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Development of Low-frequency Neural Oscillations through Adolescence

Degree Project in Medicine

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Abstract

Introduction: Schizophrenia is often conceptualized as a neurodevelopmental disorder and is characterized by psychotic and cognitive symptoms, which may be caused by altered neural networks. Adolescence is a critical period where dramatic neurodevelopmental changes occur, many of which may affect the development of oscillations. However, little is currently known about both normal and abnormal brain rhythm dynamics during this time, particularly in the low-frequency band.

Aim: To investigate how spontaneous low-frequency neural oscillations in the delta and theta band develop through the adolescent period in normal rats of both females and males.

Methods: The local field potentials (LFP) in the prefrontal cortex (PFC) and hippocampus (HC) were recorded under urethane anesthesia in 22 rats (12 females and 10 males, from two different mothers) everyday through adolescence, from P32 to P52. Signals were subjected to Fast Fourier Transform and power density spectra were acquired. Pearson's correlation coefficient (r) was calculated between peak power and age for theta and delta oscillations respectively.

Results: Both theta and delta oscillations developed early and were already present in the youngest rat at age P32. HC theta power decreased, while PFC delta increased through adolescence. Theta power stabilized with age, as indicated by a drastic reduction of standard deviation, and showed a sex difference in time of completion.

Conclusion: We have preliminary data indicating that PFC-delta and HC-theta oscillations may follow distinct developmental trajectories during adolescence with notable gender differences.

Keywords: Development, neural oscillations, prefrontal cortex, hippocampus, schizophrenia

Introduction

Background

Schizophrenia is a severe psychiatric disorder that greatly influences all major areas of life and is characterized by its complexity and chronicity (1). Estimated to affect 0.28% of the population or approximately 21 million individuals worldwide, schizophrenia is regarded as a low prevalence mental disorder (2). Although of lower occurrence, the magnitude of disease burden is inarguably immense and causes profound disruption in the lives of those affected. A systematic analysis by Salomon JA et al. reported that acute schizophrenia holds the highest disability weight of 235 health states in the Global Burden of Disease (GBD) 2013 study (3). The substantial debilitation attributable to the disorder is further validated in GBD 2016 where schizophrenia is one of the top 15 leading causes of disability among 328 diseases and injuries worldwide (4).

The impairment experienced is evident in many areas of everyday functioning and negatively affects the ability to form and maintain interpersonal relationships, perform self-care maintenance, sustain employment and live independently. The psychosocial difficulties seen socially, vocationally and residentially are present throughout the course of illness, persisting even during periods of remission from active psychosis (5). Consequently, reliance on continuous support from other parties such as family members and various mental health services is unavoidable. It becomes readily evident that the unfortunate outcomes of the illness are not solely reserved to the affected individual but also have an indisputable effect on the society as a whole. Schizophrenia is justifiably often designated as a financially catastrophic illness, accounting for a considerable share of global health expenditures (6, 7). A large swedish register-based study investigating the health care resource utilization and cost-of-illness in patients with schizophrenia, estimated the average yearly cost per patient to EUR 55 100. Economic deficits due to losses in productivity was found to account for more

than half of the total cost. Noteably, indirect costs such as informal care and primary care were not included in the calculations. Thus, the actual total cost for schizophrenia is plausibly higher than the figure suggested in the study (8).

In view of all that has been mentioned thus far, it may be asserted that schizophrenia is an extensive source of affliction, imposing a disproportionately immense burden across multiple sectors of society. However, the foremost reason for its acknowledgment as one of the most severe mental illnesses are the existing disheartening data on the associated mortality and life expectancy (9). Excess premature mortality in schizophrenic patients has been observed since the late 19th century (10). According to a 2007 systematic literature review examining published mortality studies from nearly three decades in 25 different countries, people with schizophrenia were found to have a 2.5 times higher risk of death when compared with the general population (11). Contributing factors to this increased mortality rate are numerous and are often divded into unnatural causes, such as suicides and accidents, and natural causes which include adverse effects of antipsychotic drugs, physical comorbidities, genetic effects, sedentary lifestyle and poor habits like excessive smoking and alcohol use (10, 12). The high excess mortality results in a significantly reduced life expectancy. In The Lancet Psychiatry, Carsten Hjorthoj et al. elucidated the discrepancy in life expectancy between people with schizophrenia and the general population, reporting a life expectancy of 60 and 68 years for males and females with schizophrenia respectively (13). To put these numbers into perspective, the average life expectancy in more developed countries in 2018 was 76 years for men and 82 years for females (14).

Clinically speaking, schizophrenia is a syndrome with a wide ranging array of signs and symptoms (15) which are often arranged into different groups. The characteristic

manifestations generally fall into the following three categories: positive, negative and cognitive symptoms. The labelling of a symptom as “positive” or “negative” is determined by its relation to normal functions in healthy individuals. Positive symptoms can be viewed as an excess or distortion of normal function, while negaitive symptoms contrastingly indicate a reduction or absence of normal function. Examples of positive symptoms are reality distortions such as hallucination and delusions, lack of illness awareness and disorganized behavior and speech (16-18). On the other hand, aspects of negative symptoms include asocialty, apathy, alogia (poverty of speech) avolition (loss of motivation and initiative), and anhedonia (inability to feel pleasure) (19). The third category, namely cognitive symptoms, consists of deficits in multiple neurocognitive areas such as executive functions, attention, working memory, verbal comprehension, processing speed, social cognition and verbal, aswell as visual learning and memory (20).

Although schizophrenia has been studied for over a century, its precise cause and pathophysiology remain unknown (21). However, there is a prevailing consensus on a multifactorial origin involving a complex interplay with genetic, environmental and social factors (15, 18, 22). Regarding the pathophysiology, anatomic brain abnormalities such as ventricular enlargement, decreased brain volume and reductions in grey matter, have been demonstrated in numerous neuroimaging studies (22, 23). The notion of aberrant neurotransmittor systems in schizophrenic indivduals is one of the current leading pathophysiological theories. Specifically, abnormalities in the dopaminerigc, serotonergic, glutamatergic and GABAergic systems are assumed to exist in schizophrenia (24, 25).

The management of schizophrenia requires a combination of pharmacotherapy and psychosocial treatments such as cognitive-behaviour therapy (CBT), training in illness

management skills, family psycho-education and social skills training. The feasibility and efficacy of the latter interventions is dependent on an early implementation of pharmaceutical agents, making pharmacotherapy the basis of treatment. Antipsychotic medications are the recommended first-line treatment in schizophrenia and are divded into an older ("first generation) and newer ("second generation") group (18, 22, 26). The first generation antipsychotics (FGA) are mainly dopamine D2 receptor antagonists but also affect a range of other neuroreceptors such as muscarinic, adrenergic alpha 1 and histamine-1. The second generation antipsychotics (SGA) share the D2 antagonistic properties of FGA but differ in their lower affinity for dopamine D2 receptors and higher affinity for serotonergic and adrenergic receptors. The action on multiple neuroreceptors exerted by antipsychotics is responsible for the large list of possible adverse events. FGAs are known to be associated with an increased risk of developing neurologic side effects such as tardive dyskinesia (a neurologic syndrome characterized by uncontrolled movements in the oral-facial region or in the limbs) and extrapyramidal symptoms (e.g. muscle stiffness, tremors, akathisia) (22), whereas SGAs are associated with a higher risk of metabolic side effects, consequently predisposing weight gain, hyperglycemia and dyslipidemia (27, 28).

The adverse effects of antipsychotic drugs are often so severe that discontinuation of treatment is common. In a double-blind, active-control clinical trial investigating the effectiveness of antipsychotic drugs in 1432 patients with schizophrenia, Lieberman JA et al. reported that a total of 74 percent of patients discontinued their assigned antipsychotic study medication due to intolerable side effects or lack of improvement in their symptoms (27). The high rates of non-compliance in this study highlights the existing suboptimality of the current available pharmacological agents. Little has improved since the introduction of the first antipsychotics in the 1950's despite the development of several new

agents. The reason for this lack of progress is most likely the limited knowledge regarding the etiology and pathophysiology of the disorder (29-31). However, when tolerated, treatment with antipsychotic medications has a potential to provide a substantial relief from positive symptoms for many patients (1, 32).

Although possessing the ability of efficiently limiting the impact of psychotic symptoms, these compounds have ultimately been proven unable to cure schizophrenia, indicating that the core deficits of the disorder remain unaffected (29). Among these deficiencies are disturbances in cognitive functioning, which as of late, have been of central importance in research on schizophrenia. The concept of cognitive symptoms as a key feature of schizophrenia is substantiated by several studies reporting a deterioration in cognitive functioning as much as nearly ten years prior to the onset of the more striking positive symptoms (31). Furthermore, it is now well established that cognitive symptoms are the best predictor of functional outcome, showing better correlation with clinical prognosis than psychotic symptoms (29, 31, 33).

As indicated earlier, in comparsion to the frequently treatable psychotic symptoms, cognitive deficits are not alleviated by available medication and often persist when other symptoms are in remission. In actuality, cognitive symptoms in schizophrenia account for the vast majority of the substantial disability and burden of disease described previously (29). Recognizing this fact, as a step in the attempt to ultimately find a cure for this devastating illness, the primary focus of study for the past decades has been to understand the underlying mechanisms to these impairments. Several lines of evidence suggest that neural oscillations and their synchronization are essential to various processes of healthy cognition. Cognitive functions depend on a complex interplay between various cortical and subcortical structures in the brain

where a multitude of neurons, within and across these structures, communicate by coordinating their neural activity (34). Synchronized neural oscillations are believed to enable this interplay and are considered essential for efficient interaction between neural systems in different brain regions (35-38). Abnormal oscillations are observed in patients with schizophrenia and owing to the fact that cognitive deficits appear to be a core feature, a better understanding of this neural activity may reveal important clues to the pathophysiology of the illness (39).

Neural Oscillations in the Normal Brain

"Neural oscillations", also known as "brainwaves", refers to the rhythmic, repetitive electrophysiological activity produced by neurons in the central nervous system. Oscillatory activity can be generated at various levels. Single neurons posses the ability to intrinsically produce rhytmic activity but oscillations can also emerge from interactions between neurons in local and distant neural networks. These rhythms in neural activity may be generated spontaneously or as a response to stimuli in the form of a specific sensory, motor or cognitive event (35, 40, 41). Oscillations can be recorded invasively as local field potentials (LFP) using electrodes placed within the cortex or other deep brain structures, or non-invasively by magnetoencephalogram (MEG) or electroencephalogram (EEG) where measurements are obtained outside of the skull (42).

Traditionally, neural oscillations have been characterized by the frequency range in which they occur and are usually described as low frequency oscillations at delta (0-4 Hz), theta (4-8 Hz), alpha (8-12 Hz) and beta (12-30 Hz), and as high frequency oscillations at gamma (30-80 Hz) and high gamma (> 80 Hz) (35). Other measures used in the characterization of oscillations are the amplitude and phase of the waves. The individual frequency bands are

prominent in various degree in different cortical and subcortical structures and are associated with specific brain states or behaviors (43). For example, theta oscillations are easily observed in the hippocampus (HC), sensory cortex and prefrontal cortex (PFC) (39) and are especially present during rapid eye movement sleep (REM sleep) and spatial navigation. Delta oscillations, on the other hand, are also observed in the cortex but dominate during non-rapid eye movemen sleep (NREM sleep) (35, 44).

Oscillatory synchronization is one of the most important mechanisms for coordinating neuronal firing within or across cortical networks and hence appear to play a key role in the neural processes underlying cognitive function (45). The frequency of the oscillation is particularly relevant since the length of the cycle determines the magnitude of neuronal synchronization. The short cycles of fast oscillations such as gammma, fall within the range of conduction time and synaptic delay which limits the number of neurons that can be synchronized. Thus, high-frequency oscillations primarily establish synchronization in local cortical networks. Conversely, slow oscillations such as delta and theta, have significantly longer cycles and are therefore capable of activating larger populations of neurons within the same oscillatory cycle. Since the length of the cycle is longer, low-frequency oscillations are even able to establish synchronization of neurons over longer distances (39, 46, 47). In other words, low frequencies play a particularly important role in the formation of effective communication between remote regions of the brain. Another important role of slower waves is their ability to modulate faster rhythms in a process called cross-frequency coupling (CFC). This interaction is necessary for the coordination of locally generated high-frequency (e.g. gamma) oscillation in distant neural networks (35, 44).

Different neurotransmitter systems, such as γ-aminobutyric acid (GABA), acetylcholine (ACh) and glutamate, are involved in the generation of oscillations (35). Based on findings from cellular and systems neurophysiology studies, GABA-mediated synaptic transmission is particularly believed to play a central role in the formation of synchronized neural network oscillations (48). The rhythmogenesis of oscillations is dependent on inhibitory GABAergic interneurons which exert inhibitory control over excitatory pyramidal cells. The duration of the inhibition determines the oscillatory frequency; the longer the inhibtion, the slower the frequency (35).

Abnormal Neural Oscillations in Schizophrenia

In recent years, there has been a solid rise in the interest in abnormal oscillations in schizophrenia. Aberrant oscillatory activity is found in all frequency bands and pathological alterations of the neurotransmitter systems involved in the generation and synchronization of neural oscillations is believed to be the cause to these perturbations (35, 49). The prevailing hypothesis states that alterations in GABAergic neurotransmission is central to these abnormalities and is strongly supported by postmortem examinations of patients with schizophrenia where the GABA-synthesizing enzyme GAD67 and GABA transporter 1 have been repeatedly shown to be reduced in GABAergic interneurons in the cortex and hippocampus (37, 48). In other words, an impaired synthesis and reuptake of GABA in interneurons appears to be present. The reduction of GAD67 and GABA transporter 1 is specifically noticeable in a subtype of interneurons expressing the marker parvalbumin (39). These fast-spiking parvalbumin positive (PV+) GABA interneurons have been demonstrated to be directly related to the generation gamma oscillations in optogenetic studies in mice (50).

While on the topic of gamma oscillations, due to its strong involvement in cognitive processes, these high-frequency rhythms have been the main focus of research in schizophrenia for the past decades and has resulted in a wide array of electrophysiological studies supporting a general gamma band reduction. This reduction has been corroborated in multiple studies where abnormalities in the gamma band are displayed during various sensory and cognitive tasks in schizophrenic subjects but not in healthy, non-schizophrenic subjects (34, 35, 51). Altered gamma oscillations are well established in schizophrenia and have been showing great promise as a potential endophenotype that may underlie downstream phenotypic deficits of the illness (52).

Although our knowledge is most advanced about the impairments of gamma oscillations, low frequency oscillations, such as theta and delta, also show abnormalities (53) but these are less clear and less studied. As highlighted previously, slow oscillations are essential for network interactions and their impairment will result in a dysfunctional communication between distant brain regions. This dysconnectivity is believed to be of central importance of cognitive pathology in schizophrenia (37, 39) and a better understanding of low-frequencies such as delta and theta, may be useful in deciphering the neural basis of cognitive deficits.

Adolescence - A Crucial Period for Schizophrenia and Oscillatory Development

A distincitve feature of schizophrenia is a seemingly sudden manifestation of psychotic symptoms. This first episode of psychosis is commonly accredited as the onset of the illness and occurs with an evident gender difference in a specific age range after adolescence. The age of onset of schizophrenia is defined as 15 – 30 years (13), where men tend to display the first symptoms in their early twenties and women, in their late twenties to early thirties (1). Although still clinically referred to as a psychotic disorder (15), over the past few decades schizophrenia has been increasingly conceptualized as a neurodevelopmental disorder in neuropsychiatric research. The so-called neurodevelopmental hypothesis of schizophrenia postulates that the emergence of psychosis in early adulthood is the result of aberrant neurodevelopmental processes. It emphasizes that the actual onset of the illness appears at an earlier stage referred to as the prodromal phase, and that a variety of pathological signs can be detected years before the more striking positive symptoms (1, 54). The prodromal phase occurs during adolescence (55) and consist of cognitive impairments, social abnormalities, behavioral changes and attenuated psychotic symptoms (1, 54). Evidence supporting the notion of an earlier, more subtle onset of schizophrenia has accumulated during the past years and has led to the realization that identification of predisposed individuals in the prodromal period could be highly beneficial in many aspects, especially in regards of potentially improving the prognosis of the illness. Attention has been drawn to the fact that interventions, which usually are introduced after the first psychotic episode, are currently being applied too late as the earlier deficits in the prodromal period will already have caused a considerable amount of social damage (31). This has led to a search for predictive biomarkers (1) which could enable earlier detection and intervention as well as possibly be helpful in designing novel and better targeted treatments, since these markers most likely will provide important clues to the etiopathogenesis of schizophrenia (49, 56).

As of yet, efforts to find biomarkers have mainly been directed to anatomical parameters. This trend of focus is based on the finding that macroscopic brain abnormalities, such as reduced cerebral grey matter, are present in at-risk individuals prior to the onset of psychosis (22, 23, 56). However, more recent proposals of utilizing different measurements of neuronal activity such as electroencephalography (EEG) have been made, as neural oscillations during the prodromal period may be a promising target in this search (56). Investigations involving animal models of schizophrenia during this phase could be useful in revealing different oscillatory developmental deviations.

From a neurodevelopmental perspective, adolescence is indeed a critical period where many essential refinements and alterations of brain circuits occur. Pruning of excitatory synapses, increased myelination and proliferation of inhibitory circuits are all known to take place during this time and have the potential to affect the development of neural oscillations (29). In addition to this, changes in various neurotransmitter systems associated with the emergence of neural network oscillations occur as well (57). The developmental changes do not appear with the same pace during this period and may differ between various regions and structures of the brain. The relatively more prolonged development of the prefrontal cortex, which is the last cortical region to mature, is an example of this discrepancy (29).

The adolescent development of oscillatory cortical networks that lead to improved cognition in adulthood is understudied and we do currently not have much data about the normal or abnormal development of neural oscillation during this period, in both humans and animal models. In order to acquire a frame of reference we need to begin with attaining a better understanding of the development under normal conditions. Hence, we propose a longitudinal investigation through the adolescent period of normal rats of both genders to define how

oscillations develop during this specific phase of maturation with a focus on the less-studied low-frequency oscillations in the delta and theta band in the prefrontal cortex (PFC) and hippocampus (HC). This study is intended to be an exploratory pilot experiment for future investigations where an inclusion of a schizophrenic rat model is planned. We hypothesize that the maturation of oscillatory cortical networks is an extended process appearing over the length of adolescence and into early adulthood, during which neural oscillations in different frequency bands may follow different developmental trajectories.

Aim

To investigate how spontaneous low-frequency neural oscillations in the delta and theta band develop through the adolescent period in normal rats of both sexes.

Materials and Methods

Animals and ethics

A total of 22 Sprague-Dawley rats of both genders (10 males and 12 females pups-siblings from two mothers, Charles River Laboratories in Massachusetts, USA) were employed in this study. Weaning occurred at postnatal day 21 (P21) and the animals were recorded one a day, everyday through adolescence, from P32 to P52 (body weight: 115 – 310 grams).

The housing, as well as the procedures described below, were approved and conducted in accordance with the Institutional Animal Care and Use Committee of Harvard Medical School (IACUC) and Beth Israel Deaconess Medical Center (BIDMC).

Experimental procedures

The experimental methods consisted of three components: surgical implantation of recording electrodes, electrophysiological recordings of spontaneous oscillatory activity for 2 hours and postmortem histological identification of the location of the implanted electrodes in the prefrontal cortex (PFC) and dorsal hippocampus (HC).

Non-survival Surgery

Non-survival brain surgery was performed on 22 healthy rats under urethane anesthesia (1.2-1.5 g/kg of 65-80% solution, intraperitoneal injections). Two doses were given with an hour apart. Unlike other anesthetic agents, urethane does not suppress the generation of spontaneous theta oscillations in the hippocampus (58) and was therefore a good choice for our experiments. Surgical anesthesia was confirmed by a lack of response to the pinching of the tail. In case of unobtained full anesthesia following the two injections of urethane, a small dose (3.5-5 mg/kg) of ketamine was given additionally.

Regarding the surgical set-up, the rat was placed on an isothermal pad with circulating warm water to maintain adequate body temperature and its head was fastened in a stereotaxic frame

for the electrode placements. Using a sharp surgical knive, a straight 10 mm long cut along the sagittal axis was made through the skin and fascia of the head in order to expose the bregma on the cranium. In accordance to the Rat Brain Atlas of Paxinos (Paxinos & Watson), coordinates were measured and marked out on the skull in relation to bregma and followed by drilling of small holes to allow for electrode placement. On the left side of the brain, one pair of twisted stainless steel electrode wires with a 1 mm gap between the distal ends, was implanted in the dorsal HC with one tip above the CA1 region and the other one above the dentate gyrus (DG) (3.7 mm posterior to bregma, 2.2 mm lateral to midline and 3.5 mm below the brain surface). One single stainless steel electrode wire was implanted in the right PFC and another one in the left PFC (3.2 mm anterior to bregma, 0.5 mm lateral to midline, and 5.0 mm below the brain surface for the right PFC and 4.5 mm below the brain surface for the left PFC). Furthermore, two skull screws were placed in the nasal bone and above the cerebellum to function as the reference and ground electrode respectively. To secure the positioning of the electrodes and screws during the recordings, dental cement was utilized for fixation to the bone.

Electrophysiological recordings

Electrodes were connected to an amplifier (A-M systems) and local field potentials (LFPs) were recorded via the implanted electrodes and saved in DASYLab 7.0 Acquistion System Laboratory for the data collection. Each recording session lasted approximately two hours and was followed by euthanasia of the rat using an 1 ml injection of Ketamine.

Histological procedure

After being euthanized, the rats were decapitated in order to obtain the brains. The brains were placed in glass vials containing a 10% formalin solution (Fisher Scientific) for fixation and stored at a low temperature. A freezing microtome (MICROM, HM 450) was used to thinly slice the brain at 50 microns in the coronal plane. The sections were stained with the

Nissl method and glass slides were made. Slides were analyzed with a light microscope (Nikon ECLIPSE, E400) to confirm correct placement of the electrodes.

Denomination and Grouping of rats

In order to obtain a sense of structure and to be able to differentiate the animals during the experiments and data analyses, the 22 rats were named after their family of origin and gender. As mentioned above, the rats of two different mothers were employed in this study. "So1-family" consisted of pup-siblings from one mother, while "So2-family" consisted of siblings from the other mother. The rats were further characterized by an addition of "F" (female) or "M" (male) to their name depending on their gender.

For the data and statistical analyses particularly, the 22 rats, irrespective of family origin and gender, were split into three age groups: the youngest age group (rats between the ages P32-P39), the middle-age group (rats between the ages P41-P47) and the oldest group (rats between the ages P48-P52). The rationale behind this division was primarily to facilitate the detection of potential developmental trends during the analyses.

Data and Statistical Analysis

EEG recordings were saved as ~.DDF-files in DASYLab 7.0 and extracted into CED Spike2 for filtering and signal analysis in Spike2's waveform and sonogram mode. Low-pass filter was set to pass signals under 10 Hz. Signals were subjected to Fast Fourier Transform and power density spectra were acquired. Frequencies with the greatest power (peak frequencies) were identified in the HC and PFC and corresponded to oscillations in the theta and delta frequency range respectively. Peak power values were then calculated and Microsoft® Excel formulas and functions (version 16.40) were employed for the statistical computations and data analysis. Pearson's correlation coefficient (r) was calculated between peak power and age for both theta and delta oscillations.

Result

Out of a total of 22 animals, the recording of one rat (So2-M4, age P40) was excluded due to misplacement of the hippocampal micro-electrode.

Weight

The weight of each rat was measured and documented before proceeding with the surgery the same day. The graph below (fig. 1) illustrates the progressing physical growth (115 – 310 grams) by age as well as accentuates the difference in body-mass between the genders.

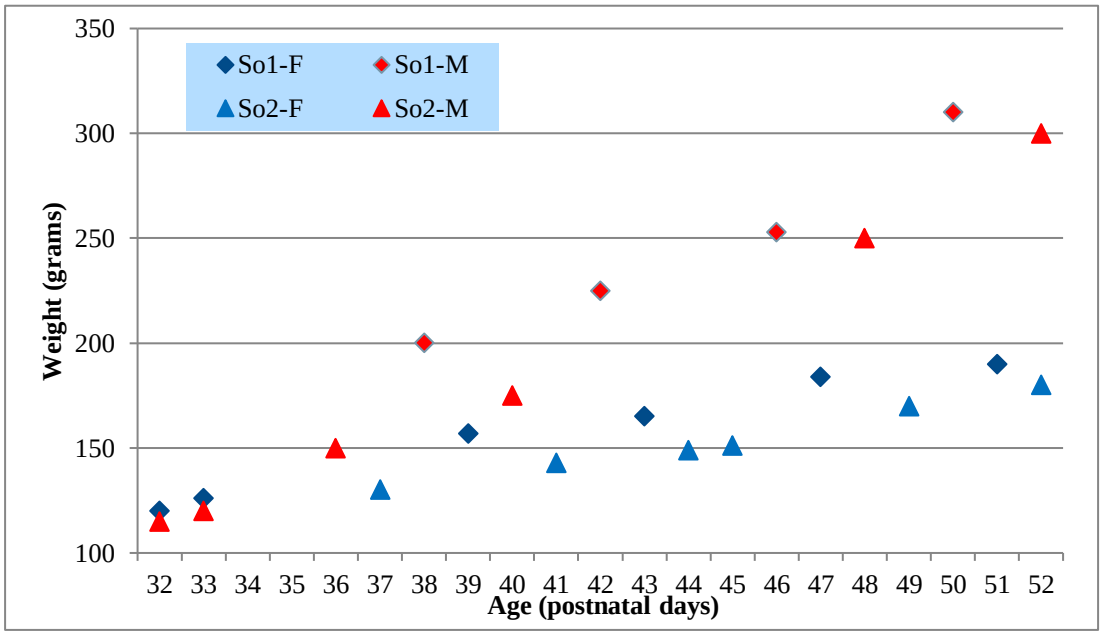

Figure 1. Weight (115 – 310 g) of the rats (n = 22) in age P32-52. The blue data points symbolize the female rats (n = 12) and the red, symbolize the male rats (n = 10). The rhombi-shaped points represent the rat-siblings from the So1-family, while the triangle-shaped represent the siblings from the So2-family.

Abbreviations*: So1-F (female rats from the So1-family), So1-M (male rats from the So1-family), So2-F (female rats from the So2-family) and So2-M (male rats from the So2-family).*

Histology

Verification of the correct positioning of the implanted wire electrodes employed for the measuring of the local field potentials (LFP) in the prefrontal cortex (PFC) and hippocampus

(HC) was made by observing the electrode-tracks in the above-mentioned structures in the histologically prepared brain sections using a microscope.

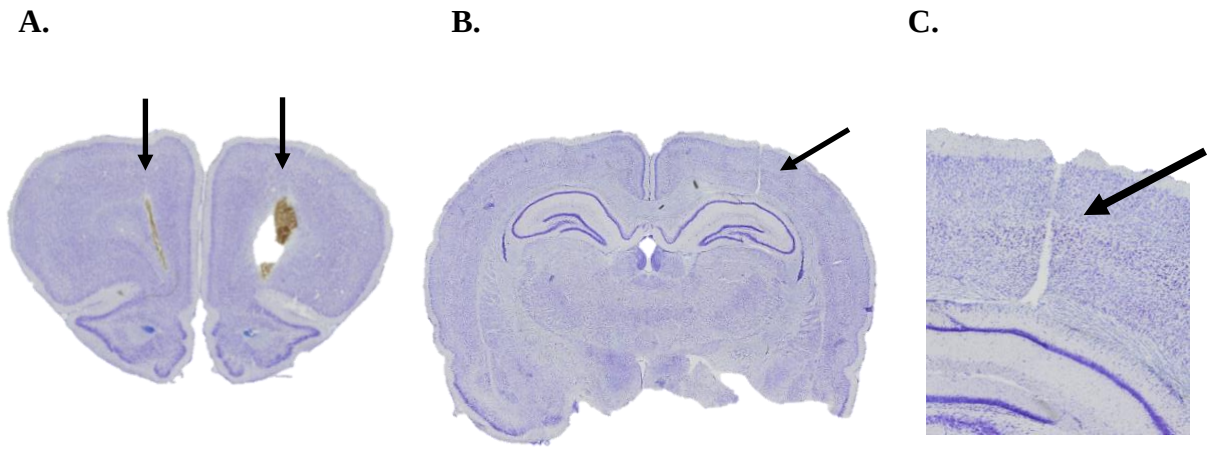

Figure 2: Placement of electrodes for local field potential (LFP) recordings in the prefrontal cortex (PFC) and hippocampus (HC). Nissl stained coronal sections of rat brains with arrows pointing at the electrode track in the left and right PFC (**A**), and in the right HC (**B**). Image (**C**) is a magnification of the electrode track marked in image (**B**).

Recordings in Hippocampus and Prefrontal cortex

As in concordance with previous studies (58), the measured neural activity in HC and PFC in urethane-anesthetized rats spontaneously alternated between two states; 1) oscillatory activity– recording segments characterized by rhythmicity and small amplitudes, and 2) wide-band activity – segments seen as irregular deflections with large amplitudes. These two types of electrical activities would occur repeatedly throughout the recording sesssion, with the oscillatory segments being the less frequent ones. The dominant rhythm in the HC were oscillations in the theta band (~4 Hz), while lower frequency oscillations in the delta band (~2 Hz) prevailed in the PFC.

The appearance of the adolescent theta and delta oscillations in HC and PFC respectively, matched the ones seen in adulthood (58) and were detected in all ages of both genders including the youngest rats at age P32-33 (n = 4, see example in fig. 3).

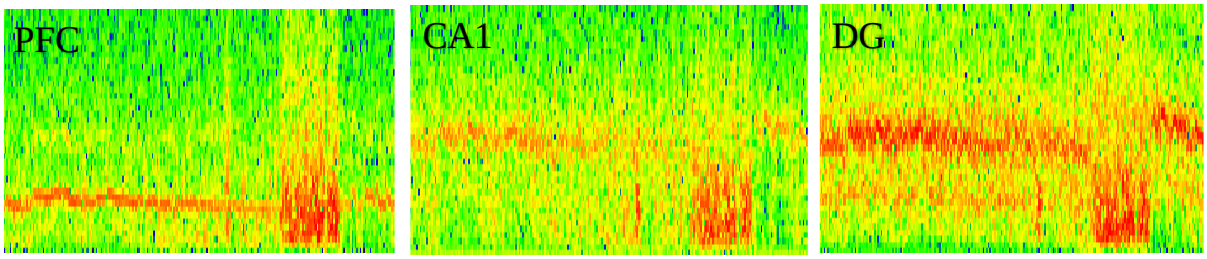

Figure 3. Example of time (500 seconds) vs. frequency (0-10 Hz) plots at age P32. Note the strong 2.5 Hz oscillation in the PFC (prefrontal cortex) and 4.6 Hz in the CA1 region and DG (dentate gyrus) in the hippocampus (HC) alternating with wide band delta, a pattern frequently seen in adulthood. The colors are an indication of the strength of the frequencies where red signifies a presence of a strong frequency and green, a weak frequency.

Hippocampal Theta oscillations in Urethane-anesthetized Rats

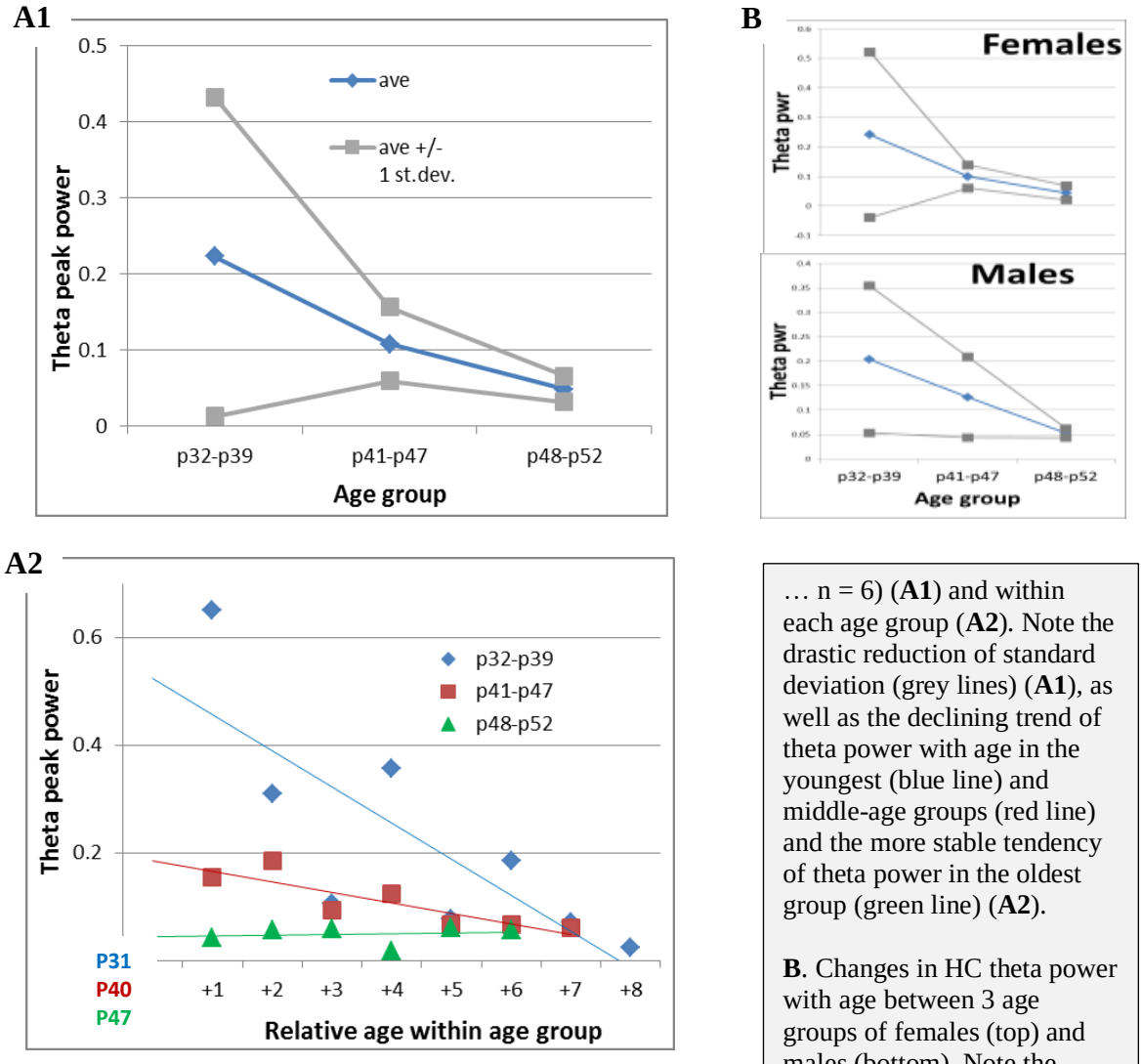

Figure 4. A. Changes in hippocampal (HC) theta power with age, between 3 age groups: the youngest group (p32-p39, n = 8), the middle-age group (p41-p47, n = 7) and oldest group (p48-p52, … n = 6) (**A1**) and within each age group (**A2**). Note the drastic reduction of standard deviation (grey lines) (**A1**), as well as the declining trend of theta power with age in the youngest (blue line) and middle-age groups (red line) and the more stable tendency of theta power in the oldest group (green line) (**A2**).

B. Changes in HC theta power with age between 3 age groups of females (top) and males (bottom). Note the different course of the reduction in standard deviation between the sexes.

HC theta power decreased through adolescence (Fig. 4. A1), shown both by significant differences between age groups (p-value < 0.05, t-test) and significant declines within the youngest and middle-aged groups (Fig 4. A2; P32–P39: r = –0.74, p-value = 0.014, n = 8; P41-P47: r = –0.87, p-value = 0.003, n= 7), to reach a stable level in the oldest group (P48-P52, n = 6). Furthermore, theta power stabilized with age, as indicated by the substantial reduction of standard deviation (Fig 4. A1) from the youngest age group (P32-P39) to the middle-aged group (P41-P47) and then to the oldest age group (P48-P52).

Both genders demonstrated this tendency of theta power reduction. The correlation between age and theta power was significant (females: r = - 0.75, p-value = 0.008; males: r = - 0.63, p-value = 0.012) for all rats (i.e. P32-P52) and in the groups of young and middle ages (r = -0.70 – -0.92, p-value < 0.014 for young females and middle-age males; n = 2 – 5 per group). Theta stabilization, that is the drastic reduction of standard deviation, was also present in both genders but differed in time of completion; in females it was finalized by the middle-aged group, while in males this process was slower or delayed (Fig 4. B).

Prefrontal cortex Delta oscillations in Urethane-anesthetized Rats

In comparsion to the HC theta oscillations, narrow-band delta oscillations in PFC followed a different course. PFC delta peak power significantly increased through adolescence (i.e. P32 – P52; r = 0.39, p-value = 0.039), with differences between genders (females: r = 0.64, p-value = 0.011; males: r = 0.31, p-value = 0.063). However, age-related tendencies within age groups and differences between age groups were not significant, although increases in delta power appeared stronger when the middle-aged group was compared with the older group (54-65% increase) than with the younger group (1-16% increase) of both female and male rats.

Standard deviations were steady in the young and middle-aged groups (10% difference) but increased in the oldest group by 62%.

Discussion

The aim of this study was to investigate how low-frequency neural oscillations in the theta and delta band develop during the adolescent period of healthy rats. We found that both theta and delta oscillations developed early as they were already present in the youngest rat at age P32. Two other main findings were that (1) HC theta peak power decreased and (2) PFC delta peak power increased through adolescence, indicating that the oscillations in the two different frequency bands most likely follow distinct developmental trajectories to reach the characteristics of oscillatory activity found in adults. But a perhaps more intriguing discovery was the age-related drastic reduction of standard deviation in theta power, possibly symbolizing maturation of oscillatory networks by the stabilization of theta toward the last third of adolescence. Furthermore, when the female and male rats were analyzed seperately, a prominent sex difference in this pattern of development became appearant: in females theta stabilization was completed in mid-adolescence, while in males it was completed in late adolescence. This dissimilarity in time of completion highlights a potential gender difference in maturation where the development of theta oscillations in the males appears to be a more extended process by being completed at the very end of the adolescent period. Our finding of a protracted maturation of theta-generating networks in late adolescence is overall consistent with the findings of longitudinal studies in human adolescents, in which oscillatory networks demonstrated a similar pattern of maturation (59, 60).

The intention was to identify some of the principles of normal neurodevelopment of oscillatory neural networks in adolescence and establish a basis for future studies. Acknowledged as the prodromal phase of schizophrenia, adolescence is a particularly vulnerable age of the illness and collecting vital information during this period is of great importance. Thus, a large emphasis was put on selecting rats within an age range analogous to

human adolescence. Evidently, there are many disparities between humans and rodents. In regard to the different stages of life, relative ages vary due to dissimilarities in development, anatomy and physiology. When correlating the entire life span, one human year equals 13.8 rat days as humans have a life expectancy of approximately 80 years and rats, 3 years. During the adolescent period, 10.5 rat days equals one human year and this comparsion is based on the time of musculoskeletal maturity (61). The use of rodent models of human adolescence is endorsed by researchers and rats are acknowledged as one of the best models of the mammalian system. The beginning of adolescence in rats is marked by the time of sexual maturation which occurs at ~P35, and ends with the start of adulthood at ~P60 (61-63). The ages employed in this study was P32 to P52, which is a suitable age range when attempting to study the characteristics of adolescence.

In general, when investigating developmental changes, a longitudinal study design is often the most advantageous choice as it allows continuous observations of variables through time and offers an opportunity to detect potential developmental trends. These benefits have been acknowledged in human studies and longitudinal observations of adolescent brain maturation has been widely utilized (64-66). This can not be stated for rodent studies of both normal animals and rodent models of disease, as longitudinal studies on development of oscillations are missing. Hence it was difficult to find previous work to compare with. However, the longitudinal character of this study is one of several major features of our research. Our results shed some light on possible developmental trends of two relatively understudied frequency oscillations (delta and theta), during an equally less explored time period of development (adolescence). Furthermore, it focused on two different forebrain structures (PFC and HC), which both are highly involved in a variety of cognitive functions and most likely develop at different pace. Finally, the inclusion of both genders is of great significance

as sex is an important factor in schizophrenia pathology and may affect brain oscillations in general.

As greatly emphasized, there is a large gap in the literature regarding the developmental processes of low-frequency oscillatory-generating networks during the adolescent period in rodents. Prior to the start of our experiments, we were prepared to spend time on fine-tuning the recording techniques developed for adult rats as we vaguely assumed that adjustments may be necessary for the significantly smaller, youngest rats. Surprisingly, this did not turn out to be the case and the experiments were successfully executed without any large adjustments to surgery and signal analysis.

As previously stated, the aim was to study the development of neural oscillations. This can ideally be achieved by using chronic recordings in freely moving rats, making multiple recordings in each rat during a 1-2 month-long period. The alternative is to use acute (non-survival) experiments under urethane anesthesia. In view of the fact that this project was intended to be an exploratory pilot study and in which we were subjected to a time constraint, the latter model was chosen for three main reasons: (1) to facilitate the experimental procedures, that is urethane anesthetized rats allow a faster turn over in the surgery-recording-histology cycle; (2) to allow recordings at earlier ages since they would be made on the same day as the surgery, compared with a 7-10 days recovery period required after survival surgery; (3) the placements of electrodes should be more accurate during the recordings as the growing brain will not be an issue, which otherwise could potentially affect the electrode location after initial insertion. However, the addition of chronic recordings in freely moving rats in future experiments is necessary. Demonstrated on a relatively large sample, the development of theta-generating networks leading to theta stabilization toward the end of adolescence appears

to be significant. Although we see clear patterns of development of theta oscillations in our acute experiments, these findings should be further explored and substantiated in chronic recordings of freely moving rats.

Regarding the following inclusion of a schizophrenic rat model, we now have data from healthy rats to use as a reference which will be helpful in identifying developmental deviations in the schizophrenic model. The MAM E17 model will be used to study the neurodevelopmental aspects of schizophrenia in rodents. In this model, Methylazoxymethanol (MAM), a DNA methylating agent, is given on embryonic day 17 (E17) and causes a non-specific disruption of cortical development which leads to neurodevelopmental defects in the cortex and hippocampus (67). As mentioned in the introduction, investigations involving animal models of schizophrenia during adolescence may help in the search for a biomarker during the prodromal phase. A better understanding of the adolescent development of abnormal brain networks responsible for the debilitating symptoms in schizophrenia, may provide valuable knowledge about this critical period of the illness.

Conclusions

We have preliminary data indicating that PFC-delta and HC-theta oscillations may follow distinct developmental trajectories during adolescence with notable gender differences.

Populärvetenskaplig sammanfattning

Utvecklingen av Lågfrekventa Hjärnvågor under Adolescensen

Schizofreni är en allvarlig, kronisk psykiatrisk sjukdom som utmärks av sin komplexitet. Sjukdomen påverkar ungefär 21 miljoner människor globalt och betraktas därmed som ett relativt lågprevalent tillstånd. Trots att förekomsten är av lägre grad orsakar schizofreni en stor sjukdomsbörda, såväl för den enskilda drabbade individen som det bredare samhället. Enligt det globala sjukdomsbördeprojektet 2016 tillhör schizofreni en av de främsta orsakerna till handikapp i världen och är förknippad med en markant ökad risk för tidig död jämfört med övriga befolkningen.

Antipsykotiska läkemedel används i behandlingen av de karaktäristiska psykotiska symptomen och kan i vissa fall vara till stor hjälp. Men dagens tillgängliga läkemedel är inte botande då de inte har en effekt på sjukdomens resterande symptom. Dessa utgörs i huvudsak av kognitiva symptom som bland annat består av inlärnings- & minnesstörningar samt en försämrad förmåga att hantera information och fatta beslut. Under de senaste åren har mycket uppmärksamhet riktats mot dessa kognitiva nedsättningar då man har börjat bekänna deras relevans och eventuella potential till att förstå de sjukdomsmekanismer som ligger till grund till sjukdomen. Bakgrunden till detta fokusskifte är bland annat upptäckten att inskränkningar i den kognitiva funktionen förekommer flera år innan debuten av de mer dramatiska psykotiska yttringarna samt att dessa symptom har en starkare koppling till sjukdomens prognos.

Hjärnvågor avspeglar hjärnans elektriska aktivitet och synkroniseringen av dessa tros vara nödvändig för normal kognitiv funktion då underliggande processer är beroende av ett effektivt samspel mellan olika delar av hjärnan. Olika hjärnvågor förekommer i olika grad

beroende på plats i hjärnan och brukar delas in efter sin frekvens. Lågfrekventa hjärnvågor utgörs av delta-, theta-, alpha- och betavågor, medan högfrekventa består främst av gammavågor. Abnorma hjärnvågor har observerats hos individer med schizofreni, framförallt inom gamma frekvensbandet. Men avvikande lågfrekventa hjärnvågor förekommer också men är betydligt mindre studerade.

Den första psykotiska episoden inträffar vanligtvis under ett specifikt tidsintervall efter adolescensen, som är en tidsperiod mellan barndomen och vuxen ålder. Som tidigare nämnt förekommer andra, mer subtila tecken på schizofreni många år före psykos. Detta har lett till att man på senare tid har försökt få fram en biomarkör som skulle kunna möjliggöra en tidigare upptäckt av sjukdomen. Avvikande hjärnvågor under adolescensen skulle kunna vara en sådan markör men vi saknar basal kunskap om utvecklingen av hjärnvågor under denna period, under normala såväl som anormala omständigheter.

Syftet med denna studie var att studera hur lågfrekventa hjärnvågor i form av delta- och thetavågor utvecklas under adolescensen vid normala förhållanden, med förhoppningen att kunna bidra med en ökad insikt till den ovisshet som föreligger inom området. I denna studie har man registrerat hjärnvågorna hos en grupp normala råttor av båda könen under adolescensen och kommit fram till att de två olika hjärnvågorna troligtvis följer olika utvecklingsbanor. Studiens observationer bör betraktas som preliminära och verifiering i form av ytterligare studier är nödvändiga för att kunna dra fler slutsatser. Observationerna kan dock användas som referens vid framtida projekt där man inkluderar en schizofren råttmodell och kan då vara till hjälp vid urskiljandet av eventuella avvikelser från den normala hjärnvågsutvecklingen. Helhetssynen är alltså att man först ska undersöka hjärnvågorna i djurmodeller för att vid ett senare tillfälle kunna studera människor med den ambitiösa

avsikten att identifiera en biomarkör. Oavsett slutligt resultat lär ett ökat forskningsfokus på detta bidra till en ökad kunskap om schizofrenins patofysiologi, vilket är en evident förutsättning för att kunna utveckla bättre behandlingar.

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