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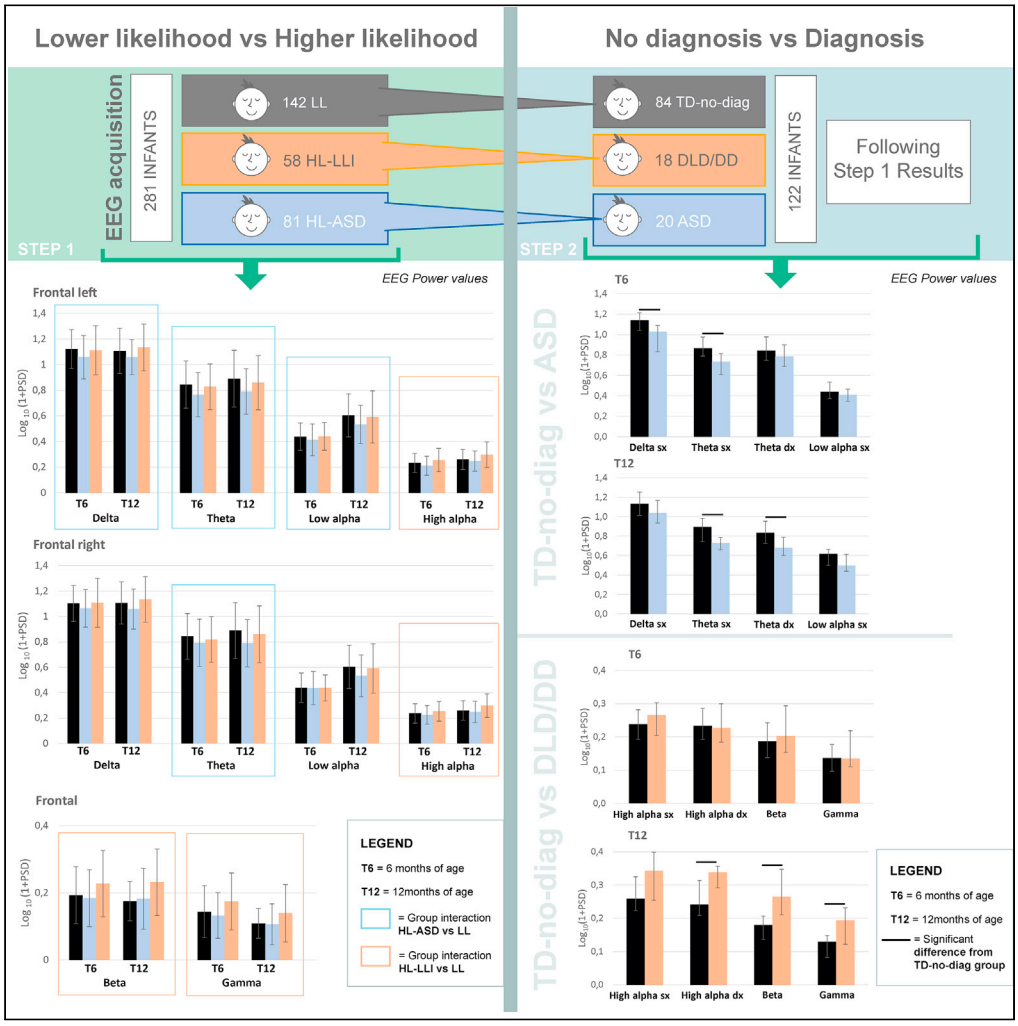

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Highlights

EEG spectral power measures represent a candidate biomarker for both ASD and LLI

Low power values in the low-frequency bands are associated with ASD outcome

High power values in the high-frequency bands are associated with LLI outcome

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Baseline EEG in the first year of life: Preliminary insights into the development of autism spectrum disorder and language impairments

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SUMMARY

Early identification of neurodevelopmental disorders is important to ensure a prompt and effective intervention, thus improving the later outcome. Autism spectrum disorder (ASD) and language learning impairment (LLI) are among the most common neurodevelopmental disorders, and they share overlapping symptoms. This study aims to characterize baseline electroencephalography (EEG) spectral power in 6- and 12-month-old infants at higher likelihood of developing ASD and LLI, compared to typically developing infants, and to preliminarily verify if spectral power components associated with the risk status are also linked with the later ASD or LLI diagnosis. We found risk status for ASD to be associated with reduced power in the low-frequency bands and risk status for LLI with increased power in the high-frequency bands. Interestingly, later diagnosis shared similar associations, thus supporting the potential role of EEG spectral power as a biomarker useful for understanding pathophysiology and classifying diagnostic outcomes.

INTRODUCTION

Neurodevelopmental disorders represent a complex clinical condition that refers to a wide group of disabilities related to some form of dysfunction in brain development, which leads to the early onset of neurocognitive deficits.[1] These disorders are characterized by multifactorial origins, heterogeneity in terms of clinical characteristics and outcomes, and an overlap of symptoms, thus making their characterization and diagnosis difficult. Early identification and intervention are critical to improving outcomes, and one of the main aims of the research in this field is to better characterize the mechanisms that trigger the disorders and to identify reliable biomarkers that can be used in the diagnosis process. Within this framework, electroencephalography (EEG) represents a powerful tool to understand neurophysiological substrates, identifying electrophysiological markers and distinguishing pathophysiologically distinct groups of patients. Indeed, it has been extensively used to study complex neuropsychiatric disorders.[2–5]

Autism spectrum disorder (ASD) and language learning impairment (LLI) are among the most common neurodevelopmental disorders, with a prevalence of 1/100[6] and 7.6/100,[7] respectively. Here, we use the term "language learning impairment" (LLI[8]) to acknowledge the continuum between spoken- and written-language impairments and to encompass children with either or both developmental language disorder (DLD) and developmental dyslexia (DD): these two disorders are often comorbid (with about one-third of children with DLD developing dyslexia by elementary school[9,10]) and aggregate in families suggesting a genetic etiology.[11] Among the symptoms that characterize both ASD and LLI, there is a limitation in the language of variable severity and with similar patterns of impairments, which make possible the existence of an overlap between their etiologies, also supported by genetic studies.[12] However, very limited works have investigated early brain development associated with the two disorders together.[13]

A number of studies have used baseline EEG, particularly EEG spectral power, to characterize brain development in infants at higher likelihood of developing ASD (HL-ASD, infants who have an older sibling with ASD), in some cases also considering the later ASD outcome.[14–17] These works suggest the hypothesis that early disruptions in the brain's oscillatory rhythms are core neural features of ASD pathophysiology. In particular, Tierney and colleagues[15] investigated developmental trajectories in EEG frontal spectral power in HL-ASD infants and those at lower likelihood of developing ASD (LL-ASD, infants who have no siblings

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with an existing ASD diagnosis) performing baseline EEG recordings when infants were 6, 9, 12, 18, and 24 months of age. They found the HL group to have lower spectral power in all of the frequency bands (delta, theta, low alpha, high alpha, beta, and gamma) at 6 months of age and different trajectories of changes in delta, alpha, beta, and gamma power. Levin and colleagues[14] tested younger infants identifying differences in frontal spectral power between HL-ASD and LL-ASD infants already at 3 months of age. Specifically, they found 3-month-old HL-ASD infants to have lower frontal high-alpha and beta power with respect to LL-ASD infants. However, no differences between groups emerged when considering LL-ASD infants, HL-ASD infants who later received the ASD diagnosis, and HL-ASD infants who did not. Huberty et al.[16] extended these results, identifying familial risk, but not a later diagnosis of ASD, to be associated with reduced frontal power at 3 months in all frequency bands, as well as with a steeper developmental power change between 3 and 36 months in delta, theta, high-alpha, beta, and gamma frequency bands, with converging trajectories at 3 years of life. Gabard-Durnam and colleagues[17] found different results. Indeed, their study shows that frontal delta and gamma power trajectories are able to distinguish infants with ASD diagnoses from others, especially using EEG power recorded within the first year after birth. Conversely, spectral power closer to the age of diagnosis did not provide additional utility for differentiating outcomes. Thus, the research conducted in the last decade supports the idea that early developmental changes in frontal EEG power may be one biomarker of the ASD familial risk and of the diagnostic outcome but with a heterogeneous pattern of results. All of the cited studies focused on frontal spectral power as it has been documented a structural and functional dysfunction in frontal regions related to ASD[18,19] and because EEG frontal power has shown to be associated with cognitive abilities that are impaired in subjects diagnosed with ASD.[20–23]

Regarding baseline EEG and language development, previous literature has focused mainly on the role of gamma activity. This is because gamma synchronization is thought to be important for the development of cortical networks,[24] and cortical activity in the gamma frequency range has been associated with various high-cognitive processes, including language.[25–28] Benasich and colleagues[29] recorded baseline EEG in HL-LLI and LL-LLI toddlers (HL-LLI, toddlers with at least one nuclear family member diagnosed with LLI; LL-LLI, toddlers with no reported family history of LLI) at age 16, 24, and 36 months, showing a positive correlation between frontal gamma power and performance on a series of behavioral tasks, including concurrent language and cognitive skills at all ages. They also found significantly lower frontal gamma power in the HL-LLI group with respect to the LL-LLI group at 24 and 36 months of age. In a later study of the same group,[30] frontal gamma power, measured at the same time points of the previous study, has been found to predict language outcomes at 4 and 5 years, thus supporting the role of early gamma activity on language development. Brito et al.[31] identified a similar correlation. Specifically, baseline parietal gamma power recorded in typically developing (TD) newborns resulted to be positively associated with linguistic abilities at 15 months of age. Moreover, our group[32] identified a positive association between central gamma power recorded in 6-month-old TD infants and language skills at 24 months of age mediated by their socioeconomic status (SES). A previous study[33] supported the idea that SES is associated with gamma power very early in development, showing that 6- to 9-month-old infants from low-income families had lower frontal gamma power than infants from high-income families and suggesting that this might be an early indicator of greater subsequent risk for poor language outcomes.

Finally, the association between baseline EEG spectral power and language development has also been investigated in HL-ASD and LL-ASD toddlers and infants. Specifically, Wilkinson et al.[34] analyzed baseline frontal gamma power and language skills in 24-month-old toddlers divided into three groups: LL-ASD, HL-ASD later diagnosed with ASD, and HL-ASD without ASD. They found a negative correlation between gamma power and expressive language ability in the HL-ASD groups and no associations in the LL-ASD group. The same group also investigated if and how the EEG power in frequency bands different than gamma is related to language development. They found that reduced frontal high-alpha power at three months was associated with poorer expressive language at 12 months, but no relations emerged with language outcome measures at 18, 24, and 36 months of age.[14] In a more recent study,[21] they used multivariate linear regression models to estimate 24-month language development with baseline EEG measures (e.g., the estimated EEG power and the slope of EEG power), recorded between 3 and 24 months. They found EEG parameters to be relevant in predicting language skills with significant interaction effects of HL and LL of developing ASD, thus supporting the idea that associations between baseline EEG and language are different depending on risk status and that baseline EEG measures are a possible predictive biomarker for language development.

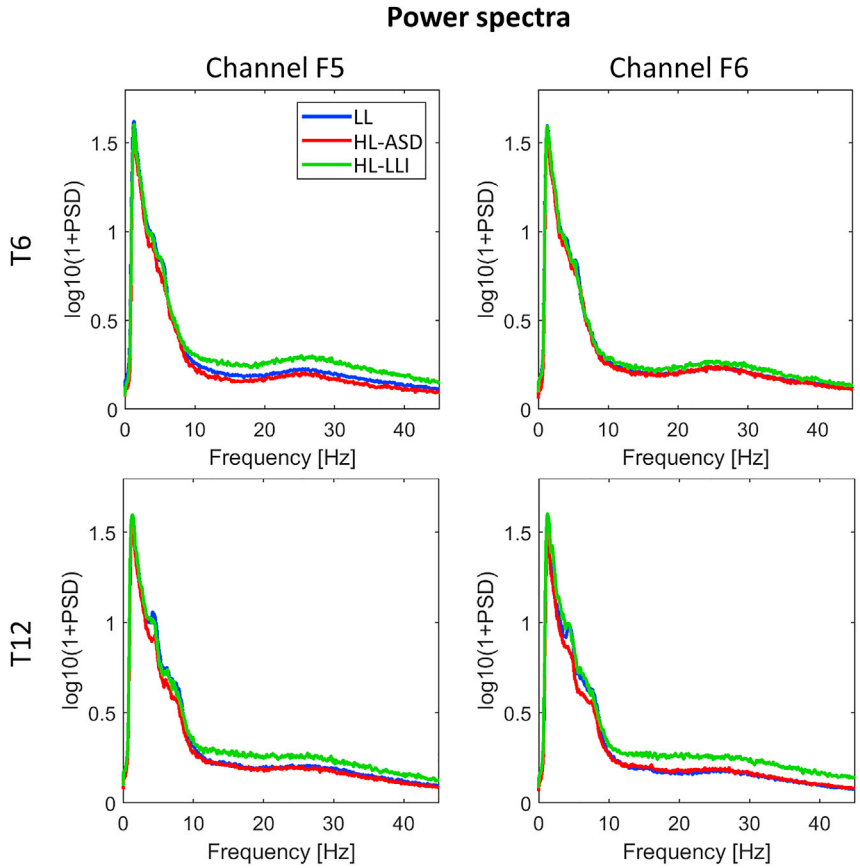

Figure 1. Power spectral density for each group (LL in blue, HL-ASD in red, HL-LLI in green) at T6 (first raw) and T12 (second raw) for channel F5 (first column) and F6 (second column)

To the best of our knowledge, no previous studies have used baseline EEG to investigate early brain development in both HL-, LL-ASD and HL-, LL-LLI infants altogether.

Due to the growing literature supporting the idea that baseline EEG spectral power recorded early in life might be used to determine the risk of developing ASD or LLI and later clinical outcomes, we focused here on baseline EEG acquired in 6- and 12-month-old HL-ASD, HL-LLI, and TD infants at lower likelihood for both ASD and LLI (LL group), also considering later clinical outcomes. The aim of the present study was 2-fold: (1) to study baseline EEG frontal spectral power recorded in the first year of life and identify differences between absolute power values in LL infants and those at HL-ASD or HL-LLI; and (2) to exploratively investigate if the identified differences also distinguish between infants with later diagnosis of ASD or DLD/DD and TD ones. We hypothesized that both HL groups would show power differences compared to LL subjects with some of these differences common to both HL groups and some specific ones. Moreover, we expect that some electrophysiological parameters sensitive to HL status would also be able to distinguish group subjects diagnosed with ASD and DLD/DD from TD ones.

RESULTS

Figure 1 shows the mean power spectral density (PSD) computed in two representative frontal channels (F5 and F6) for each group of infants (LL, HL-ASD, and HL-LLI) at 6 (T6) and 12 (T12) months of life. Subsequent analysis focused on two clusters of six electrodes positioned, respectively, in the left and right frontal hemispheres in proximity of channels F5 and F6;[32] in particular PSD values were averaged across electrodes within each cluster. Preliminary statistical analysis was performed using a series of paired t tests in order to evaluate differences between power values estimated in the right and left frontal clusters in each frequency band (delta, theta, low alpha, high alpha, beta, and gamma) at both T6 and T12. No differences

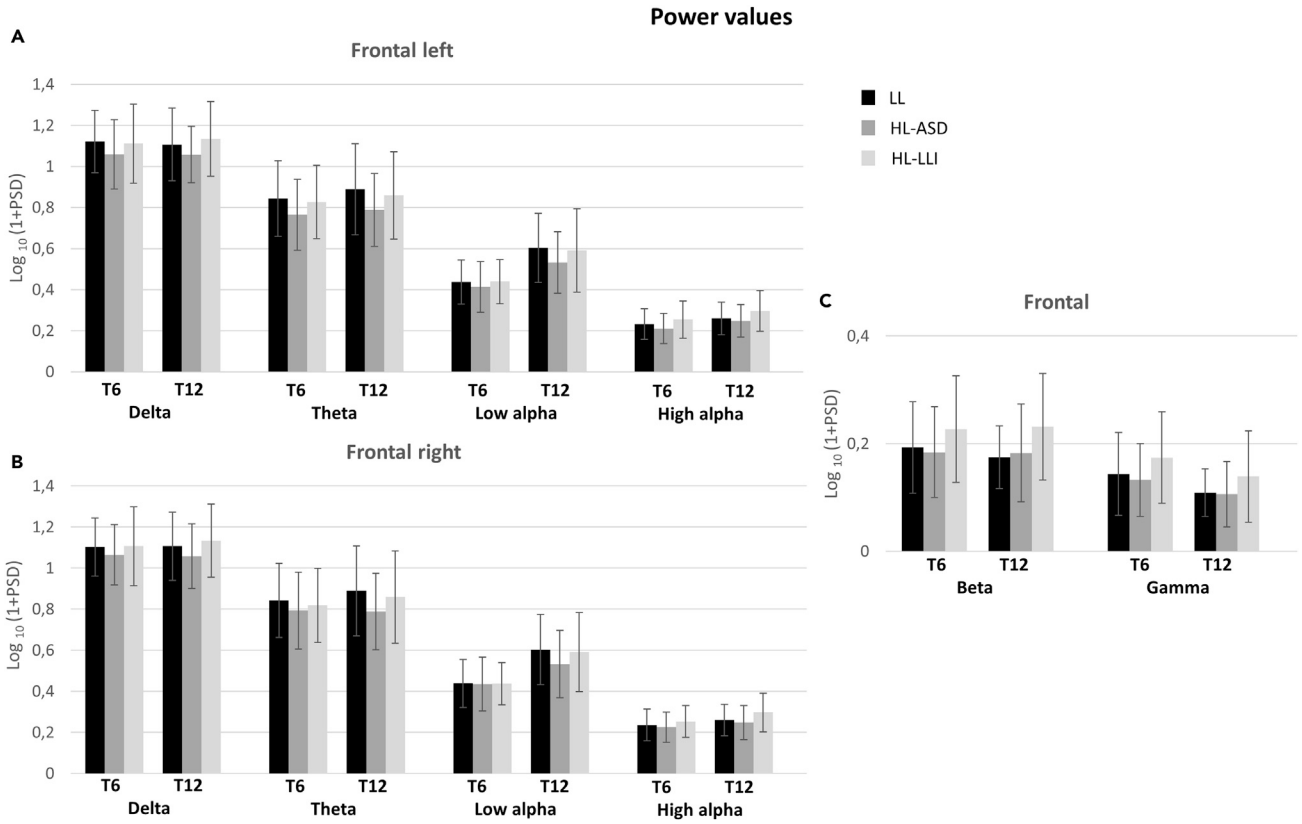

Figure 2. Mean power values for each group (LL in black, HL-ASD in dark gray, HL-LLI in light gray), time point, frequency band, and cluster

Specifically, panel A and B shows power values in delta, theta, low-alpha, and high-alpha frequency bands for the frontal left and frontal right cluster, respectively. Panel C shows power values in beta and gamma frequency bands obtained by merging left and right clusters. Errors bars represent the standard deviation.

emerged in any frequency bands at T6. Conversely, at T12, we found significant hemispheric differences in the delta, theta, low-alpha, and high-alpha frequency bands but not in the beta and gamma bands (for detailed results, see Table S4). Therefore, for subsequent analysis, beta and gamma power values computed in the left and right clusters have been averaged, obtaining a beta and gamma frontal cluster.

Figure 2 shows mean values of power for each group, time point, frequency band, and cluster. These power values were entered into linear mixed models to assess differences between groups and over time. Table 1 shows the results of the linear mixed models, whereas Table 2 reports the number of subjects used in the analysis for each group (LL, HL-ASD, and HL-LLI) and time points (T6 and T12). In the low-frequency bands (delta and theta), we found a main effect of group with the HL-ASD group, which showed lower power values than both the TD and HL-LLI groups (except for the frontal right cluster in the delta band, in which we found a significant difference only between HL-ASD and HL-LLI). In the low-alpha band, we found a main effect of time in both frontal regions, with higher power values at T12 with respect to T6, but a group effect only in the left frontal cluster, in which the HL-ASD group showed lower values of power than the LL one. In the high-frequency bands (high alpha, beta, and gamma), we found a main group effect, with the HL-LLI group, which showed higher power values than both the LL and HL-ASD groups. A significant effect of time was present only in the frontal left cluster for the high alpha power. Specifically, higher power values were recorded at T12 with respect to T6. No significant interactions between groups and time emerged at any frequency band.

Following these results, we performed an exploratory investigation of the differences in power values between TD subjects without a diagnosis (TD-no-diag group) and subjects diagnosed with ASD or DLD/DD for specific frequency bands and clusters using Mann-Whitney tests, separately for each time point (T6 and T12). Table 2 reports the details about the number of subjects for each group (TD-no-diag, ASD, and DLD/DD) and time point (T6 and T12).

Table 1. Linear mixed model and post hoc comparison results for each frequency band and cluster

	Linear mixed model		Post hoc comparisons	
Power band and cluster	F	p^a	Pairs	p^b
Delta, frontal left				
Group effect	5.048	**0.009**	LL vs. HL-ASD	**0.029**
			LL vs. HL-LLI	>0.999
			HL-ASD vs. HL-LLI	**0.012**
Time effect	0.011	0.918		
Delta, frontal right				
Group effect	6.853	**0.002**	LL vs. HL-ASD	0.133
			LL vs. HL-LLI	0.083
			HL-ASD vs. HL-LLI	**<0.001**
Time effect	3.601	0.098		
Theta, frontal left				
Group effect	6.843	**0.002**	LL vs. HL-ASD	**0.001**
			LL vs. HL-LLI	>0.999
			HL-ASD vs. HL-LLI	**0.039**
Time effect	1.867	0.247		
Theta, frontal right				
Group effect	5.558	**0.006**	LL vs. HL-ASD	**0.005**
			LL vs. HL-LLI	>0.999
			HL-ASD vs. HL-LLI	**0.037**
Time effect	0.040	0.918		
Low alpha, frontal left				
Group effect	3.823	**0.026**	LL vs. HL-ASD	**0.024**
			LL vs. HL-LLI	>0.999
			HL-ASD vs. HL-LLI	0.144
Time effect	89.187	**<0.001**		
Low alpha, frontal right				
Group effect	2.078	0.127		
Time effect	58.847	**<0.001**		
High alpha, frontal left				
Group effect	7.581	**<0.001**	LL vs. HL-ASD	0.289
			LL vs. HL-LLI	**0.020**
			HL-ASD vs. HL-LLI	**<0.001**
Time effect	14.756	**<0.001**		
High alpha, frontal right				
Group effect	5.555	**0.006**	LL vs. HL-ASD	>0.999
			LL vs. HL-LLI	**0.022**
			HL-ASD vs. HL-LLI	**0.004**
Time effect	3.768	0.098		
Beta, frontal				
Group effect	7.984	**<0.001**	LL vs. HL-ASD	>0.999
			LL vs. HL-LLI	**<0.001**
			HL-ASD vs. HL-LLI	**0.002**

(*Continued on next page*)

Table 1. *Continued*

Power band and cluster	Linear mixed model F	Linear mixed model p^a	Post hoc comparisons Pairs	Post hoc comparisons p^b
Time effect	0.349	0.694		
Gamma, frontal				
Group effect	8.328	**<0.001**	LL vs. HL-ASD	>0.999
			LL vs. HL-LLI	**0.001**
			HL-ASD vs. HL-LLI	**<0.001**
Time effect	25.327	0.098		

[a]FDR correction applied.
[b]Bonferroni correction applied.

Specifically, we assessed differences in power values between the group diagnosed with ASD and the TD-no-diag group in the delta band for the left cluster, the theta band for both right and left clusters, and the low-alpha band for the left cluster (Figure 3). Using data recorded at T6, we found significant differences between the two groups in delta and theta frontal left power ($Z = -2.921$, $p = 0.012$ and $Z = -2.405$, $p = 0.032$ $Z = -2.405$, respectively). Conversely, considering the data acquired at T12, we found significant differences between the two groups in frontal theta power in both the left and right clusters ($Z = -2.578$, $p = 0.036$ and $Z = -2.375$, $p = 0.036$, respectively). The ASD group always showed lower power values than the TD-no-diag group.

We investigated power differences between the group diagnosed with DLD/DD and the TD-no-diag group in the high-alpha band, left and right clusters, beta band, and gamma band (Figure 4). We found significant differences by only analyzing the data acquired at T12. Specifically, the DLD/DD group showed higher power in the frontal right high-alpha band ($Z = -2.464$, $p = 0.025$), beta band ($Z = -2.708$, $p = 0.025$), and gamma band ($Z = -2.342$, $p = 0.025$).

DISCUSSION

The main aims of the present study were (1) to assess differences in baseline EEG power values between LL infants and those at higher likelihood of developing ASD or LLI and (2), subsequently, to preliminarily verify if these differences are able to also distinguish between infants who have been later diagnosed with ASD or DLD/DD and TD ones.

Overall, our results showed that HL-ASD infants differed from both LL and HL-LLI infants in the EEG power associated with the low-frequency bands (delta, theta, and low alpha) showing lower values of power. Conversely, the HL-LLI infants differed from both LL and HL-ASD infants in the EEG power associated with the high-frequency bands (high alpha, beta, and gamma), showing higher values of power.

As already presented in the introduction, various studies have investigated EEG spectral power in infants at higher likelihood of developing ASD,[14–17] showing that the risk of developing ASD is associated with reduced power values in different frequency bands. Thus, our results are in line with these findings. We found significantly lower power values in delta, theta, and low-alpha bands (Table 1), but the trend is almost

Table 2. Number of subjects for each group at each time point

	Group	n at T6	n at T12
LL/HL	LL	130	62
	HL-ASD	44	67
	HL-LLI	46	34
Diagnostic outcome	TD-no-diag	79	36
	ASD	12	16
	DLD/DD	15	12

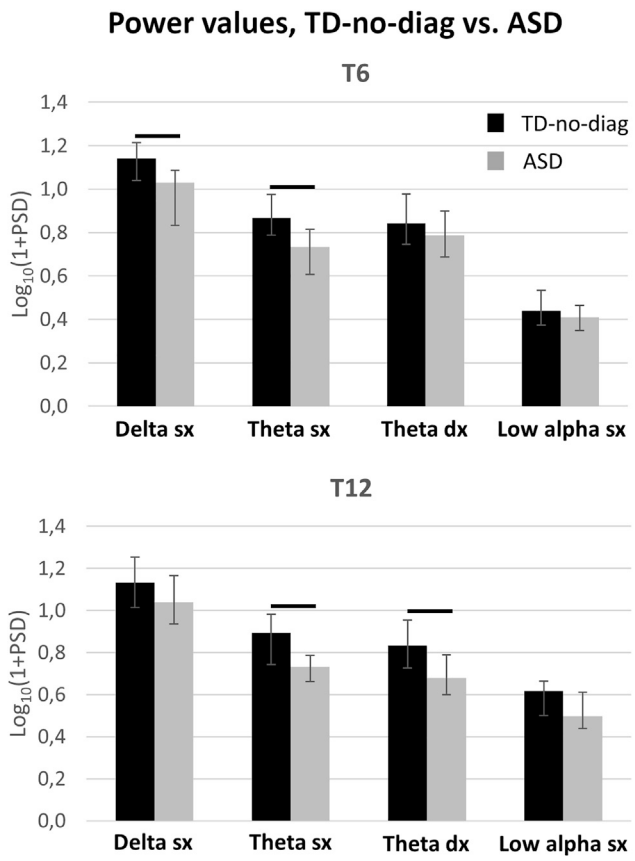

Figure 3. Median power values of TD-no-diag group (black) and ASD group (gray) at T6 (up) and T12 (bottom) in the clusters that showed a significant difference between LL and HL-ASD infants in the post hoc comparisons following the linear mixed models

Error bar represents the interquartile range. Significant differences between groups are reported with solid black line over the histograms (p <0 .05, Mann-Whitney tests).

the same in all of the frequency bands and both time points (Figure 2), thus suggesting a generally reduced neural synchrony in HL-ASD infants. Interestingly, the neuronal activity in the low-frequency bands has been linked to mechanisms that seem to be disrupted in ASD, such as neural inhibition,[35] emotional processes, and behavioral states.[36]

We found an opposite trend with regard to HL-LLI infants. Indeed, they showed significantly higher power values in high-alpha, beta, and gamma frequency bands (Table 1 and Figure 2). This result seems to be in contrast with the existing literature related to language development and frontal gamma power.[29,30] Lower frontal gamma power was identified in HL-LLI toddlers (24 and 36 months of age), and a positive correlation was found between gamma power and language and cognitive skills both concurrent[29] and successive (at 4 and 5 years).[30] This suggests that frontal gamma power may reflect cognitive skills, such as attention and working memory, fundamental for language acquisition, but it can also index higher brain maturation. The developmental trajectory of baseline gamma power shows a progressive increase in the first six months of life,[37] a decrease between 6 and 24 months of life,[15] an increase from age 3 peaking at age 4,[38] and, finally, a decrease that continues into adulthood.[39] Thus, if the relationship between gamma activity and language reflects brain maturation, the direction of this association should be different according to the period of life in which it is investigated. Tierney and colleagues[40] supported this idea, finding a negative correlation between resting gamma power and language-dependent behavioral performances in adolescents. This is also in line with our results that showed higher gamma power in HL-LLI infants between 6 and 12 months of age, when gamma power is decreasing. A similar pattern was also identified in beta and high-alpha bands, pointing out that EEG spectral power in frequency bands different than gamma might also be

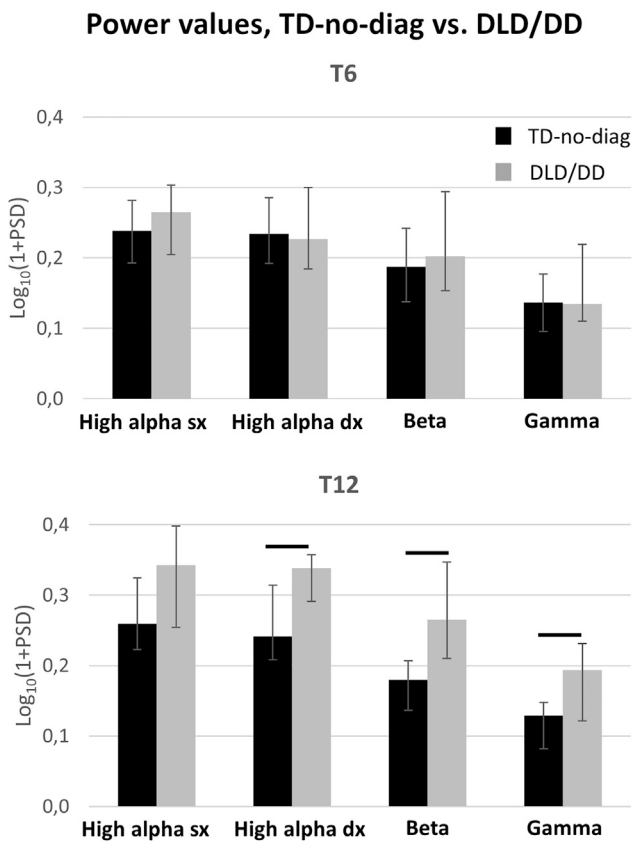

Figure 4. Median power values of TD-no-diag group (black) and DLD/DD group (gray) at T6 (up) and T12 (bottom) in the clusters that showed a significant difference between LL and HL-LLI infants in the post hoc comparisons following the linear mixed models

Error bar represents the interquartile range. Significant differences between groups are reported with solid black line over the histograms (p <0 .05, Mann-Whitney tests).

relevant in determining language development, as suggested by Levin et al.[14] and Wilkinson et al.[21] studying EEG spectral power in HL-ASD and LL-ASD infants and toddlers.

Importantly, our preliminary results support the hypothesis that EEG frontal power is not only associated with familial risk but also associated with the diagnostic outcome for both ASD and DLD/DD. Indeed, the frontal power of infants later diagnosed with ASD differed from the one of control TD infants in the delta and theta bands at six months of life and only in the theta band at 12 months of life (Figure 3). Gabard-Durnam et al.[17] found developmental changes in low-frequency power (i.e., delta) to be sensitive to both ASD risk status and diagnostic outcome. This result was not confirmed by Huberty et al.,[16] who found only the familial risk and not the diagnostic outcome to be associated with reduced frontal power and steeper developmental power changes in various frequency bands, including delta and theta. However, even if the latter study involved a large number of subjects, they pooled data across three separate sites, thus introducing a certain heterogeneity. This may be one of the reasons why they did not find associations with the diagnosis, in addition to high variability among ASD developmental outcomes in cognitive and behavioral domains.[16]

No previous studies have investigated if differences in frontal power between HL-LLI and LL-LLI infants also distinguish between subjects later diagnosed with DD/DLD and control TD ones. Our preliminary results support this hypothesis, showing that DD/DLD subjects had higher values of high-alpha, beta, and gamma frontal power at 12 months of life compared to TD control ones (Figure 4). No differences were found at T6, suggesting that neural developmental trajectories that are potentially linked to the development of DD/DLD start to bifurcate between 6 and 12 months of age.

Interestingly, despite the fact that ASD and DD/DLD show overlapping symptoms and a possible common etiology,[12] the EEG power measures associated with the development of the two disorders do not seem to overlap and have an opposite trend (lower power in the low frequencies for ASD vs. higher power in the high frequencies for DLD/DD). Further research is needed to clarify the role that these specific power modulations play as risk factors in ASD and DLD/DD development.

In conclusion, our findings support the high potentiality of the use of baseline EEG power measures in the first year of life in order to identify candidate biomarkers in both ASD and DD/DLD. Indeed, these measures resulted to be sensitive to the risk status and preliminarily also to the diagnosis outcome, with opposite trends associated with the analyzed developmental disorders. Thus, our exploratory study encourages to deepen this research line including larger groups of subjects in order to obtain reliable biomarkers.

Limitations of the study

It is important to acknowledge some limitations of the present study. First, despite the large number of subjects involved, our analyses, especially the ones performed considering the diagnosis outcome, are limited by the sample size. Indeed, due to the small number of subjects included in both the ASD and DLD/DD groups, we had only separately compared their power values with the power values of the TD-no-diag group, in specific frequency bands and electrode clusters selected *a priori* based on the results obtained from the linear mixed models. We did not have enough statistical power to use more complex and informative statistical models, and we were unable to provide reliable positive and negative predictive values as done in other works (e.g., Gabard-Durnam et al.[17]). Thus, our results related to the diagnosis are exploratory and preliminary but still promising for the characterization of both ASD and DLD/DD. Moreover, to maximize the number of data, we included in the analyses individuals with only one EEG time point. This prevented us from computing EEG parameters strictly related to the development, such as the intercept and slope of the EEG power trajectories, which are parameters that have been studied in HL-ASD populations showing promising results for the risk characterization.[16,17] Thus, future studies should be performed by increasing the number of subjects with a diagnosis in order to validate and confirm our preliminary results, as well as increasing the number of subjects with available EEG data in both time points, thus allowing the study of EEG parameters associated with the development.

Second, our sample is characterized by an SES difference between the LL group and both the HL-ASD and the HL-LLI groups. As reported in the introduction, previous studies have identified an association between SES and gamma power in early infancy.[32,33] This might affect our results and make them difficult to interpret, especially regarding the difference in gamma power between HL-LLI and LL infants. To consider SES differences, we introduced the variable SES as covariate in the linear mixed models. However, future studies are needed to better investigate the role of SES in brain maturation in both low- and high-risk infants.

Third, it should be noted that the HL-LLI group is very heterogeneous. Although all children in the HL-LLI group—even those with a first-degree relative with dyslexia only—were characterized by their higher probability to develop language impairment,[41–43] the mixed nature of this group may have affected the results. We cannot exclude that a more homogeneous sample, including only children at familial risk for DLD, might have shown higher overlap in the EEG power data with the ASD sample. Although such a specific comparison group is very difficult to recruit, this could be a goal for future studies.

Fourth, we limited our analysis to the frontal area. This was done based on the existing literature related to both ASD and LLI development as EEG frontal power has shown to be associated with several cognitive abilities,[20–23] and most of the studies that investigated the development of ASD or language have focused only on this area.[15–17,29,30] Moreover, a previous study, analyzing the interaction between EEG power measures on the likelihood of developing ASD, performed different statistical models using data from various regions and found the frontal region to be the most informative.[17] Nevertheless, in the future, the inclusion in the analysis of different scalp regions, or the analysis of source-resolved signals,[44] might be useful in better characterizing the development of both the considered neurodevelopmental disorders.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.106987.

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AUTHOR CONTRIBUTIONS

Conceptualization, C.P., V.R., and C.C.; Methodology, C.P., V.R., and C.C.; Software, C.P; Formal analysis, C.P.; Investigation, C.D. and E.M.R.; Writing – Original draft, C.P.; Writing – Review & Editing, C.P., V.R., and C.C.; Supervision, V.R. and C.C.; Funding Acquisition, C.P., V.R., and C.C.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Individuals PSD values	This paper	Zenodo: https://doi.org/10.5281/zenodo.7760605
Software and algorithms		
Matlab 2022b	http://www.mathworks.com/products/matlab/	RRID:SCR_001622
EEGLAB v2021.0	http://sccn.ucsd.edu/eeglab/index.html	RRID:SCR_007292
IBM SPSS statistics 28.0	https://www.ibm.com/products/spss-statistics	RRID:SCR_019096

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Caterina Piazza (caterina.piazza@lanostrafamiglia.it).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The dataset including all individual PSD values used to perform the statistical analysis is deposited on Zenodo (https://zenodo.org/) and publicly available as of the date of publication (Zenodo: https://doi.org/10.5281/zenodo.7760605). Raw EEG data are available from the lead contact on request.
- The code used for EEG data analysis is deposited on Zenodo (https://zenodo.org/) and publicly available as of the date of publication at (Zenodo: https://doi.org/10.5281/zenodo.7760605).
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Participants

Participants included a total of 284 infants divided into three groups: 143 infants at lower likelihood for both ASD and LLI (LL), 58 infants at HL-LLI and 83 infants at HL-ASD.

Inclusion criteria included the following: (1) gestational age $\geq$ 36 weeks; (2) Bayley Cognitive Scaled Score $\geq$ 7[45] or Griffiths developmental quotient $\geq$ 70,[46] both assessed at 6 months of age; and (3) having at least one native Italian speaker parent (95.4 % of the children with both native Italian speaker parents).

The criterion for being included in the HL-ASD group was having at least a sibling with a certified diagnosis of ASD.[47–49]

The HL-LLI group was selected based on a two-step procedure.[50,51] First, an interview was used to determine whether any of the infant's first-degree relatives received a clinical diagnosis of developmental language disorders or developmental dyslexia, and parents filled in the 'Adult Dyslexia Checklist' (ADCL) questionnaire,[52] a widespread screening tool for adults with dyslexia. Second, a clinical psychologist evaluated parents who reported reading difficulties (ADCL > 5) using standardized tests assessing word and nonword reading[53] and text reading.[54] Infants were assigned to the HL-LLI group if at least one first-degree relative (1) had a certified diagnosis of developmental language disorders and/or developmental dyslexia or (2) reported reading difficulties (ADCL > 5) and performed at least two standard deviations (SDs)

below the population mean on the two reading tasks. This group was composed of 58 children, with a parent (n = 19) or an older sibling (n = 39) with a certified diagnosis (and/or significant difficulties) of DLD (n = 24), DD (n = 22), or both (n = 12).

Infants' families were recruited from three hospitals within the Lecco and Monza Brianza area (Northern Italy). HL-ASD infants were recruited thanks to collaboration with the Italian Network for Early Detection of Autism Spectrum Disorders (NIDA Network).

All participants were informed about the methodology and duration of the study and written informed consent to participate was obtained from all parents prior to inclusion in the protocol. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Scientific Institute IRCCS E. Medea (Bosisio Parini, LC, Italy).

Three infants (1 LL and 2 HL-ASD) were excluded because they did not have usable EEG data at both time points considered in the study protocol (T6: 6 months of age; T12: 12 months of age). Thus, the total number of infants included in the present study was 281. Sample demographics are reported in Table S1. Preliminarily statistical analyses evaluated intergroup differences in sociodemographic characteristics and perinatal variables (Table S1), and a series of one-way ANOVAs and a chi-square test were applied. The three groups were matched for sex, age and gestational age. Differences between groups emerged in relation to sociodemographic variables (SES, maternal, and paternal education; Table S1). Specifically, the TD group showed higher SES and maternal education than both the HL-ASD and HL-LLI groups, as well as higher paternal education than the HL-ASD group.

Diagnostic outcome

Participants were classified into diagnostic outcome groups based on their clinical diagnosis. The TD-no-diag group included only children from the original LL group (without familiarity for neither ASD nor LLI) who at 3 years of age scored above the 30th percentile in the parent-administered Language Development Survey (LDS),[55] a questionnaire aimed at assessing expressive language development (standardized norms available for the Italian population,.[56] In addition to this criterion, all children from the LL and HL-LLI groups were included in an ongoing longitudinal study providing clinical evaluations at 3, 4.5, 6, and 8 years of age (ClinicalTrials.gov: NCT05767242). All children in the TD-no-diag group showed no difficulties in the last evaluation performed (at 8 years, n = 16; 6 years, n = 24; 4.5 years, n = 23; 3 years, n = 21). Children showing evident difficulties in one of these clinical evaluations were additionally included in a clinical in-depth evaluation performed by expert professionals. Similarly, all children from the HL-ASD group were included in an ongoing longitudinal study providing clinical evaluations until 3 years. Following these clinical assessments, two diagnostic groups of children were characterized. The DLD/DD group (n = 18) met the DSM-5 criteria for DLD or DD. Among children in the DLD/DD group, 11 had a diagnosis of DLD (61%), 6 had a diagnosis of DD (33%), and 1 had both diagnoses (6%).

The ASD group (n = 20) met DSM-5 criteria for ASD (APA, 2013) and scored above the ASD cutoff on the ADOS-2

Sample demographics are reported in Table S2. The three groups did not differ for age, gestational age, and parental education. Differences between groups emerged for sex, SES, and maternal education; Table S2). Specifically, the TD-no-diag group shows higher SES than the ASD group and higher maternal education than both the ASD and DLD/DD groups. Details about the number of children with a diagnostic outcome coming from the different LL and HL groups are reported in Table S3.

METHOD DETAILS

EEG data acquisition and processing

Infants participated in two EEG sessions at 6 (T6) and 12 months (T12). Four minutes of baseline EEG data were acquired while infants were seated on their parents' laps in a sound-attenuated and electrically shielded room. A research assistant blew bubbles in order to engage infants' attention, keeping them quit. The baseline EEG data collection was performed either before or after an experimental session (65% acquired before and 35% acquired after). Differences in the power values computed from the EEG data acquired before or after the experimental session were assessed using a series of independent t-tests. Significant differences emerged at both T6 and T12.

The recordings were made using a dense-array EGI system (Geodesic EEG System (GES) 300 or 400, Electric Geodesic, In., Eugene, Oregon, USA) equipped with 60/64-electrode caps or 128-electrode caps (HydroCel Geodesic Sensor net). Data were referenced to the vertex, sampled at 250 Hz or 1000 Hz and bandpass filtered between 0.1 and 100 Hz. Specifically, data of 29 subjects were recorded with the GES 400 system and with the 1000 Hz sample frequency. Among these subjects, six wore the 128-electrode cap.

After recording, raw EEG data were exported and processed within the open-source EEGLAB signal processing environment[57] and custom Matlab scripts (The Mathworks, Natick, MA, USA). First, data acquired with a sample frequency of 1000 Hz were down sampled at 250 Hz and the signals recorded in the channels exceeding the ones available in the 60-electrode caps were excluded from further analysis. Continuous EEG data were filtered with a 1 Hz high pass and a 45 Hz low pass FIR filter. The clean_rawdata EEGLAB plugin was used to identify bad channels (channels with flat line duration > 5 s; channels poorly correlated with their interpolated reconstruction based on neighboring channels, correlation threshold = 0.8) and to remove the bad portion of data (burst removal, SD cutoff = 20; periods with more than 25% of noisy channels). Data were further analyzed only if there was at least 1 minute of good signal (good signal: M = 184.4 s, SD = 76.2, range = 60.0-240.0), and no more than 15 channels were identified as bad (number of bad channels: M = 4.7, SD = 2.8, range = 0-14). Bad channels were then interpolated with a spherical spline and re-referenced to the common average reference. Independent component analysis (ICA) was applied by means of the RUNICA Infomax algorithm as implemented in EEGLAB.[58] The independent components (ICs) accounting for artifactual activities were then identified using the ICLabel plugin[59] (i.e. ICs with more that 80% contribution to eye activity, muscle activity, channel noise, or other noise), and removed (number of removed ICs: M = 11.9, SD = 4.0, range = 2–24).

The power spectral density (PSD) was estimated for epochs of 30 s using Welch's method (Hamming window of 10 s with 50% overlap) and then successively log transformed as follows: log PSD = log10(1+PSD). Finally, the power in the following bands was computed: delta (2–4 Hz), theta (4–6 Hz), low-alpha (6-9-Hz), high-alpha (9–13 Hz), beta (13–30 Hz), and gamma (30–45 Hz). These frequency ranges were selected based on previous infant EEG literature.[15–17] Subsequent analysis focused on frontal scalp regions. Specifically, two clusters of six electrodes were selected[32] (60/64 channel net: frontal left cluster electrodes 11, 12, 13, 14, 18, and 19; frontal right cluster electrodes 2, 56, 57, 58, 59, and 60; 128 channel net: frontal left cluster electrodes 23, 24, 27, 28, 33, and 34; and frontal right cluster electrodes 3, 116, 117, 122, 123, and 124). PSD values were then averaged across electrodes within each cluster.

QUANTIFICATION AND STATISTICAL ANALYSIS

Preliminary statistical analysis was performed using a series of paired t-tests in order to evaluate differences between power values estimated in the right and left frontal clusters in each frequency band at both T6 and T12 (Table S4). Since no differences emerged in the beta and gamma bands at both T6 and T12, for subsequent analysis beta and gamma power values computed in the left and right clusters have been averaged, obtaining a beta and gamma frontal cluster.

We used linear mixed models to assess the differences in power values between groups and over time. This allowed us to include the data of those infants who missed one of the two laboratory sessions or that did not have enough good EEG data for one session. We investigated main effects of the group (LL, HL-ASD, and HL-LLI) and time point (T6 and T12), as well as their interaction. Due to the differences between groups that emerged in relation to the sociodemographic variables and to the differences in the power values estimated from the resting acquisition performed before or after the experimental session, we included the variables SES and time of resting acquisition (before or after the experimental session) as covariates. We performed the analysis for each cluster and frequency band, and we applied the false discovery rate (FDR) adjustment[60] to correct for multiple comparisons. In addition, we investigated significant interactions using post hoc comparisons in which adjusted significance levels were set conservatively applying the Bonferroni correction.

Based on the results obtained from the linear mixed models, we assessed the differences in power values between TD-no-diag subjects and subjects diagnosed with ASD or DLD/DD for specific frequency bands

and clusters, separately for each time point (T6 and T12). Due to the low number of subjects belonging to both the ASD and DLD/DD groups (Tables S2 and 2), we used the nonparametric statistic, and we conducted a series of Mann-Whitney test. Also, we applied FDR adjustment[60] to correct for multiple comparisons.

We performed all analyses using SPSS statistics (version 28.0, Chicago, IL, USA).