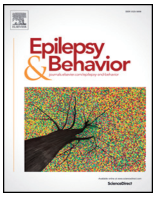
Epilepsy & Behavior 95 (2019) 87–94

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh



Functional brain connectivity in a rodent seizure model of autistic-like behavior

Philippe R. Mouchati, Jeremy M. Barry, Gregory L. Holmes *

Department of Neurological Sciences, University of Vermont College of Medicine, Burlington, VT 05405, USA

ARTICLE INFO

Article history:
Received 6 December 2018
Revised 23 March 2019
Accepted 26 March 2019
Available online 24 April 2019

Keywords:
Epilepsy
Sociability
Power spectrum
Development
Coherence
Voltage correlations

ABSTRACT

Objective: There is increasing evidence that Autism Spectrum Disorder (ASD) is a disorder of functional connectivity with both human and rodent studies demonstrating alterations in connectivity. Here, we hypothesized that early-life seizures (ELS) in rats would interrupt normal brain connectivity and result in autistic-like behavior (ALB).

Methods: Following 50 seizures, adult rats were tested in the social interaction and social novelty tests and then underwent qualitative and quantitative intracranial electroencephalography (EEG) monitoring in the medial prefrontal cortex (PFC) and the hippocampal subfields, CA3 and CA1.

Results: Rats with ELS showed deficits in social interaction and novelty, and compared with control, rats had marked increases in coherence within the hippocampus (CA3-CA1) and between the hippocampus and PFC during the awake and sleep states indicating hyperconnectivity. In addition, sleep spindle density was significantly reduced in rats with ELS. There were no differences in voltage correlations and power spectral densities between the ELS and control rats in any bandwidths.

Conclusion: Taken together, these findings indicate that ELS can result in ALB and alter functional connectivity as measured by coherence and sleep spindle density. These findings implicate altered connectivity as a robust neural signature for ALB following ELS.

1. Introduction

Autism Spectrum Disorder (ASD) is a group of complex neurodevelopmental disorders, characterized by deficits in social communication and interaction and restricted, repetitive, and stereotyped patterns of behavior. The symptoms are present from early childhood and impair everyday functioning. Individuals with ASD have co-occurring intellectual disability, language disorder, and epilepsy at higher rates than the general population [1]. A singular pathophysiological mechanism is unlikely to be responsible for the autistic phenotype. While genetics play an important role in ASD, underlying causal genetic variants have been defined in only a small subset of cases. In addition, the genes so far identified tend to be biologically pleiotropic and drive multiple diverse functions across developmental time and anatomical distribution [2]. In addition, environmental and other nongenetic factors can contribute to the development of autistic symptomatology [3]. The etiological heterogeneity associated with ASD raises the question as to how such a disparate group of etiologies can result in a recognizably consistent phenotype of impaired social communication and behavior, which can often be devastating.

There are increasing data indicating the common neurophysiologic underpinnings of individuals with ASD, regardless of etiology, is aberrant functional connectivity, defined as the “temporal correlations between remote neurophysiological events” [4–6]. While functional magnetic resonance imaging (MRI) and diffusion tensor imaging have been widely used to assess spatial connectivity between brain regions in ASD [7–12], neuroimaging studies lack temporal resolution. Electroencephalography (EEG) and magnetoencephalography have been widely used to assess connectivity over time and during different states. A particularly useful EEG feature is coherence, a measure of connectivity between brain regions. Coherence is a measure of “coupling” oscillations and therefore provides a dynamic link between brain areas required for the integration of distributed information [13,14]. In an EEG study using a large cohort of children with ASD, developmental delay without ASD and neurotypical children, brain connectivity, as measured by coherence, distinguished children with ASD from both neurotypical and developmentally delayed children [15]. Children with ASD had increased coherence which was most prominent during slow-wave sleep (SWS). In addition, this same cohort of children with ASD had reduced sleep spindle density when compared with the other groups [16].

Rodent studies have paralleled some of these findings in children. In rat pups with early-life seizures (ELS) and autistic-like behavior (ALB),

---

* Corresponding author at: 95 Carrigan Drive, Stafford 118, Burlington, VT 05405, USA.
E-mail address: gregory.holmes@med.uvm.edu (G.L. Holmes).

https://doi.org/10.1016/j.yebeh.2019.03.046
1525-5050/

EEG recordings from hippocampus and prefrontal cortex (PFC) demonstrated marked increases in coherence at all bandwidths compared with controls [17]. While alterations in coherence were seen during the awake state, alteration of coherence during sleep has not been studied in a model of ASD. Studying sleep is important since sleep EEG not only reflects the maturation of the brain [18] but also allows for examination of dynamic neural networks in the absence of external stimuli.

In addition, new insight into the function of sleep states makes it imperative that the brain's activity during sleep in neurodevelopmental disorders not be overlooked. While the exact function of sleep is unknown, the strongest evidence from human and animal experimental studies suggests that sleep's major role is to regulate brain plasticity [19]. SWS is implicated in learning and memory throughout the lifespan, and a prevailing hypothesis has been that SWS is essential for the consolidation of memories temporarily laid down during waking hours [20]. Given that there are both age- and state-dependent differential effects of sleep on neuronal responses and processes, further evaluation of sleep coherence is clearly important to our understanding of neurodevelopmental disorders.

The goals of this study were to determine if adult rats with prior ELS have ALB and alterations in functional connectivity and if hypercoherence as seen in previous rodent models and children is present across sleep and awake states. We used multisite electrophysiology in the ELS model of postnatal acquired epilepsy to study its resulting effects within and between brain regions that are critical for cognition, the PFC, and CA1 and CA3 regions of the hippocampus. To parallel the human studies, we assessed EEG measures during the awake state, SWS, and rapid eye movement (REM) sleep.

2. Material and methods

2.1. Overview of experiment

To assess seizure-induced alterations in brain oscillatory activity, male rat pups were subjected to 50 flurothyl-induced seizures from postnatal (P) day 5 to 14 (roughly corresponding to the first year of life in humans) [21]. This procedure in rats and mice has been shown to result in ALB when the animals are studied following the seizures [17,22]. To test for ALB, the sociability and social novelty tests were assessed at P60. Between P70 and P80, rats were implanted with intracranial electrodes in the right prelimbic region of the medial PFC and hippocampal subfields, CA1 and CA3. One to two weeks later, EEG recordings were carried out during epochs of sleep and wakefulness.

2.2. Animals

Sprague–Dawley rats were obtained from Charles River (Saint-Constant, QC). Animals were housed individually under United States Department of Agriculture (USDA) and Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved conditions, with a 12-h light/dark cycle, a temperature of 26 °C, and ad libitum access to food and water. Rats were weaned a postnatal (P) day 21. All procedures were approved by the University of Vermont's Institutional Animal Care and Use Committee and conducted in accordance with guidelines from the National Institutes of Health. All experiments were performed in accordance with the guidelines provided by the National Institutes of Health and University of Vermont for the humane treatment of animals. The animal protocol was approved by the Institutional Animal Care and Use Committee of the University of Vermont.

Male and female Sprague–Dawley rats ($n = 8$; 4 male, 4 female) were subjected to 5 flurothyl-induced seizures daily from P5 to P14 for a total of 50 seizures using previously described methods [23–26]. A 10% flurothyl solution (Bis(2,2,2-trifluoroethyl) ether), an inhaled convulsive agent, was delivered to the pups, which were placed in a plastic container located in an airflow hood. Flurothyl (0.1 ml) was injected slowly onto filter paper placed on the inside of the container where it evaporated. Pups were removed from the flurothyl after approximately 2 min when tonic extension of both forelimbs and hindlimbs was observed. Littermate control (CTL) pups ($n = 5$; 4 male, 1 female) were handled and removed from the dam during the time of the seizure to control for the effects of maternal separation stress.

2.3. Behavioral studies

2.3.1. Social interaction and social novelty tests

The three chamber sociability test was used to test social behavior in the seizure and CTL rats [17,27–29]. The social test apparatus consists of a wooden box with removable plastic partitions separating the box into three chambers. The size of the entire box was 122 × 41 cm (5002 cm^2) with the middle chamber area of 1148 cm^2 (28 × 41 cm) and two side chambers of 1927 cm^2 (47 × 41 cm). The height of the walls was 43 cm. An aluminum metal cylinder (11 cm in height, bottom diameter 11 cm) with grating (1 cm apart) with a lid was placed in each of the end chambers. On the first day, the rat was placed in the center chamber for 5 min, and then the doors to the side chamber were opened for 5 min to allow the rat to explore. Following this phase, the rat was removed from the chamber and returned to its home cage for 5 min. The test rat was then placed in the middle chamber with the dividers closed to allow it to explore the middle chamber for 5 min. After this 5-min habituation period, an unfamiliar rat of the same sex was placed in one cylinder box while a marble block (object) was placed in the other cylinder. The doors were then opened, and the animal was allowed 15 min to explore. Following this 15-min period, the test rat was removed from the box and placed in its home cage for 5 min. The object was then substituted with a new (novel) rat of the same sex of the test rat. The test rat was then placed in the center of the maze and allowed another 15 min to explore. Animals were videotaped during all sessions. Measurements of the corresponding amount of time spent in each chamber were done for both the sociability and social novelty tests by an investigator blinded as to group.

2.4. Electrophysiology

P70 rats underwent electrode implantation into the prelimbic cortex of the PFC and hippocampal subfields CA1 and CA3 using a custom designed head stage. Two 72-μm diameter, insulated nichrome electrodes (California Fine Wire, CA) with tips separated by ~0.5 mm were implanted at each location. The rats were anesthetized with inhaled isoflurane and placed in a stereotaxic frame. The skull was exposed, and four screws were inserted, two anterior to the left and right ends of bregma and two over the left and right cerebellum. The three pairs of electrodes were stereotaxically placed using coordinates from bregma, sagittal suture, and skull surface using Sherwood and Timiras [30]: PFC: 3.2 mm anterior to bregma; 0.8 mm lateral, 5.8 mm below skull; CA1: −3.2 mm posterior to bregma, 2.0 mm lateral, 2.1 mm below skull; CA3 −3.2 mm posterior to bregma, 3.0 mm lateral, 3.1 mm below skull. A right cerebellar screw was used for grounding while two wires were placed in over the left cerebellum and used as reference electrodes. All implants were fixed to the skull via the skull screws and grip cement. The wound was sutured, and a topical antibiotic was applied. Platinum iridium EMG electrodes were implanted in the trapezius muscles bilaterally. The interval between surgery and the beginning of the EEG recordings was a minimum of 1 week.

The implant was connected to a preamplifier with a cable connecting the rat to an amplifier and analog/digital board. Signals were filtered wideband (1–9000 Hz) and sampled at 30 K Hz and recorded on a Neuralynx (Montana) system. A light diode was attached to the head stage to allow tracking of movements. Electroencephalography recordings were obtained from the rats during foraging for food pellets in a cylindrical recording chamber for 30 min for 4 days. Rats were food deprived for 24 h prior to the foraging sessions then allowed to eat ad libitum for 1 h after the foraging. Sleep studies were done during the early afternoon for 2–3 days. The rat was placed in the recording chamber and left undisturbed for 2 h in the dark.

During foraging for food pellets, the EEG predominantly consisted of theta activity with an active EMG pattern. Slow-wave sleep (non-REM [NREM] sleep) demonstrated a high amplitude EEG consisting primarily of delta activity with intermixed faster frequencies and sleep spindles and a low-voltage EMG. Rapid eye movement sleep consisted of a lower-voltage EEG pattern with abundant theta frequencies and an inactive EMG pattern.

2.4.1. Field recordings

All EEG analyses were performed using NeuroExplore 5 software (Nex Technologies, Colorado Springs, CO). Electroencephalography bandwidths were defined as delta (Δ;: >0–<4 Hz);, theta (Θ;: 4 to <10 Hz);, alpha (α;: 10 to <20 Hz);, beta (β;: 20 to <25 Hz);, slow gamma (γ;: 25 to <50 Hz);, and fast gamma (γ;: 50 to 100 Hz) [17, 31]. Oscillatory properties using local field potentials (LFPs) were calculated for each animal during the awake state, SWS, and REM. Sleep spindles were analyzed during SWS from the PFC electrodes.

2.4.2. Coherence

Phase coherence quantifies the consistency of the relative phase between two simultaneous signals that have the same frequency [32]. Coherence was computed between two simultaneously recorded EEG signals from different brain regions in each frequency band. The fast Fourier transform (FFT) of the EEG recordings (after detrending and applying Hanning tapering) was calculated for 1–100 Hz. The number of frequency values was 65,536 with a 50% window overlap. Then, individual and cross densities were calculated: $Pxx = FFT(X) * Conj(FFT(X))$; $Pyy = FFT(Y) * Conj(FFT(Y))$; $Pxy = FFT(X) * Conj(FFT(Y))$, where X and Y refer to electrodes in different brain regions. Pxx, Pyy, and Pxy values were averaged across all intervals, and coherence values were calculated as: $Mean(Pxy) * Mean(Pxy) / (Mean(Pxx) * Mean(Pyy))$. Coherence values for delta, theta, alpha, beta, and gamma for 60 s of artifact-free epochs were calculated between i) the PFC and CA1;, ii) PFC and CA3;, and iii) CA3 and CA1.

2.4.3. EEG voltage correlations

This analysis calculates cross correlations from EEG in two areas and serves as a measure of modulation across the two electrode sites [32]. Cross-correlograms between LFPs in the PFC and CA1 and CA3 were obtained for 1 s before and after the overlap and averaged over the 60-s EEG recording (bin size = 3.4×10^{-4}). The EEG voltages within the specified bin size were calculated, and then the correlations between the electrode pairs were computed. If $x[i]$, $i = 1,\ldots N$ is the reference voltage and $y[i]$, $i = 1,\ldots,N$ is another voltage, then $c[t]$ = Pearson correlation coefficients (PCC) between vectors $\{x[1], x[2], \ldots, x[N - t]\}$ and $\{y[t + 1], y[t + 2], \ldots, y[N]\}$. To assess modulation, the mean differences from the minimum and maximum correlation values over the 2-s correlograms were calculated for each electrode pair during the three states (Fig. 1).

2.4.4. Sleep spindle density

Spindle density in the PFC was calculated using two methods:

1) Automatic detection of the spindle band (12–20) Hz band. Electroencephalography activity in the 12–20 Hz was band-filtered during 10-min epochs of SWS (with filter band specified as [Main Band Min Freq, Main Band Max Freq]). Hilbert transform was then applied to the filtered signal. The jumps of the phase of the Hilbert transform from 360° to zero were identified as starts of the oscillation cycles. The percentage of the spindle band to the remaining activity was then calculated for each second. Fig. 2 shows an example of sleep spindles with a corresponding spectrogram showing sleep spindles at ~12 Hz.
2) Manual counting. Spindle number per 10 min of SWS was calculated visually by one investigator (GLH) blinded to treatment group.

2.4.5. Histology

Following all behavioral and electrophysiological testing, rats were sacrificed; brains were removed, cut at 40 μm, and stained with thionin for verification of electrode placement as previously described [17].

2.4.6. Statistics

The analysis of variance (ANOVA) was used to compare time spent with the rat, object, or neither rat/object in the social interaction test, as well as time spent with novel or familiar rat or neither in the social novelty test. Tukey's multiple comparisons' test was used to compare differences in the three locations (rat, object, neither or novel rat, familiar rat or neither). The *t*-test was used to compare EEG measures in the ELS and CTL rats. False detection rate was assessed with the Holm–Sidak test with an alpha of 0.05. All p values reported were adjusted for false detection. Data are presented as mean ± standard error of the mean.

3. Results

All rats had tonic seizures with administration of the flurothyl. Four of the 10 (40%) rats died during or shortly after flurothyl administration

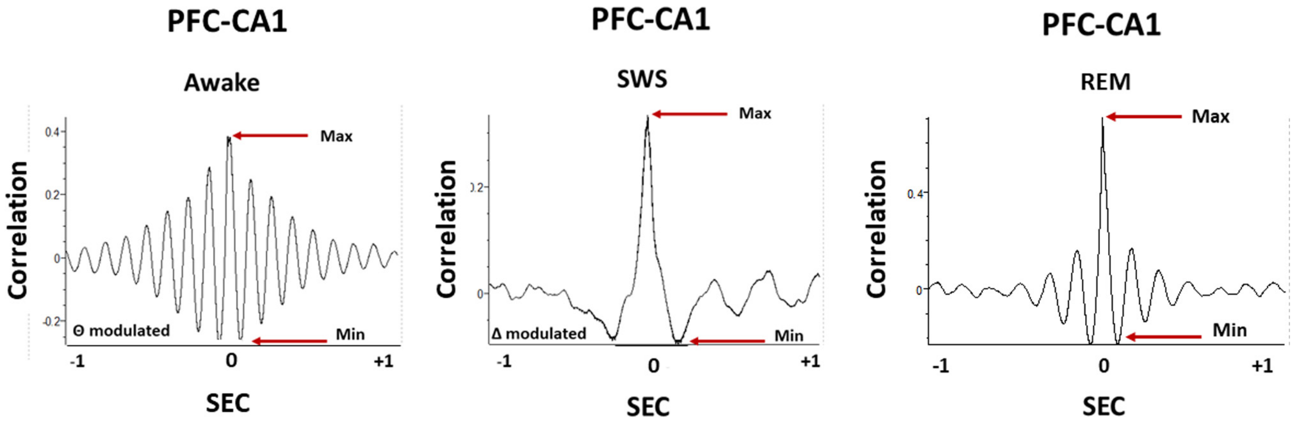


Fig. 1. Example of voltage cross correlations during the awake and sleep states over 2 s. Note the modulation by theta (~8 Hz) in the awake and REM states and by delta during SWS. Degree of voltage modulation was determined by subtracting the maximum (Max) and minimum (Min) and averaging the differences in each state and brain region. In this example, the reference variable is the PFC electrode, and the comparison variable is the CA1 electrode.

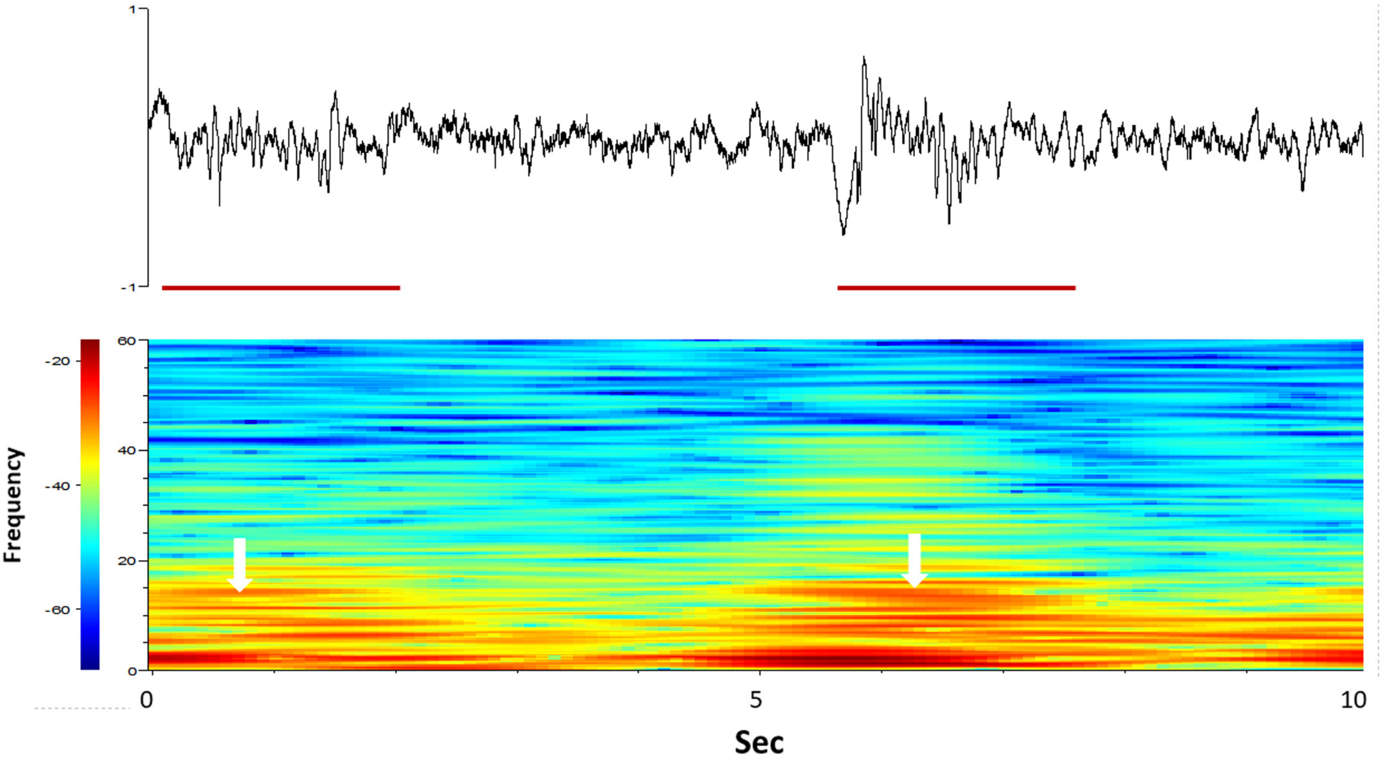


Fig. 2. EEG with spindles (underlined) corresponding with a 12-Hz band in the spectrogram (arrows).

while none of the CTL rats died. Behavioral testing was done on 6 ELS rats (3 ♂, 3 ♀) and 5 CTL (4 ♂, 1 ♀).

3.1. Behavioral testing

3.1.1. Social interaction and novelty

In the social interaction study, the ELS rats behaved differently than the CTL rats ($F(2,15) = 23.3$; $p < 0.001$), with the CTL rats spending significantly more time than the ELS rats with the rat (CTL: 634.2 ± 51.16 s; ELS: 464.5 ± 44.44 s; $p = 0.033$). The CTL rat spent significantly more time with the rat than object ($p < 0.0001$) while there was no difference in the time ELS rats spent with the rat than the object ($p = 0.181$). Control rats had significant differences in the amount of time spent with the familiar and novel rats ($F(2,15) = 12.74$; $p = 0.001$) with the CTL rats spending more time with the novel rat than the familiar rat, although the difference was not statistically significant ($p = 0.061$). In the social novelty test, group differences were noted ($F(2,15) = 12.06$; $p = 0.008$) with the ELS group spending more time with the familiar than the novel rat ($p = 0.048$). These results demonstrate that following ELS, rats have deficits in social cognition (Fig. 3).

3.2. EEG findings

Of the 6 ELS and 5 CTL rats that completed the behavioral testing, 5 ELS (3 male, 2 female) and 4 CTL (3 male, 1 female) rats had electrodes placed in the PFC, CA3, and CA1 regions. All analyses were conducted on artifact-free EEG.

3.2.1. Coherences

A marked increase in coherence was noted in the ELS compared with CTL rats in almost all frequency bands in all three states (Fig. 4). At no frequency band or behavioral state were the coherences higher in the CTL than the ELS rats. The differences in coherences were generally higher in all brain regions during SWS then during the awake and REM states.

3.2.2. Voltage correlations

Mean and peak correlations of voltage over a 2-s window were compared in the CTL and ELS rats by comparing differences between minimum and maximum correlations for each state and each electrode position. No differences between CTL and ELS rats were apparent from visual or statistical analysis of the data (Fig. 5).

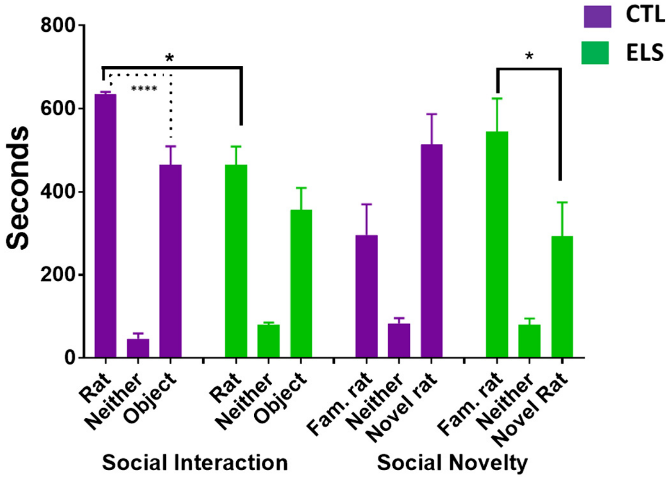


Fig. 3. Social interaction and social novelty tests. In the social interaction study, the ELS rats behaved differently than the CTL rats, with the CTL rats spending significantly more time than the ELS rats with the rat. The CTL rat spent significantly more time with the rat than object while there was no difference in the time ELS rats spent with the rat than the object. CTL rats had significant differences in the amount of time spent with the familiar and novel rats with the CTL rats spending more time with the novel rat than the familiar rat, although the difference was not statistically significant. In the social novelty test, group differences were noted with the ELS group spending more time with the familiar than the novel rat. These results demonstrate that following ELS, rats have deficits in social cognition.

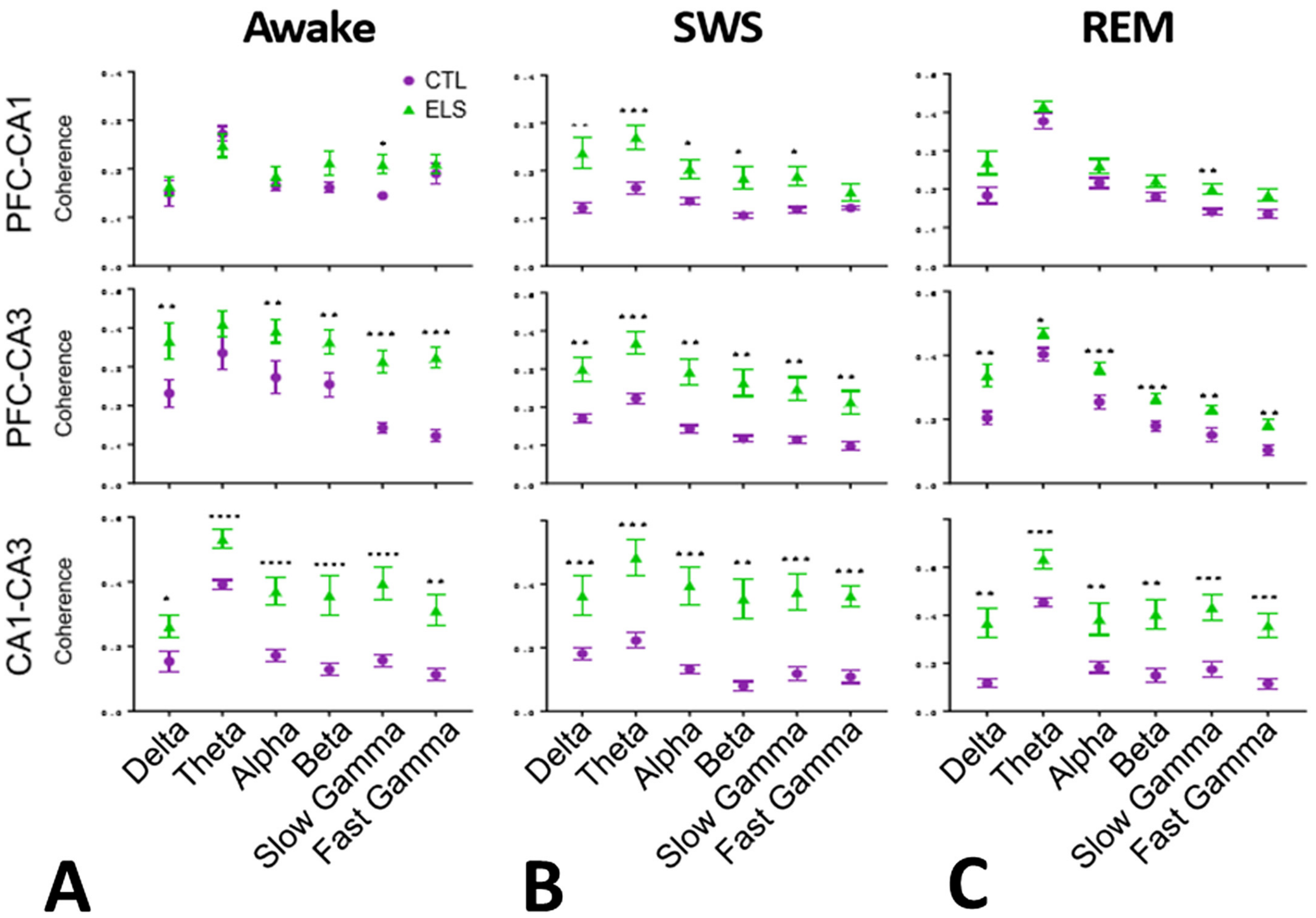


Fig. 4. Coherences (mean ± SEM) between PFC-CA1, PFC-CA3, CA1-CA3 in (A) awake, (B) SWS, and (C) REM states. (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

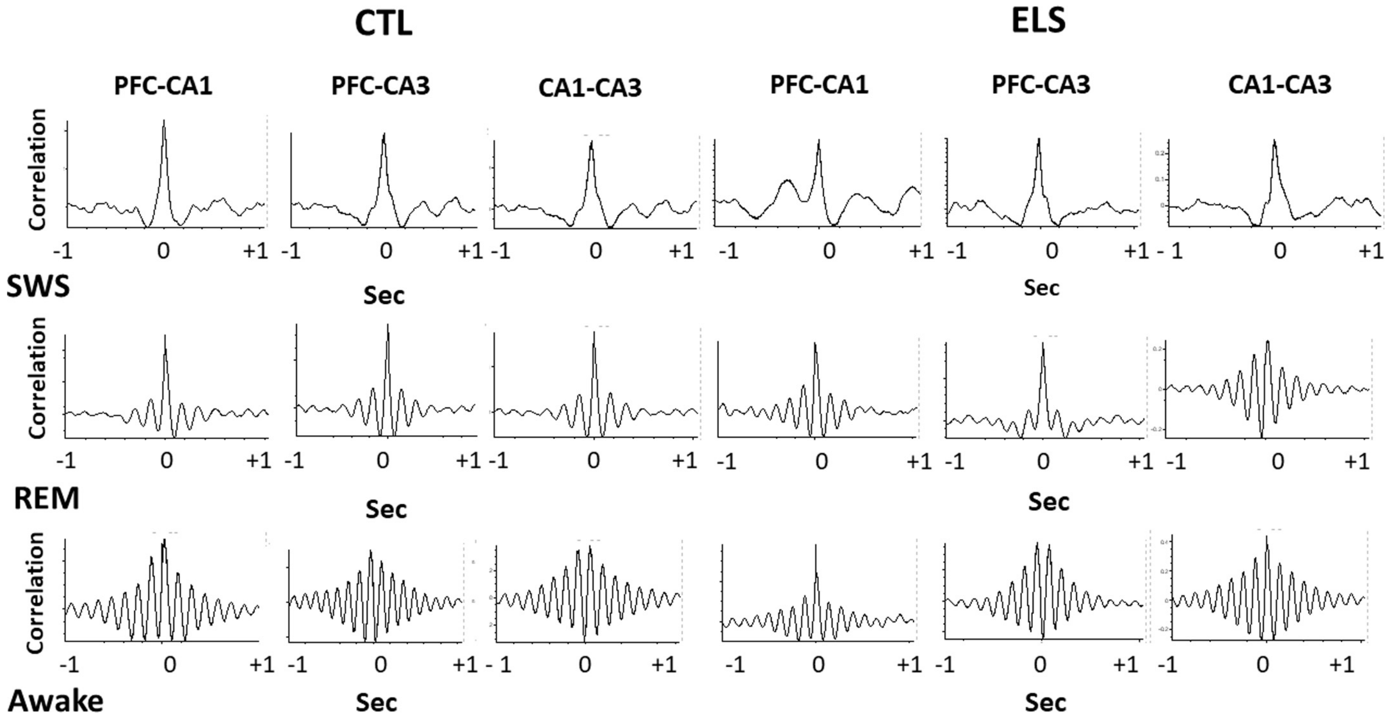


Fig. 5. Examples of voltage correlations in three brain regions (PFC, CA1, and CA3) in ELS and CTL rats during three states. No differences were noted between the ELS and CTL groups in any brain region or state.

3.2.3. Sleep spindle density

The spindle density during SWS was significantly higher in the CTL (10.41 ± 0.231%) than in the ELS (8.737 ± 0.138%) rats ($t = 6.591$; $p < 0.0001$). Likewise, a manual count of spindles showed a significantly greater number of spindles in the CTL than the ELS group (CTL: 87.2 ± 5.95; ELS: 53.4 ± 11.7; $p = 0.033$). No differences in spindle duration were found between the CTL and ELS rats (CTL: 0.846 ± 0.021; ELS: 0.891 ± 0.014; $p > 0.05$).

3.2.4. Spectral power

Other than for increased power in the theta band in ELS rats during the awake state, no significant differences in power across bandwidths in awake, SWS, and REM were noted (Suppl. Figs. 1–3).

3.2.5. Histology

Electrode placement was verified in the PFC and CA1 and CA3 regions.

4. Discussion

The primary finding in this study is that in a rodent model of ELS, adult rats demonstrate ALB with impairments in social interaction and social novelty. These ALBs occurred in rats showing significant alterations in connectivity with both intrahippocampal (CA3-CA1) and hippocampus-PFC networks having higher coherences than in CTL rats. The increases in coherence occurred at all frequencies and during all states (awake state, SWS, REM). While spectral coherence was markedly increased in the ELS rats, there were no differences between ELS rats and CTL rats in spectral power at any frequency during any of the three states. These findings show that following ELS, the brain is hyperconnected during all states. In addition, compared with the CTL groups, rats with ELS had decreases in sleep spindle density, indicating a decrease in thalamocortical information flow.

Both the hippocampus and PFC are important in learning and memory and have been implicated in the pathophysiology of ASD [33–36]. While the hippocampus stores an explicit representation of space that is used as a "cognitive map" to guide goal-based behavior [37–41], the CA1-PFC pathway is crucial for goal-directed decision-making and providing abstract representations of goals and intentions [42–45]. During the awake state, the firing of action potentials is temporally coordinated through phase of local theta in both the CA3-CA1 [46–48] and CA1-PFC pathways [49–51]. This theta-entrained activity across neuronal circuits is critical for guiding the plastic changes that underlie the dynamic "back and forth" storage and retrieval of information across these networks [52–54]. Likewise, during SWS sleep, there is delta-driven widespread synchronization of action potentials [55,56]. High coherence values during both wakefulness and sleep are an indication of strong phase linking between neuronal ensembles, indicating high connectivity between the brain regions that produce the EEG signals [57].

Since high coherence values are a marker of enhanced connectivity, it is difficult to explain why high coherences would be associated with the ALB associated with ELS. Early-life seizures can result in an imbalance of excitation/inhibition in the neocortex and hippocampus resulting in enhanced excitation that may predispose the developing brain to excessive connectivity [58–60]. High phase locking of neurons in multiple brain regions likely results in neurons in both structures firing with excessive synchrony with a diminished ability to develop localized functional ensembles [61]. In a recent study of functional MRI (fMRI) in individuals at risk for psychosis, it was found that high levels of hyperconnectivity in a circuit involving the cerebellum, thalamus, and cortex were predictive of impending full psychosis – hallucinations, delusions, and disorganized thought and behavior [62]. Neuronal synchrony in the brain is finely tuned, and it is likely that functional "over-connectivity" may be as detrimental as "under-connectivity," as a network that is overconnected at baseline may not be able to adapt to increased cognitive demand. We suggest that as with other electrophysiological processes, there is an ideal "sweet spot" for dynamic coherence and that deviations in either a positive or negative direction can alter behavior adversely.

Sleep spindles are measured by EEG as brief distinct bursts of activity in the frequency range of 12 to 20 Hz in rats. They have a characteristic waxing and waning shape and can be defined by their oscillatory frequency, amplitude, and density throughout the night. In addition, spindles are believed to play an important functional role in sleep-dependent synaptic plasticity and memory consolidation [63]. Spindles originate in the thalamic reticular nucleus [64] and reflect the integrity of the thalamocortical microcircuit. Spindle deficits have been reported in cohorts of adolescents and adults with schizophrenia [65–67] and recently in children with ASD [16]. In a sleep study including children with ASD, children with developmental delay but no ASD and neurotypical children, a strong relationship between sleep spindle density and nonverbal ratio intelligence quotient (IQ) test and the Vineland Socialization domain scores was found, suggesting sleep spindles may play a role or be a biomarker of behavior in neurodevelopment disorders [16].

Another dimension of brain connectivity, amplitude or voltage correlation, is a measure of the comodulation of the amplitude envelopes, i.e., power, of oscillations in the two brain areas [32,68]. Amplitude correlation is also referred to as "power-to-power correlation" or "amplitude-amplitude coupling". Voltage correlation marks the degree of association between amplitudes of the EEG across sites and does not calculate phase or the consistency of phase relationships seen with coherence. Amplitude correlations reflect state changes coupled across brain networks that are driven by neuromodulatory systems [69]. Studies have shown that amplitude correlations provide a measure of large-scale interactions between cortical areas that are only indirectly connected via polysynaptic pathways [70–73]. The lack of differences between the ELS and CTL indicates that voltage modulation is not altered in adult rats with ELS.

While equating rat behavior to the complex symptoms of a child with ASD is difficult, the deficits in sociability and social novelty preference in rats have some similarities to the core features that are seen in children with ASD. For example, children with ASD spend less time looking at people and longer times looking at objects compared with controls [74,75]. Both the findings of increased coherences in three brain regions during awake, SWS, and REM and decreased sleep spindles in SWS parallel findings in children with ASD [15,16]. These studies indicate that the ELS rodent model recapitulates some of the features of the ASD condition in children and can provide insights into pathophysiological mechanisms.

The results of this study need further confirmation. The small number of animals did not allow us to determine if there are gender differences and future studies are necessary to determine if gender is a biological variable in this model. It is quite possible that there are gender differences in the model. For example, it is known that the relationship of sleep spindles with cognition may be sexually dimorphic [76,77]. It is also important to note that although coherence values were higher in the ELS group than the CTL, this does not indicate cause and effect. Rather, hyperconnectivity and reduced sleep spindle density may serve as robust neural biomarkers for ALB following ELS.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yebeh.2019.03.046.

Conflict of interest

The authors note there is not conflict of interest.

Acknowledgments

This study was supported by National Institutes of Health, National Institute of Neurological Disorders and Stroke, grants NS108296 and NS108765.

References

[1] Buckley AW, Holmes GL. Epilepsy and autism. Cold Spring Harb Perspect Med 2016;6(4).

[2] Sestan N, State MW. Lost in translation: traversing the complex path from genomics to therapeutics in Autism Spectrum Disorder. Neuron 2018;100(2):406–23.

[3] Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, et al. Genetic heritability and shared environmental factors among twin pairs with autism. Arch Gen Psychiatry 2011;68(11):1095–102.

[4] Friston KJ. Functional and effective connectivity: a review. Brain Connect 2011;1(1):13–36.

[5] Stamou M, Streifel KM, Goines PE, Lein PJ. Neuronal connectivity as a convergent target of gene x environment interactions that confer risk for Autism Spectrum Disorders. Neurotoxicol Teratol 2013;36:3–16.

[6] Luckhardt C, Jarczok TA, Bender S. Elucidating the neurophysiological underpinnings of Autism Spectrum Disorder: new developments. J Neural Transm 2014;121(9):1129–44.

[7] Koshino H, Carpenter PA, Minshew NJ, Cherkassky VL, Keller TA, Just MA. Functional connectivity in an fMRI working memory task in high-functioning autism. Neuroimage 2005;24(3):810–21.

[8] Koshino H, Kana RK, Keller TA, Cherkassky VL, Minshew NJ, Just MA. fMRI investigation of working memory for faces in autism: visual coding and underconnectivity with frontal areas. Cereb Cortex 2008;18(2):289–300.

[9] Just MA, Cherkassky VL, Keller TA, Kana RK, Minshew NJ. Functional and anatomical cortical underconnectivity in autism: evidence from an FMRI study of an executive function task and corpus callosum morphometry. Cereb Cortex 2007;17(4):951–61.

[10] Kana RK, Keller TA, Cherkassky VL, Minshew NJ, Just MA. Sentence comprehension in autism: thinking in pictures with decreased functional connectivity. Brain 2006;129(Pt 9):2484–93.

[11] Cherkassky VL, Kana RK, Keller TA, Just MA. Functional connectivity in a baseline resting-state network in autism. NeuroReport 2006;17(16):1687–90.

[12] Weng SJ, Wiggins JL, Peltier SJ, Carrasco M, Risi S, Lord C, et al. Alterations of resting state functional connectivity in the default network in adolescents with Autism Spectrum Disorders. Brain Res 2010;1313:202–14.

[13] Varela F, Lachaux JP, Rodriguez E, Martinerie J. The brainweb: phase synchronization and large-scale integration. Nat Rev Neurosci 2001;2(4):229–39.

[14] Thatcher RW. Coherence, phase differences, phase shift, and phase lock in EEG/ERP analyses. Dev Neuropsychol 2012;37(6):476–96.

[15] Buckley AW, Scott R, Tyler A, Mahoney JM, Thurm A, Farmer C, et al. State-dependent differences in functional connectivity in young children with Autism Spectrum Disorder. EBioMedicine 2015;2(12):1905–15.

[16] Farmer CA, Chilakamarri P, Thurm AE, Sweto SE, Holmes GL, Buckley AW. Differences in spindle activity among young children with autism, developmental delay, or typical development. Neurology 2018;91(2):e112–22.

[17] Holmes GL, Tian C, Hernan AE, Flynn S, Camp D, Barry J. Alterations in sociability and functional brain connectivity caused by early-life seizures are prevented by bumetanide. Neurobiol Dis 2015;77:204–19.

[18] Kurth S, Achermann P, Rusterholz T, LeBourgeois MK. Development of brain EEG connectivity across early childhood: does sleep play a role? Brain Sci 2013;3(4):1445–60.

[19] Wang G, Grone B, Colas D, Appelbaum L, Mourrain P. Synaptic plasticity in sleep: learning, homeostasis and disease. Trends Neurosci 2011;34(9):452–63.

[20] Dudai Y, Karni A, Born J. The consolidation and transformation of memory. Neuron 2015;88(1):20–32.

[21] Avishai-Eliner S, Brunson KL, Sandman CA, Baram TZ. Stressed-out, or in (utero)? Trends Neurosci 2002;25(10):518–24.

[22] Lugo JN, Swann JW, Anderson AE. Early-life seizures result in deficits in social behavior and learning. Exp Neurol 2014;256:74–80.

[23] Karnam HB, Zhou JL, Huang LT, Zhao Q, Shatskikh T, Holmes GL. Early life seizures cause long-standing impairment of the hippocampal map. Exp Neurol 2009;217(2):378–87.

[24] Karnam HB, Zhao Q, Shatskikh T, Holmes GL. Effect of age on cognitive sequelae following early life seizures in rats. Epilepsy Res 2009;85(2–3):221–30.

[25] Kleen JK, Sesque A, Wu EX, Miller FA, Hernan AE, Holmes GL, et al. Early-life seizures produce lasting alterations in the structure and function of the prefrontal cortex. Epilepsy Behav 2011;22(2):214–9.

[26] Lucas MM, Lenck-Santini PP, Holmes GL, Scott RC. Impaired cognition in rats with cortical dysplasia: additional impact of early-life seizures. Brain 2011;134:1684–93 Pt 6.

[27] Nadler JJ, Moy SS, Dold G, Trang D, Simmons N, Perez A, et al. Automated apparatus for quantitation of social approach behaviors in mice. Genes Brain Behav 2004;3(5):303–14.

[28] Moy SS, Nadler JJ, Perez A, Barbaro RP, Johns JM, Magnuson TR, et al. Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. Genes Brain Behav 2004;3(5):287–302.

[29] Moy SS, Nadler JJ, Young NB, Perez A, Holloway LP, Barbaro RP, et al. Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. Behav Brain Res 2007;176(1):4–20.

[30] Sherwood NM, Timiras PS. A stereotaxic atlas of the developing rat brain. Berkeley, California: University of California Press; 1970.

[31] Colgin LL, Denninger T, Fyhn M, Hafting T, Bonnevie T, Jensen O, et al. Frequency of gamma oscillations routes flow of information in the hippocampus. Nature 2009;462(7271):353–7.

[32] Siegel M, Donner TH, Engel AK. Spectral fingerprints of large-scale neuronal interactions. Nat Rev Neurosci 2012;13:121.

[33] Ben Shalom B. The medial prefrontal cortex and integration in autism. Neuroscientist 2009;15(6):589–98.

[34] Ben Shalom D. Memory in autism: review and synthesis. Cortex 2003;39(4–5):1129–38.

[35] Varghese M, Keshav N, Jacot-Descombes S, Warda T, Wicinski B, Dickstein DL, et al. Autism Spectrum Disorder: neuropathology and animal models. Acta Neuropathol 2017;134(4):537–66.

[36] Codagnone MG, Podesta MF, Uccelli NA, Reines A. Differential local connectivity and neuroinflammation profiles in the medial prefrontal cortex and hippocampus in the valproic acid rat model of autism. Dev Neurosci 2015;37(3):215–31.

[37] O'Keefe J, Nadel L. The hippocampus as a cognitive map. Oxford: Clarendon; 1978.

[38] O'Keefe J, Black AH. Single unit and lesion experiments on the sensory inputs to the hippocampal cognitive map. Ciba Found Symp 1977;58:179–98.

[39] Becker JT, Olton DS. Cognitive mapping and hippocampal system function. Neuropsychologia 1981;19(6):733–44.

[40] Becker JT, Olton DS, Anderson CA, Breitinger ER. Cognitive mapping in rats: the role of the hippocampal and frontal system in retention and reversal. Behav Brain Res 1981;3(1):1–22.

[41] Muller R. A quarter of a century of place cells. Neuron 1996;17(5):813–22.

[42] Kim J, Delcasso S, Lee I. Neural correlates of object-in-place learning in hippocampus and prefrontal cortex. J Neurosci 2011;31(47):16991–7006.

[43] Bruni S, Giorgetti V, Bonini L, Fogassi L. Processing and integration of contextual information in monkey ventrolateral prefrontal neurons during selection and execution of goal-directed manipulative actions. J Neurosci 2015;35(34):11877–90.

[44] Bruni S, Giorgetti V, Fogassi L, Bonini L. Multimodal encoding of goal-directed actions in monkey ventral premotor grasping neurons. Cereb Cortex 2017;27(1):522–33.

[45] Saez A, Rigotti M, Ostojic S, Fusi S, Salzman CD. Abstract context representations in primate amygdala and prefrontal cortex. Neuron 2015;87(4):869–81.

[46] Barry JM, Sakkaki S, Barriere SJ, Patterson KP, Lenck-Santini PP, Scott RC, et al. Temporal coordination of hippocampal neurons reflects cognitive outcome post-febrile status epilepticus. EBioMedicine 2016;7:175–90.

[47] Dragoi G, Buzsaki G. Temporal encoding of place sequences by hippocampal cell assemblies. Neuron 2006;50(1):145–57.

[48] O'Keefe J. Hippocampus, theta, and spatial memory. Curr Opin Neurobiol 1993;3(6):917–24.

[49] Hyman JM, Wyble BP, Goyal V, Rossi CA, Hasselmo ME. Stimulation in hippocampal region CA1 in behaving rats yields long-term potentiation when delivered to the peak of theta and long-term depression when delivered to the trough. J Neurosci 2003;23(37):11725–31.

[50] Jones MW, Wilson MA. Theta rhythms coordinate hippocampal-prefrontal interactions in a spatial memory task. PLoS Biol 2005;3(12):e402.

[51] Jones MW, Wilson MA. Phase precession of medial prefrontal cortical activity relative to the hippocampal theta rhythm. Hippocampus 2005;15(7):867–73.

[52] Siapas AG, Lubenov EV, Wilson MA. Prefrontal phase locking to hippocampal theta oscillations. Neuron 2005;46(1):141–51.

[53] Fernandez-Ruiz A, Oliva A, Nagy GA, Maurer AP, Berenyi A, Buzsaki G. Entorhinal-CA3 dual-input control of spike timing in the hippocampus by theta-gamma coupling. Neuron 2017;93(5):1213–26.

[54] Fries P. A mechanism for cognitive dynamics: neuronal communication through neuronal coherence. Trends Cogn Sci 2005;9(10):474–80.

[55] Destexhe A, Contreras D, Steriade M. Spatiotemporal analysis of local field potentials and unit discharges in cat cerebral cortex during natural wake and sleep states. J Neurosci 1999;19(11):4595–608.

[56] Wolansky T, Clement EA, Peters SR, Palczak MA, Dickson CT. Hippocampal slow oscillation: a novel EEG state and its coordination with ongoing neocortical activity. J Neurosci 2006;26(23):6213–29.

[57] Srinivasan R, Winter WR, Ding J, Nunez PL. EEG and MEG coherence: measures of functional connectivity at distinct spatial scales of neocortical dynamics. J Neurosci Methods 2007;166(1):41–52.

[58] Isaeva E, Isaev D, Khazipov R, Holmes GL. Selective impairment of GABAergic synaptic transmission in the flurothyl model of neonatal seizures. Eur J Neurosci 2006;23(6):1559–66.

[59] Isaeva E, Isaev D, Khazipov R, Holmes GL. Long-term suppression of GABAergic activity by neonatal seizures in rat somatosensory cortex. Epilepsy Res 2009;87(2–3):286–9.

[60] Isaeva E, Isaev D, Savrasova A, Khazipov R, Holmes GL. Recurrent neonatal seizures result in long-term increases in neuronal network excitability in the rat neocortex. Eur J Neurosci 2010;31(8):1446–55.

[61] Voytek B, Knight RT. Dynamic network communication as a unifying neural basis for cognition, development, aging, and disease. Biol Psychiatry 2015;77(12):1089–97.

[62] Cao H, Chen OY, Chung Y, Forsyth JK, McEwen SC, Gee DG, et al. Cerebello-thalamo-cortical hyperconnectivity as a state-independent functional neural signature for psychosis prediction and characterization. Nat Commun 2018;9(1):3836.

[63] Fogel SM, Smith CT. The function of the sleep spindle: a physiological index of intelligence and a mechanism for sleep-dependent memory consolidation. Neurosci Biobehav Rev 2011;35(5):1154–65.

[64] Contreras D, Steriade M. Spindle oscillation in cats: the role of corticothalamic feedback in a thalamically generated rhythm. J Physiol 1996;490:159–79 Pt 1.

[65] Goder R, Graf A, Ballhausen F, Weinhold S, Baier PC, Junghanns K, et al. Impairment of sleep-related memory consolidation in schizophrenia: relevance of sleep spindles? Sleep Med 2015;16(5):564–9.

[66] Manoach DS, Pan JQ, Purcell SM, Stickgold R. Reduced sleep spindles in schizophrenia: a treatable endophenotype that links risk genes to impaired cognition? Biol Psychiatry 2016;80(8):599–608.

[67] Wamsley EJ, Tucker MA, Shinn AK, Ono KE, McKinley SK, Ely AV, et al. Reduced sleep spindles and spindle coherence in schizophrenia: mechanisms of impaired memory consolidation? Biol Psychiatry 2012;71(2):154–61.

[68] Bruns A, Eckhorn R, Jokeit H, Ebner A. Amplitude envelope correlation detects coupling among incoherent brain signals. NeuroReport 2000;11(7):1509–14.
[69] Munk MH, Roelfsema PR, Konig P, Engel AK, Singer W. Role of reticular activation in the modulation of intracortical synchronization. Science 1996;272(5259):271–4.
[70] de Lange FP, Jensen O, Bauer M, Toni I. Interactions between posterior gamma and frontal alpha/beta oscillations during imagined actions. Front Hum Neurosci 2008;2:7.
[71] Mazaheri A, Nieuwenhuis IL, van DH, Jensen O. Prestimulus alpha and mu activity predicts failure to inhibit motor responses. Hum Brain Mapp 2009;30(6):1791–800.
[72] Mazaheri A, Coffey-Corina S, Mangun GR, Bekker EM, Berry AS, Corbett BA. Functional disconnection of frontal cortex and visual cortex in attention-deficit/hyperactivity disorder. Biol Psychiatry 2010;67(7):617–23.
[73] Donner TH, Siegel M, Fries P, Engel AK. Buildup of choice-predictive activity in human motor cortex during perceptual decision making. Curr Biol 2009;19(18):1581–5.
[74] Swettenham J, Baron-Cohen S, Charman T, Cox A, Baird G, Drew A, et al. The frequency and distribution of spontaneous attention shifts between social and nonsocial stimuli in autistic, typically developing, and nonautistic developmentally delayed infants. J Child Psychol Psychiatry 1998;39(5):747–53.
[75] Bhat AN, Galloway JC, Landa RJ. Social and non-social visual attention patterns and associative learning in infants at risk for autism. J Child Psychol Psychiatry 2010;51(9):989–97.
[76] Ujma PP, Konrad BN, Gombos F, Simor P, Potari A, Genzel L, et al. The sleep EEG spectrum is a sexually dimorphic marker of general intelligence. Sci Rep 2017;7(1):18070.
[77] Ujma PP, Sandor P, Szakadat S, Gombos F, Bodizs R. Sleep spindles and intelligence in early childhood-developmental and trait-dependent aspects. Dev Psychol 2016;52(12):2118–29.