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The GABA Developmental Shift Is Abolished by Maternal Immune Activation Already at Birth

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Abstract

Epidemiological and experimental studies suggest that maternal immune activation (MIA) leads to developmental brain disorders, but whether the pathogenic mechanism impacts neurons already at birth is not known. We now report that MIA abolishes in mice the oxytocin-mediated delivery γ-aminobutyric acid (GABA) shift from depolarizing to hyperpolarizing in CA3 pyramidal neurons, and this is restored by the NKCC1 chloride importer antagonist bumetanide. Furthermore, MIA hippocampal pyramidal neurons at birth have a more exuberant apical arbor organization and increased apical dendritic length than age-matched controls. The frequency of spontaneous glutamatergic postsynaptic currents is also increased in MIA offspring, as well as the pairwise correlation of the synchronized firing of active cells in CA3. These alterations produced by MIA persist, since at P14–15 GABA action remains depolarizing, produces excitatory action, and network activity remains elevated with a higher frequency of spontaneous glutamatergic postsynaptic currents. Therefore, the pathogenic actions of MIA lead to important morphophysiological and network alterations in the hippocampus already at birth.

Key words: apical arborization, excitatory–inhibitory imbalance, hippocampal network, Poly(I:C), pyramidal neurons.

Introduction

Epidemiological studies indicate that maternal infection and inflammatory processes during pregnancy are risk factors for developmental disorders in offspring (Patterson 2002, 2009; Atladóttir et al. 2010, 2012; Brown 2012; Knuesel et al. 2014; Lyall et al. 2014; Estes and Mcallister 2016; Zerbo et al. 2015). Specifically, a bacterial infection during the second trimester of pregnancy or a viral infection in the first trimester have been correlated with the diagnosis of autism spectrum disorders (ASD) in children (Atladóttir et al. 2010). To investigate the effects of in utero exposure of the fetus to a maternal immune activation (MIA), various animal models have been designed (Dean et al. 2015; Giovanoli, Notter, et al. 2015; Solek et al. 2018; Bilbo et al. 2018).
bacterial cell wall endotoxin lipopolysaccharide (LPS) (Custódio et al. 2018; Kirsten and Bernardi 2017), or the viral mimic double-stranded RNA polyinosinoc-polycytidylic acid (Poly(I:C)) generate offspring with a variety of autistic-like behavioral impairments (Smith et al. 2007; Meyer et al. 2008; Hsiao and Patterson 2011). Indeed, pregnant mice exposed to Poly(I:C) on embryonic day 12.5 (E12.5) deliver offspring that in adulthood display the 3 core symptoms of autism: reduced sociability, impaired communication skills, and stereotypic behaviors (Malkova et al. 2012). Experimental studies attempting to decipher the pathophysiological mechanisms triggered by MIA have highlighted the wide diversity of brain areas impacted by the prenatal insult, such as the neocortex (Richetto et al. 2014; Faylor et al. 2016; Kim et al. 2017; Shin Yim et al. 2017), hippocampus (Giovannoni, Weber-Stadlbauer, et al. 2016; Wolff and Bilkey 2015), and cerebellum (Shi et al. 2009), producing a variety of alterations. Particularly, Poly(I:C)-induced MIA offspring was shown to exhibit pleiotropic pathophysiological disturbances. This included a loss of neuronal layer identity with somatosensory cortical disorganized patches (Kim et al. 2017; Shin Yim et al. 2017); a reduction of parvalbumin interneurons’ perineuronal nets (Paylor et al. 2016) and an altered GABAergic (γ-aminobutyric acid (GABA)) transcriptome (Richetto et al. 2014) leading to decreased GAD67 (glutamate decarboxylase) expression in the prefrontal cortex; and in the hippocampus, synaptic deficits (Giovannoni, Weber-Stadlbauer, et al. 2016) and alterations of place cell firing (Wolff and Bilkey 2015). However, whether MIA hinders developmental sequences of ionic currents is not known. This is important as perturbation of developmental sequences might deviate their normal course, leading to misplaced/misconnected neurons and aberrant networks, a concept known as neuroarcheology (Ben-Ari 2008). Indeed, neurons with immature features might constitute a leading pathogenic mechanism perturbing relevant brain network oscillations. In keeping with this hypothesis, Corradini and colleagues have recently shown that the developmental GABA excitatory to inhibitory shift is not present in cortical neurons of MIA offspring, leading to paradoxical excitatory actions of GABA in prewean juvenile offspring (Corradini et al. 2018). However, whether these alterations are present earlier and in particular at birth is not known.

Birth is a highly complex and stressful event with a high degree of vulnerability (Boksa et al. 2006; Chudal et al. 2014; Say et al. 2016; Brander et al. 2017; Wang et al. 2017). We reported in earlier studies that birth is associated with a neuroprotective oxytocin-mediated abrupt reduction of intracellular chloride ([Cl\(^-\)]\(_i\)) allowing for hyperpolarizing actions of GABA (Tyzio et al. 2006). This is abolished in Fragile X (FRX) and intrauterine valproic acid (VPA) rodent models of ASD, and the effects are persistent with GABA exciting neurons also in prewean (P14–15) juvenile offspring (Tyzio et al. 2014). In addition, maternal administration of the NKCC1-specific antagonist bumetanide shortly before and during delivery restored the hyperpolarizing and inhibitory actions of GABA in prewean juvenile offspring and attenuated behavioral deleterious sequel (Eftekhari et al. 2014; Tyzio et al. 2014). These results suggest that GABA alterations already present at birth could lead to long-term consequences and that restoring the correct polarity of GABA actions might constitute an early preventive treatment.

We now report that Poly(I:C)-induced MIA alters the transient excitatory to inhibitory switch of GABA at birth due to higher [Cl\(^-\)]\(_i\) levels as attested by measures of the GABA\(_A\)R driving force and its restoration by bumetanide. In addition, pyramidal neurons of the hippocampal CA3 region at birth present longer and abnormally sinuous apical tree in MIA offspring. Hippocampal network activity is also altered, with increased frequency of glutamatergic postsynaptic currents and a higher pairwise correlation of firing onset in CA3 pyramidal neurons. These effects are persistent, with excitatory GABA action at P14–15, and higher frequency of glutamatergic postsynaptic currents leading to a long-lasting inhibitory versus excitatory imbalance in MIA offspring. Altogether, our work shows that an in utero insult such as Poly(I:C)-induced MIA produces major physiological, morphological, and hippocampal network changes already at birth.

### Material and Methods

#### Animals and MIA Induction

All animal experiments were carried out according to the guidelines set by the European Communities Council Directive (2010/63/EU). Time-pregnant female C57BL6/J mice at embryonic day 6–8 (E6–E8; Charles River Laboratories) were housed on arrival in an ABSL2 animal-housing facility with ad libitum access to food and water under a 12 h/12 h light/dark cycle. Mice were left undisturbed until the day of MIA induction. Poly (I:C) (20 mg/kg, polyinosinic-polycytidylic acid potassium salt, Sigma-Aldrich) or an equivalent volume of sterile saline solution was administered to the pregnant dams at E12.5 by intraperitoneal injection as described previously (Hsiao and Patterson 2011). For the injection, Poly (I:C) was dissolved in sterile saline to a final concentration of 5 mg/mL.

#### Acute Brain Slices

Brains from P0 and P14–15 pups were removed and immersed into ice-cold solution containing (in mM): 132.5 chloride chloride, 2.5 KCl, 1.23 NaH2PO4, 3 MgCl2, 0.7 CaCl2, 25 NaHCO3, 8 glucose. Horizontal hippocampal brain slices (300–400 μm thick) were cut on a vibratome (VT1200 Leica Microsystems). Slices were then left to recover for 1 h at room temperature (22–25 °C) in artificial cerebrospinal fluid (aCSF) containing (in mM): 126 NaCl, 3.5 KCl, 1.2 NaHPO4, 2 CaCl2, 1.3 MgCl2, 11 glucose, 25 NaHCO3 (pH 7.4). All solutions were bubbled with 95% O2/5% CO2.

#### Electrophysiology

For electrophysiological recordings of CA3 pyramidal neurons, slices were placed in a conventional fully submerged chamber superfused with aCSF at a rate of 2–3 mL/min at room temperature.

#### Single-channel Voltage Clamp Recordings

The driving force of γ-aminobutyric acid type A receptor (GABA\(_A\)R) was estimated (DF\(_{\text{GABA}}\)) by single-GABA\(_A\)R channel recordings in cell-attached configuration using an EPC-10 amplifier (HEKA Elektronik Dr. Schulze GmbH). Patch pipette solution contained (in mM): 120 NaCl, 5 KCl, 20 TEA-Cl, 5 4-aminopyridine, 0.1 CaCl2, 10 MgCl2, 10 glucose, 10 HEPES-NaOH, pH 7.2–7.3 (with GABA at 5 μM). Abnormal NKCC1-dependent intracellular chloride accumulation was tested with acute application of the specific NKCC1 antagonist bumetanide. Briefly, DF\(_{\text{GABA}}\) was determined on pyramidal cells before and after a 15 min bath application of bumetanide (10 μM, Sigma-Aldrich). Recordings were digitized and analyzed as described previously (Khazipov et al. 1995; Tyzio 2003).

#### Whole-cell Recordings

Whole-cell recordings were performed with a MultiClamp 700B amplifier (Molecular Devices) and EPC-10 amplifier (HEKA Elektronik Dr. Schulze GmbH).
Electronika Dr. Schulze GmbH). Data were acquired at 10 kHz and filtered at 2.4 kHz. Patch pipette solution contained (in mM): 130 K-glucuronate, 10 Na-glucuronate, 7 NaCl, 4 Mg-ATP, 10 HEPES, 4 phosphocreatine, 0.3 Na-GTP, pH 7.3 with KOH. 0.5–1% biocytin (Sigma-Aldrich) was added to the pipette solution for post hoc morphological reconstruction at P0 (see below). Pipette resistance was 6–8 MΩ. At P15, spontaneous glutamatergic postsynaptic currents (sGlu PSCs) were recorded for 15 min at the reversal potential for GABAergic currents (−75 ± 2 mV). Actual reversal potential for GABA currents was detected experimentally by changing the holding potential step by step (around Vh = −75 mV) with increment 1 mV, and the potential at which the sGABA current (outward) was zero was taken as a holding potential for recording of sGlu PSCs. At P0, experimentally detected actual reversal potential was around −70 mV.

Spontaneous GABAergic postsynaptic currents (sGABA PSCs) were recorded for 15 min at +10 ± 2 mV where the glutamatergic currents are reversed or negligible. Reversal potential for GABA currents was detected experimentally by changing the holding potential step by step (around Vh = +10 mV) with increment 1 mV, and the potential at which the sGlu currents (inward currents) was zero was taken as a holding potential for recording of sGABA PSCs.

After recording, data were filtered using a Bessel low-pass filter to reduce the root-mean-square (RMS) noise and improve the signal-to-noise ratio and analyzed using Clampfit 10.4 (Molecular Devices) and Mini Analysis 6.0 (Synaptosoft Inc). The threshold amplitude for event detection was set as 4 pA for sGlu PSCs and 30 pA for sGABA PSCs. Cumulative distribution was obtained using Mini Analysis 6.0 and averaging was performed in OriginPro (OriginLab).

The following experimental parameters were similar between groups: animal’s age, cell depth, number of events analyzed for P14–15 sPSCs (400 events) and recording duration analyzed for P0 sPSCs (5 min).

**Intrinsic Properties**

The membrane capacitance (Cm) and resting membrane potential (i.e., membrane potential at I = 0, Vrest) were measured immediately after establishing the whole-cell configuration. Action potential firing pattern of the cell was recorded at least 5 min after the start of the dialysis to allow intracellular medium diffusion into the cell. The holding current (Ih) and the series resistance (Rs) in response to a 5 mV pulse were monitored throughout the recording. Criteria for data inclusion for analysis were leakage current <−50 pA at Vhold = −70 mV, Rs ≤ 25 MΩ, and Rs change over recording time ≤20%. Additionally, cells were included for analysis if their firing pattern was typical of a pyramidal cell. Post hoc biocytin revelation provided the cells’ morphology and location within the hippocampal CA3 pyramidal cell layer at P0.

**Cell-attached Recordings**

Cell-attached recordings of action potential firing were performed with a Multiclamp 700B amplifier (Molecular Devices) on P14–15 hippocampal slices using 7–10MΩ borosilicate glass pipettes filled with artificial aCSF. After a baseline period of at least 10 min, isoguvacine (specific GABA A R agonist, 10 μM, Sigma-Aldrich) was bath applied for 120–140 s and washed for 15 min. Baseline spiking frequency was defined as 100% of spiking activity. Spiking frequency during the application of isoguvacine was calculated as a percentage of the baseline spiking frequency. Firing activities were analyzed offline with Mini Analysis 6.0 (Synaptosoft Inc). To rule out the possibility of artifactitious spiking variations, only cells showing a maximum difference of 30% spiking from the baseline period compared with wash were included in the analysis. The threshold amplitude for detecting action potentials was set at twice the baseline noise.

**Calcium Imaging**

**Recordings**

After 1 h recovery, P0 slices were incubated for 25–30 min in a lightproof oxygenated chamber heated at 37 °C filled with 2.5 mL aCSF with 25 μL Fura-2AM solution containing: 50 μg Fura-2AM (Molecular Probes, Life Technologies), 48 μL DMSO (Sigma), 2 μL pluronic acid (Life Technologies). Slices were then left to recover for 30 min to 1 h before recording under continuously superfused oxygenated aCSF (O2/CO2—95%/5%; 3 mL/min) heated at 37 °C. Slices were imaged using a multibeam 2-photon laser scanning apparatus (Trimscope-LaVision Biotec) coupled to an Olympus microscope (wide numerical aperture 20X, NA 0.95, Olympus) as described previously (Crépel et al. 2007; Dehoret 2011). Laser scanning was performed with a 475 × 355 μm scanfield using a CCD camera (LaVision Imager 3QE) with a 4 × 4 binning, time resolution of 137 ms, and a duration of 1000 frames per recorded movie.

**Analysis**

Calcium imaging recordings were preprocessed using an upgraded version of the custom designed MATLAB (MathWorks) software “Hippo” previously described (Crépel et al. 2007; Bonifazi et al. 2009) to identify the cell contours, extract the average calcium signals from the detected cell areas, and mark the onsets–offsets of the calcium events.

For each movie, active cells were defined as those exhibiting calcium events with a minimum 5% fluorescence deflection within the recording time. Calcium plateaus were defined as long-lasting events during which fluorescence deflection was sustained for at least 30 frames (Crépel et al. 2007; Ferrari et al. 2012).

To calculate the pairwise correlation between active cells, the calcium signal of each cell was converted into a binary time series preserving the temporal resolution, with the ones marking the time window between onset and offset of each calcium event. The correlation between time series was calculated according to the following equation:

\[
C_{ij} = \frac{\sum_{t=1}^{T} S_i(t) \times S_j(t)}{\sqrt{N_i \times N_j}}
\]

where \( t \) is the frame number, \( S_i \) and \( S_j \) are the time series of cell i and j, respectively, T is the total number of frames, \( N_i \) and \( N_j \) are the total number of ones in the time series of neuron i and j, respectively. Note that this is the equivalent of calculating the cosine between the 2 vectors representing the binary time series.

To compute the correlation between the plateau cells, similarly to what was described by Crépel et al., 2007, the binary time series of the event’s onsets for the plateau cells was smoothed with a Gaussian (σ = 1 frame). The correlation was then calculated according to equation 1 and the significance of each correlation value with an accuracy of P < 0.001 was estimated from surrogate data sets, in which the events were randomly reshuffled in time.
Biocytin-filled CA3 Pyramidal Neurons Morphological Reconstruction

After whole-cell patch clamp recording, P0 slices were immediately immersed in Antigenfix (DiaPath) for overnight fixation. Slices were then rinsed 3 times for 10 min in 1X-PBS before being stored at 4°C in 1X-PBS 0.01% azide until biocytin revelation. For biocytin revelation, slices were first rinsed as described above, incubated in blocking solution (1X-PBS, 0.3% Triton, 5% Normal Goat Serum) at room temperature for 1 h under gentle agitation, and incubated overnight at 4°C in Streptavidin-Alexa 555 (1/1000, Thermo Fisher-Invitrogen) in blocking solution under gentle agitation. The following day slices were rinsed as previously, incubated for 10 min in 1X-PBS with 1/1000 Hoechst (Sigma-Aldrich), and rinsed before mounting (Fluoromount-G™ Slide Mounting Medium, Electron Microscopy Sciences). Neurons were stack-imaged with a Leica SP5-X confocal microscope (Leica Microsystems) at a 400 Hz frequency, 1024 x 555 (1/1000, Thermo Fisher-Invitrogen) in blocking solution (1X-PBS, 0.3% Triton, 5% Normal Goat Serum) at room temperature for 1 h under gentle agitation, and incubated overnight at 4°C in Streptavidin-Alexa 555 (1/1000, Thermo Fisher-Invitrogen) in blocking solution under gentle agitation. The following day slices were rinsed as previously, incubated for 10 min in 1X-PBS with 1/1000 Hoechst (Sigma-Aldrich), and rinsed before mounting (Fluoromount-G™ Slide Mounting Medium, Electron Microscopy Sciences).

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MIA Enhances Glutamatergic Postsynaptic Currents at Birth

We further evaluated glutamatergic and GABAergic activity using whole-cell voltage clamp recordings to investigate spontaneous glutamatergic and GABAergic postsynaptic currents (sGlu PSCs and sGABA PSCs, respectively). In P0 CA3 pyramidal neurons of MIA offspring, the frequency of sGlu PSCs was enhanced compared with saline ones (0.57 ± 0.132 Hz vs. 0.23 ± 0.049 Hz; Mann-Whitney test, P < 0.05; Fig. 2A–C), with no difference of amplitude (8.03 ± 1.162 pA vs. 7.48 ± 0.784 pA; Mann-Whitney test, not significant; Fig. 2A,B,D). In contrast, the frequency and amplitude of sGABA PSCs at P0 was not different between MIA and control pyramidal neurons (0.34 ± 0.116 Hz; 41.06 ± 2 pA vs. 0.34 ± 0.116 Hz; 41.06 ± 2 pA vs. 0.18 ± 0.03 Hz; 43.01 ± 2.94 pA; Mann-Whitney test, not significant; Fig. 2E–H). Therefore, Poly(I:C)-induced MIA increases glutamatergic spontaneous activity of CA3 pyramidal neurons at birth.

At Birth the Pairwise Correlation of Active Cells is Increased After MIA

Hippocampal CA3 early correlated neuronal activity was further investigated in MIA offspring by 2-photon calcium imaging of P0 acute brain slices (Fig. 3A.1). Semiautomated detection of cells (Fig. 3A.2) and evaluation of calcium activity (Fig. 3B) revealed that the percentage of active cells was not different in MIA CA3 networks compared with saline (39.77 ± 3.32% vs. 36.41 ± 3.19%; Mann-Whitney test, not significant; Fig. 3C). However, the hippocampal networks of MIA offspring presented a significantly increased correlation in the calcium events occurrence in active cellular pairs (15 saline networks, 14 MIA networks; Kolmogorov-Smirnov test, P < 0.001; Fig. 3D). Despite a tendency, no significant difference was found in the percentage of cells presenting a plateau calcium activity (22.72 ± 2.21% vs. 16.35 ± 1.76%; Mann-Whitney test, not significant; Supplementary Fig. 2A), and the distance-dependent pairwise correlation of calcium plateau cells (Kolmogorov-Smirnov, P = 0.06, not significant; Supplementary Fig. 2B–E) between groups. Hence, more neurons generate correlated activity at birth in MIA than control hippocampal networks.
The Depolarizing Actions of GABA are Persistent in MIA Offspring and Associated with an Excitatory Activity

We further evaluated if the GABA polarity alterations observed on the day of birth persisted in prewean juvenile MIA offspring. The $D_{GABA}$ continued to be depolarizing in MIA CA3 pyramidal neurons at P14–15 (11.7 ± 5.1 mV), whereas it was slightly hyperpolarizing in control neurons (−3.2 ± 4.1 mV; 2-tailed T-test, P < 0.001; Fig. 4A,B). We next assessed if the depolarizing action of GABA was associated with neuronal excitation using the specific GABA$_A$R agonist isoguvacine in cell-attached configuration. As expected, isoguvacine decreased the basal spiking frequency of neurons recorded in control animals (67.24 ± 12.87% of basal spiking; paired T-test, P < 0.05; Fig. 4C,D), indicative of an inhibitory action of GABA at P14–15. Spiking activity was similar before the application of isoguvacine and after washout of the drug under both MIA and saline conditions (Fig. 4D, right). Consequently, MIA induces persistent GABA-depolarizing and -excitatory activities in pyramidal hippocampal neurons of prewean juvenile pups.

Glutamatergic PSCs Frequency and Amplitude are Enhanced in Prewean MIA Offspring

Spontaneous PSC recordings were performed in pyramidal neurons at P14–15 to assess network activity in CA3 after MIA. The frequency of sGlu PSCs was increased in MIA compared with saline neurons (21.45 ± 1.83 Hz vs. 13.4 ± 1.54 Hz; Mann–Whitney test, P < 0.005; Fig. 5A–C) and so was the amplitude (14.59 ± 0.91 pA vs. 11.8 ± 0.69 pA; Mann–Whitney test, P < 0.05; ***
**Figure 2.** Enhanced frequency of glutamatergic postsynaptic currents at birth after MIA. (A) Representative traces of sGlu PSCs and (E) sGABA PSCs recorded in whole-cell voltage clamp configuration of P0 pyramidal cells of saline and MIA offspring. (B) The frequency of sGlu PSCs is increased in MIA \((n = 11\) cells) compared with saline \((n = 10\) cells). (C) Averaged cumulative distribution of sGlu PSCs interevent interval \((\text{iei})\) and (D) amplitude for the pooled dataset represented in (B). (F) The frequency and amplitude of sGABA PSCs is similar between saline \((n = 10\) cells) and MIA \((n = 12\) cells) pyramidal neurons. (G) Averaged cumulative distribution of sGABA PSCs iei and (H) amplitude for the pooled dataset represented in (F). Mann–Whitney test, \(* P < 0.05\).

**Figure 3.** MIA-induced increased pairwise correlation of active cells in CA3 hippocampal networks at birth. (A.1) Representative image of a P0 hippocampal slice loaded with the Fura-2AM fluorescent calcium sensor and (A.2) corresponding semiautomated cells contours detection. Dashed lines on picture define the CA3 area in which further steps of analysis were carried out. (B) Representative traces of fluorescence emitted by cells loaded with Fura-2AM. Each trace corresponds to fluorescence variations emitted by a single cell. (C) The percentage of active cells in P0 CA3 networks is similar in saline \((n = 15\) networks) and MIA conditions \((n = 14\) networks). Mann–Whitney test not significant. (D) Correlation between the calcium events timing in pairs of active cells. Only pairs showing correlations above zero, i.e., with at least one overlapping event, were pooled and included in the plot. Kolmogorov–Smirnov test, \(P < 0.001\).
Dysregulated developmental sequences with the presence in adult networks of neurons endowed with immature features.

Birth marks the developmental transition from a stabilized environment to a continuous flow of unpredictable stimuli, therefore initiating major physiological adaptations. The fetal brain must then quickly overcome the major ongoing physiological changes, without being affected by the harmful side effects of the acute stressors during the delivery process (Lagercrantz and Bistotelli 1977). Furthermore, dysregulated GABAergic polarity on the day of birth has been identified as a critical signature of developmental brain disorders (Ben-Ari 2015). Indeed, we previously showed that the protective inhibitory action of GABA at birth was abolished in hippocampal neurons of VPA and FRX rodent models of ASD (Tyzio et al. 2014), accompanied by lasting imbalanced sGlu PSCs and maintained depolarizing and excitatory GABA. The present study reveals that the environmental insult of MIA similarly alters GABA action at birth by dysregulating intracellular chloride concentration, as suggested by the acute application of the NKKC1 antagonist bumetanide that rescued the polarity of GABA. In addition, our present results show hyperactive developing hippocampal networks after MIA already at birth and a similar lasting alteration of sGlu PSCs accompanied of sustained depolarizing and excitatory GABA actions.

Several reports have shown alterations in the excitatory versus inhibitory balance after MIA as well as increased susceptibility for seizures in stimulated conditions. Indeed, adult offspring from PolyI:C MIA subjected to an epilepsy protocol produced by focal electrical stimulation in the hippocampus (i.e., kindling model of epilepsy) was shown to exhibit increased hippocampal excitability following kainic acid treatment was also reported by (Pineda et al. 2013), reduced number and density in the dentate gyrus (Piontkiewitz et al. 2012; Zhang and van Praag 2015) respectively; Mann–Whitney test, not significant; Fig. 5E–H). Therefore, MIA enhances sGlu PSCs at P14–15.

**Discussion**

Epidemiological studies have stressed the importance of gestational maternal infection and inflammatory processes as risk factors for developmental disorders in offspring (Patterson 2002, 2009; Atladóttir et al. 2010, 2012; Brown 2012; Knuesel et al. 2014; Lyall et al. 2014; Zerbo et al. 2015). However, whether and how MIA hinders developmental sequences is not known. To the best of our knowledge, our results provide the first evidence that MIA produces already at birth important physiological and morphological changes in the hippocampus of mice. These results shed new light on the deleterious effects of intrauterine insults lead to long-term changes by deviating developmental sequences with the presence in adult networks of neurons endowed with immature features.

To the best of our knowledge, our results provide the first evidence that MIA produces already at birth important physiological and morphological changes in the hippocampus of mice. These results shed new light on the deleterious effects of intrauterine insults lead to long-term changes by deviating developmental sequences with the presence in adult networks of neurons endowed with immature features.

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Several reports have shown alterations in the excitatory versus inhibitory balance after MIA as well as increased susceptibility for seizures in stimulated conditions. Indeed, adult offspring from PolyI:C MIA subjected to an epilepsy protocol produced by focal electrical stimulation in the hippocampus (i.e., kindling model of epilepsy) was shown to exhibit increased hippocampal excitability and accelerated kindling rate, with increased seizure susceptibility persisting 2 weeks later (Pineda et al. 2013). Increased susceptibility to seizures following kainic acid treatment was also reported in 3-month-old MIA offspring (Corradini et al. 2018). Interestingly, parvalbumin GABAergic neurons are impacted by MIA with decreased GAD67 protein levels in the dorsal hippocampus (Dickerson et al. 2014), reduced number and density in the dentate gyrus (Piontkiewitz et al. 2012; Zhang and van Praag 2015) respectively; Mann–Whitney test, not significant; Fig. 5E–H). Therefore, MIA enhances sGlu PSCs at P14–15.

**Discussion**

Epidemiological studies have stressed the importance of gestational maternal infection and inflammatory processes as risk factors for developmental disorders in offspring (Patterson 2002, 2009; Atladóttir et al. 2010, 2012; Brown 2012; Knuesel et al. 2014; Lyall et al. 2014; Zerbo et al. 2015). However, whether and how MIA hinders developmental sequences is not known. To the best of our knowledge, our results provide the first evidence that MIA produces already at birth important physiological and morphological changes in the hippocampus of mice. These results shed new light on the deleterious effects of intrauterine insults lead to long-term changes by deviating developmental sequences with the presence in adult networks of neurons endowed with immature features. Birth marks the developmental transition from a stabilized environment to a continuous flow of unpredictable stimuli, therefore initiating major physiological adaptations. The fetal brain must then quickly overcome the major ongoing physiological changes, without being affected by the harmful side effects of the acute stressors during the delivery process (Lagercrantz and Bistotelli 1977). Furthermore, dysregulated GABAergic polarity on the day of birth has been identified as a critical signature of developmental brain disorders (Ben-Ari 2015). Indeed, we previously showed that the protective inhibitory action of GABA at birth was abolished in hippocampal neurons of VPA and FRX rodent models of ASD (Tyzio et al. 2014), accompanied by lasting imbalanced sGlu PSCs and maintained depolarizing and excitatory GABA. The present study reveals that the environmental insult of MIA similarly alters GABA action at birth by dysregulating intracellular chloride concentration, as suggested by the acute application of the NKKC1 antagonist bumetanide that rescued the polarity of GABA. In addition, our present results show hyperactive developing hippocampal networks after MIA already at birth and a similar lasting alteration of sGlu PSCs accompanied of sustained depolarizing and excitatory GABA actions.

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and decreased input in the medial prefrontal cortex (Canetta et al. 2016). A developmental study by Paylor and colleagues highlighted an age-dependent and region-specific reduction of perineuronal nets in the medial prelimbic cortex and amygdala of MIA offspring (Paylor et al. 2016). In mature dentate granule cells recorded after MIA, intrinsic properties are altered and the frequency of miniature inhibitory postsynaptic currents (mIPSCs) is reduced (Zhang and van Praag 2015). Increased sEPSCs amplitude has also been reported in neurons of the basolateral amygdala in MIA mice (Li et al. 2018). Thus, in utero exposure to MIA disrupts the inhibitory/excitatory balance from birth and into adulthood.

Our results further show that the apical morphology of hippocampal CA3 pyramidal neurons from MIA newborn offspring is hypertrophied and strongly altered in shape. Whether these alterations are permanent remains to be investigated. In addition, these morphological alterations might be structure dependent and depend on the developmental stage at which the MIA insult occurred (Meyer 2006; Meyer et al. 2008). Indeed, LPS-induced MIA led to reorganized apical dendritic tree architecture and to increased apical dendrites complexity in P14–21 mouse pyramidal cells from layer II primary visual cortex (Gao et al. 2015), also see (Fernández de Cossío et al. 2017). In contrast, in adult Poly(I:C)-induced MIA mouse offspring, the total
number and length of dendritic intersections of hippocampal dentate granule cells are decreased (Li et al. 2014; Zhang and van Praag 2015), and dendritic architecture of medial prefrontal cortex pyramidal cells is altered (Li et al. 2014).

Altered dendritic architecture and increased spanning of apical arbors might result from MIA-induced dysregulation of neurotrophic cues. Indeed, depolarizing GABA actions at birth are likely to contribute to the apical dendritic overgrowth by modulating neuritic outgrowth via calcium-dependent mechanisms (Barbin et al. 1993; Owens and Kriegstein 2002; Represa and Ben-Ari 2005; Wang and Kriegstein 2009). This in turn can regulate brain-derived neurotrophic factor (BDNF) release and play a role on neuronal morphology (Marty et al. 1996; Fiorentino et al. 2009; Kuczewska et al. 2011). Therefore, from a network perspective, MIA might enhance GABAergic neurons growth and synaptogenesis (through depolarizing GABA at birth) (Fiorentino et al. 2009), thereby increasing the probability of synaptic contacts between interneurons and pyramidal cells and facilitating synchronous activity between active cellular pairs.

Depolarizing GABA action at birth might also facilitate glutamatergic NMDA receptors-mediated currents by removing the intracellular magnesium block from NMDA receptors (Leinekugel et al. 1997) leading to the uncovered increase in glutamatergic input after MIA. In addition, depolarizing GABA facilitates the formation of excitatory synapses in neocortical neurons (Owens and Kriegstein 2002; Wang and Kriegstein 2009) and an enhanced glutamatergic synaptogenesis. Thus, the absence of the switch of GABA action at birth after MIA could lead to an enhanced glutamatergic synaptogenesis and activity. This is in accord with our previous report, in which restoring the depolarizing to hyperpolarizing switch of GABA at birth in 2 rodent models of autism also restored normal spontaneous glutamatergic activity at P15 in the offspring (Tyzio et al. 2014).

Although not fully understood, a general picture is starting to emerge on the likely sequence of events that occur after MIA. Choi and colleagues have shown that MIA activates the interleukin-17 pathway (through IL-17a) leading to an abnormal cortical development as early as E14.5 in mice (Choi et al. 2016). The MIA-induced resulting dyspastic cortical patches are responsible of the abnormal behaviors observed in adult mice (Shin Yim et al., 2017). Furthermore, several other intermediate steps occurring after the MIA insult have been identified. Specifically, in utero Poly(I:C) injection increases interleukin-1 (IL-1), interleukin-6 (IL-6), and TNF-α levels (Giovanoli, Notter, et al. 2015; Giovanoli, Weber-Stadlbauer, et al. 2016; Corradini et al. 2018). Exposure to IL-1β and TNF-α upregulates the Na+/K+ cotransporter NKCC1 gene mRNA and protein expression in human endothelial cells of umbilical vein (Topper et al. 1997) and cultured astrocytes in a dose-dependent manner (Huang et al. 2014). In addition, Poly(I:C) alters the ratio of NKCC1 and KCC2 protein and mRNA levels in cortical homogenates of P20 mice in a cytokine-dependent way (viz. IL-6 and IL-1β) (Corradini et al. 2018) leading to an abolishment of the excitatory to inhibitory shif of GABA. Therefore, our results on the Poly(I:C)-dependent bumetanide-sensitive alteration of GABA polarity in the hippocampus at birth and at the end of the second postnatal week suggest that an MIA-elicited inflammatory response might trigger an early dysregulation in the expression of chloride cotransporters leading to an alteration of developing networks. Yet, the exact links between an event restricted to labor and birth and the long-term deleterious sequel remains elusive. The fact that maternal bumetanide treatment during that precise short period is sufficient to attenuate the behavioral and electrical features of autism illustrates the importance of this critical period that can reduce or aggravate the effects of intrauterine insults (Tyzio et al. 2014).

In conclusion, CA3 hippocampal neurons of MIA offspring show already at birth changes that are reminiscent of those occurring in animal models of ASD and other disorders (Ben-Ari 2017). Hence, despite their different behavioral, physiological, and clinical features, neurodevelopmental disorders seem to share some pathophysiological commonalities, notably early alterations of the polarity of GABAergic activity.

Supplementary Material

Supplementary material is available at Cerebral Cortex online.

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Competing financial interests

Y. Ben-Ari, D.C. Ferrari, N. Burnashev, R. Tyzio and N. Lozovaya are shareholders of Neurochlore, a biotech company dedicated to the development of treatments for children with autism. Y. Ben-Ari is also the CEO of the company.

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