Neuroinflammation alters voltage-dependent conductance in striatal astrocytes

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Karpuk N, Burkovetskaya M, Kielian T. Neuroinflammation alters voltage-dependent conductance in striatal astrocytes. J Neurophysiol 108: 112–123, 2012. First published March 28, 2012; doi:10.1152/jn.01182.2011.—Neuroinflammation has the capacity to alter normal central nervous system (CNS) homeostasis and function. The objective of the present study was to examine the effects of an inflammatory milieu on the electrophysiological properties of striatal astrocyte subpopulations with a mouse bacterial brain abscess model. Whole cell patch-clamp recordings were performed in striatal glial fibrillary acidic protein (GFAP)-green fluorescent protein (GFP)+ astrocytes neighboring abscesses at postinfection days 3 or 7 in adult mice. Cell input conductance (Gi) measurements spanning a membrane potential (Vm) surrounding resting membrane potential (RMP) revealed two prevalent astrocyte subsets. A1 and A2 astrocytes were identified by negative and positive Gi increments vs. Vm, respectively. A1 and A2 astrocytes displayed significantly different RMP, Gi, and cell membrane capacitance that were influenced by both time after bacterial exposure and astrocyte proximity to the inflammatory site. Specifically, the percentage of A1 astrocytes was decreased immediately surrounding the inflammatory lesion, whereas A2 cells were increased. These changes were particularly evident at postinfection day 7, revealing increased cell numbers with an outward current component. Furthermore, RMP was inversely modified in A1 and A2 astrocytes during neuroinflammation, and resting Gi was increased from 21 to 30 nS in the latter. In contrast, gap junction communication was significantly decreased in all astrocyte populations associated with inflamed tissues. Collectively, these findings demonstrate the heterogeneity of striatal astrocyte populations, which experience distinct electrophysiological modifications in response to CNS inflammation.

NEUROINFLAMMATION LEADS TO astrocyte activation and the initiation of complex cellular alterations referred to as reactive astrogliosis. The hallmarks of astrogliosis are astrocyte hypertrophy and a robust increase in glial fibrillary acidic protein (GFAP) expression (Sofroniew and Vinters 2010). Since astrocytes are critical for maintaining central nervous system (CNS) homeostasis, including K+ and glutamate buffering and redistribution (Takahashi et al. 2010; Wallraff et al. 2006), nutritional support for neurons via glucose delivery (Rouach et al. 2008), gap junction-mediated communication by Ca2+ waves and ATP release (MacVicar and Thompson 2010; Scemes and Giaume 2006), and information processing through tripartite synapses (Halassa and Haydon 2010; Hamilton and Attwell 2010), it is important to understand how reactive astrocytes adapt their activity to maintain tissue homeostasis. Electrophysiological techniques have been widely used to study ion exchange through cell membranes as well as gap junctions. Several models have been utilized to study the electrophysiological properties of activated astrocytes in situ (Anderova et al. 2004; Bordey et al. 2001; Schroder et al. 1999; Takahashi et al. 2010; Wang et al. 2008); however, the consequences of a rapid, fulminate neuroinflammatory stimulus on astrocyte electrophysiological properties remain incompletely understood. Previous studies from our laboratory have utilized a well-established model of bacterial brain abscess (Kielian et al. 2001, 2007a, 2007b) to investigate the effects of local inflammation on astrocyte gap junction communication (GJC) and hemichannel (HC) activity (Karpuk et al. 2011). Specifically, we reported distance-dependent alterations in GJC, HC opening, modulations in connexin and pannexin expression, as well as upregulation of glutamate transporters. We also noted correlations between general electrophysiological parameters of astrocytes in relation to their distance from the brain abscess margins. However, our prior study did not identify the nature of astrocytic electrophysiological modifications in the striatum or explain how a potent inflammatory milieu, modeled by acute bacterial infection, can alter inward and outward currents in activated astrocytes.

Previous studies in the stratum of young rats demonstrated that >95% of astrocytes displayed passive properties, which do not exhibit time- or voltage-dependent currents and therefore possess typical linear voltage-current (I-V) relationships (Adermark and Lovinger 2008). Passive astrocyte properties are thought to be mediated by extensive GJC that can distort whole cell recordings by currents escaping to neighboring cells. In contrast, earlier studies in hippocampal slices indicated that the passive profile of astrocytes is intrinsic and not influenced by GJC (Schools et al. 2006; Wallraff et al. 2006). However, voltage-dependent currents that are characteristic of complex astrocytes (Matthias et al. 2003) can also be revealed in reactive astrocytes after the application of various channel inhibitors and/or leak subtraction (Anderova et al. 2004; Perillan et al. 1999). Among them, K+ voltage-dependent currents are considered essential contributors to resting conductance (Bordey and Sontheimer 1997; Seifert et al. 2009).

The percentage of passive astrocytes can be altered during astrogliosis, which agrees with the idea of alterations in ionic membrane currents following astrocyte activation (Anderova et al. 2004). Indeed, inward-rectifying K+ (KIR) currents were reduced in activated astrocytes after an entorhinal cortex lesion (ECL) (Schroder et al. 1999) and in layer I astrocytes during cortical freeze lesion (CFL) (Bordey et al. 2001), whereas an increase in KIR current occurred in astrocytes after a cortical stab wound (CSW) (Anderova et al. 2004). However, astrocyte delayed-rectifying outward K+ current was enhanced in CFL and CSW models but not in ECL. These discrepancies may result from the diverse nature of the injury models, which elicit different degrees of astrogliosis (Bordey et al. 2001; Liberto et
Most electrophysiological studies have investigated astrocytic currents over a wide range of membrane potentials. However, in vivo experiments revealed minor 1- to 2-mV deviations of astrocytic resting membrane potential (RMP) associated with neuronal electrical activity (Amzica et al. 2002; Mishima and Hirase 2010). An interpretation of these findings is that astrocytic functioning in situ occurs over a very narrow membrane potential range but is associated with large currents passed through the astrocytic membrane. Regarding this issue, in the present study we investigated electrical events of astrocytes with a narrow range of membrane potentials surrounding RMP. This demonstrates the feasibility of our approach to query changes in intrinsic astrocyte properties in the face of neuroinflammation.

The objective of the present study was to identify alterations in astrocytic inward and outward membrane currents surrounding RMP in the inflammatory milieu generated during bacterial brain abscess formation. Whole cell voltage- and current-clamp recordings were used to measure properties of striatal astrocytes at varying distances from the abscess in acute brain slices from GFAP-green fluorescent protein (GFP) mice. We report that the number of astrocytes expressing preferentially inward or outward current components (referred to as A1 and A2 astrocytes, respectively) was altered during the course of neuroinflammation. The increased voltage-dependent conductance and capacitance in all astrocytes surrounding the inflammatory milieu coincided with increased HC activity at postinfection day 3. The possible functional role of A1 and A2 populations is discussed. Collectively, these studies identify novel adaptations of astrocytes to an inflammatory milieu that may impact CNS homeostasis and neuronal integrity in neighboring tissues.

MATERIALS AND METHODS

Generation of experimental brain abscesses. Brain abscesses were established in GFAP-GFP mice (8–12 wk of age: The Jackson Laboratory, Bar Harbor, ME), by the intracerebral inoculation of live Staphylococcus aureus (strain USA300 CAV1002) (Sifi et al. 2007) encapsulated in agarose beads as previously described (Kielian et al. 2001). A rodent stereotaxic apparatus equipped with a Cunningham mouse adaptor (Stoelting, Kiel, WI) was used to implant S. aureus-encapsulated agarose beads into the dorsal striatum, with the following coordinates relative to bregma: +1.0 mm rostral, +2.0 mm lateral, and −3.0 mm deep from the surface of the brain. A burr hole was made, and a 5-μl Hamilton syringe fitted with a 26-gauge needle was used to slowly deliver 2-μl beads (10⁷ colony-forming units) into the brain parenchyma over a 30-s period. The needle remained in place for 2–3 min after injection to prevent the efflux of injected material. Earlier studies from our laboratory have established that the introduction of sterile agarose beads does not induce detectable inflammation or peripheral immune cell infiltrates (Baldwin and Kielian 2004; Kielian et al. 2001). In addition, at the time points evaluated in the present study (i.e., postinfection days 3 and 7) the tissue within 1–2 mm from the initial stab wound is completely obliterated by necrosis, and, as such, comparisons with sham-injected animals (i.e., sterile agarose beads) are less relevant. Since astrocyte electrophysiological measurements were conducted adjacent to the brain abscess margins, it can be concluded that responses are elicited by inflammation originating from bacterial infection and not from the original stab site used to inoculate bacteria into the brain parenchyma, since this region no longer exists. Therefore, comparisons between the intact parenchyma surrounding brain abscesses and identical regions from uninfected brains were considered the most accurate comparisons to reveal changes elicited by the inflammatory milieu after bacterial exposure. We utilized a similar strategy for our recent report, in which comparisons were made between infected and noninfected tissues (Karpuk et al. 2011). The animal use protocol, approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee, is in accord with National Institutes of Health guidelines for the use of rodents.

Acute brain slice preparation. At the appropriate time points after infection, mice were killed by cervical dislocation and immediately decapitated. The brain was quickly removed and bathed in ice-cold artificial cerebrospinal fluid (ACSF, in mM: 124 NaCl, 26 NaHCO₃, 3 KCl, 2 MgCl₂, 2 CaCl₂, 0.4 ascorbic acid, 10 glucose) for slice preparation. Next, horizontal slices (300–400 μm thick) were prepared with a Leica VT1000S vibrating-blade microtome (Leica Microsystems) and immediately placed in ACSF at 32°C. After a 20- to 30-min incubation period at 32°C, slices were held in ACSF at room temperature for at least 1 h before further use. All incubation solutions were equilibrated and continuously bubbled with carbogen (95% O₂-5% CO₂). In some experiments, slices were incubated with sulforhodamine 101 (SR101 acid chloride, 0.2–0.4 μM; Sigma, St. Louis, MO) as an additional marker for astrocyte identification (Nimmerjahn et al. 2004). In other experiments, brain slices were incubated with ethidium bromide (EtBr) for 10 min to identify cells with increased HC activity (Karpuk et al. 2011) that could be used for patch-clamp recordings.

Electrophysiology. Electrophysiological recordings were performed in a submerged chamber (RC-27, Warner Instruments, Hamden, CT) continuously perfused with ACSF at a rate of 1–2 ml/min at 30°C. Patch pipettes for recording electrodes were pulled from borosilicate glass capillaries (1.5/0.84 mm OD/ID) with a micropipette puller (P-97, Sutter Instruments, Novato, CA). Recording electrodes were filled with a solution containing (in mM) 130 KCl, 0.2 CaCl₂, 1 MgCl₂, 5 EGTA, 10 HEPES, pH 7.2–7.3 with an electrical resistance of 6–10 MΩ. In other experiments, K-glutamate or CsCl (Sigma) was substituted in equimolar concentrations for K⁺ in the intracellular recording solution. The gap junction permeant dye Alexa Fluor 350 (0.5 mM, Invitrogen, San Diego, CA) was added to the intracellular recording solution to calculate the degree of astrocyte GJIC. The following drugs were used (in mM): 1 CsCl, 6 KCl, and 0.1 gap junction/HC inhibitor carbenoxolone disodium salt (CBX) (all from Sigma).

Whole cell patch-clamp recordings were performed on GFAP-GFP⁺ striatal astrocytes with a computer-controlled amplifier (Multiclamp 700B, Molecular Devices, Sunnyvale, CA) and a video setup equipped with a motorized Axio Examiner Z1 fluorescent microscope, a high-sensitivity and -resolution digital camera (Axiocam MRm), and AxioVision software (all from Zeiss). Analog signals from the amplifier were digitized at a 5-to-10 kHz sampling rate with a 16-bit resolution Digidata-1440A acquisition system and pCLAMP-10 software (both from Molecular Devices), which allowed detection of picocammere changes in macroscopic currents passed through low-resistance astrocytes in living brain tissue. It was shown earlier (Zhou et al. 2009) and confirmed in this study that access resistance (Rᵣ) exceeds the membrane resistance (Rₘ) of astrocytes up to 5 times, which can cause voltage-clamping errors of ~80% depending on Rᵣ/Rₘ, where Rᵣ is total membrane resistance (Rᵣ + P). Using the membrane test function integrated in pCLAMP-10, we estimated the astrocyte Rᵣ/Rₘ as 5.2 ± 0.74 (n = 18) and 4.9 ± 0.42 (n = 27) in uninfected and infected brain slices, respectively. However, the remaining voltage [~20% of command voltage (Vc)] applied to the cellular membrane can generate still large passive currents, which obscures voltage-dependent currents. The linear I-V relationship is a prominent characteristic of passive currents. Therefore, I-V differentiation negates passive conductance and may reveal voltage-depen-
dent conductances, in particular, if the actual membrane potential \( V_m \) is taken into account [i.e., \( V_m = V_c \times (R_m/R_t) \)].

Electrophysiological parameters of astrocytes were calculated offline with Clampfit-10 and custom-designed software (Karpuk and Hayar 2008). The total resting membrane conductance \( G_t \) [\( G_m \) is estimated membrane conductance, i.e., \( G_m = G_i \times (R_t/R_m) \)] and resistance \( R_t \) were calculated as the linear I-V slope by using 3–5 data points near the value of RMP (Fig. 1B). Utilizing this method, cell input conductance \( G_i \) was calculated at every \( V_c \) used in the recordings (on average, from −140 to +60 mV). Thus \( G_i \) is only one point within the numerous \( G_i \) range calculated at different \( V_c \). Since \( G_i \) values (nS) were dependent on \( V_c \) as shown in this study, the relationship between \( G_i \) and \( V_c \) revealed both positive and negative \( G_i \) increments or \( G_i \) slopes measured in picosiemens per millivolt or picoamperes per millivolt square (Fig. 1C). The latter indicates that real current changes at the cell membrane should be adjusted by the factor \( R_t/R_m \) to compensate for the voltage drop on \( R_m \). Since I-V tails usually fluctuate because of inward and outward current activation at negative (i.e., from −90 to −140 mV) or positive (i.e., 0 to +60 mV) membrane potentials, respectively, we defined the basic \( G_i \) slope, which was calculated within the linear segment of the \( G_i \) plot within −90 to 0 mV of \( V_c \) and always included the RMP point (i.e., zero holding current) (Fig. 1C). The negative and positive \( G_i \) slopes (i.e., \( -G_i \) and \( +G_i \), respectively) averaged from three or four consequential voltage-clamp recordings were the main criteria for grouping astrocytes into different subsets. This method enabled the

**Fig. 1. Methods for calculating astrocyte input conductance parameters.** A: whole cell voltage-clamp recording in a striatal glial fibrillary acidic protein (GFAP)-green fluorescent protein (GFP) astrocyte located near an abscess at postinfection day 7. Holding potential (HP) and applied voltage steps (not shown) were −75 and 5 mV, respectively. B: voltage-current (I-V) relationship constructed from the current and voltage points that were obtained by averaging the corresponding segments (a–b) (70–90 ms) delineated in A. The total resting conductance \( G_i \) was taken as the linear I-V slope including 3–5 points near zero current (bold line, curved arrows, 60 nS). The dashed line was drawn to emphasize linear I-V. C: plot of cell input conductance \( G_i \) vs. holding voltages. Dashed lines indicate negative and positive linear slopes (−90 ± 4 pS/mV and 170 ± 13 pS/mV, respectively). Left dashed line indicates the basic negative \( G_i \) slope [calculated at the linear segment of \( G_i \) plot within the voltage range −90 to 0 mV including the resting membrane potential (RMP) value (zero holding current)] that was used as a criterion to identify astrocyte subsets. Right dashed line indicates the positive \( G_i \) slope. The absolute differences between maximal (max, star) and minimal (min, circle) \( G_i \) found at the same voltage range (−90 to 0 mV) were defined as voltage-dependent conductance \( G_v \) (straight arrows). The minimal \( G_i \) was used for passive current subtraction (see D–F). D: voltage-dependent currents were revealed in the recording depicted in A after current subtractions. E: I-V was calculated from current subtractions in D by the same method used in B. F: \( G_v \) plot calculated from I-V in E reveals identical \( G_v \) patterns compared with the original \( G_i \) pattern in C, which supports the conclusion that calculation of \( G_i \) slopes represents an efficient tool to explore \( G_v \).
identification of A1 astrocytes typified by $-G_i$ as well as A2 astrocytes that were classified by $+G_i$. Cells that exhibited complex $G_i$ patterns were included in appropriate subsets as dictated by their basic $G_i$ slope (Fig. 1C; Fig. 2).

The minimal $G_i$ detected at the $V_c$ range from $-90$ to $0$ mV was considered passive conductance and was used for current subtractions to visualize active current components (Fig. 1, D–F). The absolute difference between maximal and minimal $G_i$ found in the $V_c$ range from $-90$ to $0$ mV was considered the voltage-dependent conductance ($G_v$) (Fig. 1C). It should be noted that maximal $G_i$ for A1 cells was located at more negative potentials than minimal $G_i$, and opposing maximal/minimal $G_i$ locations were characteristic for A2 cells (Fig. 2D). Cell membrane capacitance ($C_m$) values were taken from the amplifier readout in voltage-clamp mode as compensated capacitance. In current-clamp mode, $C_m$ was calculated from $I-V$ recordings with the time constant definition $t_m = R_m C_m$, where $t_m$ is the time constant calculated by the declining membrane potential at 5–20 mV from RMP (i.e., time point at 63% of maximal declination) when negative rectangular current steps were injected into cells by patch pipette.

Quantitation of astrocyte coupling. The gap junction permeant dye Alexa Fluor 350 was included in the patch pipette to visualize the degree of GJC in GFAP-GFP+ striatal astrocytes. Calculations of astrocyte coupling were performed by enumerating the number of superimposed cell images under appropriate filters for GFAP and Alexa Fluor 350 in microscopic fields of view (220 × 165 μm). Cell coupling was confirmed by the measurement of optical intensities with AxioVision software. Cells were considered coupled if the peak optical intensities for Alexa Fluor 350 overlapped with GFAP-GFP intensities and exceeded 10% of background levels. Astrocyte soma area was measured by manually outlining cell body images ( distinguishable by GFAP-GFP and SR101 fluorescence) with appropriate tools in AxioVision.

Statistical analyses. A Student’s two-tailed $t$-test was used for data analyses (MS Excel 2007), with values reported as means ± SE compiled from independent experiments.
RESULTS

Characterization of striatal astrocyte hypertrophy during brain abscess formation. Representative fluorescent images of brain slices from GFAP-GFP mice harboring brain abscesses at postinfection day 3 and day 7 (Fig. 3, A and B, respectively) depict a central necrotic core delineated by nonspecific SR101 uptake, surrounded by intense astrocyte activation as demonstrated by robust GFAP expression. In general, brain abscesses were larger at postinfection day 3 with irregular margins, whereas at day 7 lesions were more compact and exhibited a structured organization. By day 7 after infection, a narrow zone devoid of GFP signal was evident surrounding the central abscess core, which represents the region where fibrotic encapsulation occurs (Fig. 3B). Astrocytes stained by Alexa Fluor 350 during whole cell recordings revealed different morphologies based on their distance from the brain abscess margins (Fig. 3, C–J). To measure the degree of astrocyte hypertrophy, we next quantitated soma area. In agreement with our observations of distinct morphological changes nearest the inflammatory site, astrocytic soma were significantly larger closest to the brain abscess margins at both postinfection days 3 and 7 (Fig. 4A). Collectively, these morphological findings support the possibility of astrocyte heterogeneity emanating from a site of active inflammation, which supports recent work in our laboratory using the experimental brain abscess model (Karpuk et al. 2011).

Effects of neuroinflammation on basic astrocyte electrophysiological parameters. Next, we evaluated basic electrophysiological parameters of GFAP-GFP+ astrocytes in acute brain slices from both uninfected GFAP-GFP mice and animals harboring S. aureus-induced brain abscesses at days 3 and 7 after infection. In our previous study, we found that some electrophysiological parameters of GFAP-GFP+ striatal astrocytes, as well as GJC and HC activity, were significantly modified immediately surrounding inflamed tissues (Karpuk et al. 2011). The depolarized RMP was due to an increased number of astrocytes with depolarizing potentials immediately surrounding abscesses, mainly in the range of −50 to −30 mV (i.e., 16% in uninfected slices vs. 33% at day 3 after infection). However, in the present study we restricted our analysis to astrocytes that displayed RMP values from −90 to −50 mV, since these ranges fall within the values typical for astrocytes under resting conditions with minimal activation of inward/outward currents (Perillan et al. 1999). Additionally, the V_m range was narrowed because of the effect of R_C in whole cell voltage-clamp configuration. This approach revealed both negative and positive G_L increments in response to depolarizing voltage steps that allowed us to divide GFAP-GFP+ astrocytes into two subsets, which we refer to as A1 and A2 astrocytes, respectively (Fig. 2; also refer to MATERIALS AND METHODS for how these two astrocyte subtypes were classified). Since the frequency of complex astrocytes in the striatum was rather low (~15% of the total cells analyzed at postinfection day 3), they were not defined as a separate category because statistical analysis could not be applied.

Impact of neuroinflammation on intrinsic properties of A1 and A2 astrocytes. A1 and A2 astrocytes demonstrated different electrophysiological parameters in uninfected brain slices (Table 1). Specifically, A2 astrocytes had more hyperpolarized RMP compared with A1 astrocytes (−76.6 ± 1.12 and −69.2 ± 1.30 mV, respectively, P < 0.001), whereas A1 astrocytes had higher G_L compared with A2 cells (33.6 ± 1.64 and 21.1 ± 1.69 nS, respectively, P < 0.001). Accordingly, C_m was significantly higher in A1 compared with A2 astrocytes (37.9 ± 2.16 and 29.3 ± 2.43 pF, P < 0.05), since t_m did not differ (Table 1). In addition, a positive relationship was ob-
served between astrocyte soma area and $G_t$ for both A1 and A2 subsets (slope = 540 ± 120 pS/µm²; $P < 0.05$, Fig. 4B). $G_t$ values were decreased within 0–200 µm from the abscess margins and increased between a distance of 200 and 800 µm for both astrocyte subsets at postinfection day 3 (25.8 ± 1.8 nS; $n = 15$) and 36.1 ± 5.4 nS ($n = 9$), respectively; $P < 0.05$. In contrast, astrocyte soma area was increased in close proximity to the abscess (Fig. 4A) and approached values characteristic of astrocytes from uninfected slices with increasing distance from the lesion (data not shown). Therefore, the opposing changes in $G_t$ and soma area distorted their relationship as depicted in Fig. 4B.

Compared with uninfected slices, A1 and A2 astrocytes surrounding the inflammatory brain abscess margins exhibited differences in several electrophysiological parameters (Table 1 and Fig. 5). For example, RMP was inversely modified in A1 and A2 astrocytes at postinfection day 3. Specifically, membrane hyperpolarization was observed in A1 cells (−73.3 ± 1.15 mV) vs. −69.2 ± 1.30 mV in day 3 and uninfected slices, respectively; $P < 0.001$), whereas depolarization occurred in A2 astrocytes (−64.2 ± 1.61 mV vs. −76.6 ± 1.12 mV in day 3 and uninfected slices, respectively; $P < 0.001$). In parallel with RMP depolarization, $G_t$ was increased only in A2 astrocytes (21.1 ± 1.69 to 29.9 ± 2.25 nS; $P < 0.01$), which was expected since these cells possess positive $G_t$ increments under depolarizing conditions, which was observed in A2 cells during inflammation. However, $G_t$ did not increase in A1 cells with negative $G_t$ increments despite RMP hyperpolarization. As a result, averaged $I$-$V$ were almost indistinguishable between A1 and A2 astrocytes at postinfection day 3 but did differ at day 7, reflecting major changes in astrocyte intrinsic properties at this time point (Fig. 5 and Table 1).

Despite the finding that $G_t$ ($G_m$) was increased only in A2 astrocytes after infection, $C_m$ and time constants were altered in both A1 and A2 subsets (Table 1). These measurements were significantly increased at postinfection day 3 and returned to values typical of uninfected slices by day 7 after bacterial exposure. Collectively, these data suggest that astrocyte membrane surface area was increased at postinfection day 3, since $C_m$ is proportional to membrane area, and are in agreement with the astrocyte hypertrophy observed near the abscess margins (Fig. 3A). However, this relationship was not always observed, since astrocyte soma area was also increased at postinfection day 7, whereas $C_m$ values returned to levels in uninfected slices at this time point (Table 1). This disconnect between $C_m$ values and astrocyte hypertrophy may be explained by the lack of astrocytic fine processes (based on visual observations) in close proximity to the brain abscess margins at postinfection day 7.

Neuroinflammation alters percentages and distribution patterns of A1 and A2 astrocytes in time-dependent manner. In uninfected slices, ~60% of GFAP-GFP⁺ astrocytes displayed

![Fig. 4. Astrocytes undergo hypertrophy over the course of brain abscess evolution. A: area of GFAP-GFP⁺ astrocyte soma was determined in uninfected slices (67.7 ± 2.2 µm²) as well as at postinfection days 3 (94.5 ± 4.2 µm²) and 7 (134.5 ± 5.6 µm²) (***$P < 0.001$ vs. uninfected). B: a positive correlation between astrocyte soma area and resting conductance was only observed in astrocytes from uninfected slices (slope = 540 ± 120 pS/µm², correlation coefficient = 0.66, $n = 26$, $P < 0.05$)](http://jn.physiology.org/ by doi:10.1152/jn.01182.2011 • www.jn.org)

### Table 1. Electrophysiological parameters of different GFAP-GFP⁺ astrocyte subsets

<table>
<thead>
<tr>
<th>Astrocyte Group</th>
<th>RMP, mV</th>
<th>$G_t$, nS</th>
<th>$G_m$, nS</th>
<th>$C_m$, pF</th>
<th>$t_{m}$, ms</th>
<th>$n$</th>
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<tbody>
<tr>
<td>A1 Uninfected</td>
<td>−69.2 ± 1.30</td>
<td>33.6 ± 1.64</td>
<td>168 ± 8</td>
<td>37.9 ± 2.16</td>
<td>1.05 ± 0.04</td>
<td>28</td>
</tr>
<tr>
<td>Day 3 PI</td>
<td>−73.3 ± 1.15a</td>
<td>30.9 ± 1.26</td>
<td>154 ± 6</td>
<td>68.9 ± 6.95c</td>
<td>2.22 ± 0.24c</td>
<td>18</td>
</tr>
<tr>
<td>Day 7 PI</td>
<td>−67.2 ± 4.63</td>
<td>42.1 ± 5.44</td>
<td>210 ± 27</td>
<td>28.4 ± 3.08</td>
<td>0.91 ± 0.05b</td>
<td>8</td>
</tr>
<tr>
<td>A2 Uninfected</td>
<td>−76.6 ± 1.12e</td>
<td>21.1 ± 1.69e</td>
<td>105 ± 8</td>
<td>29.3 ± 2.43d</td>
<td>0.97 ± 0.05</td>
<td>21</td>
</tr>
<tr>
<td>Day 3 PI</td>
<td>−64.2 ± 1.61e</td>
<td>29.9 ± 2.25b</td>
<td>149 ± 11c</td>
<td>66.7 ± 4.84a</td>
<td>2.45 ± 0.28a</td>
<td>15</td>
</tr>
<tr>
<td>Day 7 PI</td>
<td>−69.9 ± 1.71b</td>
<td>21.3 ± 2.09c</td>
<td>106 ± 10c</td>
<td>34.6 ± 7.6c</td>
<td>1.24 ± 0.19</td>
<td>14</td>
</tr>
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Values are means ± SE. GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; PI, postinfection; RMP, resting membrane potential; $G_t$, total resting membrane conductance; $G_m$, estimated membrane conductance; $C_m$, cell membrane capacitance; $t_{m}$, time constant. a$P < 0.05$; b$P < 0.01$; c$P < 0.001$ vs. uninfected slices; d$P < 0.01$; e$P < 0.001$ vs. A1 cells at same time point.
A1 characteristics, whereas 40% exhibited features of the A2 subset (Fig. 6). The percentages of A1 astrocytes were decreased at both days 3 and 7 after infection (i.e., 51% and 37.5%, respectively), whereas A2 cells were increased at both time points (i.e., 49% and 62.5%, respectively; Fig. 6A). We also observed that A1 and A2 astrocytes exhibited different distribution patterns in the context of inflammation. In general, A1 astrocytes that displayed only negative $G_\text{i}$ increments were located farther from the abscess margins compared with A2 cells at postinfection day 3 (280 ± 67 vs. 161 ± 30 μm; $P < 0.05$; Fig. 6B). Conversely, A1 astrocytes were more prominent closer to the inflammatory site than A2 cells at day 7 after bacterial exposure (243 ± 86 vs. 421 ± 86 μm; $P = 0.05$; Fig. 6B). Therefore, astrocyte activation was associated with a redistribution of A1 and A2 populations, whereas increased percentages of A2 astrocytes were observed during brain abscess development.

**Neuroinflammation modulates astrocyte electrophysiological reactivity.** The differential distribution of A1 and A2 astrocytes from the brain abscess margins compared with A2 cells at postinfection day 3 (280 ± 67 vs. 161 ± 30 μm; $P < 0.05$; Fig. 6A). Conversely, A1 astrocytes were more prominent closer to the inflammatory site than A2 cells at day 7 after bacterial exposure (243 ± 86 vs. 421 ± 86 μm; $P = 0.05$; Fig. 6B). Therefore, astrocyte activation was associated with a redistribution of A1 and A2 populations, whereas increased percentages of A2 astrocytes were observed during brain abscess development.

The rate of $G_\text{i}$ changes was dependent upon $G_\text{v}$ amplitude (data not shown) and the distance from the inflammatory site, with maximal expression within 200 μm of the abscess at postinfection day 3 ($-124 ± 21$ vs. $-66 ± 6$ for A1 and $129 ± 36$ vs. $32 ± 3$ pS/mV for A2; $P < 0.05$ vs. uninfected). The amplitude of both $G_\text{i}$ slopes reached control values at a distance of 300–400 μm from the abscess margins at postinfection day 3 but revealed even smaller values at day 7 (35 ± 11 and 21 ± 9 pS/mV, respectively; Fig. 7). Overall, the negative $G_\text{i}$ slope was more exaggerated at postinfection day 3 near the abscess but returned to levels observed in uninfected slices by day 7 farther from the abscess.

Of note, ~15% of the total GFAP-GFP$^+$ astrocytes examined possessed both $+G_\text{i}$ and $-G_\text{i}$ and were only found within 100 μm of abscesses at postinfection day 3. These cells were classified as complex astrocytes based solely on electrophysio-

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**Fig. 5.** $I$-$V$ relationships in A1 and A2 astrocytes. The differences between the $I$-$V$ curves of A1 and A2 astrocytes were minimal at postinfection day 3 and maximal in uninfected brain slices as well as at day 7 after infection. $I$-$V$ were averaged (using a bin value of 10 mV) for all appropriate astrocyte subsets presented in Table 1. Numbers with arrows indicate the extrapolated values of RMP for A1 (downward arrows) and A2 (upward arrows).

**Fig. 6.** Percentage and distribution of A1 and A2 astrocytes are affected by the inflammatory milieu. 

**A:** % of A1 and A2 astrocytes in uninfected slices vs. abscess tissues reveal that the former are more abundant during early infection (i.e., day 3), whereas A2 astrocytes are more prevalent during later stages of inflammation (i.e., day 7). 

**B:** inverse relationship between the distribution patterns of A1 and A2 astrocytes relative to distance from the abscess margins. 

$*P < 0.05$. 

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Fig. 7. Rate of cell conductance changes is heightened in both A1 and A2 astrocytes during brain abscess development. Both negative and positive \( G_i \) slopes were increased at day 3 postinfection nearest the brain abscess margins (i.e., 0 to 200 \( \mu \text{m} \)), while only positive slopes were increased at day 7 farther from the abscess (201 to 800 \( \mu \text{m} \); \( *P < 0.05 \) vs. values from uninfected slices) with calculations presented in Table 2.

Table 2. Parameters of \( G_i \) increments in GFAP-GFP\(^+\) striatal astrocytes

<table>
<thead>
<tr>
<th>Astrocyte Subset</th>
<th>Treatment Group</th>
<th>Distance from Brain Abscess Margin</th>
<th>( V_c ), pS/mV</th>
<th>( V_m ), nS/mV</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0–200 ( \mu \text{m} )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>Uninfected</td>
<td>34</td>
<td>-67 ± 7</td>
<td>-1.67 ± 0.17</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Day 3 PI</td>
<td>14</td>
<td>-124 ± 22*</td>
<td>-3.10 ± 0.55*</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Day 7 PI</td>
<td>6</td>
<td>-35 ± 12</td>
<td>-0.87 ± 0.30</td>
<td>6</td>
</tr>
<tr>
<td>A2</td>
<td>Uninfected</td>
<td>20</td>
<td>32 ± 6</td>
<td>0.80 ± 0.15</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Day 3 PI</td>
<td>9</td>
<td>129 ± 36*</td>
<td>3.22 ± 0.90*</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Day 7 PI</td>
<td>5</td>
<td>21 ± 9</td>
<td>0.52 ± 0.22</td>
<td>5</td>
</tr>
</tbody>
</table>

Values are means ± SE. Results were calculated at the command voltages (\( V_c \)) and adjusted for membrane potentials (\( V_m \)) by the factor \( [R/R_m]^2 \), where \( R \) is total membrane resistance and \( R_m \) is membrane resistance. \( *P < 0.05 \) vs. uninfected slices.

We predicted that astrocytes with increased \( G_v \) neighboring an abscess would also display increased HC activity for two reasons. First, we have recently reported that astrocyte HC activity was increased in close proximity to abscesses at postinfection day 3 and was reduced at day 7 (Karpuk et al. 2011), in agreement with the redistribution of \( G_v \) shown in this study (Fig. 8). Second, astrocytes that displayed activated HCs also revealed increased \( G_v, [8.97 ± 1.25 \text{ nS} (n = 5); P < 0.05 \) vs. uninfected]. \( G_v \) steadily decreased at a distance up to 600 \( \mu \text{m} \) from the abscess at postinfection day 3 with a negative slope (\(-24 ± 4 \text{ pS}/\mu \text{m}; P < 0.05\); Fig. 8B), in accordance with the progressive reduction in inflammatory stimulus intensity farther from the lesion site. Conversely, resting conductance was increased at the same distance from the abscess at postinfection day 3, reflecting the passive current amplification. However, the opposite relationship between \( G_v \) and distance from the abscess was observed at postinfection day 7, an interval when inflammation is beginning to resolve (\( 12 ± 3 \text{ pS}/\mu \text{m}; P < 0.05\); Fig. 8B). Collectively, these electrophysiological data suggest that astrocytic \( G_v \) modifications surrounding an abscess coincide with the extent of inflammation.

**Neuroinflammation modulates astrocyte voltage-dependent conductance.** Because \( G_v \) amplitudes were not significantly different between A1 and A2 astrocytes, we combined \( G_v \) parameters from both groups to simplify additional calculations. With this approach, the \( G_v \) of astrocytes revealed distribution patterns similar to those observed for \( +/− G_c \) measurements. Specifically, maximal \( G_v \) amplitude was found within 200 \( \mu \text{m} \) of the abscess margins at postinfection day 3 (11.4 ± 1.02 vs. 6.3 ± 0.5 nS in uninfected; \( P < 0.001 \)), whereas \( G_v \) approached values representative of uninfected tissues at farther distances from the abscess margins (Fig. 8A). In addition, a positive relationship between \( G_v \) and astrocyte soma area was observed at postinfection day 3 (slope = 30 ± 10 \text{ pS}/\mu \text{m}^2; \( P < 0.05 \)) (Fig. 9), which suggests that voltage-dependent currents are activated in hypertrophied astrocytes. In contrast, \( G_v \) was reduced over twofold at postinfection day 7 (4.47 ± 1.31 nS; \( P < 0.05 \) vs. day 3; Fig. 8) but was still significantly higher farther from the abscess margins (8.37 ± 0.86 nS; \( P < 0.05 \) compared with uninfected), mainly because of outward currents (Fig. 8). These \( G_v \) modifications indicate that astrocyte activation was reduced in regions immediately surrounding the abscess at postinfection day 7, whereas astrocytes located at farther distances from the inflammatory milieu remained affected. Here \( G_v \) was calculated from original \( I-V \) with \( V_c \) values. The estimated \( G_v \) values on \( V_m \) had severalfold higher amplitude depending on \( R/R_m \). Overall, the \( G_v \) percentage represented a significant portion of \( G_m \) in uninfected tissues (i.e., 23%), whereas the maximal \( G_v \) percentage was observed at postinfection day 3 near the abscesses (i.e., 37%; Table 3).

**Table 3.** Changes in astrocyte conductances at postinfection day 3 and day 7 in both uninfected and postinfection day 3 mice. Shown are values for astrocytes that have been activated by inflammation (A1) or have not been activated (A2). Values are means ± SE. ANOVA (3-way) and Bonferroni’s post hoc test. (\( *P < 0.05 \) vs. uninfected, 0–200 \( \mu \text{m} \)).

**Table 3.** Changes in astrocyte conductances at postinfection day 3 and day 7 in both uninfected and postinfection day 3 mice. Shown are values for astrocytes that have been activated by inflammation (A1) or have not been activated (A2). Values are means ± SE. ANOVA (3-way) and Bonferroni’s post hoc test. (\( *P < 0.05 \) vs. uninfected, 0–200 \( \mu \text{m} \)).
targets for intracellular Cs\(^+\) voltage-dependent currents, not passive currents, are the first CBX, respectively). Collectively, these results suggest that characteristics negative and positive \(G_i\) increments identified two astrocyte subsets classified by their characteristic negative and positive \(G_i\) increments. We referred to these as A1 and A2 populations, respectively, which is a relatively new approach for determining astrocyte electrophysiological profiles. Specifically, A1 and A2 astrocytes were characterized by dominating inward and outward voltage-dependent currents, respectively, at a \(V_c\) range of \(-90\) to \(0\) mV and the percentages of each population changed dynamically during the course of infection. More A2 astrocytes with outward currents were observed at postinfection day 7, which was the final time point investigated in this study. All activated astrocytes demonstrated increased membrane capacitance and hypertrophy during the course of infection, whereas increased resting \(G_i\) was only evident in A2 astrocytes.

In this study we applied a novel method for the analysis of \(I-V\) relationships in astrocytes. This method takes into account the derivatives of currents recorded at all applied \(V_c\), which allowed us to identify and quantify subtle \(I-V\) declinations evoked by voltage-gated channels. The derivatives of currents were calculated as \(I-V\) slopes, which represented the values of cell input conductance (i.e., \(G_i\)). The negative or positive \(G_i\) changes versus \(V_c\) determined by the sign of basic \(G_i\) slope indicated the direction of the summary current vector through the cell membrane, which was used to distinguish A1 from A2 astrocytes (Fig. 2D). It is highly probable that A1 and A2 current components include K\(^+\) channels underlining the passive conductance shown for hippocampal astrocytes (Seifert et al. 2009; Zhou et al. 2009). If this is taken together with the intrinsic properties presented in Table 1, it can be suggested that the different electrophysiological attributes of A1 and A2 astrocytes may be indicative of their functional specialization in the glial syncytium.

To our knowledge, this is the second study describing detailed electrophysiological parameters of striatal astrocytes in brain slices of adult mice during normal and pathological conditions (Wang et al. 2008). In prior reports, A1 and A2 astrocytes were identified in brain slices of young rats by visual analysis of \(I-V\) tails in the gliotic cortex (Anderova et al. 2004) and striatum (Adermark and Lovinger 2008). The method used in this study should facilitate A1 and A2 astrocyte as well as complex astrocyte identification using a standard mathematical algorithm.

Since \(G_i\) of A1 and A2 astrocytes was altered in opposite directions in response to incremental holding potential, it is probable that similar events may occur during natural fluctuations of astrocytic \(V_m\) in situ. In support of this possibility,
spontaneous astrocytic $V_m$ oscillations evoked by $K^+$ concentration ($[K^+]$) modifications in the extracellular space due to neuronal activity were observed in deeply anesthetized animals (Amzica et al. 2002; Mishima and Hirase 2010). Under such conditions, A1 and A2 astrocytes may reveal temporally distinct $K^+$ permeabilities with identical $V_m$, which would be expected to increase transjunctional voltage and contribute to $V_m$ oscillations. Thus we propose that, if directly connected, A1 and A2 astrocyte pairs may serve as an electrical drive participating in $K^+$ buffering as well as synchronized or desynchronized astrocytic oscillations, although additional studies are needed to test this possibility. When GJC is attenuated during neuroinflammation, the productivity of “electrical drives” should be increased to fit the metabolic needs of the inflamed tissue. By extension, the amplitude and rate of astrocyte membrane currents should be markedly altered, which was observed in the present study at the height of the inflammatory response (i.e., postinfection day 3). The RMP of A1 and A2 populations were quite distinct under similar resting conductance (Table 1), probably reflecting their extreme transitional states that could occur during natural fluctuations. In particular, the amplitude of $G_v$ was dramatically increased in both A1 and A2 astrocytes in closest proximity to the abscess margins. This reflects inward and outward current activation on a “passive” $V_m$ range, which has been observed in other studies, although the entire $V_m$ range was not examined by those investigators (Bordey et al. 2001; Schroder et al. 1999; Wang et al. 2008).

Both astrocytic RMP and $C_m$ returned to values observed in uninfected slices at postinfection day 7, indicating that recovery processes have been initiated at this stage. This timing coincides with a decrease in inflammatory mediator expression within the abscess (Baldwin and Kielian 2004) and the genesis of the fibrotic capsule (Fig. 3B) that serves to sequester the lesion and protect surrounding parenchyma from further destruction (Liberto et al. 2004). A1 and A2 astrocytes displayed preferentially inward and outward voltage-dependent currents, respectively, and their frequencies were inversely correlated at postinfection days 3 and 7 (Fig. 6), which is in agreement with previous studies that utilized other models of brain injury (Anderova et al. 2004; Wang et al. 2008). Given the pronounced degree of parenchymal necrosis during abscess development, it is likely that regional neuronal loss and vascular damage affected A1 and A2 activation. However, at day 7 after infection, the major neuronal source of extracellular $K^+$ and glutamate is expected to be dramatically diminished because of clearance in the extracellular space. The $G_v$ (mainly $K^+$ conductance) decrease observed near the inflammatory site at postinfection day 7 supports the interpretation that decreased extracellular $[K^+] (K^+_e)$ may provoke the reduction in voltage-dependent currents (Fig. 8). Accordingly, fewer A1 cells were observed at this time point in close proximity to the abscess (Fig. 6). At greater distances from the abscess site, the observed changes in A2 astrocytes during inflammation persisted until day 7 after bacterial challenge (i.e., $G_v$ increase), suggesting that membrane modification of ion channels had stabilized in concordance with changes in the abscess microenvironment. On the basis of our findings, we propose that A1 and A2 astrocytes exert different functional properties during neuroinflammation, namely, A1 astrocytes facilitate $K^+$ clearance whereas A2 cells are critical for transporting or propagating ions/metabolites to the vasculature or surrounding brain regions. However, these possibilities remain speculative and should be examined in future studies. Another unresolved issue is whether A1 and A2 astrocytes represent unique populations or, alternatively, the same population that can express different complements of ion channels. We have not observed any evidence of astrocyte proliferation associated with brain ab-

Table 3. Estimated $G_v$ of GFAP-GFP$^+$ striatal astrocytes at different distances from abscess

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>$G_v$ nS</th>
<th>n</th>
<th>% of $G_m$</th>
<th>$G_v$ nS</th>
<th>n</th>
<th>% of $G_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>32.0 ± 2.50</td>
<td>41</td>
<td>23</td>
<td>26.5 ± 3.55</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Day 3 PI</td>
<td>57.0 ± 5.10*</td>
<td>18</td>
<td>37</td>
<td>42.0 ± 4.30</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>Day 7 PI</td>
<td>22.5 ± 6.55</td>
<td>6</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. $G_v$, voltage-dependent conductance. *$P < 0.001$ vs. uninfected slice. 

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cesses in BrdU incorporation studies, whereas significant proliferation of Iba-1+ microglia was detected (unpublished observations). By extension, this supports the conclusion that the same astrocytes are present throughout the course of infection. Because the percentages of A1 and A2 astrocytes differed at days 3 and 7 after \textit{S. aureus} exposure without any changes in the total astrocyte pool, we propose that some A1 cells may acquire A2 characteristics as the inflammatory milieu changes. Since there is currently no reliable way to discriminate between A1 and A2 astrocytes besides their electrophysiological properties, it would be challenging to isolate these subsets and study their responses in vitro. In addition, our data indicate that A1 and A2 phenotype is influenced by the local microenvironment, such that removal of cells would likely change the intrinsic electrophysiological parameters of A1/A2 cells from what is observed in intact slices.

Attenuation of GJC/HC activity by CBX application into the bath solution had no effect on the passive profile of astrocytes, change the intrinsic electrophysiological parameters of A1/A2 microenvironment, such that removal of cells would likely differ at

\begin{itemize}
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