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Maternal immune activation impairs hippocampal pyramidal neuron excitability in newborn rat offspring: Implications for neurodevelopmental disorders

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Maternal infection during pregnancy is associated with an increased risk of neurodevelopmental disorders, including depression, schizophrenia, and autism spectrum disorder. The hippocampus plays a critical role in these disorders, but the impact of maternal immune activation (MIA) on early hippocampal neuron function remains poorly understood. We investigated the effects of lipopolysaccharide-induced MIA in pregnant rats (20–80 μg/kg on gestational days 15–19) on the electrophysiological properties of hippocampal pyramidal neurons from newborn offspring. Primary neuronal cultures were prepared from the hippocampi of newborn rats and maintained for 13 days in vitro (DIV13). Whole-cell patch-clamp recordings assessed neuronal excitability parameters between DIV4-13. MIA significantly altered action potential characteristics in offspring hippocampal neurons, including: (1) increased latency time, threshold potential, and repolarization potential; (2) decreased peak potential, ascend and descend velocities; and (3) reduced spontaneous and evoked firing frequencies. These alterations suggest impaired glutamatergic neurotransmission in the hippocampus of MIA offspring, with potential sex-specific effects observed for spontaneous activity. Our findings demonstrate that MIA significantly decreases the excitability of hippocampal pyramidal neurons in newborn offspring. This reduced glutamatergic neurotransmission may contribute to the pathophysiology of neurodevelopmental disorders associated with maternal infection during pregnancy. This study provides novel insights into early neurophysiological changes following prenatal immune challenge that may inform therapeutic interventions targeting hippocampal function.

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Keywords: Electrophysiology, glutamate, hippocampus, lipopolysaccharide, maternal immune activation, neurodevelopmental disorders, neuronal excitability

Introduction

The recent COVID-19 pandemic demonstrated that our civilization is more vulnerable to acute infectious diseases than it was thought before. Since acute contagious diseases are not avoiding pregnant women, the investigation of long-term consequences of maternal infection on the offspring is strongly needed. It has been reported that the children of women suffering from an acute infectious disease during pregnancy were found to have a higher risk of future development of depression (1), schizophrenia (2–6), and autism (7). However, knowledge of the mechanisms mediating the neurodevelopmental effects of maternal infectious illness is limited.

An acute infectious illness increases blood concentrations of inflammatory and anti-inflammatory cytokines and stress hormones, such as corticosteroids. These factors can pass the placenta, as well as the blood–brain barrier, and affect embryonal neurodevelopment. Studies in laboratory animals involving maternal immune activation (MIA) with a bacterial (lipopolysaccharide or LPS) (8) or viral (polyinosinic: polycytidylic acid or Poly I:C) (9) antigens showed that the MIA primarily affected central serotonergic (5-HT) and dopaminergic pathways, as well as the hippocampus.

Our previous study showed that the MIA with LPS altered the rates of the spontaneous firing activity of central 5-HT and dopamine-secreting neurons in offspring in a sex-dependent way. The former was decreased in both sexes, and the latter increased in males only (8). Similar effects on the excitability pattern of dopaminergic neurons were observed after the MIA with immune activation by Poly I:C (9). Offspring of LPS-treated dams had also lower 5-HT and dopamine levels in the medial prefrontal cortex, nucleus accumbens (10), striatum (11), amygdala (12), and hypothalamus (13). The decrease in brain 5-HT and dopamine levels was accompanied by a reduced density of 5-HT neurons in the dorsal raphe nucleus and dopamine neurons in the substantia nigra (13).

With regards to the hippocampus, MIA decreased local 5-HT and dopamine levels (14), densities of serotonin-1A (5-HT_{1A}) and glucocorticoid receptors (10, 15), concentrations of the brain-derived neurotrophic factor (BDNF), and impaired adult neurogenesis in the hippocampus (16). To the authors' best knowledge, the effects of MIA on the excitability of hippocampal neurons in offspring have not yet been investigated. The knowledge of the excitability pattern of hippocampal neurons under normal and maternal stress–induced conditions is, however, important for understanding the mechanisms of hippocampal neuronal plasticity. The present study is, therefore, aiming to test the hypothesis that MIA leads to impaired functioning of hippocampal pyramidal neurons isolated from the hippocampi of newborn offspring. The novelty of the present study is that it is the first to assess the effect of the MIA on the excitability of the individual offspring hippocampal pyramidal neurons very early after birth.

Results

Prenatal LPS Does Not Alter V_{rest}

MIA by LPS did not affect V_{rest} of hippocampal neurons isolated from newborn offspring, as measured at the DIV4-13 of their cultivation. At DIV10, however, a statistically significant interaction between sex and prenatal LPS treatment was observed ($F_{1,63} = 10.40$, $p = 0.002$). Prenatal LPS tended to increase V_{rest} in females and decrease it in males; nevertheless, the Tukey post-hoc test did not reveal any between-group differences. At DIV13, statistically significant sex differences in V_{rest} were observed ($F_{1,29} = 9.06$, $p = 0.005$); it was higher in females compared to the males, regardless of prenatal LPS treatment (not shown).

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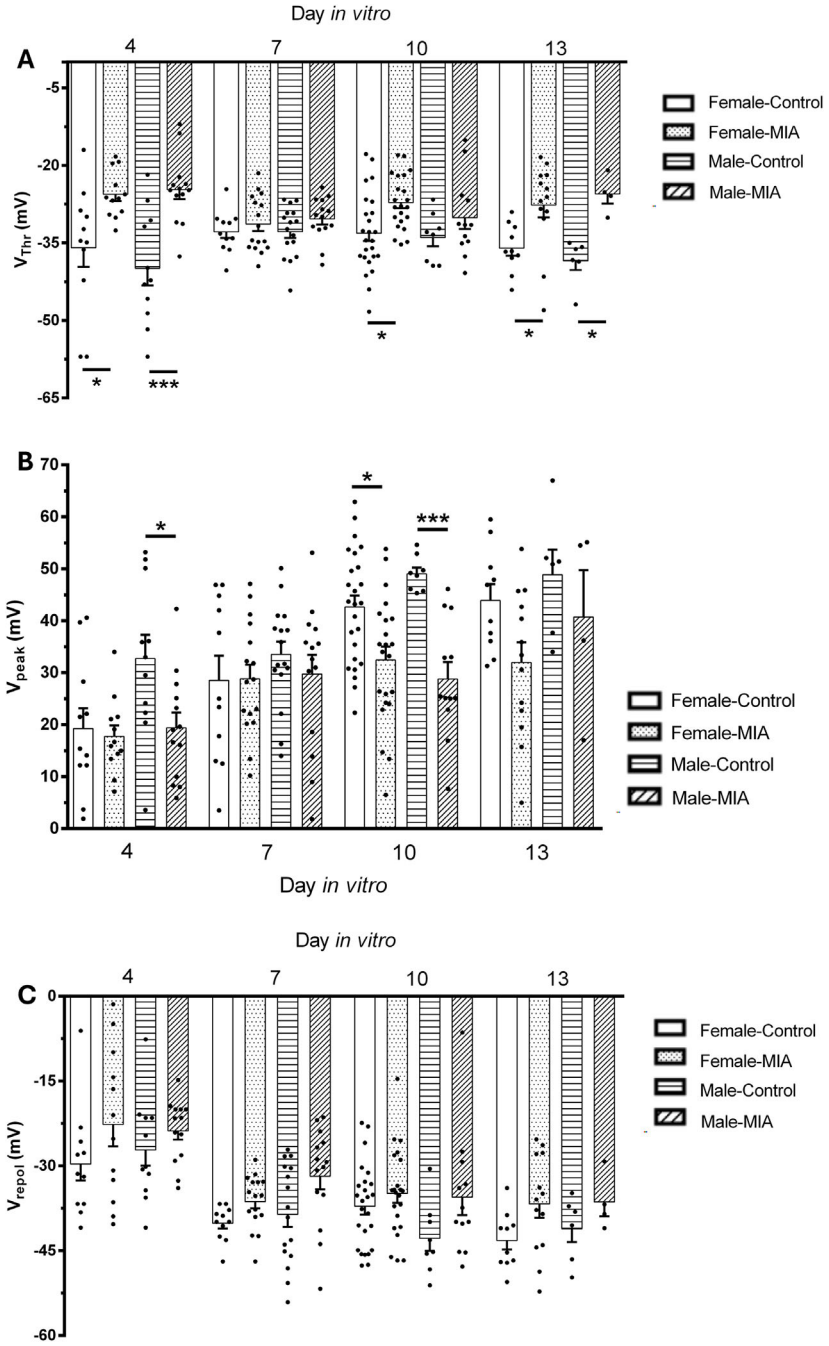

Figure 1. Effect of MIA on the membrane action potential threshold (B), peak (C), and repolarization (D) values potential of the offspring hippocampal neurons. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ in comparison with controls, Tukey post-hoc test.

Prenatal LPS Increases V_{thr} and Vrep and Decreases Vpeak

Prenatal LPS had a statistically significant increasing effect on the V_{thr} as measured at DIV4 ($F_{1,43} = 23.64$, $p < 0.0001$), DIV10 ($F_{1,63} = 10.83$, $p = 0.007$), and DIV13 ($F_{1,29} = 18.41$, $p = 0.0002$; Figure 1A). Prenatal LPS had also a statistically significantly decreased V_{peak}, as measured at DIV4 ($F_{1,43} = 4.73$, $p = 0.04$) and DIV10 ($F_{1,63} = 29.96$, $p < 0.0001$, Figure 1B) and increased V_{rep}, as measured at DIV7 ($F_{1,53} = 7.87$, $p = 0.007$), DIV10 ($F_{1,63} = 4.67$, $p = 0.03$), and DIV13 ($F_{1,29} = 4.44$, $p = 0.04$, Figure 1C). At DIV4, V_{peak} in females was higher compared to the males ($F_{1,43} = 4.94$, $p = 0.03$). At DIV10, the V_{peak} difference between control and prenatally LPS-treated rats was higher in females compared to the males ($F_{1,43} = 4.21$, $p = 0.046$). No sex differences and no sex × treatment interaction for these parameters were observed. The values from the individual neurons/recordings are shown in the figure using the dots.

Prenatal LPS Increases T_{lat} and Decreases $V_{max\text{-}ascend}$ and $V_{max\text{-}descend}$

Prenatal LPS had a statistically significant increasing effect on the T_{lat}, as measured at DIV4 ($F_{1,43} = 234.70$, $p < 0.0001$) and DIV7 ($F_{1,53} = 42.54$, $p < 0.0001$; Figure 2A). Prenatal LPS statistically significantly suppressed the $V_{max\text{-}ascend}$, as measured at DIV4 ($F_{1,43} = 74.59$, $p < 0.0001$), DIV7 ($F_{1,53} = 47.23$, $p < 0.0001$), DIV10 ($F_{1,63} = 4.98$, $p = 0.03$), and DIV13 ($F_{1,29} = 9.93$, $p = 0.004$; Figure 2B), and $V_{max\text{-}descend}$, as measured at DIV4 ($F_{1,43} = 127.00$, $p < 0.0001$), DIV7 ($F_{1,53} = 46.31$, $p < 0.0001$), and DIV13 ($F_{1,29} = 6.99$, $p = 0.01$, Figure 2C). At DIV4, $V_{max\text{-}ascend}$ was higher in

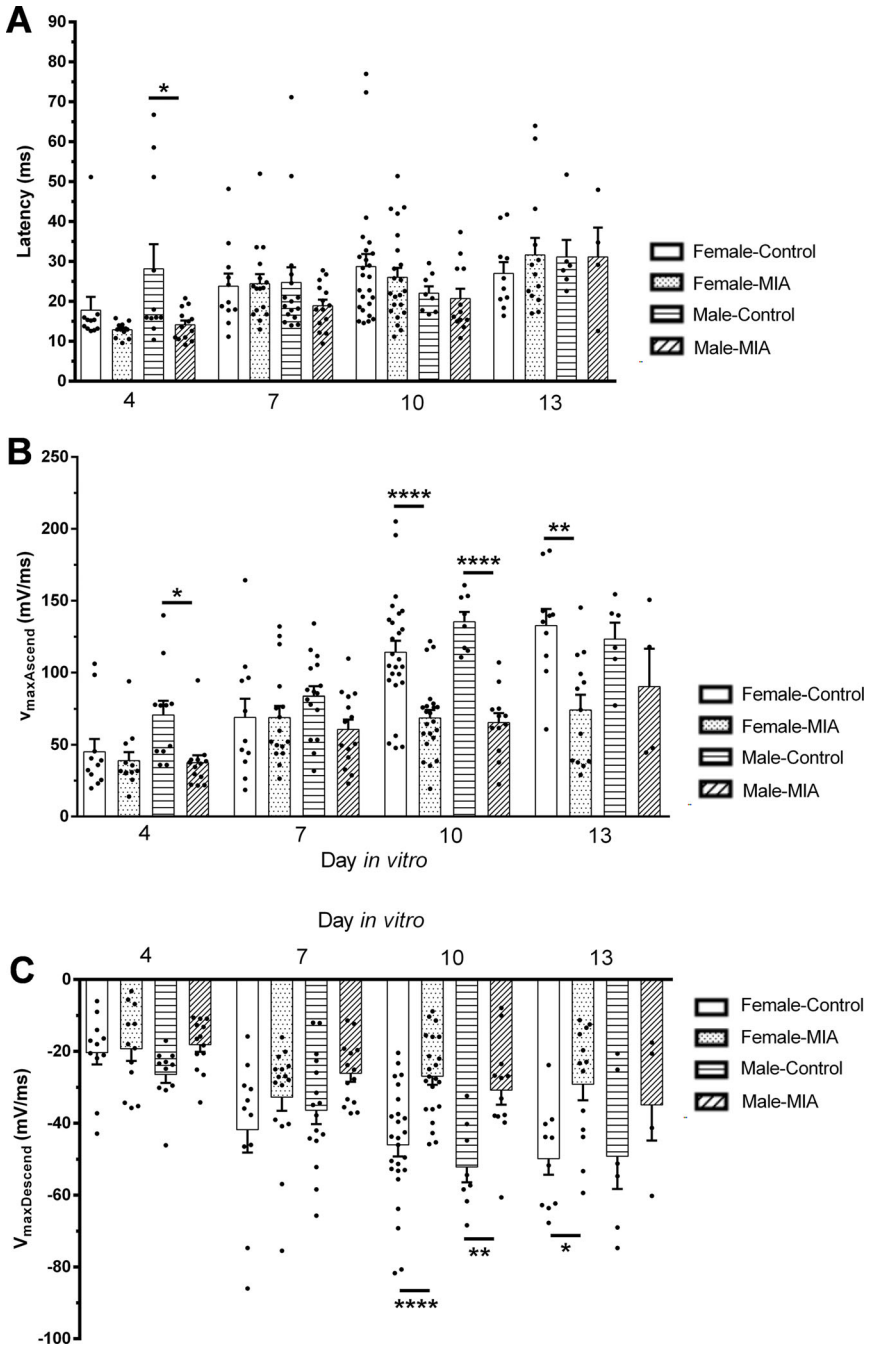

Figure 2. Effect of the MIA on the latency time (A) and ascend (B) and descend (C) velocities of the APs generated by the offspring hippocampal neurons. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ in comparison with controls, Tukey post-hoc test. The numbers of recordings from individual neurons are shown on the bars.

females compared to males ($F_{1,43} = 4.11$, $p = 0.049$). No sex differences in the action potential (AP) threshold potential and latency time and no sex × treatment interaction for these parameters were observed. The values from the individual neurons/recordings are shown in the figure using the dots.

Prenatal LPS Suppressed the Depolarizing Current Pulse–induced and Spontaneous Activity of Hippocampal Neurons

Prenatal LPS significantly suppressed the number of APs within the depolarizing current pulse (DCP)-induced AP series, as measured at DIV10 ($F_{1,63} = 22.06$, $p < 0.0001$) and DIV13 ($F_{1,29} = 15.36$, $p = 0.005$; Figure 3A). No sex differences and no sex × treatment interactions were observed, as measured at DIV10-13. The spontaneous activity was significantly decreased as well, but only at DIV13 and only in male rats (Figure 3B). A significant effect of prenatal LPS treatment ($F_{1,19} = 5.60$, $p = 0.03$) and significant sex × treatment interaction ($F_{1,19} = 6.38$, $p = 0.02$) was detected. The values from the individual neurons/recordings are shown in the figure using the dots.

Discussion

The results of the present study show that maternal MIA induced significant alterations in the waveform of the APs generated by offspring hippocampal neurons. Thus, the APs generated by hippocampal neurons isolated from the offspring of LPS-treated dams are characterized by decreased threshold, peak, and repolarization potentials, decreased AP ascend and descend speeds, and increased latency time, compared to the

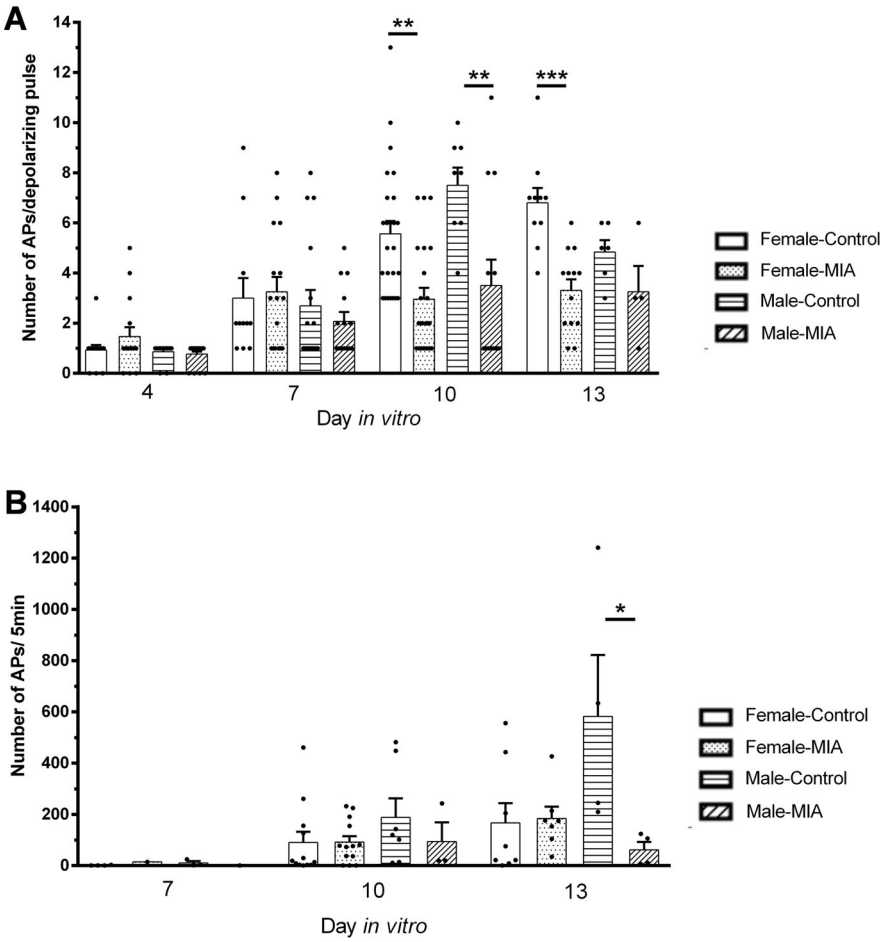

Figure 3. Effect of the MIA on the evoked (A) and spontaneous (B) activity of the offspring hippocampal neurons. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ in comparison with controls, Tukey post-hoc test. The numbers of recordings from individual neurons are shown on the bars.

APs generated by hippocampal neurons isolated from offspring of control dams. MIA also led to decreased DCP-induced and spontaneous activity of hippocampal neurons in offspring. The summary of the effects of prenatal LPS on the excitability of hippocampal neurons is shown in Table 1.

We found that the MIA did not alter the resting membrane potential of neurons isolated from the hippocampi of newborn offspring, as measured during the early (DIV3-7) and late (DIV10-13) stages of *in vitro* maturation. In our previous study (17), however, we reported that maternal stress led to an enhancement of V_{rest} of the neurons isolated from the hippocampi of newborn offspring, as measured at DIV4-10. The difference between the results of the present and previous studies might be explained by the nature of the stress situation and the time of its application. Our previous study examined the effect of pregestational chronic unpredictable stress. The present study studied the effect of stress associated with MIA during the third trimester of gestation.

It was found that MIA led to the increased threshold potential of AP generation in the offspring hippocampal neurons. As a result, the AP latency time was increased, and the number of APs observed during the DCP application decreased. Other intrinsic AP characteristics affected by the MIA decreased ascend and descend speeds and increased repolarization potentials. Therefore, maternal stress likely alters the voltage-dependent ion channels' expression and/or activity in the AP generation. Indeed, stress-induced changes in the expression of certain voltage-dependent ion channels, such as Kv1 (18) and Kv7 (19) voltage-dependent potassium channels, were reported. The exact mechanism mediating the effect of maternal stress on the expression and/or activity of the voltage-dependent ion channels in the offspring neurons is not yet known. However, it is likely to be based on the interactions between maternal and embryonal stress hormones (e.g., corticosteroids), pro- (e.g., interleukins-1 and 12: IL-1/12, tumor necrosis factor-alpha: TNF-α, and interferon-gamma: IFNγ) and anti-inflammatory (e.g., interleukins-4, 6 and 10: IL-4/6/10), cytokines, and neurotrophic factors (e.g., brain-derived neurotrophic factor: BDNF) (20–22).

Table 1. Summary of the statistically significant effects of the MIA on the excitability of glutamate neuron isolates from the offspring hippocampi

	DIV4	DIV7	DIV10	DIV13
Resting potential	0	0	0^{1}	0^{2}
AP threshold potential	↑	0	↑	↑
AP latency time	↑	↑	0	↑
AP maximum ascend speed	$\downarrow^{2}$	↓	↓	↓
AP maximum descent speed	↓	↓	0	↓
AP peak potential	$\downarrow^{2}$	0	$\downarrow^{1}$	0
AP repolarization potential	0	↑	↑	↑
Evoked activity	0	0	↓	↓
Spontaneous activity	n/e	0	0	$\downarrow^{1}$

AP, action potential; DIV, day-in-vitro; 0, np effect; ↑, increasing effect; ↓, decreasing effect; n/e, not evaluated; [1], significant interaction between sex and prenatal LPS treatment; [2], significant sex difference.

Another interesting finding of the present study is that prenatal LPS decreased the average peak value of the APs generated by hippocampal neurons isolated from the newborn offspring. This finding is similar to that of a previous study (23). However, Grigoryan and Segal found that prenatal stress tended to decrease the V_{peak} potential of APs generated by offspring hippocampal neurons, but the effect was not statistically significant. In the present study, the effect of the MIA was significant. These differences might be explained by the nature of the stressors being applied: forced swimming and cage declination in Grigoryan and Segal's *versus* MIA in this study.

We found that MIA decreased the depolarization pulse–induced and spontaneous generation of the APs in the pyramidal neurons isolated from newborn male hippocampi. The firing activity of the neurons is the most fundamental characteristic determining the neurotransmitter release from the nerve terminals (24). It is, therefore, likely that the MIA led to decreased glutamate neurotransmission in the offspring hippocampi and perhaps in other brain areas as well.

As explained in our previous studies, the depolarization pulse–induced firing activity of the cultivated hippocampal neurons results from the direct activation of the voltage-dependent sodium channels. It is, therefore, primarily dependent on the intrinsic properties of the neurons. The spontaneous activity, however, depends on external excitatory synaptic inputs. It is consequently determined by the whole neuronal network (17, 25). MIA-induced decrease in the evoked activity of the hippocampal neurons isolated from the neonatal offspring brain might thus result from the increased AP threshold potential, which may, in turn, be caused by the abnormal expression of some voltage-dependent ion, for example, potassium channels (18, 19).

As in our previous studies (17, 25), spontaneous activity of the cultivated hippocampal neurons could be detected during the late stages of the cultivation (DIV10-13), when interneuronal synaptic connections are developed. Thus, the MIA-induced changes in the spontaneous activity of hippocampal neurons may be due to abnormal neuronal network development. It has been reported that stress may alter the hippocampal network development, for example, it may reduce the number of synaptic connections between the neurons *via* a mechanism involving various neurotrophic factors, such as BDNF (26, 27). Even though the actual networks were formed in *in vitro* conditions, which were similar for both groups, the previous exposure of the neurons or their precursors to the stress hormone and/or inflammatory cytokines putatively affected their future ability to create the networks.

Excitatory glutamatergic and inhibitory GABAergic synaptic inputs mediate the spontaneous activity of cultivated hippocampal neurons (28). Prenatal stress likely decreased the strength of the excitatory and/or increased the strength of the inhibitory synaptic inputs of the offspring hippocampal neurons. It has indeed been reported that prenatal stress increased the frequency of the miniature inhibitory postsynaptic currents recorded in the offspring hippocampal neurons (23).

While our present study reported that MIA suppressed the spontaneous activity of the offspring hippocampal neurons, a stimulatory effect of maternal stress exposure on the cultivated neurons isolated from the offspring hippocampi was observed in our previous experiments (17). As stated above, the difference between the present and previous studies' results might be explained by the stressor's nature and the time of its application.

Hippocampal glutamate circuits are fundamental in memory consolidation, retrieval, and cognitive performance (29). They also have a critical role in anxiety and depressive-like behavior (30). Decreased excitability of the hippocampal glutamate neurons, observed in this study, may explain, at least in part, decreased cognitive performance (12) and increased anxiety (31) and depressive-like behavior (16) in the offspring of the MIA dams reported in previous studies. It is known that children of women suffering from an infectious disease during pregnancy have a higher risk of future development of depression (1), schizophrenia (2–6), and autism (7). The results of the present study suggest that the early postnatal suppression of the excitability of hippocampal neurons might underline these epidemiological observations. Early-life therapeutic interventions, stimulating neurotransmission within the hippocampal area, applied using noninvasive, low-risk techniques such as transcranial magnetic stimulation (32), may, therefore, reduce the risk of future-life psychopathologies in the children of women who experienced infectious disease during the pregnancy. However, the risks and benefits of these interventions must be very carefully assessed. Animal models can be used in these assessments; however, the limitation of these models, which results from the difference between rodents and human brains, must be considered. Speaking specifically about the LPS-based model of the MIA, its major limitation is the fact that it primarily models bacterial infection. At the same time, from the epidemiological point of view, higher risk might be expected from viral infections.

In conclusion, our study's results, together with the results of previous studies from our (17) and other (23) groups, indicate that maternal stress induces significant alterations in the excitability pattern of the offspring hippocampal neurons. The nature of the maternal stress–induced alterations depends on the type of stressors applied (e.g., physical stressors versus immune activation) and the time of the application (e.g., pregestational versus prenatal). Interactions between maternal and embryonal stress hormones, cytokines, neurotrophic factors, embryonal ligand-(glutamate and GABA), and voltage-dependent ion channels might underline the nature of the maternal stress–induced alterations of the excitability of the offspring hippocampal neuronal circuits.

Materials and Methods

Animals

Adult female (200–250 g) and male (250–300 g) Wistar rats were ordered from the Animal Breeding facility of the Institute of Experimental Pharmacology and Toxicology, Centre for Experimental Medicine, Slovak Academy of Sciences (Dobra Voda, Slovakia). Animals were housed under standard laboratory conditions (temperature: 22 $\pm$ 2°C, humidity: 55% $\pm$ 10%) with a 12-h light/12-h dark cycle (lights on at 7 a.m.). Pelleted food and tap water were available ad libitum. All experimental procedures were approved by the Animal Health and Animal Welfare Division of the State Veterinary and Food Administration of the Slovak Republic (Permit number Ro 3592/15-221) and complied with the Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes.

MIA

After an acclimatization period of 1 week, the animals were placed in cages with one male and three females in each. Vaginal smears were taken daily between 7 and 8 a.m. and examined under light microscope. The day sperm cells were detected was considered as day 0 of gestation. During days 15–19 of the gestation, LPS-treated dams were once per day subcutaneously injected with LPS at increasing doses of 20, 20, 40, 40, and 80 μg/kg, as described previously (8, 12). The third trimester of gestation was chosen for the LPS administration because it is a critical developmental period for specific central nervous system (CNS) structures (33). Control dams were injected with saline. On day 20 of gestation, dams were placed in individual cages. The birth usually occurs on day 21 of gestation.

Primary Culture of Hippocampal Neurons

As previously described, hippocampal neurons were isolated from newborn Wistar rats of both sexes at the first postnatal day (25, 34). Hippocampi were removed and transferred to ice-cold isolation solution containing (in mM): 137 NaCl; 5.4 KCl; 1.1 $Na_2HPO_4 \times 2H_2O$; 1.1 KH_2PO_4; 6.1 Glucose and 1 Kynurenic acid; pH 7.3 with NaOH. Tissue was chopped and incubated in a predigestion solution (Leibovitz L-15 Medium; 25 U/mL Papain; 2 mM Kynurenic acid) for 25 to 30 min at 37°C in an atmosphere containing 5% CO_2. Digested tissue was washed with cold STOP solution (isolation solution mixed with a heat-inactivated fetal bovine serum in a ratio of 3:1). Finally, hippocampi were transferred into incubation media containing high glucose (4.5 g/L) Dulbecco's Modified Eagle's (DMEM) Medium supplemented with 10% heat-inactivated fetal bovine serum, antibiotics (75,000 IU/L penicillin and 75 mg/L streptomycin), 2 mM $MgCl_2$, 10 nM progesterone, 100 μM putrescine, and 12.5 mg/mL ITSS (25 mg insulin, 25 mg transferrin, and 25 μg Na-selenite). In incubation media, hippocampi were consecutively triturated with three glass Pasteur

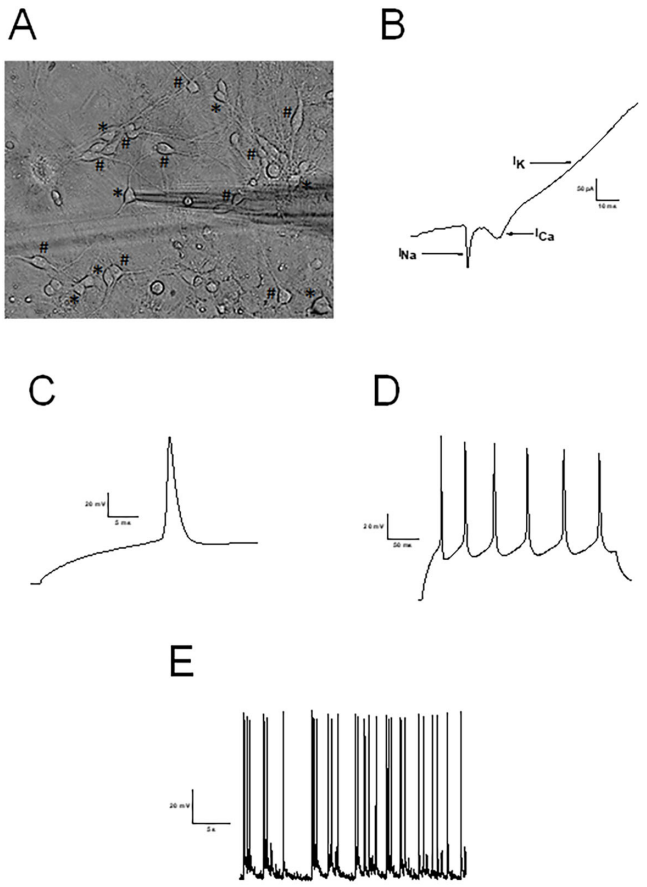

Figure 4. Identification of glutamate pyramidal neurons in primary cultures prepared from neonatal rat hippocampi and their evoked and spontaneous activity. (A) Characteristic morphology of pyramidal (*) and nonpyramidal (#) neurons. (B) Sodium (Na^+), calcium (Ca^{2+}), and potassium (K^+) currents, characteristic for hippocampal pyramidal neurons, recorded immediately after the opening of the neurons, under the whole-cell voltage-clamp using voltage ramp from −80 to +80 mV with a speed of 1.6 mV/1 ms and holding potential of −70 mV. (C) Single AP generated by a DCP of 30 pA in DIV7 cultivated neurons. (D) AP series generated by a DCP of −10 pA in DIV10 cultivated neuron. (E) Spontaneous AP generated by a neuron in DIV13 culture.

pipettes with decreasing diameters. From the final single-cell suspension, hippocampal neurons were seeded at a density of 5×10^4 cells/cm^2 on 35 mm plastic Petri dishes containing glass coverslips (Sarstedt, Slovakia) coated with poly-D-lysine (50 μg/1 mL/1 cm^2) in incubation media containing penicillin/streptomycin. After 24 h, the medium was exchanged for an antibiotics-free medium. At days 4 to 5 *in vitro* (DIV4-5) Cytosine β-D-arabinofuranoside (1 μM) was added to reduce the proliferation of nonneuronal cells. Neurons were incubated in a humidified incubator in an atmosphere containing 5% CO_2 at 37°C up to DIV14. Unless mentioned otherwise, all chemicals were purchased from Sigma Aldrich, Slovakia.

Electrophysiological Recordings

Recordings were done in the whole-cell patch-clamp configuration at room temperature using a HEKA EPC10 amplifier (HEKA Electronics, Lambrecht, Germany). Patch pipettes with resistance ranging from 4 to 5 MΩ were fabricated from borosilicate glass capillaries (Sutter Instruments, Novato, CA). Pyramidal neurons were identified by their characteristic physical appearance (triangular soma; Figure 4A) and the presence of potassium ($I_{K)}$, sodium (I_{Na}), and calcium (I_{Ca}) currents, measured immediately after the opening of the cell (Figure 4B). As previously described, AP firing was measured under the current clamp conditions (25, 34). The intracellular solution contained (in mM): 120 K-gluconate; 20 KCl; 2 $MgCl_2$; 2 Na_2ATP; 0.25 Na_2GTP; 10 HEPES; pH 7.3 (with KOH). The osmolarity of an intracellular solution was approximately 290–300 mOsmol/L in all cases (measured by Osmomat 030-Gonotec, Germany). The osmolarity of an extracellular solution was set to a value of 2–3 mOsm/L lower than the osmolarity of the corresponding intracellular solution. DCP can activate a single AP (Figure 4C) or AP series (Figure 4D) in cultured hippocampal neurons after DIV4-5. We have used this protocol on the days DIV8-9, when the expression of voltage-dependent I_{Na}, I_K, and I_{Ca} is developed. In these experiments, resting membrane potential was maintained at −70 mV. Evoked AP series were activated by a series of six 300-ms-long DCPs with amplitudes increasing with a step of +50 pA. Longer maturation was necessary for the development of spontaneous activity. After DIV10-12, hippocampal neurons in a primary culture become spontaneously active (Figure 4E). The experiments were done on DIV13-14 when about 80% of all tested cells were spontaneously active. Spontaneous activity was recorded for 5 min at an intrinsic membrane potential of each cell. The mean membrane potential corrected for a liquid junction potential was -65.2 ± 0.4 mV.

Data and Statistical Analysis

The following characteristics of the AP waveform and neuronal excitability were analyzed: membrane resting potential (V_{rest}), AP latency time (t_{lat}) and threshold (V_{thr}), peak (V_{peak}), and repolarization (V_{rep}) potentials, and maximal ascend ($V_{max\text{-}ascend}$) and descent ($V_{max\text{-}descend}$) velocities. V_{thr} was detected as an electrical potential value at the time point where its second derivation by time reaches the local maximum. The t_{lat} was measured as the time between the start of the DCP and the detection of V_{thr}, as described previously. $V_{max\text{-}ascend}$ and descent $V_{max\text{-}descend}$ were detected as the local minimum and maximum of the derivative of membrane electrical potential by time, respectively, as described previously (35). The effects of sex, prenatal LPS treatment, and sex × treatment interaction differences for each parameter were assessed using the two-way analysis of variance (ANOVA), followed by the Tukey post-hoc test. Before the analysis by ANOVA, data normality and homoscedasticity were verified using Shapiro–Wilk's and Levene's tests, respectively. The probability of $p < 0.05$ was considered as significant.

Data Availability Statement

The original research data are available upon request.

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Authors Contribution

E.D., L.M., D.J., and L.L. planned the study and formulated the working hypothesis. L.M., R.M., and K.C. conducted experiments. L.M., R.M., and E.D. analyzed the results. L.M., L.L., and E.D. wrote the manuscript. All authors critically proofread the manuscript and approved it for publication.

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Authors Disclosures

The authors declare no financial interest in publishing this study.

References

1. Markham JA, Koenig JI. Prenatal stress: role in psychotic and depressive diseases. Psychopharmacology (Berl). 2011;214(1):89–106. DOI: 10.1007/s00213-010-2035-0. PMID: 20949351; PMCID: PMC3050113
2. Suvisaari J, Mantere O. Inflammation theories in psychotic disorders: a critical review. Infect Disord Drug Targets. 2013;13(1):59–70. DOI: 10.2174/18715265112129990032. PMID: 23713669
3. Brown AS. Exposure to prenatal infection and risk of schizophrenia. Front Psychiatry. 2011;2:63. DOI: 10.3389/fpsyt.2011.00063. PMID: 22131978; PMCID: PMC3222883

4. Canetta SE, Brown AS. Prenatal infection, maternal immune activation, and risk for schizophrenia. Transl Neurosci. 2012;3(4):320–7. DOI: 10.2478/s13380-012-0045-6. PMID: 23956839; PMCID: PMC3744366
5. Khandaker GM, Zimbron J, Lewis G, Jones PB. Prenatal maternal infection, neurodevelopment and adult schizophrenia: a systematic review of population-based studies. Psychol Med. 2013;43(2):239–57. DOI: 10.1017/S0033291712000736. PMID: 22717193; PMCID: PMC3479084
6. Kneeland RE, Fatemi SH. Viral infection, inflammation and schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2013;42:35–48. DOI: 10.1016/j.pnpbp.2012.02.001. PMID: 22349576; PMCID: PMC3408569
7. Patterson PH. Maternal infection and autism. Brain Behav Immun. 2012;26(3):393. DOI: 10.1016/j.bbi.2011.09.008. PMID: 22001185
8. Csatlosova K, Bogi E, Durisova B, Grinchii D, Paliokha R, Moravcikova L, et al. Maternal immune activation in rats attenuates the excitability of monoamine-secreting neurons in adult offspring in a sex-specific way. Eur Neuropsychopharmacol. 2021;43:82–91. DOI: 10.1016/j.euroneuro.2020.12.002. PMID: 33341344
9. De Felice M, Melis M, Aroni S, Muntoni AL, Fanni S, Frau R, et al. The PPARα agonist fenofibrate attenuates disruption of dopamine function in a maternal immune activation rat model of schizophrenia. CNS Neurosci Ther. 2019;25(5):549–61. DOI: 10.1111/cns.13087. PMID: 30461214; PMCID: PMC6488881
10. Lin YL, Lin SY, Wang S. Prenatal lipopolysaccharide exposure increases anxiety-like behaviors and enhances stress-induced corticosterone responses in adult rats. Brain Behav Immun. 2012;26(3):459–68. DOI: 10.1016/j.bbi.2011.12.003. PMID: 22198119
11. Kirsten TB, Chaves-Kirsten GP, Chaible LM, Silva AC, Martins DO, Britto LR, et al. Hypoactivity of the central dopaminergic system and autistic-like behavior induced by a single early prenatal exposure to lipopolysaccharide. J Neurosci Res. 2012;90(10):1903–12. DOI: 10.1002/jnr.23089. PMID: 22714803
12. Bakos J, Duncko R, Makatsori A, Pirnik Z, Kiss A, Jezova D. Prenatal immune challenge affects growth, behavior, and brain dopamine in offspring. Ann N Y Acad Sci. 2004;1018:281–7. DOI: 10.1196/annals.1296.033. PMID: 15240379
13. Wang S, Yan JY, Lo YK, Carvey PM, Ling Z. Dopaminergic and serotoninergic deficiencies in young adult rats prenatally exposed to the bacterial lipopolysaccharide. Brain Res. 2009;1265:196–204. DOI: 10.1016/j.brainres.2009.02.022. PMID: 19236855
14. Depino AM. Early prenatal exposure to LPS results in anxiety- and depression-related behaviors in adulthood. Neuroscience. 2015;299:56–65. DOI: 10.1016/j.neuroscience.2015.04.065. PMID: 25943476
15. Zager A, Andersen ML, Tufik S, Palermo-Neto J. Maternal immune activation increases the corticosterone response to acute stress without affecting the hypothalamic monoamine content and sleep patterns in male mice offspring. Neuroimmunomodulation. 2014;21(1):37–44. DOI: 10.1159/000355466. PMID: 24216750
16. Lin YL, Wang S. Prenatal lipopolysaccharide exposure increases depression-like behaviors and reduces hippocampal neurogenesis in adult rats. Behav Brain Res. 2014;259:24–34. DOI: 10.1016/j.bbr.2013.10.034. PMID: 24177209
17. Bogi E, Belovicova K, Moravcikova L, Csatlosova K, Dremencov E, Lacinova L, et al. Pre-gestational stress impacts excitability of hippocampal cells in vitro and is associated with neurobehavioral alterations during adulthood. Behav Brain Res. 2019;375:112131. DOI: 10.1016/j.bbr.2019.112131. PMID: 31377253
18. Miyata S, Taniguchi M, Koyama Y, Shimizu S, Tanaka T, Yasuno F, et al. Association between chronic stress-induced structural abnormalities in Ranvier nodes and reduced oligodendrocyte activity in major depression. Sci Rep. 2016;6:23084. DOI: 10.1038/srep23084. PMID: 26976207; PMCID: PMC4791682
19. Friedman AK, Juarez B, Ku SM, Zhang H, Calizo RC, Walsh JJ, et al. KCNQ channel openers reverse depressive symptoms via an active resilience mechanism. Nat Commun. 2016;7:11671. DOI: 10.1038/ncomms11671. PMID: 27216573; PMCID: PMC4890180
20. Idunkova A, Lacinová Ľ, Dubiel-Hoppanova L. Impact of depression on first and second generation: from biochemistry to electrophysiology. Gen Physiol Biophys. 2023;42:107–122. DOI: 10.4149/gpb_2023001. PMID: 36896941
21. Singh S, Fereshetyan K, Shorter S, Paliokha R, Dremencov E, Yenkoyan K, et al. Brain-derived neurotrophic factor (BDNF) in perinatal depression: side show or pivotal factor? Drug Discov Today. 2022;28(2):103467. DOI: 10.1016/j.drudis.2022.103467. PMID: 36528281
22. Pavlovicova M, Lacinova L, Dremencov E. Cellular and molecular mechanisms underlying the treatment of depression: focusing on hippocampal G-protein-coupled receptors and voltage-dependent calcium channels. Gen Physiol Biophys. 2015;34(4):353–66. DOI: 10.4149/gpb_2015013. PMID: 25926550
23. Grigoryan G, Segal M. Prenatal stress affects network properties of rat hippocampal neurons. Biol Psychiatry. 2013;73(11):1095–102. DOI: 10.1016/j.biopsych.2013.02.003. PMID: 23541001
24. Wozny C, Maier N, Fidzinski P, Breustedt J, Behr J, Schmitz D. Differential cAMP signaling at hippocampal output synapses. J Neurosci. 2008;28:14358–62. DOI: 10.1523/JNEUROSCI.4973-08.2008. PMID: 19118168; PMCID: PMC6671250
25. Moravcikova L, Moravcik R, Jezova D, Lacinova L, Dremencov E. Delta-opioid receptor-mediated modulation of excitability of individual hippocampal neurons: mechanisms involved. Pharmacol Rep. 2021;73(1):85–101. DOI: 10.1007/s43440-020-00183-2. PMID: 33161533
26. Duman RS, Sanacora G, Krystal JH. Altered connectivity in depression: GABA and glutamate neurotransmitter deficits and reversal by novel treatments. Neuron. 2019;102(1):75–90. DOI: 10.1016/j.neuron.2019.03.013. PMID: 30946828; PMCID: PMC6450409
27. Schmidt HD, Duman RS. The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. Behav Pharmacol. 2007;18(5-6):391–418. DOI: 10.1097/FBP.0b013e3282ee2aa8. PMID: 17762509
28. Blankenship AG, Feller MB. Mechanisms underlying spontaneous patterned activity in developing neural circuits. Nat Rev Neurosci. 2010;11(1):18–29. DOI: 10.1038/nrn2759. PMID: 19953103; PMCID: PMC2902252
29. Osborne DM, Pearson-Leary J, McNay EC. The neuroenergetics of stress hormones in the hippocampus and implications for memory. Front Neurosci. 2015;9:164. DOI: 10.3389/fnins.2015.00164. PMID: 25999811; PMCID: PMC4422005
30. Femenía T, Gómez-Galán M, Lindskog M, Magara S. Dysfunctional hippocampal activity affects emotion and cognition in mood disorders. Brain Res. 2012;1476:58–70. DOI: 10.1016/j.brainres.2012.03.053. PMID: 22541166
31. Enayati M, Solati J, Hosseini MH, Shahi HR, Saki G, Salari AA. Maternal infection during late pregnancy increases anxiety- and depression-like behaviors with increasing age in male offspring. Brain Res Bull. 2012;87(2-3):295–302. DOI: 10.1016/j.brainresbull.2011.08.015. PMID: 21893170
32. Mori F, Nisticò R, Nicoletti CG, Zagaglia S, Mandolesi G, Piccinin S, et al. RANTES correlates with inflammatory activity and synaptic excitability in multiple sclerosis. Mult Scler. 2016;22(11):1405–12. DOI: 10.1177/1352458515621796. PMID: 26733422
33. Stolakis V, Liapi C, Zarros A, Kalopita K, Memtsas V, Botis J, et al. Exposure to ethanol during neurodevelopment modifies crucial offspring rat brain enzyme activities in a region-specific manner. Metab Brain Dis. 2015;30(6):1467–77. DOI: 10.1007/s11011-015-9730-9. PMID: 26380981
34. Ondacova K, Moravcikova L, Jurkovicova D, Lacinova L. Fibrotic scar model and TGF-beta1 differently modulate action potential firing and voltage-dependent ion currents in hippocampal neurons in primary culture. Eur J Neurosci. 2017;46(6):2161–76. DOI: 10.1111/ejn.13663. PMID: 28833693
35. Lacinova L, Moosmang S, Langwieser N, Hofmann F, Kleppisch T. Cav1.2 calcium channels modulate the spiking pattern of hippocampal pyramidal cells. Life Sci. 2008;82(1-2):41–9. DOI: 10.1016/j.lfs.2007.10.009. PMID: 18045623