Altered oscillation frequencies in the lateral geniculate complex in the rat model of absence epilepsy

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ARTICLE INFO

Keywords:
Electrophysiology gamma frequency
Infra-slow oscillation
Lateral geniculate complex
WAG/Rij rats

ABSTRACT

Absence epilepsy (AE) is a neurological disease that manifests in spike-wave discharges not present in healthy neuronal circuits. Mutations in ion channels directly underlying this rhythmic discharge may additionally affect rhythms in multiple brain centres which disturbances contribute to the epileptic phenotype. Malfunctioning of the light detection system (from retina to subcortical visual structures), heavily dependent on oscillatory activities, could partially explain severe problems with sleep and arousal observed in epileptic patients. Therefore, the aim of our study was to evaluate characteristics of retinal-derived oscillations in the lateral geniculate complex of the thalamus; a major gateway for the light information flow for the brain. Extracellular recordings in vivo were performed on urethane-anaesthetised WAG/Rij and Wistar rats from single units in the identified parts of lateral geniculate complex to test their basic oscillatory features as well as reaction to transient and sustained changes in ambient light conditions. Here, we show altered rhythmic activity of the lateral geniculate neurons in the absence epilepsy model with the increase of both the infra-slow and fast oscillatory frequencies. Further, we describe their disturbed reaction to sustain change in ambient light and provide evidence for major changes in intergeniculate leafllet neuronal firing; a part of the lateral geniculate complex implicated in the circadian timekeeping. Altogether, our results are the first to show a malfunctioning of light detection mechanisms in the absence epilepsy that may in turn underpin sleep-promoting system insufficiencies and other arousal disturbances contributing to epileptic phenotype.

1. Introduction

Rhythmic processes in the nervous system occur at multiple levels of organisation – from single pacemaking cells to complex neuronal networks. Not only do they include cycling expression of a variety of genes, but also oscillations of membrane potential that lead to patterned action potential firing by excitable cells. To sustain and synchronise oscillations within the network, neurons communicate their firing pattern via the rhythmic release of neurotransmitters. However, increased synchronisation amongst cells as well as between whole neuronal structures may lead to pathological states such as epilepsy (Buzsáki et al., 2013; Buzsáki and Chrobak, 1995; Buzsaki and Draguhn, 2004).

Absence epilepsy (AE) is a type of generalised epilepsy characterised by a sudden behavioural arrest with spike-wave discharges (SWD) seen throughout the electroencephalogram. Recent advances in understanding this neurological disease have been aided by a well-validated model of AE - WAG/Rij (Wistar Albino Glaxo from Rijswijk) rats. This AE model shares pharmacological, electrophysiological and behavioural similarities with human patients (Epps and Weinshenker, 2013), and in keeping with clinical characteristics, the onset of seizure in this model may occur spontaneously, but may also be evoked by sensory stimulation as flashing light or hyperventilation (Guerrini and Genton, 2004). SWDs are also correlated with the level of wakefulness and display a clear circadian rhythmicity (Smyk et al., 2011; Stewart et al., 2006). Typical SWDs are well-structured, characterised by a high-amplitude 2–4 Hz frequency and they have been shown to arise from...
excessive synchronisation of the thalamo-cortical loop. The genetic mechanism of SWD generation is complex, however there are some mutations common amongst patients and animal models such as in low-voltage activated T-type calcium channels (Crunelli and Leresche, 2002) or GABA receptors and transporters (Brockhaus and Pape, 2011; Li et al., 2006; Liu et al., 2007). Considering the wide expression of these genes throughout the nervous system and their role in various physiological processes (including those of a rhythmic nature), multiple pathologies that are not directly linked to SWD generation were reported in AE models (Aker et al., 2006; Sacharz et al., 2018a, 2018b; Suntsov et al., 2009).

The mammalian visual system is one of the best studied sensory systems that generates and conveys multiple oscillatory processes of various frequencies. It consists of the retina, subcortical visual structures and the visual cortex, all of which are reported to possess rhythmic properties. Many rhythms in the visual system initiate in the retina and code information about the perceived object (Zoefel and VanRullen, 2017). Previous studies from our laboratory and others demonstrated that the retinal network generates infra-slow oscillatory (ISO, < 0.01 Hz) activity (cat: (Ascoli and Maffei, 1964; Rodieck, 1967), rat: (Freeman et al., 2008)) which is projected upon the firing pattern of subcortical visual structures such as the lateral geniculate nucleus of the thalamus (LGN) (Albrecht et al., 1998; Albrecht and Gabriel, 1994; Chrobok et al., 2018; Filipov and Frolov, 2005; Lewandowski and Blasja, 2004), the olivary pretectal nucleus (OPN) (Szkudlarek et al., 2012, 2008) or the suprachiasmatic nucleus of the hypothalamic (SCN) (Miller and Fuller, 1992). As a consequence of its expression in the OPN, ISO pattern is reflected in rhythmic changes of pupil diameter (Blasja et al., 2013; Yüzgeç et al., 2018). In rodents, the LGN is a complex of three functionally and anatomically distinct nuclei that utilise light information to serve different physiological purposes: 1) the dorso-lateral geniculate (DLG) is a part of primary visual pathway, directly linking the retina with the primary visual cortex, 2) the intergeniculate leaflet (IGL) is a part of the biological timing system, conveying photic and non-photic cues to phase-shift the SCN and 3) the ventro-lateral geniculate (VGL) which is known to permit visuomotor functions (Harrington, 1997; Monavarfeshani et al., 2017). Recent reports show that the firing of subcortical visual neurons in the LGN, OPN and SCN is additionally governed by gamma-band oscillation that may be visualised as a harmonic distribution pattern (HDP) of inter-spike intervals (ISI) (Chrobok et al., 2018; Storchi et al., 2017; Tsuji et al., 2016). While the ISO pattern encodes sustain characteristics of ambient light, gamma oscillation carries information of transient light changes (Chrobok et al., 2018). Growing evidence show, that retinal gamma may be transmitted through LGN to reach visual cortex (Saleem et al., 2002).

We have focused our investigations on the possible malfunctioning of the subcortical visual system in the absence epilepsy, as it heavily relies on oscillatory activities disturbed in this neurological disease. Additionally, the system of light detection and processing is involved in the regulation of non-visual photic functions as the regulation of circadian rhythms, sleep and arousal; all of which are implicated in the epileptic phenotype (Suntsova et al., 2009). Our previous studies on the subcortical visual system of WAG/Rij rats were focused on a small part of the LGN – the IGL which forms part of the mammalian time keeping system. We showed clear disinhibition of IGL neurons reflected in the increased firing rate and blunted reaction to changes in sustained lighting conditions, accompanied by a decreased GABAergic synaptic tone (Chrobok et al., 2017). Moreover, we have reported the reduction of T-type calcium current amplitude in the WAG/Rij rat IGL and VLG, when compared to control (Wistar rats) (Chrobok et al., 2016). However, it remains unclear if the observed disturbances in oscillatory properties in WAG/Rij rats are constrained to the ISO activity in the IGL or arise from the retina and are common in the whole LGN on multiple frequency bands.

The aim of this study was to evaluate oscillatory properties of ISO neurons in three parts of the LGN in the absence epilepsy model. Based on single-unit electrophysiological recordings in vivo performed on urethane-anaesthetised WAG/Rij and Wistar rats, our results show malfunction of the rhythmic neuronal discharge in the infra-slow and gamma-band frequencies with increased frequency of both rhythms. Our study provides novel evidence for disturbances in the basic functioning of the visual system in absence epilepsy.

2. Methods

2.1. Animals

33 male WAG/Rij and 29 male Wistar rats were bred at the Institute of Zoology and Biomedical Research Animal Facility at the Jagiellonian University in Krakow with food and water ad libitum. Animals were housed 2–6 per cage under a standard light-dark regime (12:12 light-dark cycle; lights on at 07:00 am) with controlled temperature and humidity (temperature: 23°C, humidity: 67%). Experiments were designed in accordance with Polish regulations and the European Communities Council Directive of 24 November 1986 (86/609/EEC), to minimise animal suffering. All protocols were conducted on adult rats, weighing 215–390 g. To minimise the number of animal used, control recordings from Wistar rats were replicated from our previously published study (Chrobok et al., 2018).

2.2. Anaesthesia and surgery

All surgical procedures performed were previously described in detail (Chrobok et al., 2018). Briefly, animals were injected with urethane (1.5 g/kg, i.p.) diluted in saline. In some cases, to ensure deep anaesthetic state, to two additional doses (10% of the initial dose) were supplied. Next, animals were secured in the stereotoxic frame (Advanced Stereotoxic Instruments, Warren, Michigan, USA) and maintained on a heating pad (37°C). Throughout the experiment, electrocardiogram and electrocorticogram were constantly monitored. Then, craniotomy was executed to expose brain tissue above LGN (4.0 mm lateral and 4.2–5.2 mm posterior to bregma) (Paxinos and Watson, 2007). Eyes were kept open and atropinised. Both the brain surface and eyes were covered with mineral oil to minimise tissue drying.

2.3. Single-channel recordings

Borosilicate electrodes were pulled using a horizontal puller (Sutter Instruments, CO P-97, USA), filled with 4% Chicago Sky Blue dissolved at 2 M NaCl solution and placed in the LGN. Extracellular signals were amplified with the preamplifier and CyberAmp 380 amplifier (Axon Instruments, U.S.A.) and filtered at 300–3000 Hz. Signal was converted with CED Micro mkII converter and recorded with Spike2 software (Cambridge Electronic Design Inc., Cambridge, UK). All recordings were initialised in bright ambient light conditions (~300 lx), after infra-slow oscillatory (ISO) activity was found. Stable ISO activity was recorded for a minimum of 10 min in light-on conditions, after which lights were turned off and Faraday’s cage was masked with light-impermeable foil. Light pulses were performed using LED diode (brightness: 280 lx, duration: 5 s, interval: 30 s, repetition: 8x) presented to the contralateral eye of the recording site after minimum 30 min of darkness. At the end of each experiment, a negative current (20 μA) was applied for ten minutes to deposit dye markings at the recording site.

2.4. Histological verification

After electrophysiological experiments, animals were euthanized with an overdose of barbiturates and brain tissue carefully excised from the skull. Subsequently, brains were immersed in 4% paraffin-formaldehyde in 0.1 M phosphate buffered saline (pH = 7.4) for 24–48 h. Fixed tissue...
was cut on the vibroslicer (Leica VT1000S, Heidelberg, Germany) into 100 μm thick coronal slices. Chicago Sky Blue marks were visualised using light microscopy and further classified as located in the DLG, IGL or VLG.

2.5. Data analysis and statistics

Single units were sorted manually in Spike2 via principal components analysis. Firing frequency histograms (1 s) and instantaneous frequency plots were prepared in Spike2. ISO pattern properties were analysed in MATLAB (MathWorks Inc., Natick, MA, USA) with the use of a custom made script. ISO pattern frequency was assessed by Fast Fourier Transform. ISI histograms and peri-stimulus histograms were calculated in NeuroExplorer 5 (Nex Technologies, Madison, AL, USA). To compare light-evoked changes in ISO pattern characteristics, paired t-test and Wilcoxon test were used. Inter-species comparisons were made with unpaired t-tests and Mann-Whitney tests. Proportions were compared using chi-square tests. All statistical tests were performed in Prism 6 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Infra-slow oscillatory properties of the LGN neurons in WAG/Rij rats

Previous studies performed in Wistar rats report the expression ISO pattern in all three parts of the lateral geniculate complex (Albrecht et al., 1998; Chrobok et al., 2018; Lewandowski and Blasik, 2004). As our previous research in WAG/Rij rats was focused on the ISO activity in the IGL only (Chrobok et al., 2017), we first aimed to perform single-channel extracellular experiments to characterise ISO neurons in all three parts of the LGN in this model.

For this investigation we recorded from 50 ISO neurons (mean period: 130.9 ± 55 s; range: from 49 to 271 s): 20 of which were localised in the DLG, 15 in the IGL and 15 in the VLG of WAG/Rij rats. All neurons recorded responded to a light pulse presented to the contralateral eye at the beginning of the protocol. Recordings were started in the bright ambient light conditions (3’00 lx). Once 10 min of sustained ISO recordings had been obtained, lighting conditions were switched to record in total darkness (< 1 lx). In all three parts of the LGN, the ISO activity remained stable in darkness (Fig. 1A,C,E), which was also true for the IGL (as shown previously (Chrobok et al., 2017)). However, properties of the ISO pattern were during different lighting conditions as the periods of oscillations in all three structures were shortened in darkness (DLG: p = 0.0002; IGL: p = 0.0022; VLG: p = 0.0001, Fig. 1B,D,F). The shortening of intraburst rather that extraburst phase was responsible for this effect (intraburst length - DLG: p = 0.0037; IGL: p = 0.0022; VLG: p = 0.0159, Fig. 1B,D,F).

Next, we compared the ISO properties of WAG/Rij rat LGN with the control recordings of 67 ISO neurons in 29 Wistar rats (reported previously in (Chrobok et al., 2018)). The comparison between two rat strains showed that there was a generally shorter period of ISO in WAG/Rij rats both for the ambient light-on (DLG: p = 0.2797; IGL: p = 0.0413; VLG: p < 0.0001, Fig. 2) and light-off conditions (DLG: p = 0.6942; VLG: p = 0.0016, Fig. 2) due to a significantly shorter intraburst phase (light-on - DLG: p = 0.0339, IGL: p = 0.0025; VLG: p = 0.0007; light-off - DLG: p = 0.0014; VLG: p = 0.0429, Fig. 2). Moreover, neurons in the IGL were characterised by higher firing rates in the absence epilepsy model during both the intraburst (p = 0.024) and extraburst phases of the oscillation (p = 0.041). ISO activity in the IGL was not detected or was very sparse in Wistar rats during constant darkness, thus inter-strain comparisons were not possible.

3.2. Firing regularity of the ISO LGN neurons in WAG/Rij rats

To further assess the pattern of the action potential firing by the ISO neurons in the WAG/Rij rat LGN, we measured individual inter-spike intervals (ISI) for each unit recorded. The regularity of firing was examined by a coefficient of variation (Cv) for ISI, based on Young’s regularity criterion (Young et al., 1988). All but one LGN neurons were classified as non-regular (Cv exceeded 0.35). Moreover, the comparison with ISO neurons recorded in Wistar rats showed that IGL units were significantly less regular in spiking in the absence epilepsy model (p = 0.0019, Fig. 3A).

3.3. Gamma-band oscillation in the WAG/Rij rat LGN

Recent reports from our group (Chrobok et al., 2018), as well as others (Saleem et al., 2017; Storchi et al., 2017; Tsuji et al., 2016), demonstrate a gamma frequency oscillation of retinal origin that governs the firing pattern for a subset of neurons in the rodent subcortical visual system. In keeping with this, we next calculated ISI histograms for each ISO neuron recorded from the LGN of WAG/Rij rats to observe the characteristic harmonic distribution pattern (HDP) that reflects the shaping of neural firing by a gamma oscillations (Fig. 3B). Gamma patterning can also be depicted as apparent “bands” in the instantaneous frequency plots (Fig. 1A,C,E). The mean frequency of gamma oscillation in the LGN of WAG/Rij rats was 39 ± 1 Hz, ranging from 32.3 to 44.4 Hz. In WAG/Rij rats, this fast oscillation was found in 55, 40 and 53% of ISO neurons recorded in DLG, IGL and VLG, respectively. Interestingly, the abundance of fast oscillatory neurons amongst all ISO neurons was higher for the IGL in absence epilepsy model, in comparison to Wistar rats (p = 0.04, Fig. 3D). Further comparison between strains depicted a significantly higher frequency of gamma oscillation in the absence epilepsy model than in Wistar rats (39 ± 1 Hz vs. 36.2 ± 1 Hz, p = 0.041, Fig. 3C).

3.4. Responsiveness to light stimulation in the absence epilepsy model

ISO neurons in the subcortical visual system are generally light-responsive, as this oscillatory pattern originates from retinal activity and is further projected on the activity of retinorecipient neurons. Therefore, to assess the responsiveness of ISO neurons to light stimulation and its possible alteration in the absence epilepsy model, we recorded neural responses to retinal illuminations in the LGN. Before each stimulation, retinas were dark-adapted for at least 30 min. Brief light pulses (brightness: 280 lx, duration: 5 s, interval: 30 s) were presented to the contralateral eye in eight repetitions. Then, peri-stimulus time histograms (PSTH) were administered to each neuron tested, enabling us to classify responses as ON (type 1) and OFF (type 2), based on the 95% confidence limit. Type 2 responses were characterised by a drop of firing rate during the light pulse with rebound activity after pulse termination, whereas the activity in type 1 responses was increased during the stimulation. Type 1 responses were further divided into two groups based on the activity after the presentation of a light stimulus; type 1a - where the response was confined within the stimulus duration, and type 1b - displaying a delayed return to baseline-like activity (Fig. 4A). Light-evoked changes in the firing rate were analysed for 30 ISO LGN neurons in the WAG/Rij rats (13 in DLG, 8 in IGL and 9 in VLG) and for 49 neurons in Wistar rats (15 in DLG, 17 in IGL, 17 in VLG). The proportion of response types did not vary between strains for either of the structures examined (Fig. 4B). Interestingly, no type 2 responses were found in the ISO IGL neurons in both rat strains.

Next, we aimed to examine the possible inter-strain differences in the amplitude of type 1 and type 2 responses in each structure. For this, the firing rate change in the transient phase (first 0.5 s) and sustained phase of the PSTH (0.5–5 s) were calculated and compared separately. No major disturbances of the light response magnitude were noted in WAG/Rij rats, despite the significantly higher amplitude of the sustained phase of a response to light in the VLG (p = 0.038, Fig. 4C).
4. Discussion

Here, we show that oscillatory activity in the subcortical visual system can be reliably recorded from the rat absence epilepsy model, however the characteristics of these rhythmic activities differ from healthy controls. Further, we demonstrated elevated frequencies of both the ISO activity and gamma-band oscillation in the LGN of WAG/Rij rats with an altered reaction to sustained change in lighting conditions observed only in the IGL. Recently, we showed that the IGL of WAG/Rij rats is characterised by the disinhibition of neuronal network caused by dampened GABAergic transmission, astrogliosis and decreased amplitude of T-type calcium conductance (Chrobok et al., 2017, 2016). This study strengthens these previous observations, as IGL units recorded in WAG/Rij rats generated significantly higher firing rates to such an extent that this increased neuronal activity remained after induction of darkness, which typically causes IGL activity to vanish in healthy controls (Chrobok et al., 2018; Lewandowski and Blasjak, 2004). Additionally, we found that the regularity of action potential generation in the IGL is distorted in WAG/Rij rats, further hinting at altered synaptic mechanisms in the neuronal network of the IGL. Intriguingly, differences in the frequencies of oscillatory activities were not accompanied by striking disturbances in the acute responsiveness to presentation of transient light pulses, with the exception of the VLG, where neurons were more excited by light stimulation in the WAG/Rij strain.

Growing evidence suggests that oscillatory activities in the subcortical visual system arise from the retina and are not intrinsically generated by distinct retinorecipient areas due to their lack in ex vivo recordings and potent synchronisation in vivo in sites innervated by one eye (Blasjak and Lewandowski, 2004; Chrobok et al., 2018; Tsuji et al., 2016). Therefore, any disturbances in the oscillation frequency must stem from changes in the retinal network itself. Retinal pathologies that exceed the ones seen in albino strains were described in the WAG/Rij...
with higher occurrence of gamma-oscillating cells in the IGL in WAG/Rij rats. Wis - Wistar rats (in grey); WR - WAG/Rij rats (in red); DLG - dorsolateral geniculate; IGL - intergeniculate leaflet; VLG - ventrolateral geniculate; \(*p < 0.01, *p < 0.05.

As they are both highly implicated in the generation of retinal oscillations (Arai et al., 2004; Petit-Jacques et al., 2005; Petit-Jacques and Bloomfield, 2008), we hypothesise that the increase in oscillation frequencies may stem from changed kinetics in the activity of these channels.

Studies performed in frogs and mice report that the gamma-band oscillation in the retina is likely to be generated by amacrine cells and can be modulated via light-regulated input from bipolar cells (Hartveit, 1999; Petit-Jacques et al., 2005; Petit-Jacques and Bloomfield, 2008). The frequency of gamma oscillation presumably depends on the kinetics of inhibitory postsynaptic currents (IPSC), as faster currents would increase the oscillation frequency, and IPSC kinetics are strongly shaped by the subunit composition of the GABAA channel (Buzsáki and Wang, 2012). On the other hand, the mechanism of ISO activity generation remains elusive. Similarly to fast oscillations, the ISO pattern reflected in the synchronised oscillation of subcortical sites (Chrobok et al., 2018) arises from tightly-coupled rhythmic output of the retinal network. Therefore, horizontal mechanisms are most likely to play part in its generation or synchronisation amongst output activity of retinal ganglion cells. Intriguingly, we show the increase in frequency of both oscillatory bands in WAG/Rij rats suggesting a common mechanism or interdependence of these phenomena.

The consequences of pathologically altered frequencies of retinal oscillations observed in the WAG/Rij rats remain largely unknown. However, because sleep-promoting brain centres are retinoreceptive (Chou et al., 2002) and light information is crucial for optimal function of various brain networks and behaviours, change in many brain-controlled functions may arise as a consequence of retinal dysfunction. Absence epilepsy is often accompanied by sleep-promoting system insufficiencies and sleep architecture changes (Halász, 1991; Suntsova et al., 2009). These may stem from a variety of disruptions in the neurophysiology seen in the epileptic phenotype, including direct disturbances of the sleep promoting neurons of the ventrolateral preoptic area (Suntsova et al., 2009). Moreover, circadian structures such as the IGL and most importantly the SCN may rely on ultradian time cues encoded in ISO and/or gamma frequency transmitted from the retina (Chrobok et al., 2018; Tsuji et al., 2016). Therefore, it can be hypothesised that the temporal disruption of these signals in the absence epilepsy model may provide a cause of disturbance to the sleep/wake cycle in WAG/Rij rats.

Altogether, our study provides evidence for the increased frequencies in the oscillatory activities in the subcortical visual system in WAG/Rij rats which may arise from neurophysiological changes in the retinal network. Due to the extensive retinal innervation of many arousal and sleep-related brain centres, this disruption in basic functioning of neuronal networks may contribute to the epileptic phenotype of this absence epilepsy model. However, further behavioural and pharmacological studies on the involvement of the visual system in the epilepsy are needed to directly link abnormal oscillation frequencies in sensory systems with the generation of SWDs.
Author contributions
LC and KP-C conceived the project. LC, JSJ-L, KP-C and MHL contributed to the design of experiments. LC and KP-C performed experiments. LC, JSJ-L and KP-C analysed and interpreted electrophysiological data. LC wrote the paper. MHL supervised the project. All authors revised the manuscript critically and approved the final version.

Declaration of Competing Interest
The authors declare no competing financial interests.

Acknowledgements
This study was supported by the Polish Ministry of Science and Higher Education project "Diamontry grant III" No. 0001/DIA/2014/ 43, 2014-2017. LC was additionally supported by the Polish National Science Centre doctoral scholarship "Etudia IV" 2016/20/T/NZ4/ 00273. Authors would like to thank Rebecca C. Northeast, PhD for her support. This study was supported by the Polish Ministry of Science and Higher Education, DI/2017/12. LC was additionally supported by the Polish National Science Centre grant, grant number 2016/20/T/NZ4/00273. Authors would like to thank Rebecca C. Northeast, PhD for her support. This study was supported by the Polish Ministry of Science and Higher Education, DI/2017/12. LC was additionally supported by the Polish National Science Centre grant, grant number 2016/20/T/NZ4/00273. Authors would like to thank Rebecca C. Northeast, PhD for her support. This study was supported by the Polish Ministry of Science and Higher Education, DI/2017/12. LC was additionally supported by the Polish National Science Centre grant, grant number 2016/20/T/NZ4/00273. Authors would like to thank Rebecca C. Northeast, PhD for her support. This study was supported by the Polish Ministry of Science and Higher Education, DI/2017/12. LC was additionally supported by the Polish National Science Centre grant, grant number 2016/20/T/NZ4/00273. Authors would like to thank Rebecca C. Northeast, PhD for her support. This study was supported by the Polish Ministry of Science and Higher Education, DI/2017/12. LC was additionally supported by the Polish National Science Centre grant, grant number 2016/20/T/NZ4/00273. Authors would like to thank Rebecca C. Northeast, PhD for her support. This study was supported by the Polish Ministry of Science and Higher Education, DI/2017/12. LC was additionally supported by the Polish National Science Centre grant, grant number 2016/20/T/NZ4/00273. Authors would like to thank Rebecca C. Northeast, PhD for her support.


