of connections that significantly differ between groups, significant difference components for the comparison yoga practitioners > controls were found, comprising three nodes, with the caudate nucleus as the central node, connected to the parahippocampal gyrus and inferior temporal gyrus. The comparison meditators vs. controls and yoga vs. meditators did not yield significant results; however, significant higher-degree centrality for both meditators and yoga practitioners was observed compared to controls. Furthermore, compared to controls, meditators and yoga practitioners revealed equally stronger connectivity to a large number of brain regions (Gard et al., 2014).

Effects of Yoga on Brain Metabolites and Neurotransmitters

So far, only one PET study investigated endogenous striatal dopamine release during yoga meditation using ^{11}C-raclopride PET in experienced yoga Nidra practitioners (n = 8), during rest and relaxation meditation on two separate days, in random

order (Kjaer et al., 2002). A significant 8% decrease in binding potential was observed during meditation in the ventral striatum, associated with the experience of reduced readiness for action, indicating an increased dopaminergic signaling in the striatal regions most closely associated with reward and pleasurable effects (Kjaer et al., 2002).

In the first MRS study, Streeter et al. (2007) instructed one group of eight experienced yoga participants to practice a yoga session, especially focusing on yoga physical postures, while the control group (n = 11) had to read for 60 min before the MRS scan. MRS voxels were placed in the cortex and deep GM structures (exact location not specified). In yoga practitioners, an increase in GABA levels was found after a yoga practice while no changes were observed in the control subjects after the reading exercise (Streeter et al., 2007). In a subsequent interventional study, the same group measured MRS GABA levels in the left thalamus in healthy subjects that were randomly assigned to a 60-min yoga (n = 19) or walking (n = 15) intervention, three times a week for 12 weeks (Streeter et al., 2010). The yoga subjects reported a greater improvement in mood and anxiety, compared to the walking group. Although no significant changes in thalamic GABA levels between groups were found, a significant positive correlation between changes in mood scales (revitalization, tranquility, state-trait anxiety trait) and changes in thalamic GABA levels was found in the yoga group (Streeter et al., 2010).

One study investigated the effects of a yoga intervention in patients with type 2 diabetes on N-acetyl aspartate (NAA) and myoinositol (MI) brain metabolites in the right dorsolateral frontal lobe by means of MRS (Nagothu et al., 2015). In this case–control study, a total of 68 patients, were assigned to either a yoga group (n = 34) or a control group (n = 34). The yoga group did yoga (including physical postures and breathing exercises) for six months, 6 days a week, for 45–60 min under daily supervision of a qualified yoga teacher. The control group was not on any specific exercise regimen. Neither exact dropout numbers nor the exact age and number of females vs. males of each group are reported by Nagothu et al. (2015). Higher NAA and lower MI levels were found in the yoga group compared to the control group in the right dorsolateral frontal lobe, pointing toward higher neuronal integrity and lower neuroglial functioning, respectively. No baseline values were reported (Nagothu et al., 2015). In another MRS study, with the same study design and intervention as the previously mentioned research, no differences in NAA and MI values were found between the control (n = 5) and yoga (n = 5) groups (Santhakumari et al., 2016).

In addition to VBM effects, Yang et al. (2016) also acquired MRS in 25 MCI patients subjects, randomized to either a yoga group (n = 14) or a memory enhancement training group (n = 11). The MRS study was conducted with ROIs placed in the hippocampi and dorsal anterior cingulate cortex. A significant interaction effect between time and group was detected for choline and *post hoc* analysis showed that choline decreased in the hippocampi after memory training, but remained unchanged in the yoga group. Of note, at baseline choline levels were greater in the memory training group compared to the yoga group (Yang et al., 2016).

A summary of significant results related to these functional neuroimaging studies in yoga is provided in **Tables 2A–D** and illustrated in **Figure 1B** (effects on CBF and glucose metabolism, showing mainly increases in frontal regions and a decrease in posterior regions; however, each yoga meditative content showed a distinctive pattern), **Figure 1C** (neuronal activation, showing mainly increased activation in frontal regions during yoga and decreases in posterior regions), **Figure 1D** (functional connectivity, showing increased functional connectivity in mainly prefrontal regions within DMN), and **Figure 1E** (neurotransmitters and metabolites, showing widespread effects depending on the yoga intervention and measured metabolite).

DISCUSSION

Although the published studies on neuroimaging effects of yoga have been characterized by various forms of yoga, mostly smaller groups and heterogeneous target assessment, relatively consistent cerebral structural and functional changes have been found. These may be linked to presumed beneficial physical and psychological effects of yoga reported in a growing body of clinical research studies (Field, 2011, 2016; Vancampfort et al., 2012; Villemure et al., 2015).

Overall, morphological neuroimaging findings are consistent with an increase in regional GM density or volume in yoga practitioners compared to controls. Even though the insular cortex was not employed as an ROI in the included studies, higher GM density in the insular cortex was most consistently reported in the whole-brain analyses (Froeliger et al., 2012b; Villemure et al., 2015; Hernández et al., 2016; **Figure 1A**). These results are congruent with morphometric neuroimaging of meditation practitioners, where it was found that increases in the insular cortex were the most consistent structural alterations across meditation styles (Fox et al., 2014). In addition to morphological effects in the insula, multiple functional studies included in this review (Kalyani et al., 2011; Wang et al., 2011; Froeliger et al., 2012a; Hernández et al., 2015, 2018) showed differences in the activation or connectivity of the insula in yoga practitioners (**Figures 1C,D**). Importantly, many of these did not investigate the full triad of yoga but only included yoga styles focusing on meditation without physical postures (*asana*). In parallel, a meta-analysis and systematic review on the functional effects of meditation, including mindfulness-based interventions, reported the consistent recruitment of the insula across multiple styles of meditation (Fox et al., 2016; Young et al., 2018), suggesting an important role for the insula across meditative processes. The insula is central in interoceptive body awareness, the sensitivity toward the psychological conditions of one's own body (e.g., sensing increased heart rate, sweaty skin, tense stomach, etc.), and empathy and possibly even metacognition (Gu et al., 2012; Simmons et al., 2013). The insula is involved

during meditation but also upon postural changes and slow breathing (Critchley et al., 2015). Various neuroimaging studies have shown that higher interoceptive awareness is associated with increased ability of effective stress coping, whereas low interoceptive awareness has been observed in patients with depression (Avery et al., 2014). Yoga may induce an increase in functional connectivity between the insula and brain regions involved in regulation of affective and sensory processing (such as the PFC), which may induce higher GM density in the insular cortex.

Furthermore, increased GM volumes or density in the hippocampus were consistently observed in different morphological studies (Froeliger et al., 2012b; Hariprasad et al., 2013; Villemure et al., 2015; Gothe et al., 2018), including yoga studies containing the full triad of yoga. In line with these results, several functional and structural neuroimaging studies of meditation also reported differences in the hippocampal formation (Fox et al., 2014, 2016). These effects could be linked to increased BDNF levels, a marker for neuroplasticity, as observed after a yoga intervention (Naveen et al., 2016). BDNF may play a role in the morphological effects observed in the hippocampus as it is found in high concentrations within this region (Murer et al., 2001). In contrast, in a large population study in yoga and meditation practitioners (without making a distinction between both), decreased hippocampal volumes were observed at a cross-sectional level, compared to controls, which might be attributed to early life stressors. Practicing meditation and yoga had no significant relation over time with hippocampal volume (Gotink et al., 2018).

The amygdala was explicitly investigated in two morphological structural studies, where decreases in density and volume were observed (Hölzel et al., 2009; Gotink et al., 2018). A significant correlation was found between changes in perceived stress scores and the amygdala volume (Hölzel et al., 2009). Indeed, extensive evidence showed neural correlates of stress-induced modulation of structure and function of both amygdala and hippocampus. It therefore seems plausible that the smaller amygdala and increased hippocampal volumes are due to less experienced stress through yoga practice (Roozendaal et al., 2009; McEwen et al., 2016; McEwen, 2017; Gotink et al., 2018).

Functional neuroimaging studies investigating differences in neural activation during yoga meditation, in comparison to an emotional task or cognitive functioning, found yoga-induced effects in various cortical and subcortical brain regions. However, heterogeneous results across all studies were obtained, which may be due to the heterogeneous nature of the study design, as well as multiple yoga styles and tasks during the functional activity imaging acquisition.

From the five studies focusing on CBF/perfusion and glucose metabolism changes during a yoga meditation practice, it was shown that CBF is influenced by yoga meditation (Herzog et al., 1991; Lou et al., 1999; Cohen et al., 2009; Khalsa et al., 2009; Wang et al., 2011), but influences on CBF may depend heavily on the type and focus of the meditation (Fox et al., 2016; **Figure 1B**). Eight studies investigated neural activation changes during yoga meditation, with a majority of the studies showing increased activation in the frontal regions during yoga meditation (Froeliger et al., 2012c; Hernández et al., 2015; Lin et al., 2017; Mishra et al., 2017). Functional connectivity studies (Froeliger et al., 2012a; Gard et al., 2015; Eyre et al., 2016; Hernández et al., 2018; Sevinc et al., 2018; Dodich et al., 2019; Santaella et al., 2019) observed improved connectivity in yoga subjects, compared to controls, in multiple networks and regions. Half of the studies on functional connectivity focused on the DMN, using seed-based analyses with seeds in the PFC (Hernández et al., 2018; Sevinc et al., 2018; Santaella et al., 2019). Studies investigated different yoga styles, and heterogeneous results were found, but with a consistent increased connectivity toward the PFC. In general, neural activity within the DMN is known to correlate with mind-wandering, which is in return associated with lower levels of happiness. A possible way to reduce mind-wandering is yoga meditation. Meditation has previously been linked to stronger coupling between the DMN and regions implicated in self-monitoring and cognitive control (Brewer et al., 2011).

Only one study investigated the effect of yoga (*yoga nidra meditation*) on dopaminergic function using the dopamine $D_{2/3}$ receptor radioligand ^{11}C-raclopride, demonstrating a significant increased dopaminergic release during the Yoga Nidra meditation practice in the ventral striatum (Kjaer et al., 2002). The ventral striatum plays an important role in the circuitry underlying goal-directed behaviors, behavioral sensitization, and changes in affective states (Ito et al., 2004), as well as in the reward/motivation circuitry (Volkow et al., 2012). It should also be noted that the dopaminergic system, via the basal ganglia, participates in regulating the subcortical-prefrontal interactions. In total, five studies used MR spectroscopy to investigate changes in metabolite concentrations in the brain. Nearly all studies employed different ROIs. Increased GABA levels, associated with stress reduction, decreased MI, and increased NAA levels have been reported after yoga (Streeter et al., 2007, 2010; Nagothu et al., 2015). Increased GABA levels might be the result of the activated PFC during or after yoga, as observed in multiple yoga studies (Froeliger et al., 2012c; Hernández et al., 2015; Lin et al., 2017; Mishra et al., 2017), which is believed to increase glutamate, stimulating the reticular nucleus of the thalamus to increase secretions of GABA (Guglietti et al., 2013).

A postulated neurobiological model comprising underlying mechanisms on central yoga effects is that yoga induces increases in CBF, cerebral activity, and glucose metabolism, reflecting recruitment of additional higher demand of cortical substrates, while decreases suggest that the yoga practice can be carried out using fewer neuronal resources as learning proceeds (Dayan and Cohen, 2011). Repeated functional activation might induce morphological changes affecting white and GM through dendritic arborization/synaptogenesis, neurogenesis (limited to the hippocampus), myelin remodeling, and fiber reorganization (Dayan and Cohen, 2011; Zatorre et al., 2012; Lövdén et al., 2013). In return, changes in functional connectivity can occur. Furthermore, multiple bottom-up (e.g., controlled breathing and physical postures) and top-down (e.g., focused attention and interoception) processes also play an important role in

mind–body interventions such as yoga (Muehsam et al., 2017). The beneficial effects of yoga practice is that the combination of its three main components—deep breathing, meditation, and physical postures—can activate the parasympathetic nervous system and thereby increase the GABA concentration, the main inhibitory neurotransmitter in the brain, consistent with the findings of Streeter et al. (2007, 2010). An augmenting effect of parasympathetic tone can occur through vagal nerve afferents caused by the baroreflex response that is mainly elicited by the deep and slow breathing, and by activated baroreceptors through different yoga poses (chin lock, inversion, and chest opening poses) during a yoga session (Streeter et al., 2012). This may also be a basis for the anxiety- and stress-relieving effects of yoga. Counteracting stress-related corticosteroid release reduces GABA, which can lead to neurological morphological changes that could have long-term consequences (Roozendaal et al., 2009; McEwen et al., 2016).

In this review, we attempted to summarize the application of neuroimaging (including MRI, PET, and SPECT) to study the neurobiological effects of yoga. Nevertheless, a formal meta-analysis of the results was not considered appropriate because of the relatively small and heterogeneous number of consistently designed studies. These relatively small sample sizes may also have limited the sensitivity to identify effects. Additionally, 38% of the studies (13/34) did not include a control group. The selection of a control group and control conditions is quite heterogeneous and varies from cognitive to motor task, making it difficult to compare studies. Physical exercise, or matching based on physical exercise level, was in general most consistently used as control group or condition, respectively. This is in line with the results of a systematic review regarding comparison groups in yoga research (Park et al., 2014). Physical exercise studies allow to establish whether the yoga effect is mainly determined by exercise or whether yoga provides an additional effect. Given its importance in order to control for non-specific effects of group participation, attention, and activity (Park et al., 2014) but scarcity of studies using an active control group, we strongly advocate incorporation in future study designs. In studies on effects of physical activity on brain structure and function, several similarities have been found. In general, physical activity (or aerobic training) has also been associated with increased GM density. Areas that were most consistently observed included the hippocampus, PFC, and motor-related areas such as cerebellum and motor cortex (for a review, see Thomas et al., 2012 and Erickson et al., 2014). In addition, a review of Sexton et al. showed that a relation between physical activity and WM structure has been suggested in multiple studies (Sexton et al., 2016). Regarding functional activity changes, Herold et al. (2020) reported profound changes especially in the frontal lobe, cerebellum, and hippocampus in response to exercise. Therefore, especially regarding cognitive, executive, and motor effects that can be associated with yoga practice, future study designs should clearly try to demonstrate whether structural and functional effects are different from a pure physical exercise component. This is a complex effort, as also physical exercise can be modulated by many variables, including exercise intensity, duration, aerobic vs. anaerobic, and a range of regional or global exercise levels.

Similarly, variations in style and intensity of yoga interventions were present in the reviewed studies, which makes it difficult to find clear common ground for the findings. Moreover, half of the yoga-related neuroimaging studies included in this review investigated the effects of yoga styles that mainly focus on meditation, whereas the other half investigated yoga including physical postures (*asana*). The latter is the type of yoga that is mostly practiced in the Western world nowadays, with even a primary focus on the physical postures. The different yoga styles may have different effects on the brain, since each yoga style differentially emphasizes the subcomponents of yoga. The study by Villemure et al. (2015) indicated that postures, breathing exercises, and meditation contribute differently to the structural changes observed after a series of yoga practices (Villemure et al., 2015). About three-fourths of the study subjects were female; however, this in line with the observations that lifetime yoga practitioners are more likely female (Cramer et al., 2016). Most studies did not use randomization. Four studies were of a mere exploratory design and did not control for multiple comparisons (Lou et al., 1999; Cohen et al., 2009; Hariprasad et al., 2013; Hernández et al., 2016), increasing the chance of false-positive findings.

Future research should be aimed at disentangling the effects of each yoga subcomponent on specific neuronal patterns and the interaction between these subcomponents, in larger and better-defined populations. Finally, the general issue of publication bias is likely also present, where negative findings may remain unreported (Egger et al., 1997).

CONCLUSION

Based on the relatively scarce but expanding neuroimaging evidence of yoga practice in predominantly healthy subjects, it has been shown that yoga has both a structural and functional effect on brain areas involved in interoception, posture, motivation, and higher executive functions. Overall, most consistent structural effects were observed in the hippocampus and insular cortex, while functional studies showed mainly increases in frontal executive and attention areas. However, the number of studies is still limited and heterogeneous and several inconsistencies are present due to the heterogeneity among the different yoga styles included and the great variability in the applied research protocols.

More extensive, well-designed, and multimodal/multiparametric research studies with the control group preferably including physical exercise should be performed to further investigate the potential beneficial effects of yoga not exclusively on the healthy brain but also in disease state, for example in mood and anxiety disorders such as major depression, PTSD, or anxiety states. The integration of both neuroimaging and neurophysiological techniques

(EEG, EMG, etc.) will further allow to investigate and bridge imaging findings with neurophysiological and behavioral assessment/improvements in well-being.

AUTHOR CONTRIBUTIONS

JvA performed the PubMed search and wrote the review. JC and KVL wrote the review and critically revised the manuscript for intellectual content. KD and SS critically revised the manuscript for intellectual content. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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frontiers in Integrative Neuroscience

REVIEW
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Gut-Brain Axis in the Early Postnatal Years of Life: A Developmental Perspective

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Emerging evidence suggests that alterations in the development of the gastrointestinal (GI) tract during the early postnatal period can influence brain development and vice-versa. It is increasingly recognized that communication between the GI tract and brain is mainly driven by neural, endocrine, immune, and metabolic mediators, collectively called the gut-brain axis (GBA). Changes in the GBA mediators occur in response to the developmental changes in the body during this period. This review provides an overview of major developmental events in the GI tract and brain in the early postnatal period and their parallel developmental trajectories under physiological conditions. Current knowledge of GBA mediators in context to brain function and behavioral outcomes and their synthesis and metabolism (site, timing, etc.) is discussed. This review also presents hypotheses on the role of the GBA mediators in response to the parallel development of the GI tract and brain in infants.

Keywords: gut-brain axis, brain, gastrointestinal tract, postnatal development, cognition, metabolites, microbiota

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INTRODUCTION

The early years of childhood form the basis for physical, metabolic, emotional, cognitive, and social development and have a lasting impact on adult life. Although development starts *in utero*, the developmental events from birth up to 2–3 years of life are equally crucial. This period of life is termed the early postnatal period, where infants undergo rapid developmental maturation in a intrauterine-independent environment. Environmental factors (e.g., diet and early life experiences) are crucial determinants of postnatal development, lifelong health, and wellness.

There is a rapid brain development (e.g., synaptogenesis and myelination) (Knickmeyer et al., 2008) and establishment of cognitive behavioral outcomes in the first 2 years of life

Abbreviations: 5-HT, serotonin; AHR, aryl-hydrocarbon receptor; BBB, blood–brain barrier; EEC, enteroendocrine cells; ENS, enteric nervous system; GABA, gamma-aminobutyric acid; GBA, gut-brain axis; GF, germ-free; GI, gastrointestinal; GLP, glucagon-like peptide; IDO, indoleamine-2,3-dioxygenase; LPS, lipopolysaccharides; PYY, peptide YY; SCFA, short-chain fatty acids; SPF, specific pathogen-free; TPH, tryptophan hydroxylase; TRP, tryptophan; VN, vagus nerve.

(Nelson et al., 2007). The GI tract also undergoes profound growth, morphological changes, and functional maturation, including the establishment of a stable GI microbiota (Xu, 1996; Koenig et al., 2011). Other systems, such as the immune, nervous, skeletal, and circulatory systems, also continue to develop in the early postnatal years of life (Sgarbieri and Pacheco, 2017).

The development phases of the GI tract and the brain are interdependent and occur in a parallel timeline (Carlson et al., 2018; Gao et al., 2019), but are not necessarily synchronous. The developmental interdependency between the GI tract and brain can be attributed to the GBA; a complex, bidirectional communication, incorporating neural, endocrine, immune, and metabolic mediators. The GBA is increasingly recognized as having a role both in physiological and pathological conditions. However, the development of the communication between the GI tract and brain via the GBA remains poorly understood, and more research is required to define better strategies to improve cognitive outcomes, particularly in the early postnatal period.

This review provides an overview of major developmental events in the brain and GI tract in the early postnatal period and their parallel developmental trajectories under physiological conditions. Current knowledge of GBA mediators in context to brain function and behavioral outcomes and their synthesis and metabolism (site, timing, etc.) is discussed. Evidence and hypothesis on GBA mediator's development in the early postnatal period are also provided.

EARLY LIFE BRAIN DEVELOPMENT

The development of the brain is an organized, predetermined, and highly dynamic multistep process. It begins *in utero* following fertilization and continues postnatally into adolescence in humans (Gibb and Kovalchuk, 2018). During the early postnatal period, brain architecture is shaped and the foundation is set for perceptual, cognitive, and emotional abilities (Paterson et al., 2006). It is increasingly recognized as crucial for the establishment of cognitive and behavioral abilities that last a lifetime (Nelson et al., 2007). Recently, an emphasis has been given to the first 1,000 days, as an opportunity to influence cognitive outcomes in the child (Cusick and Georgieff, 2016). Studies elucidating brain development over this period are vital for research, clinical, educational, and social outcomes. For instance, data on brain development may be relevant for early diagnosis of behavioral disorders like autism (Keehn et al., 2013; Wolff et al., 2015).

The critical brain developmental events include neurulation, neurogenesis, gliogenesis, neural migration, synaptogenesis, myelination, and regressive events like apoptosis and synapse pruning [see reviews by Andersen (2003); Tau and Peterson (2009); Davis (2018)]. In the prenatal period, the development of the brain is mostly influenced by genetic determinants, but in the early postnatal period environmental factors take precedence. Hence, brain developmental events in the early postnatal period are of particular importance, as less favorable environmental conditions can compromise the foundation of brain development, and can have adverse impacts on later stages of life (McCrory et al., 2010).

In the following section, cellular, structural, and functional development of the brain in the early postnatal period are discussed.

Postnatal Development

In the postnatal period, neurogenesis (formation of neurons) continues to a limited degree in the olfactory bulb (Bergmann et al., 2012) and hippocampal dentate gyrus throughout life (Boldrini et al., 2018). Unlike neurogenesis, gliogenesis (formation of glia) peaks during the first year of life and continues until adolescence (Semple et al., 2013; Reemst et al., 2016; Allswede and Cannon, 2018). Glia has three significant cell subtypes within the brain, namely microglia, astrocytes, and oligodendrocytes, each with different developmental timelines. The microglia regulates neurogenesis, and synaptic refinement (c.f., section "Immune Mediators") astrocytes support formation and plasticity of the synapse while the oligodendrocytes form myelin (Eroglu and Barres, 2010). The proliferation of microglia peaks in the first 2 weeks after birth and continues until the first month after birth (Budday et al., 2015). The proliferation of astrocytes and oligodendrocytes peak before birth and continue until 15 months of age and adulthood, respectively (Allswede and Cannon, 2018; Davis, 2018). Apoptosis of neuronal cells is largely completed *in utero*, however, apoptosis of the glial cell population continues to occur in the first few months of after birth (Tau and Peterson, 2009; Stiles and Jernigan, 2010). Oligodendrocytes undergo apoptosis to control myelin production during the initial stage of myelination (Caprariello et al., 2015).

Synaptogenesis (formation of the synapse) begins *in utero* but peaks across most of the regions of the brain in the early years of postnatal life (Huttenlocher and Dabholkar, 1997). Synaptogenesis peaks at different times in different regions of the brain, such as in the areas of the cerebral cortex where heterogeneity in synaptogenesis is well documented (Huttenlocher and Dabholkar, 1997). The infant's brain has almost double the number of synapses compared to the adult brain, and their abundance is reduced by the process of synaptic pruning, which is pronounced during the period of childhood to adolescence (Huttenlocher, 1979; Huttenlocher and Dabholkar, 1997). Together the formation and retraction of synapses shape the neural connections in the brain.

The cerebral cortex is divided into three functionally distinct areas, namely, sensory areas (e.g., visual cortex and auditory cortex), motor areas (e.g., motor cortex), and association areas (e.g., prefrontal cortex). Synaptogenesis in the visual cortex (present in the occipital lobe) peaks at around 6 months of age (Huttenlocher, 1999), whereas in the auditory cortex (temporal lobe) it peaks around 3 months of age, and in the prefrontal cortex (present in the frontal lobe) around 3 years of age (Huttenlocher and Dabholkar, 1997). Hence, this developmental pattern indicates that synaptogenesis peaks first in the sensory and later in the association areas, from a posterior to an anterior direction (Huttenlocher and Dabholkar, 1997; Giedd et al., 1999). The communication across synapses is facilitated by neurotransmitters (c.f., section

"Neurotransmitters") whose abundance increases concomitantly with synaptogenesis (Herlenius and Lagercrantz, 2004).

Myelination is a critical cellular event for the development of the brain, particularly for enhanced neuronal activity and communication. This process consists of the wrapping of axons of neurons with a myelin sheath. Myelination begins in the prenatal period, peaks during the first 3 years of life and continues until the second and third decade years of life (Giedd et al., 1999). Like synaptogenesis, myelination occurs first in the sensory areas followed by association areas of the brain from a posterior to an anterior direction (Volpe, 2000; Barkovich, 2005). Hence, the developmental pattern of synaptogenesis and myelination is indicative of areas with functions that are critical in early life, thus necessitating an earlier requirement for maturation (Huttenlocher and Dabholkar, 1997; Barkovich, 2005).

The brain undergoes significant structural development in the first 2 years of life (Casey et al., 2000). At birth, the total brain volume is 36% of an adult brain, and it reaches around 70% by the first year of age, and 80% by the second year (Knickmeyer et al., 2008). The cortical volume also increases by 88% in the first year and 15% in the second year (Knickmeyer et al., 2008). Cortical volume is determined by the cortical thickness and surface area, and these determinants also change in the first 2 years of life. The increase in cortical thickness and surface area is 31 and 76.4% in the first year of life, and 4.3 and 22.5% in the second year (Lyall et al., 2015). Regional differences in cortical thickness and surface areas are also observed (Shaw et al., 2008; Lyall et al., 2015; Remer et al., 2017). The volume of thalamus and amygdala increases by 130 and 14% in the first and second year, respectively (Knickmeyer et al., 2008). The hippocampus grows slowly in the first year but increases rapidly in the second year, likely linked to the increasing complexity of spatial working memory and path integration when a 2 years child becomes more mobile (Wolbers et al., 2007; Gilmore et al., 2012).

Concurrent with a rapid cellular and structural brain growth is an equally rapid development of the brain functions in the first years of postnatal life (Gilmore et al., 2012). The brain's functional networks are present *in utero*, but continue to develop in the early postnatal period (Gao et al., 2015): primary sensory-motor and auditory networks are the first to develop, followed by visual, attention, and default mode networks, and finally, the executive control networks begin to emerge (Gao et al., 2015). Different functional networks are activated during different cognitive tasks performed by infants, such as distinguishing different voices, recognizing faces, object permanence, etc. (Paterson et al., 2006).

Changes in both the structural and functional networks of the brain contribute to the development of cognitive abilities (e.g., perception, memory) in the first years after the birth of infants (Gilmore et al., 2018). These developmental events are mainly affected by external factors (diet and early life experiences) (Nelson et al., 2007; Deoni et al., 2018). Any positive and negative alterations of these external factors can either enhance or compromise the development of the brain.

Within the body, the early life development of the brain is co-dependent on the development and appropriate functioning of many organs. It is recognized that the GI tract plays one of the most significant roles in shaping the development of the brain.

EARLY LIFE GASTROINTESTINAL TRACT DEVELOPMENT

In utero, the fetus gets nutrients from the maternal blood via the placenta (Salafia et al., 2007) but after birth, the infant begins enteral nutrition with the uptake of breast-milk (Sangild et al., 2000). This shift from parenteral to enteral nutrition requires a developed GI tract before birth (Sangild et al., 2000). At birth, the tube is fully formed with the required motility functionality to ensure the survival of infant on mother's breast-milk, independent of placental nutrition (Grand et al., 1976). Details of the GI tract developmental events in the prenatal period have been reviewed elsewhere (Grand et al., 1976; Montgomery et al., 1999; Dimmitt et al., 2018). The GI tract continues to mature in structure and function postnatally, and early life foods are one of its crucial determinants. For instance, mother's milk and increasingly complex foods after weaning influence the maturation of GI tract (Kelly et al., 1991; Zhang et al., 1998; Jensen et al., 2001) to digests food, absorbs nutrients, and deliver nutrients to the body's cells for growth development, and maintenance.

Postnatal Development

The GI tract cellular features are primarily established prenatally followed by structural and functional maturation postnatally in response to early life food (breast-milk and/or formula) (Zhang et al., 1998; Jensen et al., 2001). The structural maturation of the GI tract includes changes in terms of size and anatomical features. Postnatally, the esophagus, stomach, and small and large intestines continue to grow in size (Weaver et al., 1991; Xu, 1996). The postnatal period is also marked by a decline in epithelial permeability (Jakoi et al., 1985; Jakobsson et al., 1986; Drozdowski et al., 2010). After birth, the small intestine is permeable to macromolecules (e.g., immunoglobulin G) present in breast-milk (Jakoi et al., 1985). Within the first few days, the small intestine's permeability to macromolecule is reduced, which results in cessation of macromolecule transport paracellularly (Jakoi et al., 1985). The exact timing of permeability reduction in humans remains unknown, but studies in piglets and rats suggest that barrier closure happens in the first 2 days after birth (Weström et al., 1984), and by postnatal week 3 (Arévalo Sureda et al., 2016), respectively. Villi development is largely completed at birth, whereas a rapid increase in crypt depth and crypt cell proliferation in the small intestine also occurs in the first years of life, increasing the surface area for nutrient absorption (Thompson et al., 1998; Cummins and Thompson, 2002).

Unlike other peripheral organs, the GI tract has a dedicated nervous system called the enteric nervous system (ENS). The regulation and coordination of muscular and secretory activity by the ENS are required for digestion and absorption (Rao and Gershon, 2016). The ENS is embedded along the wall of the GI tract and consists of a network of neurons that mainly resides within two major ganglionated plexuses (Furness, 2012). The myenteric plexus lies in the muscular propria layer, and the submucosal plexus is in the submucosa layer. In mice, the maturation of ENS in terms of neuronal morphology

(e.g., dendritic and axonal structure), types of neurons (e.g., cholinergic and nitrergic), neurally mediated motility patterns in different regions of GI tract occurs during the postnatal period, as reviewed by Foong (2016). For detailed information on ENS development, readers are redirected to the extensive compilation by Rao and Gershon (2018).

In utero, the GI tract of the fetus is exposed to amniotic fluid, which contains 98% water and 2% protein, sodium, chloride, and CO_2 (i.e., low nutrient content) (Bonsnes, 1966). Immediately after birth, the infant is introduced to colostrum, which is rich in proteins (e.g., lactoferrin and lactoperoxidase), immunoglobulins, and growth factors (e.g., epidermal growth factor and vascular endothelial growth factor) (Ballard and Morrow, 2013; Godhia and Patel, 2013). The infant GI tract undergoes further functional development to adapt to complex and more diverse nutrient profiles postnatally (Hampson, 1986; Thompson et al., 1998). The activity of the enzymes enterokinase (protein hydrolysis), gastric lipase (lipid hydrolysis), and lactase (carbohydrate hydrolysis) increases gradually after birth (Antonowicz and Lebenthal, 1977; Moreau et al., 1988; Shulman et al., 1998) to facilitate the digestion of complex food structures. Functional maturation of the GI tract in the postnatal period also includes the establishment of the microbiota.

Microbial Colonization

The colonization of microbes in the GI tract begins at birth and continues until about 3 years of age when the composition becomes adult-like (Koenig et al., 2011; Yatsunenko et al., 2012). However, the literature suggests the presence of microbes *in utero*. This view arises from the fact that microbes have been detected in the meconium (i.e., the first stool of infant after birth), amniotic fluid, and placenta (Aagaard et al., 2014; Ardissone et al., 2014; Urushiyama et al., 2017; Shi et al., 2018). A study by Ardissone et al. (2014) showed that approximately 61% of the microbial population in meconium was found to be similar to that of the amniotic fluid, suggesting that microbes in the meconium originate by swallowing of amniotic fluid by the fetus (Ardissone et al., 2014). The viability of microbes *in utero* remains debated in the scientific community, and the problem of contamination artifacts is an issue discussed among researchers. However, recent mouse studies showed viable bacteria in the fetal gut, uterus, and placenta, suggesting the possibility of the presence of viable bacteria in a human fetus (Younge et al., 2019). Therefore, more studies on *in utero* colonization are warranted to challenge the accepted sterile womb paradigm.

In the postnatal period, the microbial colonization of the infant GI tract follows a succession of steps. Studies of the GI microbiota in the infant are limited to fecal samples. Stool samples are a proxy for the microbial population of the large intestine but may not represent it accurately. During the first few weeks after birth, the GI microbiota of infants is dominated by facultative anaerobes like members of the *Enterobacteriaceae* family (Palmer et al., 2007; Matsuki et al., 2016; Nagpal et al., 2017), which are likely coming from the mother's vagina and skin (Palmer et al., 2007; Lozupone et al., 2013). At around 6 months, strict anaerobes, including bacteria of the *Bifidobacterium*, *Clostridium*, and *Bacteroides* genera, dominate the composition (Nagpal et al., 2017). At around 3 years of age, the microbiota profile shows a high degree of resemblance to that of adults (Palmer et al., 2007; Koenig et al., 2011; Yatsunenko et al., 2012) and is represented almost entirely by strict anaerobes like the *Clostridium coccoides* group, *Clostridium leptum* subgroup, and *Prevotella* (Nagpal et al., 2017).

However, the GI microbial community consists not only of bacteria but also include phage, archaea, and fungi. Most studies have focused on bacterial colonization of the GI tract in infants, and much less is known about other kingdoms of life. According to the available knowledge, bacteriophage, mainly of the Caudovirales order and *Microviridae* family, archaea *Methanobrevibacter smithii*, and fungi *Candida albicans* are the most predominant non-bacterial organism in the infant GI tract during the first years of life (Palmer et al., 2007; Smith et al., 2013; Heisel et al., 2015; Lim et al., 2015, 2016; Schei et al., 2017; Ward et al., 2017).

The transition from milk to solid food is one of the influential factors of the colonization process in infants (Fallani et al., 2011; Koenig et al., 2011; Turroni et al., 2012). More studies where the analysis of the bacteria, phage, archaea, and fungi composition and function are needed to fully understand the colonization patterns and their temporal changes during the transition from milk to solid foods.

PARALLEL DEVELOPMENT BETWEEN THE GI TRACT AND BRAIN

The majority of the development of the GI tract and brain occur in parallel, but their development is asynchronous in terms of attaining peak and maturity. For instance, microbial colonization, tissue structural maturation, and ENS maturation coincide with the refinement and remodeling of brain neural circuits and cognitive development in the first years of life (**Figure 1**). There is increasing evidence that the colonization of the GI tract by the microbiota appears to have a parallel developmental trajectory to the brain for up to 3 years of age. A study by Carlson et al. (2018) showed that infants with a high relative abundance of *Bacteroides* in their stools had better cognitive performance in terms of receptive language and expressive language. In contrast, infants with a high level of *Faecalibacterium* in their stools had lower cognitive performance (Carlson et al., 2018). Another study in infants showed a positive association of the alpha diversity of the fecal microbiota and the functional connectivity between the supplementary motor area and the inferior parietal lobule (areas associated with cognitive outcomes) of the brain (Gao et al., 2019).

Evidence from rodent studies has also provided insights into the correlation between changes in the GI microbiota and brain function in early postnatal life. Germ-free (GF) mice displayed altered anxiety responses, abnormal motor activities, enhanced stress responses, and memory dysfunction (Sudo et al., 2004; Gareau et al., 2011; Heijtz et al., 2011). Interestingly, when GF mice are conventionalized with microbiota obtained from specific pathogen-free (SPF) mice in adulthood rather than early life, anxiety-like behavior associated with altered synaptic related

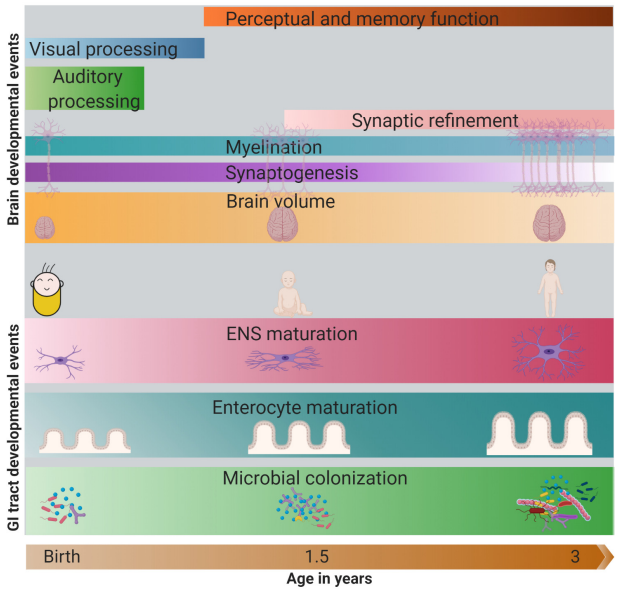

FIGURE 1 | Parallel development of the GI tract and the brain in first 3 years of life. In the GI tract, increase in microbial abundance and diversity, enterocyte maturation (change in crypt and villi structure) and ENS maturation (change in nerve density, type of neurons) occurs rapidly in the first 3 years of life. Meanwhile, brain also develops rapidly, with the change in its volume (peak in the first year), synaptogenesis, myelination, synaptic refinement, and establishment of cognitive abilities like auditory and visual processing, perception, and memory. The darkness of the color represents the intensity/peak of the developmental event. GI, gastrointestinal; ENS, enteric nervous system. Note: The developmental timing of the cellular events may vary across different regions of the brain.

proteins and neurotransmitter turnover persist (Sudo et al., 2004; Heijtz et al., 2011). These findings suggest that specific changes in brain structure and function cannot be reversed beyond a critical window in the early postnatal period (Sudo et al., 2004; Heijtz et al., 2011).

Additionally, adult GF mice exhibit a decreased production of the neurotransmitter serotonin (5-HT) in the GI tract, as compared to conventionally raised and SPF adult mice (Reigstad et al., 2015; Yano et al., 2015). 5-HT is produced both in the brain and the GI tract (c.f., section "Tryptophan Metabolites"). It is well known that brain-derived 5-HT is associated with mood regulation, learning, and memory (Cowen and Sherwood, 2013; Carhart-Harris and Nutt, 2017), but whether changes in GI-derived 5-HT regulate these brain functions, remains to be confirmed.

A study by Collins et al. (2014) showed that, at 3 days of age, the development of myenteric plexus of the ENS was structurally abnormal in GF mice compared to that of SPF mice. The myenteric plexus showed decreased nerve density and ganglionic size but increased nitrergic neurons in the GF mice (Collins et al., 2014). Whether these functional changes in the GI tract translate into cognitive outcomes, remain unknown, but it is plausible that there is an interdependency between the establishment of the GI microbiota, the ENS and the development of the brain.

It is important to note that studies in rodent models may not be reproducible in humans, as there is a marked difference between rodents and humans in terms of the developmental patterns of the GI tract and brain. Rodents are born with a relatively underdeveloped GI tract, and most of the functional development occurs in the postnatal period (Searle, 1995; Drozdowski et al., 2010; Guilloteau et al., 2010). The timing of brain developmental events is also different between humans and rodents (Pressler and Auvin, 2013). The anatomy and physiology of the GI tract, brain growth, and developmental patterns of both organs in piglets share a greater similarity to humans than other non-primate models like rodents (Guilloteau et al., 2010; Mudd and Dilger, 2017).

Most studies of GI and brain development have been mainly focused on the role of the GI microbiota. The GI tract undergoes developmental changes not only in terms of microbiota but also enzyme activity, gastric secretions, small intestinal permeability, and increased surface area for absorption of nutrients (i.e., crypt-villi structural modification) (c.f., section "Postnatal Development"). How these changes in the GI mucosa affect brain outcomes remains mostly unknown. For instance, an increase in the surface area of absorption of nutrients over this period could result in increased availability of nutrients for the host and less for the microbiota. The result could be a profile of different neuroactive metabolites in the GI tract contributing to specific cognitive outcomes. However, no studies have been conducted to relate structural and functional modifications in the GI tract to brain developmental events in the early years of postnatal life.

GUT-BRAIN AXIS

The GI tract and the brain are connected through a complex network of signaling pathways collectively termed as the GBA (Carabotti et al., 2015). In the last decade, the role of GI microbiota in the GBA has been extensively assessed, and the term has been extended to microbiota-GBA. Here, the term GBA includes the microbiota. The communication between the GI tract and brain is bidirectional and is mediated by neural, endocrine, immune, and metabolic mediators.

The GBA has been studied using top-down and bottom-up approaches. The modulation of the GI functions by the brain (top-down approach) is well established by preclinical and clinical evidence. For instance, modulation of motility, secretion (HCl acid in the stomach, bicarbonates in pancreatic juice, and mucus by goblet cells), and mucosal immune responses in the GI tract are controlled by the brain as reviewed by Rhee et al. (2009). The modulation of brain functions by GI-derived molecules (bottom-up approach) involves different signaling pathways (**Figure 2**). The importance of the GBA is increasingly recognized both in physiological (e.g., GI homeostasis) and pathological conditions (e.g., mood disorders, obesity, and autism) and have been extensively reviewed in Mayer (2011); Agustí et al. (2018); Liu and Zhu (2018); Martin et al. (2018). However, the understanding of GBA during the co-development of the GI tract and the brain in the early postnatal period is limited.

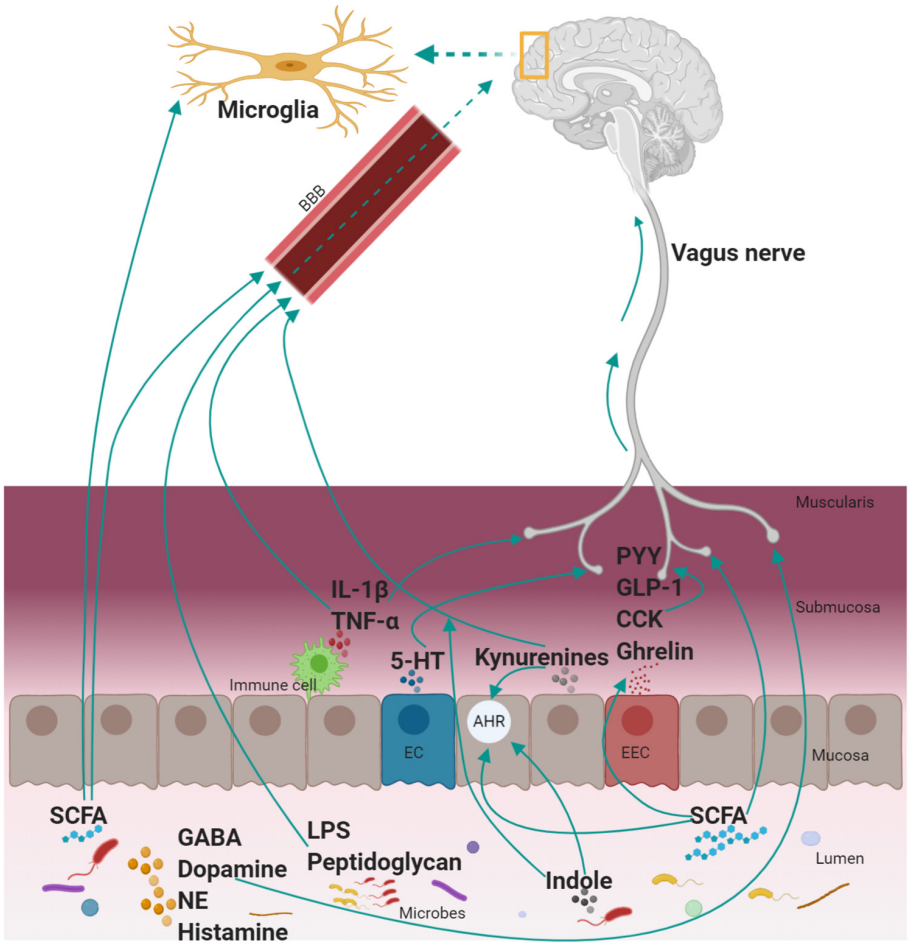

FIGURE 2 | Mechanism of communication between the GI tract and the brain. A myriad of mediators is involved in the complex communication between the GI tract and the brain. These include neural (vagus nerve), endocrine (hormones; PYY, GLP-1, CCK, and ghrelin), immune [cytokines (IL-1β and TNF-α), microglia, microbial antigenic component (LPS, peptidoglycan), and metabolic (TRP metabolites (kynurenines, 5-HT, and indole), SCFA, neurotransmitters (GABA, dopamine, NE, and histamine)] mediators. The mode of action of these mediators is by: activating the vagus nerve or crossing the BBB to communicate with the brain directly. SCFA regulates other mediators (EEC to produce hormones, microglia maturation, AHR activation; an essential receptor for TRP metabolites (produced both by the host and microbiota). GI, gastrointestinal; GLP-1, glucagon-like peptide-1; PYY, peptide YY; CCK, cholecystokinin; TNF-α, tumor necrosis factor-α; IL-β, interleukin-β; GABA, gamma-aminobutyric acid; NE, norepinephrine; SCFA, short-chain fatty acids; EEC, enteroendocrine cells; TRP, tryptophan; LPS, lipopolysaccharides; BBB, blood–brain barrier; AHR, aryl hydrocarbon receptor; EC, enterochromaffin cell; 5-HT, serotonin. Note: Kynurenines include kynurenine and downstream metabolites of the kynurenine pathway and not necessarily all the kynurenines can cross the blood–brain barrier.

Neural Mediators

The vagus nerve (VN) is the longest nerve in vertebrates and innervates many visceral organs like the heart, lungs, and GI tract (Bonaz et al., 2018). It has a vital role in many functions such as digestion, immune responses, heart rate, and controlling mood (Breit et al., 2018). The VN also plays a crucial role in facilitating neural signals between the GI tract and the brain (Bravo et al., 2011). It is the principal component of the parasympathetic nervous system and is composed of 80% afferent and 20% efferent fibers (Bonaz et al., 2018). The afferent fiber carries information from the GI tract to the brain, and the efferent nerve fiber carries information from the brain to the GI tract. The efferent fiber mainly regulates motility and glandular secretion in the GI tract, possibly by interacting with the ENS, mainly by cholinergic activation via nicotinic receptors (Garza et al., 2009; de Jonge, 2013). Over the last decade, the vagal afferent pathways have been increasingly recognized as sensors of hormones, cytokines, and metabolites produced in the GI tract with potential consequences for brain function and behavior. The afferent pathway is also involved in the activation and regulation of the hypothalamic-pituitary adrenal axis, a principal component of the physiological stress system, and a key mediator of the GBA during stress as reviewed by De Weerth (2017).

Vagal afferent fibers are located in all layers of the GI tract but do not cross the mucosal layer outwardly (Wang and Powley, 2007). Thus, they cannot sense the luminal contents directly, but indirectly through the diffusion of microbial metabolites such as short-chain fatty acids (SCFA) (Lal et al., 2001) or via enteroendocrine cells (EEC) (Li et al., 2000). The EEC represent about 1% of epithelial cells and form the largest endocrine organ

of the body (Mayer, 2011). These cells are capable of sensing luminal contents, and in response they produce and release molecules (e.g., a variety of hormones and 5-HT) that bind to receptors expressed on afferent endings (Egerod et al., 2012, 2018). A study showed that the administration of *Lactobacillus rhamnosus* improved depression and anxiety-like behavior in mice (Bravo et al., 2011). These effects were not observed in vagotomized mice, suggesting the importance of the VN in delivering improving brain functions in response to a specific bacterium (Bravo et al., 2011).

In the early postnatal life, the VN is not fully functional. Infants are born with the VN only partially myelinated (Porges and Furman, 2011). As discussed before (c.f., Section "Postnatal Development"), nerve myelination continues in the postnatal period, and that also holds for the VN. Development from partially myelinated to fully myelinated VN starts at approximately 24 weeks of gestation and continues until adolescence (Sachis et al., 1982; Porges and Furman, 2011). However, a faster VN myelination rate was observed from 32 weeks of gestation until 6 months after birth (Sachis et al., 1982), suggesting accelerated transmission of signals between the GI tract and brain during this period, likely due to the consumption of breast-milk by infants. Milk is an essential source of long-chain polyunsaturated fatty acids (e.g., docosahexaenoic acid and arachidonic acid), sphingolipids (e.g., sphingomyelin), phospholipids (e.g., phosphatidylcholine), and cholesterol, which are all essential for myelin sheath synthesis and development (Deoni et al., 2018). However, the effects of breast-milk or substitutes on the myelination of the VN is poorly understood.

Endocrine Mediators

The hormones produced by EEC are essential mediators of the GBA. Ghrelin, glucagon-like peptide (GLP)-1, cholecystokinin and, peptide YY (PYY) are produced and released by EEC in response to the food intake and composition (Egerod et al., 2012; Latorre et al., 2016). These hormones regulate food intake, satiety, gastric emptying, and energy balance by transmitting signals between the GI tract and the brain, reviewed in Raybould (2007); Cong et al. (2010); Holzer and Farzi (2014). Ghrelin is mainly released by the stomach, and it stimulates gastric emptying, regulates appetite, and increases the release of growth hormone (Kojima et al., 1999; Sun et al., 2004). Cholecystokinin and GLP-1 are produced in the small intestine and inhibit gastric emptying and reduces food intake (Liddle, 1997; Holst, 2007). The site of production of PYY is the ileum and the colon, and it decreases gastric motility, improves glucose homeostasis, and induces satiety (De Silva and Bloom, 2012).

Studies have shown that GI hormones also play a crucial role in regulating emotion and mood. For instance, ghrelin reduces anxiety-like and depressive-like symptoms of chronic stress (Lutter et al., 2008), whereas high PYY, mimicking its postprandial plasma concentration, promotes hedonic behavior (Batterham et al., 2007). It remains to be proven that these effects occur in physiological conditions. A variety of GI hormones are produced in normal physiological conditions, and the effect of one hormone is possibly counterbalanced by others. For instance, GLP-1 enhance anxiety-like behavior (Möller et al., 2002; Gulec et al., 2010), whereas GLP-2 could attenuate depression-like behavior (Iwai et al., 2009). These hormones regulate the signaling between the GI tract and the brain, most likely by activating the receptors present in the vagal afferent fiber (Egerod et al., 2018; Okada et al., 2018).

The type of feeding is known to influence the production of GI hormones. Infant fed infant formula during the first 6 months of age had higher ghrelin and lower PYY blood concentrations compared with infants fed breast- milk over the same period (Breij et al., 2017). However, there are no studies that report associations between changes in GI hormones and behavior over the developmental phase of both tissues and in response to feeding types in infants. Additionally, the signals from endocrine hormones may be altered during VN myelination in early postnatal life (c.f., section "Neural Mediators").

Immune Mediators

The constituents of the immune system, immune cells and signaling molecules, act as an important intermediary in the GBA. Microglia, the tissue-resident immune cells in the brain, has increasingly been recognized as a significant neuroimmune player of the GBA and in early life brain development (Erny et al., 2015). For instance, the microglia regulates neurogenesis and synaptic refinement (c.f., section "Postnatal Development") by phagocytosing excess neurons and synapses (Schafer et al., 2012; Cunningham et al., 2013). Regulation of neurogenesis is crucial for ensuring that this process does not exceed neuron's demand of the developing brain, and ultimately aides in brain organization (Cunningham et al., 2013). Synaptic refinement is essential for shaping the neural circuitry by eliminating the redundant synapses during postnatal brain development (Wu et al., 2015). A study by Erny et al. (2015) showed that the microglia in adult GF mice have abnormal morphology and density, altered cell proportions (e.g., dendrite length), and immature phenotype when compared with SPF mice. These adverse effects were partially rectified when adult GF mice were colonized with complex microbiota, suggesting a role for the microbiota in microglia maturation and function (Erny et al., 2015). It is important to note that the oral administration of a mixture of SCFA (acetate, propionate, and butyrate) (c.f., section "Short-Chain Fatty Acids") was sufficient to drive the maturation of the microglia in GF mice (Erny et al., 2015). However, the mechanism underlying the maturation of effects of SCFA remains to be determined. Evidence from these studies points out to a relationship between the microbiota and the microglia that could be important in the immune-mediated aspects of the GBA and brain development in the early postnatal life.

The signaling molecules of the immune system (e.g., cytokines) also participate in the GBA, possibly by two mechanisms: binding to VN receptors or transport across the BBB. Evidence shows that the afferent VN fiber has receptors for the cytokine interleukin-1β (Ek et al., 1998). This cytokine is capable of triggering its production and other proinflammatory cytokines that induce neuroinflammation (Shaftel et al., 2007). Tumor necrosis factor-α can cross the BBB (Gutierrez et al., 1993) and results in neuroinflammation

and dysfunction in the brain (Seleme et al., 2017). Bacterial peptidoglycan (outermost covering of Gram-positive bacteria) derived from resident commensals could also cross the BBB under physiological conditions, thereby influencing the brain development and the social behavior in 3-day-old mice (Arentsen et al., 2016).

Another study in rats has shown that lipopolysaccharides (LPS), from the surface of Gram-negative bacteria, can also cross the BBB (Vargas-Caraveo et al., 2017). Studies in mice have shown that intraperitoneal injection of LPS resulted in a decrease in novel object exploratory behavior by impairing continuous attention and curiosity toward objects (Haba et al., 2012). LPS can bind to the toll-like receptor 4 expressed on the microglia (Laflamme and Rivest, 2001) and afferent VN (Hosoi et al., 2005). However, the relationship between LPS-driven immune activation and alteration of behavior remains to be established.

The immune system in the early postnatal period undergoes the most rapid and radical changes compared with other systems in the body (Goenka and Kollmann, 2015). Commensal microbiota is essential for driving normal immune stimulation and maturation (Kamada et al., 2013; Olin et al., 2018). In infants, the cells of the innate immune system (e.g., monocytes and macrophages) are mostly developed prenatally, but their functions remain less developed in newborns (Simon et al., 2015). This lower activity could be to avoid unnecessary immune reactions during the period of continuous developmental remodeling (Prabhudas et al., 2011; Franchi et al., 2012). The cells of the adaptive immune system (e.g., B and T cells) are low in number and are functionally immature in infants (Tasker and Marshall-Clarke, 2003; Haines et al., 2009), which is most likely due to limited exposure to antigens required to develop an immune memory (Prabhudas et al., 2011). With the development of immune cells in early life, the level of their secretory products (i.e., cytokines) can also change over time (Corbett et al., 2010). This dynamic nature of immune mediators in the early postnatal life is likely to contribute to the development of the brain and associated behavior.

Metabolic Mediators

Metabolites are low molecular weight compounds, typically under 1,000 Da, which are reactants, intermediates, or products of enzyme-mediated biochemical reactions (Fanos et al., 2012). Metabolites play essential roles in the GBA and can have either direct or indirect (e.g., interaction with a neural mediator) effects on brain function. Metabolites can be produced either by the host, the GI microbiota, or the interactions in between them. Among various metabolites produced in the body, tryptophan (TRP) metabolites, SCFA, and neurotransmitters are increasingly recognized as potential mediators of the GBA.

Tryptophan Metabolites

Tryptophan is an essential amino acid for the synthesis of body proteins, and it is a precursor to several metabolites. Once absorbed, TRP can be metabolized in enterocytes and hepatocytes, thereby reducing its availability to the rest of the body, including the brain (Waclawiková and El Aidy, 2018). TRP is metabolized through different pathways (hydroxylation and kynurenine) in the GI mucosa, producing neuroactive compounds (Bender, 1983) that are of importance for the GBA.

The hydroxylation pathway generates two important metabolites, 5-HT and melatonin that participate in the GBA (Bender, 1983). The neurotransmitter 5-HT is involved in GI functions such as gastric secretion and motility (Gershon and Tack, 2007), and in the brain it regulates mood and is involved in cognitive and behavioral functions (Cowen and Sherwood, 2013; Carhart-Harris and Nutt, 2017). About 95% of total 5-HT in the body is synthesized by enterochromaffin cell, a subtype of EEC, and 5% is synthesized in the central nervous system (Gershon and Tack, 2007). So far, there is no evidence for the production of 5-HT by the GI microbiota, but studies have shown that microbiota mediates 5-HT synthesis in EEC, which could account for up to 50% of GI-derived 5-HT (Reigstad et al., 2015; Yano et al., 2015).

There is no evidence supporting that 5-HT produced in the GI tract can cross the BBB. Nakatani et al. (2008) showed that brain-derived 5-HT could cross the BBB to reach the peripherical circulation in rats. Interestingly, microbes in the GI tract have shown to influence the brain 5-HT level in a mouse model (Clarke et al., 2013). More studies are required to evaluate the bi-directional transport of 5-HT across the BBB and the potential regulatory role by the GI microbiota. Recently, studies have shown that certain commensal microbes and probiotic strains can uptake luminal 5-HT via specific transporters, which in turn can influence the microbial colonization of the GI tract (Lyte and Brown, 2018; Fung et al., 2019). By linking these findings, it could be inferred that the GI microbiota both requires 5-HT produced in the GI tract and regulates the concentration of 5-HT both in the GI tract and brain. Hence, the role of microbiota in the host serotonergic system warrants further attention.

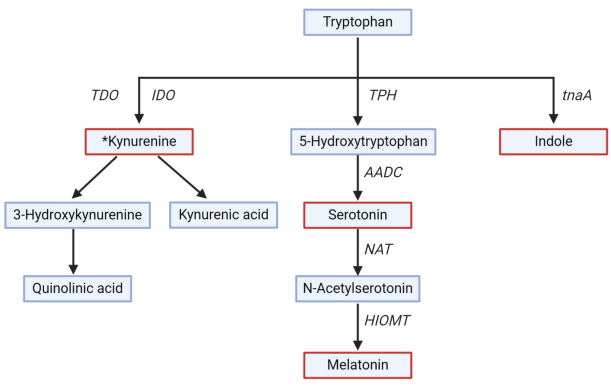

FIGURE 3 | Tryptophan metabolism along different pathways. Key metabolites (serotonin, melatonin, kynurenine, and indole) are in red. Rate-limiting enzymes shown are Trpytophan-2,3-dioxygenase (TDO), indoleamine-2,3-dioxygenase (IDO), tryptophan hydroxylase (TPH), Aromatic L-amino acid decarboxylase (AADC), *N*-acetyl transferase (NAT), hydroxyindole-*O*-methyl transferase (HIOMT), tryptophanase (tnaA). *All the downstream metabolites and enzymes of the kynurenine pathway have not been shown for simplicity.

TRP is metabolized to 5-HT in a two-step process (**Figure 3**). TRP hydroxylase (TPH), a rate-limiting enzyme in the biosynthesis of 5-HT, exists in two isoforms in the GI tract (TPH1) and the brain (TPH2) (Bender, 1983; Badawy, 2019). The conversion of 5-HT to melatonin is another two-step process and is catalyzed by two limiting enzymes: *N*-acetyl transferase and hydroxyindole-*O*-methyltransferase (Bender, 1983; Zagajewski et al., 2012). Melatonin is produced both in the GI mucosa and the pineal gland (Zagajewski et al., 2012). Melatonin regulates circadian rhythms of behavior, physiology, and sleep patterns, and also regulates GI motility (Richard et al., 2009).

The kynurenine pathway is gaining interest due to the role of kynurenine and downstream metabolites (collectively called kynurenines) on the GI tract and brain functions, and thus on GBA signaling (Cervenka et al., 2017). The kynurenine pathway is responsible for around 90% of TRP degradation (Badawy, 2017). Kynurenine is produced from TRP by the action of TRP-2,3-dioxygenase and indoleamine-2,3-dioxygenase (IDO) (Platten et al., 2019). Kynurenine is further metabolized into downstream metabolites, of which kynurenic acid, 3-hydroxykynurenine, and quinolinic acid are of particular interest for their neuroactive effect on the brain (Badawy, 2017). The metabolite kynurenic acid has a neuroprotective effect, while 3-hydroxykynurenine and quinolinic acid have a neurotoxic effect (Schwarcz and Stone, 2017). The enzyme TRP-2,3-dioxygenase is expressed in the liver, and IDO is widespread in numerous tissues, including the GI tract and the brain (Le Floc'h et al., 2011). The activity of IDO is regulated by proinflammatory cytokines (e.g., interferon-γ) released by toll-like receptor activation (Mahanonda et al., 2007), suggesting that the kynurenine pathway is more active in periods of immune activation or pathological conditions (Clarke et al., 2012). Unlike 5-HT, kynurenine, and 3-hydroxykynurenine produced in the GI tract can cross the BBB and can be further metabolized in the brain (Fukui et al., 1991).

An increase in TRP metabolism along the kynurenine pathway can result in a reduced availability of TRP for 5-HT synthesis and increased production of harmful kynurenine metabolites in the brain, contributing to mood disorder (Maes et al., 2011). This may also imply decreased melatonin levels which are associated with circadian malfunctioning and can increase the risk of mood disorders (Quera-Salva et al., 2011). Interestingly, melatonin appears to promote the expression of IDO, suggesting a negative feedback loop through which melatonin regulates the balance between kynurenine and 5-HT pathways (Li et al., 2017).

The GI microbes can also metabolize TRP (Wikoff et al., 2009; Zheng et al., 2011; Waclawiková and El Aidy, 2018). The primary metabolite produced by microbial metabolism of TRP is indole, which is catalyzed by the enzyme tryptophanase (Jaglin et al., 2018; Waclawiková and El Aidy, 2018). Recently, Jaglin et al. (2018) have shown that administration of indole directly in the rat's cecum, where microbes metabolizing TRP to indole are highly abundant, was associated with decreased motor activity and anxiety-like behavior. However, the effect of indole on the human brain and behavior has not been studied yet.

It is important to note that TRP metabolites: kynurenine, kynurenic acid, indole, and indole- derivatives are important ligands for aryl hydrocarbon receptor (AHR) (DiNatale et al., 2010; Mezrich et al., 2010; Jin et al., 2014). The AHR is a cytoplasmic ligand-induced receptor, which is ubiquitously expressed on almost all tissues (Yamamoto et al., 2004) and contributes to immune homeostasis by having an antimicrobial and anti-inflammatory effect (Zelante et al., 2013, 2014). For instance, lactobacilli utilize TRP to produce indole-3-aldehyde, an AHR ligand, which has shown to activate innate lymphoid cells that provide mucosal resistance against the pathogen *C. albicans* (Zelante et al., 2013). Interestingly, microbial metabolites such as SCFA were found to regulate AHR and its target genes in the intestine, which in turn influence the microbial composition, highlighting the bi-directional communication of AHR and the GI microbiota (Korecka et al., 2016). Evidence on the role of AHR in brain development and function is limited. A study by Latchney et al. (2013) showed altered hippocampus neurogenesis and contextual fear memory in AHR deficient adult mice, suggesting a role of AHR in brain development. Whether the regulation of neurodevelopment by AHR is due to TRP metabolites is yet to be proven.

The combined increase in surface area for nutrient absorption (Thompson et al., 1998) and diversity of the commensal microbiota (Nagpal et al., 2017) during the maturation of the GI tract in the early postnatal period, means that more TRP is absorbed and/or more TRP metabolites are produced and released in the peripheral circulation. However, the impact of GI tract maturation on TRP metabolism in the early postnatal life is poorly understood. Interestingly, a study in infants showed that cereals enriched with TRP increased plasma concentrations of melatonin and improved sleep quality (Cubero et al., 2009). As the sleep-wake cycle is controlled by TRP-derived melatonin (Brown, 1994) and more melatonin levels resulted in better sleep (Cubero et al., 2009). This evidence could be indicative of more TRP metabolism through the hydroxylation pathway than other pathways. The role of the TRP pathways and resulting neuroactive metabolites in brain development and function in early postnatal life is a fertile area of research.

Short-Chain Fatty Acids

The organic acids SCFA are saturated fatty acids with a chain length from one to six carbon atoms. They are the primary end-products of bacterial fermentation and are produced in the GI tract depending on the content of dietary (e.g., fiber) (Bergman, 1990), and non-dietary components (e.g., mucins) (Hoskins and Boulding, 1981; Montoya et al., 2017). The most abundant SCFA produced in the human GI lumen are acetate, butyrate, and propionate (Dalile et al., 2019). The majority of SCFA produced are absorbed (Ruppin et al., 1980; Hoogeveen et al., 2020) and utilized by enterocytes as an energy source at different ratios (Huda-Faujan et al., 2010; Dalile et al., 2019). Acetate is the most abundant SCFA, and it is produced by most microbes, while butyrate and propionate are produced by fewer GI tract bacterial species (Cummings et al., 1987; Morrison and Preston, 2016).

The SCFA regulate various GI functions. For instance, butyrate, acetate, and propionate help to maintain barrier

integrity protect from inflammation, and affect mucous production in the GI tract (Dalile et al., 2019). Recently, SCFA are gaining attention for their potential role in the GBA. Studies have found that GLP-1 and PYY secreting EEC, co-expressed SCFA receptors like free fatty acid receptor 2 and 3 (Karaki et al., 2008; Tolhurst et al., 2012), and deletion of these SCFA receptors in EEC in a mouse model has resulted in impaired PYY expression (Samuel et al., 2008) and reduced GLP-1 blood concentration (Tolhurst et al., 2012). Collectively, these findings suggest that SCFA may stimulate the release of these GI hormones that act as an essential mediators of GBA function, as discussed above. SCFA have been shown to promote TPH1 expression in a human carcinoid cell line derived from pancreatic tissues that share functional similarities with EEC, suggesting that SCFA can regulate production of 5-HT by EEC (Reigstad et al., 2015). However, caution must be exercised while translating cell lines result on humans, as these cell divides continuously and may express unique gene patterns that are absent in cells *in vivo* (Kaur and Dufour, 2012). Further evidence of SCFA importance in the GBA comes from a study where butyrate administration by intraperitoneal injection has been shown to attenuate social behavior deficiency in rodents (Kratsman et al., 2016). Butyrate and propionate can also activate tyrosine hydroxylase, the rate-limiting enzyme for catecholamine synthesis (c.f., section "Neurotransmitters")(Nankova et al., 2014).

Other studies showed that SCFA could also directly influence the GBA. Brain uptake of SCFA was reported following the injection of a mix of ^{14}C-SCFA into the carotid artery, which suggests that BBB might be permeable to SCFA (Oldendorf, 1973). SCFA might also directly activate vagal afferents. Luminal perfusion of sodium butyrate into the jejunum of anesthetized male rats evoked vagal efferent nerve responses that were abolished following vagotomy (Lal et al., 2001). Therefore, SCFA can participate in GBA both directly and indirectly; however, further studies are required to understand their role in GBA under physiological conditions.

In the early postnatal period, SCFA production and proportion are expected to change in response to microbial colonization of the GI tract (Midtvedt and Midtvedt, 1992; Norin et al., 2004; Bergström et al., 2014). For instance, exclusively breastfed infants had relatively more acetate in their stools as compared to non-breastfed infants (Bridgman et al., 2017), likely due to the fermentation of oligosaccharides present in human breast-milk by members of the *Bifidobacterium* genus (Azad et al., 2016). The introduction of solid food results in the establishment of different microbial colonizers, which change the SCFA profile in the fecal sample (Differding et al., 2020). However, direct and indirect effects of SCFA production in the early postnatal period on GBA and subsequent consequences for the development of the brain and behaviors are poorly understood.

Neurotransmitters

Chemical substances that carry information between neurons are called neurotransmitters. There are about 100 different neurotransmitters produced in the body and each with different functions. Based on chemical composition, neurotransmitters are mainly classified as amino acids and biogenic amines. Functionally, neurotransmitters can be classified as excitatory (increase action potential firing), inhibitory (decrease action potential firing), or modulatory (fine-tune the action of both excitatory and inhibitory neurotransmitters).

Dietary amino acids are precursors for the synthesis of 5-HT, gamma-aminobutyric acid (GABA), norepinephrine, dopamine, and histamine. The synthesis of 5-HT is exclusively from dietary TRP. In contrast, dietary phenylalanine (an essential amino acid) serves as a precursor to tyrosine (a non-essential amino acid), which is essential for the synthesis of norepinephrine and dopamine, and histidine (an essential amino acid) serves as a precursor for histamine (reviewed in Fabisiak et al., 2017; Mittal et al., 2017; Fernstrom and Fernstrom, 2018).

Genes responsible for metabolizing amino acids to neurotransmitters (or precursors of thereof) have been identified in some bacteria, *in vitro*. For instance, *Lactobacillus* and *Klebsiella* spp. possess a histidine decarboxylase gene that converts histidine to produce histamine (Kim et al., 2001; Lucas et al., 2008). *Legionella pneumophila* and *Pseudomonas* spp. have a phenylalanine hydroxylase gene that facilitates the conversion of phenylalanine to tyrosine (precursor of dopamine and norepinephrine), which has been demonstrated *in vitro* (Letendre et al., 1975; Flydal et al., 2012). From the above evidence, it could be speculated that neurotransmitter production by the GI microbes might be modulated by dietary amino acids and contributes to GBA signaling. A list of neurotransmitters and their production by microbial species and their amino acid precursors are shown in **Table 1**. However, the uptake and metabolism of dietary amino acid by the GI microbiota for neurotransmitter synthesis has not been studied.

Some studies report evidence of the metabolism of neurotransmitters by the microbiota. Pathogenic *Escherichia coli* O157:H7 has an increasing growth rate in the presence of norepinephrine and dopamine (Freestone et al., 2002). An extract of peel and pulp of banana, which is rich in neurochemicals (e.g., norepinephrine, dopamine, and 5-HT), has been shown to promote the growth of both pathogenic and non-pathogenic bacteria (Lyte, 1997). The mechanisms by which the GI microbiota can metabolize neurotransmitters *in vivo* are yet to be understood.

There is accumulating evidence *in vivo*, suggesting that the GI microbiota plays a role in modulating the abundance of neurotransmitters. For instance, GF mice have reduced levels of norepinephrine in cecal content (Asano et al., 2012), and of GABA in feces and plasma (Matsumoto et al., 2013). The turnover rate of norepinephrine, dopamine, and 5-HT was higher in the striatum (part of the brain) of GF mice compared with the SPF mice (Heijtz et al., 2011). These reduced levels of neurotransmitters are in line with the altered anxiety-like response in the GF phenotype, suggesting the role of microbiota in the modulation of behavior (Heijtz et al., 2011; Neufeld et al., 2011). However, no studies have yet reported whether the microbiota directly affects the level of neurotransmitters in the body or modulates host production of neurotransmitters. There is also no evidence whether neurotransmitters from the GI tract can cross the BBB to

TABLE 1 | Potential neurotransmitters in the gut-brain axis.

Neurotransmitter	Amino acid precursor	Microbial species[1]	Gastrointestinal tract role	Brain role
Serotonin	Tryptophan	*Escherichia coli* (K-12), *Klebsiella pneumoniae* (Özoğul, 2004; Shishov et al., 2009)	Regulates gastric secretion and motility (Misiewicz et al., 1966)	Mood regulation by decreasing anxiety and stress (Williams et al., 2006)
GABA[2]	Glutamine[3]	*Lactobacillus brevis* and *Bifidobacterium dentium* (Barrett et al., 2012)	Regulates gastric emptying, secretion, and motility (Hyland and Cryan, 2010)	Process sensory information and regulates memory and anxiety (Kalueff and Nutt, 1996)
Dopamine	Phenylalanine	*Escherichia*, and lactic acid-producing bacteria such as *Lactococcus* and *Lactobacilli* spp. (Shishov et al., 2009; Özogul, 2011)	Regulates motility (Li, 2006)	Voluntary movement, induces feeling of pleasure (Juárez Olguín et al., 2016)
Norepinephrine	Phenylalanine	*Escherichia*, *Bacillus*, and *Saccharomyces* spp. (Shishov et al., 2009; Lyte, 2011)	Regulates blood flow (Schwarz et al., 2001)	Motor control, emotion and endocrine modulation (Kobayashi, 2001)
Histamine	Histidine	*Lactobacillus* and *Pediococcus* spp. (Landete et al., 2007; Özogul et al., 2012)	Modulation of motility, enhancement of gastric acid production (Kano et al., 2004; Kim et al., 2011)	Regulates wakefulness, and motivation (Brown et al., 2001; Torrealba et al., 2012)

[1]The list of bacterial strains is mostly based on in vitro studies and may not be present in the gastrointestinal tract and are provided as examples. [2]Gamma aminobutyric acid (GABA) is the only inhibitory amino acid neurotransmitter and all others are modulatory biogenic neurotransmitters. [3]Glutamine is the only non-essential amino acid precursor, whereas all other precursors of neurotransmitters are essential amino acids.

reach the brain. Interestingly, the vagal afferent nerve express receptors for 5-HT, GABA, and dopamine (Egerod et al., 2018), suggesting the possibility of an alternative route for communication between the GI tract and brain. Therefore, GI derived neurotransmitters appear to be a potential mediator of the GBA, and further studies are required to confirm their potential.

In the early postnatal period, histological (e.g., crypt depth) and functional (e.g., enzyme) GI tract changes can result in different rates of amino acid uptake and host neurotransmitter production. The increased relative abundance and diversity of the GI microbiota could also influence neurotransmitter production. For instance, *Bifidobacterium* strains have shown to dominate the GI tract of breastfed infants (Kato et al., 2017; Nagpal et al., 2017; Lawson et al., 2020) and also one of the strain *Bifidobacterium brevis* has shown the ability to produce GABA (Barrett et al., 2012). Change in abundance of different *Bifidobacterium* strains postnatally (Kato et al., 2017) could result in an alteration of the GABA level in the GI tract. Changes in the production of neurotransmitters (type and amount) and their role in the GBA in response to early postnatal developmental remain to be established.

CONCLUDING REMARKS

The early postnatal years of life are marked by rapid developmental changes both in the GI tract and brain. The process of microbial colonization and cognitive development coincide in the first years of life. Sophisticated complex communication systems involving mediators such as VN, GI hormones, cytokines, and the GI-derived metabolites are known to govern the crosstalk between the GI tract and the brain. The establishment of microbes in the GI tract can influence immune (e.g., microglia) and metabolic (e.g., neurotransmitters and TRP metabolites) mediators that ultimately may have an impact on the brain development and behavioral outcomes. Early life foods (breast-milk, formula, and complementary foods) are crucial determinants of GBA mediators in the early postnatal period. Breast-milk could have a potential role in the development of the myelination pattern of VN and the production of hormones in the GI tract, which acts as an essential intermediary between the GI tract and the brain. Overall, the role the GBA mediators during the critical period of development is ill-defined.

It should be noted that many studies relating to the GBA have been carried out on rodent animal models, but considerable differences in developmental patterns of the GI tract and the brain between humans and rodents exist. The use of animal models with more comparable anatomy and physiology (e.g., piglets and primates) to that of humans is desirable to gain a better understanding of the mechanistic pathways of GBA and improve the translation of research to infants. Future research is required to understand whether the expected changes in GBA mediators occur during the critical period of GI tract and brain development and how they can be related to cognitive behavioral outcomes that are the manifestation brain development in infants. For this, longitudinal studies of postnatal life are required. Insights in this area can be targeted via dietary interventions to optimize the communication between the GI tract and the brain to improve cognitive outcomes in infants.

AUTHOR CONTRIBUTIONS

AJ, CM, JM, RD, WY, WM, and NR have contributed to the work. AJ conceived and wrote the manuscript. CM and NR helped in structuring the paper and critically reviewed the paper. All other

authors advised and critically reviewed versions of the paper. All authors approved the manuscript for publication.

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