Glial cells and neuronal function in Alzheimer's disease

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Chapter 2

Modulating astrocyte function in acute hippocampal slices from the mouse, a technical note

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Modulating astrocyte function in acute hippocampal slices

Abstract

Astrocytes are involved in a wide range of homeostatic and supportive brain functions. They become reactive subsequent to activation of microglia upon brain damage and in many neurological diseases. Reactive astrocytes are characterized by an increased expression of GFAP and genes involved in immune activation. Astroglial dysfunction, as a result from their reactive state, could directly impact synaptic transmission. The functional consequences of the changes in reactive astrocytes on surrounding neurons are incompletely understood but they could ultimately affect cognition.

In this study we used acute hippocampal slices from the mouse and applied the bacterial protein lipopolysaccharide (LPS, 1 µg/ml), a strong inflammatory stimulus, to investigate how it interfered with astrocyte function. In this technical note we demonstrate that slicing by itself already induced a large inflammatory response as measured by gene expression of TNF-α and IL-1β; application of LPS did not have an additional effect on reactive gliosis.

In a second series of experiments we employed fluoroacetate (5 mM) a drug that inhibits astrocyte metabolism. We investigated its effects on neuronal excitability as determined by depolarization induced firing and on synaptic plasticity quantified as spike-timing dependent plasticity (STDP). In STDP causally paired activity in the pre- and post-synapse enhances synaptic efficacy. Fluoroacetate did mildly enhance neuronal excitability and reduced or even prevented STDP, indicating that it might be useful as a modulator of glia-neuronal communication in future studies.
1. Introduction

Astrocytes are involved in a wide range of homeostatic and supportive brain functions (Allaman, Bélanger, & Magistretti, 2011; Nedergaard & Verkhratsky, 2012). As part of the tripartite synapse, they are actively involved in the regulation of synaptic transmission (Araque, Parpura, Sanzgiri, & Haydon, 1999). Astrocytes receive input from neurons (Perea, 2005) and can influence synaptic transmission by the release of gliotransmitters (Araque, Parpura, Sanzgiri, & Haydon, 1998; Henneberger, Papouin, Oliet, & Rusakov, 2010), regulation of the extracellular potassium concentration (Kofuji & Newman, 2004), and up-take of neurotransmitters (Danbolt, 2001). When brain homeostasis is compromised, astrocytes undergo morphological and functional changes. Under pathological conditions astrocytes adopt a reactive state that is characterized by an increased expression of glial fibrillary acidic protein (GFAP) (Hol & Pekny, 2015; Pekny et al., 2016). Reactive astrocytes are found in numerous central nervous system (CNS) diseases, including Alzheimer’s disease (AD), stroke, and Huntington’s disease (Osborn, Kamphuis, Wadman, & Hol, 2016; van Dijk, Vergouwen, Kelfkens, Rinkel, & Hol, 2016; Khakh et al., 2017). Astrocyte dysfunction, resulting from their reactive state, could directly impact synaptic functions (Ortinski et al., 2010; Wu, Guo, Gearing, & Chen, 2012; Jo et al., 2014) and potentially cognition. The functional consequences of reactive astrocytes on synaptic transmission are as yet incompletely understood.

In this study we investigated the suitability of two modulators of astroglial function for further studies in acute hippocampal slices: the inflammatory stimulus lipopolysaccharide (LPS) and the specific astroglial metabolic inhibitor fluoroacetate (FA).

LPS is a component of gram-negative bacterial cell walls and induces a robust inflammatory response in glial cells. LPS activates the toll-like receptor 4 (TLR4), which is predominantly expressed in microglia. TLR4 stimulation induces the activation of microglia, characterized by an increased release of pro-inflammatory factors including interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α), and interleukin-6 (IL-6) (Subhramanyam, Wang, Hu, & Dheen, 2019; Zhang et al., 2019). The release of IL-1β and TNF-α by microglia activates astrocytes (Holm, Draeby, & Owens, 2012; Chen et al., 2015; Jha, Jo, Kim, & Suk, 2019). LPS exposure also increases the expression of IL-1β, TNF-α, and IL-6 by astrocytes (Pang, Cai, & Rhodes, 2001; Tarassishin, Suh, & Lee, 2014).

Fluoroacetate was already introduced in 1987 as an astrocyte specific metabolism inhibitor (Fonnum et al., 1997; Paulsen et al., 1987), and more recent studies indicate the inhibitory effect of FA on glia-neuronal transmission (Henneberger, Papouin, Oliet, & Rusakov, 2010; Pabst et al., 2016; Kaczor & Mozrzymas, 2017). Which transporter is responsible for the astrocyte specific uptake of FA remains unclear, although a Na+-dependent aspartate transporter has been suggested (Gonda & Quastel, 1966; Szerb & Issekutz, 1987). Astrocytes likely convert FA into fluorocitrate, inhibiting the tricarboxylic acid cycle (Szerb & Issekutz,
1987). Studies suggest that the metabolic effect of FA on glia is mediated by an impairment of the carbon flux through the tricarboxylic acid cycle, and not by impairment of oxidative ATP production (Swanson & Graham, 1994). The astrocyte specificity and the effects of FA on astrocyte function are still not completely understood and controversial findings suggest different ways of action. Szerb and Issekutz, 1987 found that FA treatment in acute hippocampal slices reduced the glutamine synthesis in astrocytes and reduced glutamate uptake (Szerb & Issekutz, 1987). While the effect on glutamine production was also found in astroglial cultures, the reduced glutamate uptake was not (Swanson & Graham, 1994).

Here we investigate whether the application of LPS could be used as a tool to induce reactive gliosis in acute hippocampal slices; we determined the expression levels of known markers for reactive astrocytes (GFAP and vimentin) and the expression of the genes coding for the cytokines IL-1β and TNF-α. Such a tool would be extremely useful to study how reactive astrocytes and activated microglia affect synaptic transmission.

In a second series of experiments we investigate the functional consequences of the glial metabolic inhibitor FA on neuronal excitability and spike-timing dependent plasticity (STDP) also in acute hippocampal slices. The latter pharmacological tool would be helpful in unraveling the mechanisms involved in glia-neuronal communication.

2. Methods

2.1 Mice

C57BL/6 mice (Harlan, the Netherlands) were housed under standard conditions and were provided food and water ad libitum. All experiments were performed in accordance with protocols and guidelines that were approved by the Institutional Animal Care and Use Committee of the University of Amsterdam operating under standards set by EU Directive 2010/63/EU.

2.2 Hippocampal slice preparation and experimental protocols

Horizontal slices (300 μm) were obtained from the hippocampus of mice at postnatal day 30-43 or from 4-month-old mice. The mouse was decapitated and its brain rapidly removed and kept in ice-cold modified artificial cerebrospinal fluid (mACSF) containing: 60 mM sucrose, 87 mM NaCl, 2.5 mM KCl, 1.25 mM NaH2PO4, 7 mM MgCl2, 0.5 mM CaCl2, 10 mM D-glucose, 25 mM NaHCO3. mACSF was oxygenated with 95% O2 - 5% CO2 and reached a pH of 7.4. Slices were cut with a vibratome (VT1000S, Leica, Germany) and transferred to mACSF of 35 °C for 20 min, to recover. Finally, slices were transferred to oxygenated ACSF containing: 126 mM NaCl, 3 mM KCl, 2 mM CaCl2, 2 mM MgSO4, 1.25 mM NaH2PO4, 10 mM D-glucose, 26 mM NaHCO3, and kept at room temperature until used.
**LPS stimulation**

After at least one hour of recovery at room temperature, half the slices of a young mouse were incubated in ACSF with 1 µg/ml lipopolysaccharide (LPS; L4391-1MG, E.coli 0111:B4, Sigma-Aldrich) to activate the microglia and induce reactive astrocytes. The other half remained in ACSF and was used as animal-matched controls. From both groups, slices were taken at fixed time points after the start of the LPS application and snap frozen on dry ice. Samples were stored at -80 °C until used for RNA isolation.

**Incubation with fluoroacetate**

For electrophysiological experiments, slices of 4-month-old animals were prepared and treated the same way as the younger ones described above. After recovery the slices were incubated in ACSF with 5 mM fluoroacetate (FA; Aldlab Chemicals, LLC) for 1 hour before recording started. During recording, they were continuously perfused with ACSF (2.5 ml/min) and kept at 30 °C.

### 2.3 RNA isolation, cDNA synthesis, and quantitative real-time PCR

For RNA isolation, tissue sections were thawed and total RNA was isolated with TRizol (Life Technologies) according to the manufacturer’s protocol, and the RNA was subsequently precipitated in 2-propanol and 20 µg/µl glycogen (Roche) overnight at -20 °C. Samples were centrifuged (12,000 x g for 30 minutes) at 4 °C, washed twice with cold 75% ethanol, and the RNA pellet was dissolved in MilliQ. The RNA concentration was determined using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Total RNA (500 ng) was treated with DNasel (gDNA wipe out buffer, Qiagen) at 42 °C for 2 min and cDNA was synthesized using Quantic Reverse Transcription kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Quantitative PCR (qPCR) was performed using the 7500 Real-Time PCR System (Applied Biosystems, USA). For the qPCR reaction 1 µl of 1:20 diluted cDNA in MilliQ was used with 1 µl primer mix (forward and reverse primers, 2 pmol/µl, Table 1), 5 µl FastStart Universal SYBR Green Master mix (Roche, Basel, Switzerland) and 3 µl MilliQ. The reaction mix was added to a 96 well plate. The following cycling conditions were used: 2 min 50 °C, 10 min 95 °C, and 40 cycles of 15 sec 95 °C and 1 min 60 °C. A dissociation curve was obtained by ramping the temperature from 60 °C to 90 °C. Applied Biosystems 7500 Real-Time PCR software (Applied Biosystems) was used for the analysis of amplification curves. Threshold was set at 0.2.

Gene expression was normalized to three reference genes: β-actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and hypoxanthine phosphoribosyltransferase (HPRT). The mRNA expression levels of GFAP, vimentin, IL-1β, and TNF-α were normalized to timepoint zero.
Modulating astrocyte function in acute hippocampal slices

### Table 1. Primer sequences

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward sequence</th>
<th>Reverse sequence</th>
</tr>
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<tbody>
<tr>
<td>GFAP</td>
<td>GGAGATGCGGGATGGTGAG</td>
<td>ACCACGTCTTGTGCTCCTG</td>
</tr>
<tr>
<td>IL-1β</td>
<td>CAAACAACAGTATATTTCTC</td>
<td>GATCCACACTCTCCAGCTGCA</td>
</tr>
<tr>
<td>TNF-α</td>
<td>CAAATTCGAGTGACAAGGCTG</td>
<td>GGGTGGTCAGCACCAC</td>
</tr>
<tr>
<td>Vimentin</td>
<td>TCCAGGAGAGGAGACGCGAGA</td>
<td>GCAAGGATTCCACTTCCGTT</td>
</tr>
<tr>
<td>β-actin</td>
<td>GCTCTCCCTGTACCCGCAAG</td>
<td>CATCTGCTGAAAGGTGACA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>TGCAACACCAACTGTTAGCC</td>
<td>GCATGGACTGGTGTCATGA</td>
</tr>
<tr>
<td>HPRT</td>
<td>ATGGAGGGCCATCACATTGT</td>
<td>ATGTAATCCAGGGGATGAC</td>
</tr>
</tbody>
</table>

### 2.4 Electrophysiology

Voltage and current clamp recordings in the whole cell configuration were obtained with an Axopatch 200B (Axon Instruments, Molecular Devices, CA, USA) from CA1 pyramidal neurons in hippocampal slices with or without FA incubation. Recording pipettes were pulled from borosilicate glass (Science Products, Hofheim, Germany) and had a resistance of 4-7 MΩ when filled with pipette solution containing: 131.25 mM potassium D-gluconate, 8.75 mM KCl, 10 mM 2-[4-(2-hydroxy-ethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), 0.5 mM ethylene glycol-bis(2-amino-ethyl)ether)-N,N,N′,N′-tetraacetic acid (EGTA), 4 mM Mg-ATP, 0.4 mM Na2-GTP, pH adjusted to 7.3, osmolarity was adjusted with sucrose to 300 mOsmol. Series resistance was compensated for at least 70%. The liquid junction potential (-14 mV) was corrected online.

Neurons were visualized with an upright microscope (SliceScope Pro 6000, Scientifica, Uckfield, UK) equipped with oblique illumination and a 40X water immersion objective. Data acquisition and analysis were performed with in-house software running in Matlab (MathWorks, Natick, MA USA). Signals were low-pass filtered at 5 kHz and sampled at 20 kHz using a NI USB-6259 data acquisition system (National Instruments, Austin, TX, USA).

Neurons were held in current-clamp at -70 mV by a slow feedback loop that stabilized membrane voltage by injecting the appropriate holding current outside the time periods where the protocols were run. Cell firing was induced by a series of depolarizing current steps (-100 pA until +500 pA in steps of 75 pA, lasting 500 ms). At the beginning of each sweep the input resistance was determined with a 50 pA hyperpolarizing current injection that lasted 50 ms.

Synaptic plasticity was determined as spike-timing dependent plasticity (STDP), which enhances synaptic efficacy by causally pairing the presynaptic stimulus with a postsynaptic action potential at an optimal time interval of 10 ms. These experiments started with a baseline recording during which the excitatory postsynaptic potential (EPSP) had to be stable for at least 10 minutes. EPSPs were evoked with 7.5 seconds interval by a 0.2 ms
bi-phasic current pulse (DS4, Digitimer, Welwyn Garden City, UK) given through a bipolar tungsten electrode (WPI, Berlin, Germany) located at the Schaffer Collaterals about 300 μm away from the recorded neuron. Stimulus intensity (100-1000 μA) was adjusted to evoke 40-60% of the maximal EPSP amplitude. STDP was induced by pairing of the stimulus with a single postsynaptic action potential evoked by a short somatic current depolarization (3 ms, 0.5-1.5 nA) via the recording pipette given 10 ms after the stimulus. Pairing (60 trials) was followed by a test sequence similar to the baseline period which lasted for at least 15 minutes. The input resistance of the cell was continuously monitored; experiments were excluded if it changed more than 30% over the duration of an experiment. The maximal amplitude of each EPSP was determined and the amplitude after pairing was normalized to the mean baseline value. For illustration purposes (Figure 2) the amplitude was averaged over five successive time points. The relative change in EPSP amplitude, quantifies the change in synaptic efficacy induced by the paired stimulation train.

2.5 Statistics
All statistical analysis was performed with Prism 5.0 (GraphPad Software, San Diego, CA, USA). Mean and pooled values are reported with standard error of the mean (± SEM) for number of mice: N and number of cells: n. Multiple comparison within and between groups was performed with two-way ANOVA followed by the appropriate post-hoc test. Unless otherwise mentioned two-group comparisons were made with Student’s t-test. We assume that $p < 0.05$ rejects the null hypothesis.

3. Results

LPS modulation of a reactive astrocyte phenotype in acute hippocampal slices
The first series of experiments was designed to investigate the effect of LPS application (1 µg/ml) on astrogliosis in acute hippocampal slices from young mice. After 80 minutes recovery from the slicing procedure, half of the slices obtained from each mouse were incubated with LPS while the animal-matched control slices remained in ACSF. At fixed time points after the start of the LPS stimulation (data points in Fig. 1), slices were taken from each condition and snap frozen. We performed qPCR analysis on mRNA isolated from the snap frozen samples of four repeated experiments that used four different animals. In controls, the mRNA expression of the astroglial markers GFAP and vimentin remained almost constant over the five hours following slicing (Fig. 1a, open circles). The application of LPS (horizontal bar in Fig. 1) had no systematic effect on the expression of GFAP but induced a mild 50% increase of the astroglial marker vimentin over the subsequent 3-4 hours ($p =$
0.056 for the whole experimental period). This is in strong contrast to the mRNA expression levels of the two genes coding for the cytokines, IL-1β and TNF-α obtained from the same experiments (Fig. 1b). The mRNA expression of both cytokines increased sharply (>50 fold) during the first 80 minutes after slicing, where after it gradually declined back to lower levels in the following five hours. The application of LPS did not induce any additional increase in inflammatory cytokine expression.

![Figure 1. mRNA expression of reactive astroglial markers and cytokines in control and in LPS stimulated slices.](image)

**Fluoroacetate modulation of intrinsic neuronal excitability and STDP induction**

Astrocytes modulate neuronal function and neuronal communication. In a second series of experiments we incubated hippocampal slices of 4-month-old mice in 5 mM of the astrocyte specific metabolic inhibitor FA for 1 hour before recordings started. We then investigated
whether this treatment affected three aspects of neuronal excitability: 1) basic parameters of the neuronal membrane, 2) firing properties of the neurons, and 3) efficacy of synaptic communication and its plasticity. Pyramidal neurons in the CA1 region of the hippocampus were recorded under current clamp in the whole-cell configuration. The resting membrane potential and input resistance of neurons in control and in FA preincubated slices were not different (Fig. 2a, b).

From a well-defined and controlled membrane voltage (-70 mV) current injection of 500 ms duration and increasing amplitude (50-500 pA) evoked trains of action potentials in the neurons. The firing rate of neurons in the FA incubated slices was about 10% higher than the one in controls (Fig. 2c) suggesting a somewhat higher excitability after astroglial inhibition. Intracellular excitatory postsynaptic potentials (EPSPs) were evoked by extracellular stimulation of the Schaffer collaterals (interval of 7.5 seconds). An optimal time interval of 9-10 ms between the presynaptic stimulus and the depolarization induced postsynaptic action potential was chosen. Peak EPSP amplitudes were determined during baseline and after 60 pairings. Pairing reliably induced STDP when the EPSP amplitude after pairing was larger than before. In control slices STDP (150 ± 20%, n = 5 from 3 mice, \( p = 0.02 \)) could be easily induced, while it was suppressed or even prevented in FA incubated slices (85 ± 20%, n = 5 from 3 mice, \( p = 0.36 \)). We also directly tested that the level of STDP induced in control slices was higher than in FA treated slices (\( p = 0.02 \)).

Figure 2. Fluoroacetate modulates excitability and spike-timing dependent plasticity in hippocampal CA1 pyramidal neurons.

a. Resting membrane potential was not different in neurons in control and in FA treated slices (n = 10 from 4-5 mice per condition, \( p = 0.45 \)). b. Input resistance was also not affected by preincubation in FA (n = 10 from 4-5 mice per condition, \( p = 0.19 \)). c. Mean number of action potentials evoked by depolarizing current steps of 50-500 pA demonstrated an excitability increase by FA preincubation (treatment x current steps two-way ANOVA: n = 10 from 4-5 mice per condition, \( F_{1,126} = 5.10, p = 0.03 \)). d. Time course of EPSP amplitude before and after STDP induction (grey area) obtained from control or FA treated neurons. A higher level of STDP was induced in control compared to FA incubated neurons (n = 5 from 3 mice per condition, \( p = 0.02 \)). Vertical bars represent SEM.
4. Discussion

In this technical note we first investigated whether LPS application to acute hippocampal slices is a valid tool to study astrogliosis ex vivo. Numerous techniques have been used to investigate the consequences of disturbed astrocyte function for synaptic transmission. Acute slices present a preparation with pretty intact neuronal morphology and connectivity, despite the heavy dissection damage. Interventions to rescue and/or unravel underlying mechanisms of astrogliosis can be tried out. In the acute slice we observed an almost stable mRNA expression of the classic markers for reactive astrocytes GFAP and vimentin. In contrast, qPCR analysis indicated a strong (50 fold) transient upregulation of the expression of the genes coding for the cytokines IL-1β and TNF-α for at least 1 hour after slicing where after it gradually declined. The strong increase in mRNA levels of both IL-1β and TNF-α is likely mediated by a quick microglial activation. The application of LPS hardly affected GFAP expression, it slightly enhanced vimentin and was not able to further enhance the mRNA expression of both cytokines.

Takano et al., 2014 indicate that astrocytes do not tolerate the traumatic injury and hypoxia well, that are unavoidable in preparing acute brain slices. Changes in immunolabeling of GFAP, nestin and connexin 43 were found as quickly as 90 minutes after slicing (Takano et al., 2014). Sodium ions in the cutting solution are often exchanged for sucrose or N-methyl-d-glucamine (NMDG) (Takano et al., 2014), which reduces the driving force for Na⁺, and thus neuronal firing and reduces excitatory injury. However, changes in ion concentrations in slicing solutions may induce more stress on astrocytes, which are sensitive to interstitial ion concentration (Nedergaard & Verkhratsky, 2012). That is why we only partially substituted sodium (60 mM) for sucrose in our cutting solution. We also implemented a 20 min recovery step at 35 °C after slice preparation. A sucrose cutting solution and recovery step are often used in electrophysiological studies for recordings of astrocytes and microglia (Wallraff et al., 2006; Plescher et al., 2018). These modifications in our slicing protocol might underlie the stable mRNA expression of GFAP and vimentin after slicing. After acute trauma or hypoxia, microglia can rapidly be activated (Kettenmann, Hanisch, Noda, & Verkhratsky, 2011). Other groups use sodium-kynurenate in their slicing solution to block glutamate receptors and prevent the activation of microglia (Madry et al., 2018). Since we wanted to obtain neuronal recordings, we did not use the glutamate receptor blocker kynurenic acid which would have interfered with our read-out measurements.

Microglia and astrocytes release several cytokines upon activation, including IL-1β and TNF-α (Pang, Cai, & Rhodes, 2001; Kettenmann, Hanisch, Noda, & Verkhratsky, 2011; Orre et al., 2014; Tarassishin, Suh, & Lee, 2014; Subhramanyam, Wang, Hu, & Dheen, 2019; Zhang et al., 2019). These cytokines are highest expressed by microglia (Zhang et al., 2014). Our experiments demonstrated that the activation of microglia and astrocytes induced by slicing cannot be further upregulated by LPS application.
Acute hippocampal slices of 4-month-old mice were treated with the glial metabolic inhibitor FA in order to assess its effect on intrinsic neuronal excitability and synaptic plasticity. We observed, without further quantification, that neurons seemed to be more fragile: some died directly after obtaining the whole-cell patch, while others demonstrated substantial fluctuations in input resistance and membrane voltage after FA incubation. However, neurons that could be stably recorded in the whole-cell configuration after FA incubation did have a similar resting membrane potential and input resistance as controls. Under these circumstances intrinsic excitability defined as the number of action potentials induced by well-defined depolarizing current steps, was increased by about 10%. Karus et al. 2015 reported epileptic like burst activity and found an increased neuronal sodium level in hippocampal slices after FA application. Their experiments suggest that reduced glutamate uptake and hampered astroglial potassium buffering by FA treatment underlie these effects (Karus, Mondragão, Ziemens, & Rose, 2015). Whether the increase in excitability, found in our experiments, is a direct effect of FA on neurons or indirectly mediated by the inhibition of astroglial glutamate uptake or potassium buffering could not be distinguished based on our data.

From previous studies it is known that FA affects glia-neuronal communication in the CA1 region (Henneberger, Papouin, Oliet, & Rusakov, 2010) and dentate gyrus of the hippocampus (Pabst et al., 2016). Spike-timing dependent depression is mediated by astrocytes at synapses in the neocortex (Min & Nevinan, 2012), the striatum (Valtcheva & Venance, 2016), and the hippocampus (Andrade-Talavera, Duque-Feria, Paulsen, & Rodriguez-Moreno, 2016). The latter study also showed that the inhibition of calcium signaling in astrocytes or FA application did not affect STDP in P12-18 mice (Andrade-Talavera, Duque-Feria, Paulsen, & Rodriguez-Moreno, 2016). In contrast to these findings, our protocol induced STDP in 4-month-old mice and the application of FA prevented its expression which strongly suggests that astrocytes are involved in STDP.

The incomplete understanding of the mechanisms and specificity of FA (Henneberger, Papouin, Oliet, & Rusakov, 2010; Pabst et al., 2016), as well as the controversial findings about its direct inhibitory function make it difficult to directly assign the findings after FA treatment to either astrocyte specific functions or to a network effect. Studies by Kaczor and colleagues, however, indicate that FA specifically affects astrocytes and not neurons. They showed in a hippocampal astrocyte-neuronal co-culture that the induction of inhibitory long-term potentiation (iLTP) is blocked by FA treatment (Kaczor & Mozrzymas, 2017). Also, the frequency of miniature inhibitory postsynaptic currents (mIPSCs) was reduced after treatment. However, no effect of FA on iLTP induction or mIPSC frequency was found in an astrocyte-free neuronal culture (Kaczor, Rakus, & Mozrzymas, 2015; Kaczor & Mozrzymas, 2017).
These findings indicate that FA could be a valid first tool to determine a potential role for astrocytes in neuronal function and to further unravel the specific role for astrocytes at different synapses. The effect of FA on STDP induction in hippocampal slices was for us an indication that astrocytes play a role in the enhancement of CA3-CA1 synaptic efficacy. We hypothesize that reactive astrocytes, found in numerous CNS diseases, including AD, stroke, and Huntington's disease (Osborn, Kamphuis, Wadman, & Hol, 2016; van Dijk, Vergouwen, Kelfkens, Rinkel, & Hol, 2016; Khakh et al., 2017) might also influence STDP and ultimately cognition.

In conclusion, in this technical note we showed that slicing induced an inflammatory response, likely mediated by a quick activation of microglia. The expression of the reactive astroglial markers GFAP and vimentin remained stable and was hardly affected by LPS; the application of LPS was not able to further increase the mRNA expression levels of the cytokines. FA treatment which inhibits glial metabolism, did mildly enhance neuronal excitability and suppressed or even prevented stimulation induced synaptic plasticity, indicating that FA might be a suitable tool to unravel mechanisms involved in the glia-neuronal communication.
References


Modulating astrocyte function in acute hippocampal slices


