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Gap junction-mediated calcium waves define communication networks among murine postnatal neural progenitor cells

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Abstract

In the postnatal neurogenic niche, two populations of astrocyte-like cells (B cells) persist, one acting as neural progenitor cells (NPCs, B1 cells) and one forming a structural boundary between the neurogenic niche and the striatum (B2 cells, niche astrocytes). Despite being viewed as two distinct entities, we found that B1 and B2 cells express the gap junction protein connexin 43 and display functional coupling involving 50–60 cells. Using neonatal electroporation to label slowly cycling radial glia-derived B1 cells, which send a basal process onto blood vessels, we further confirmed dye coupling between NPCs. To assess the functionality of the coupling, we used calcium imaging in a preparation preserving the 3-dimensional architecture of the SVZ. Inter-cellular calcium waves were observed among B cells. These waves travelled bidirectionally between B1 and B2 cells and propagated on blood vessels. Inter-B cell calcium waves were absent in the presence of a gap junction blocker but persisted with purinergic receptor blockers. These findings show that privileged microdomains of communication networks exist among NPCs and niche astrocytes. Such functional coupling between these two cell types suggests that niche astrocytes do not exert a mere structural role, but may play an active role in shaping the behavior of NPCs.

Keywords

subventricular zone; adult neurogenesis; ependymal cells; astrocytes

Introduction

The adult SVZ is one of two neurogenic zones that persist in the adult brain of all mammalian species examined. The SVZ is the largest neurogenic zone and is located along the wall of the lateral ventricle beneath a layer of ependymal cells (E cells). This neurogenic region contains several cell types, including neural progenitor cells (NPCs), intermediate progenitors, neuroblasts, and a specialized type of astrocytes. Both NPCs and astrocyte-like cells (also called niche astrocytes) express glial fibrillary acidic protein as well as other antigenic markers of astrocytes and are referred under the umbrella term B cells (B1 and B2 cells, respectively) to acknowledge their shared antigenic properties (Liu *et al.*, 2006; Mirzadeh *et al.*, 2008; Wang and Bordey, 2008). However, they appear as distinct entities based on their location and cytoarchitecture. B1 cells exhibit their cell body beneath the

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ependymal layer while B2 cells are located at the SVZ-striatal border (Doetsch *et al*, 1997). B1 cells display epithelial properties including an apical process intercalated between ependymal cells and a basal process ending on blood vessels. The basal processes are of variable length depending on the proximity of the vessels to the ependyma and confer a radial glia-like morphology to NPCs (Mirzadeh *et al*, 2008). B2 cells display a more stellate appearance (Mirzadeh *et al*, 2008) although some have an elongated morphology creating a physical boundary between the SVZ and the striatum (Liu *et al*, 2005).

B1 cells also display intimate contacts with ependymal cells. More specifically, B1 cells and ependymal cells express one astrocytic gap junction protein, connexin 43 (Liu *et al*, 2006; Mirzadeh *et al*, 2008). Staining for connexin 43 was observed at the interface between B1-B1 and B1-E cells. It is, however, unclear whether these homotypic and heterotypic junctions are functional. It is also unknown whether B2 cells express connexins and form a functional coupling network between each other.

We thus explore whether B cells and E cells display functional gap junction coupling. We report dye coupling between B1-B1 and B1-E cells, and unexpected coupling between B1-B2 cells. In addition, connexin 43 expression is enriched in the SVZ in particular at the striatum border where B2 cells are located. We adapted a slice preparation for calcium imaging preserving the 3-dimensional (3-D) architecture of the SVZ. Using this preparation, we found that gap junction-dependent calcium waves travelled between B cells (B1-B1 and B1-B2 cells). These waves also involved propagation on blood vessels. These findings show unexpected large communication networks between B cells, including between NPCs and niche astrocytes. This finding suggests that these two types of B cells can exchange information as well as metabolites through their connectivity to the blood vessels. Further investigation of the role of this functional connectivity between NPC and niche astrocytes cells is warranted.

Materials and Methods

Mice

Research protocols were approved by the Yale University Institutional Animal Care and Use Committee. Experiments were performed in CD1 mice (Charles River Laboratories, MA) and two lines of transgenic mice: human glial fibrillary acidic protein (*hGFAP*)-GFP mice (Jackson Labs) and *hGFAP-tTA/TetO*-MrgA1:GFP mice (noted *hGFAP*-MrgA1:GFP, a gift from Dr. K. McCarthy, Univ. North Carolina at Chapel Hill). In the absence of doxycycline, astrocytes express GFP fused to the MrgA1 receptors (Fiacco *et al*, 2007). All the experiments were performed in postnatal day (P) 17 to P54 in at least three mice.

Connexin 43 immunostaining

Mice were deeply anesthetized with pentobarbital (50 mg/kg). The brain was then quickly removed and placed in 4% paraformaldehyde overnight at 4°C, then washed in 1x PBS. The next day, 100- μ m-thick slices were prepared using a vibratome (Leica VTS 1000). Immunostaining was as previously described (Platel *et al*, 2009). Free-floating sections were blocked in TBS containing 0.1% Triton X-100, 0.1% Tween-20, and 2% BSA and incubated in mouse anti-connexin (1:500, Chemicon, CA) overnight at 4°C. After several washes in TBS containing 0.1% Tween-20, slices were incubated with a secondary antibody (Alexa Fluor series at 1:1,000, Invitrogen). Staining in the absence of the primary antibody did not yield any signal. Staining was replicated at least in 3-4 slices from three different mice. Z-section images were acquired on a confocal microscope (FluoView 1000) with a 20x dry objective (N.A. 0.75) or a 60x oil objective (N.A. 1.42). Images were reconstructed using Imaris 4.0 (Bitplane AG) and Photoshop CS3.

Acute brain slice preparation

Mice were deeply anesthetized with sodium pentobarbital (50 mg/kg). After dissection, sagittal brain slices (250-300 μ m) were prepared in chilled (4°C) dissecting solution (in mM): 25.2 NaCl, 176 sucrose, 2.5 KCl, 5 MgCl₂, 1.2 CaCl₂, 1.25 NaH₂PO₄, 10 Glucose, 26 NaHCO₃, pH 7.4 bubbled with 95% O₂/5% CO₂. Slices were incubated for >1 hr in oxygenated artificial cerebrospinal fluid (aCSF) at room temperature (in mM): 125 NaCl, 2.5 KCl, 1 MgCl₂, 2 CaCl₂, 1.25 NaH₂PO₄, 10 Glucose, and 26 NaHCO₃, pH 7.4. Slices were then transferred to a flow-through chamber and superfused (~1 ml/min) with oxygenated aCSF. The chamber was mounted on the stage of an upright microscope (Olympus BX61) equipped with a laser-scanning confocal head (Olympus Fluoview 300) and a water-immersion Nomarski and fluorescence 60x objective (Numerical aperture 0.9). Experiments were performed at room temperature.

Patch clamp recordings and dye-coupling analysis

Whole-cell patch-clamp recordings were obtained as previously described (Liu *et al.*, 2006; Liu *et al.*, 2005; Young *et al.*, 2010). Patch pipettes pulled from thin-walled borosilicate glass (WPI) had resistances of 6-8 M Ω when filled with an intracellular solution containing (in mM): KCl 110, MgCl₂ 3, EGTA 2, Hepes 10, phosphocreatine kinase 310 units/mg, Na₃-GTP 0.3, Mg-ATP 4, phosphocreatine 10, and 20 μ M Alexa fluor dye 488 or 568. The pH and the osmolarity were adjusted to 7.3 and 290 mOsm, respectively. Whole-cell recordings were performed using an Axopatch-200B amplifier and current signals were low-pass filtered at 2-5 kHz and digitized on-line at 5-20 kHz using a Digidata 1320 digitizing board (Axon Instruments). Cells were held in voltage clamp with a constant -5 pA current injection for 13-17 minutes to allow for dye diffusion.

Z-stack images were acquired on a confocal microscope (FluoView 300) with a 60x water immersion objective (N.A. 0.95). Images were analyzed using Imaris 4.0 (Bitplane AG) or ImageJ 1.39t (Freeware, Wayne Rasband, NIH, USA) and reconstructed in Photoshop CS3. To count the number of coupled cells, we used Cell Counter plugin in ImageJ. The Regions of Interest (ROI) were placed in the center of the cell bodies in the z-section.

Sulforhodamine 101 (SR101) loading and co-localization analysis

SR101 (200-400 μ l) was transcardially perfused in *hGFAP*-MrgA1:GFP mice at 350 μ M prior to slice preparation. For analysis, cells were counted ~10-20 μ m below the slice surface. Cells stained within blood vessels were excluded from the analysis. The ImageJ plugin Cell Counter was used to quantify cells that had fluorescent signals in one or more than one channel.

Calcium imaging and analysis

SVZ cells were loaded by pressure application (<3 psi) of Fluo-4 AM (250 μ M in aCSF, 0.4% Pluronic acid F-127) inside the tissue (Lacar *et al.*, 2010). Time-lapse movies were acquired at 0.30-0.89 Hz with FluoView 300 acquisition software. Most recordings lasted between 3.33 and 5.5 min. Calcium activity was analyzed with custom-coded software CalSignal (written by JCP) (Platel *et al.*, 2007). F_0 (*i.e.* baseline) and F are the mean fluorescence intensities measured throughout all of the ROI and in each ROI, respectively. A change in fluorescence was considered to be a calcium increase if it was >15% F/F_0 .

Time-lapse color representation (TLCR) for calcium waves

Frames in a movie were selected depicting calcium activity propagation. TLCR allows for temporal coding of a movie in a single image. This was performed as follows with a custom-coded script in MATLAB (written by B.L.). Every pixel in a time-lapse movie (typically

512 × 512 resolution) was treated as its own signal. For every pixel, the 8-bit intensity value (0-255) was re-scaled to 0-1. The average signal intensity of all time frames was then subtracted from each individual frame. When there is a salient calcium event that occurs at a particular time point (and only that time point), the resulting intensity would be very high (e.g. 0.9 on the 0-1 scale). If there is no calcium activity, the intensity would be low. The intensity for the individual pixels was then multiplied by RGB values, each representing particular time points, to assign that pixel a color. RGB values are comprised of three numbers, each represented on a 0-1 scale. (Red would be 1 0 0, blue would be 0 0 1, etc.) All color-coded time-series frames were then merged into a single image. Frames that depict high intensity (representing a salient event) will be shown within the spectrum of colors (blue to red), representing when it occurs in the movie (early to late, respectively). Non-salient signals, while still assigned an RGB value, will not be evident by virtue of their resulting low intensity. When salient signals do not appear more than once in the same place, this method provides an accurate representation of the pattern of activity in a movie.

Drugs

Pressure application (<3 psi) was performed using a computer-driven Picospritzer II (General Valve, NJ). Antagonists were bath applied. Suramin and MRS2179 were purchased from Tocris (MO). Fluorescent dyes were purchased from Molecular Probes (USA). All other chemicals except antibodies were purchased from Sigma (MO).

Statistical analysis

Data were presented in Origin 8.0. Statistical significance was determined using the Fisher's Exact test with $p < 0.05$ for significance (KyPlot 2.0). Data are presented as mean $\pm$ standard error of the mean (SEM) unless otherwise indicated. The box plot is represented as: Box = SEM and median, open circle = mean.

Results

Connexin 43 expression is enriched in E and B cells

Using immunostaining in *hGFAP*-GFP mice, we examined connexin 43 expression in the dorso-lateral SVZ. Connexin 43 was highly expressed in E cells along the ventricle, in B1 cells (in particular their radial process, arrow, Fig 1A and B), and in B2 cells (arrowhead, Fig. 1A-D). Connexin 43 staining has recently been reported in ependymal cells and B1 cells further validating our finding (Mirzadeh *et al*, 2008). Cells were identified as B1 based on GFP expression, their location beneath the ependymal layer, and the presence of a radial process extending through the SVZ. B2 cells were identified based on their location in the SVZ and away from the ependymal layer. A region enriched in connexin 43 staining was also visible between the SVZ and striatum (Fig. 1A and C).

Gap junction coupling between B1, B2 and E cells

To explore gap junction coupling among B and E cells, we obtained whole-cell patch clamp recordings to fill B cells with the fluorescent dye Alexa fluor 568 (molecular weight, 730). This dye has limited diffusion through gap junctions and remains more concentrated in the recorded cells than a lower molecular weight dye, thus allowing better visualization of the recorded cell morphology. B cells were recorded in the dorso-lateral SVZ of acute slices from *hGFAP*-GFP mice. Figure 2A and B illustrates several planes above and below the recorded B1 cell (red, arrow) in a coronal section showing dye coupling to GFP⁺ cells in the SVZ. About 80% of the dye coupled cells (excluding ependymal cells) expressed GFP identifying them as B cells (Fig. 2C). 3-D perspectives and drawing in coronal view illustrate the location of the dye filled cells with respect to the ventricular surface (Fig. 2D-

E, G, and H). Alexa 568-coupling is visible between B1 cells (arrows) as well as E cells (blue arrow) and B2 cells (arrowheads) and involved a mean of ~32 B cells (n=4 slices, Fig. 2F). B1 cells were beneath the ependyma and sent a radial glia-like process inside the SVZ. B2 cells were inside the SVZ and at the SVZ-striatal border zone. The location of dye-filled B2 cells is better appreciated on the top-down projection from the 3-D coronal perspective (Fig. 2H). A 3-D perspective drawing illustrates the coupled cells in different views (Fig. 2I).

To further validate that NPCs cells were coupled, we used *in vivo* electroporation of radial glial cells at postnatal day 0-1 located along the lateral ventricle (Fig. 3A). Radial glial cells generate neuroblasts that integrate as interneurons in the olfactory bulb and progressively transform into B1 cells, i.e. the NPCs of the SVZ (Merkle *et al.*, 2004). Because B1 cells are slow cycling, they are expected to retain the electroporated CAG-GFP plasmid while fast cycling cells dilute it and lose GFP expression after successive divisions. Indeed, most GFP⁺ cells at 3-4 weeks post-electroporation displayed a B1 cell morphology characterized by a long radial glia-like process terminating with endfeet on blood vessels (Fig. 3B and D). Patch clamp recordings of GFP⁺ cells with such morphology and beneath the ependymal layer revealed the presence of Alexa fluor 488-coupling to B1 and B2 cells (Fig. 3C and E, schematic diagram in Fig. 3F). A high-power image of the coupled cells inside the tissue illustrates the extensive blood vessel coverage formed by the dye-coupled cells (Fig. 3G). Collectively, these data show that B1 and B2 cells are coupled and form a communication network that bridges the ventricular surface to blood vessels.

An en-face view of the SVZ in acute slices to image B1 cell coupling and calcium activity

To better image the extent of B1 cell coupling, we prepared acute sagittal slices that contain an “en-face” view of the dorso-lateral SVZ with the ependymal layer on top (Fig. 4A and 5A). In addition, to better visualize the extent of coupling we used the Alexa fluor 488 dye, which diffuses better than the Alexa fluor 568 due to its smaller molecular weight (570). A B1 cell just underneath the ependymal layer was filled with the Alexa fluor 488 (arrow, Fig. 4A and D). Three individual sections and a 3-D-en face view with projections are shown in Figure 4A and B, respectively. Coupling involved a mean of ~60 B cells in a 40-50 μ m-thick optical section (n=4 slices, Fig. 4C). The dye diffused to other B1 cells located close to the ventricular surface presumably through their lateral processes, which are visible on the individual section (Fig. 4A). The coupling faded as a function of the distance as illustrated using pseudo-coloring (Fig. 4D). In addition, coupled B1 cells form clusters along the ventricular surface (Fig. 4D).

Such an en-face preparation was chosen to image calcium activity as it preserves the 3-D architecture of the SVZ. We previously reported preferential B-cells loading using the calcium indicator Fluo-4 AM pressure applied inside the tissue (Lacar *et al.*, 2010; Young *et al.*, 2010). To further identify Fluo-4-loaded cells as B cells, we used sulforhodamine 101 (SR101) in either CD1 wild-type mice or *hGFAP*-MrgA1:GFP mice, in which the outlines of B cells are GFP-fluorescent (Holm *et al.*, 1997; Kafitz *et al.*, 2008; Platel *et al.*, 2010a; Young *et al.*, 2010). In acute slices, the majority (74.3%) of the Fluo-4-fluorescent cells were SR101-positive (n=4 slices, n=404 cells) (Supplemental Fig. 1A-C). In *hGFAP*-MrgA1:GFP slice, 90% of SR101-positive SVZ cells were GFP-fluorescent, identifying SR101-positive cells as B cells (n=5 slices, n=338 cells, Supplemental Fig. 1D and E). Ependymal cells were SR101-positive, but they were morphologically distinguishable from B cells and did not display spontaneous calcium activity (Genzen *et al.*, 2009). Thus the majority of Fluo-4-loaded SVZ cells were identified as B cells.

Intercellular calcium waves engage B1 and B2 cells

A representative en-face view of Fluo-4-loaded B cells in the SVZ is shown in Figure 5A. Fluo-4-labelled B1 cells, which are located underneath the ependymal layer, were seen in proximity to Texas red-dextran (TR-dextran)-loaded capillaries as illustrated in the 3-D perspective and projections (Fig. 5B). Calcium waves occurred between B1 cells adjacent to a capillary and lasted ~10 s (Fig. 5C, white box in 5B). These waves travelled at a mean speed of 4 $\mu\text{m/s}$ (n=28 waves, 11 slices, Fig. 5E) and involved ~5 B cells in a single optical plane (Fig. 5F).

In addition to B1-B1 waves, calcium propagations between B1 and B2 cells were also visible (Fig. 6). B1 (arrow) and B2 cells (arrowheads) are visible in the 3-D en-face perspective, which also contains red fluorescent capillaries and lipid-droplet-containing ependymal cells (Fig. 6A and B). A calcium wave travelled from a cell beneath the ependymal layer to B1 and B2 cells in ~10 s (Fig. 6C-E). We created time lapse color representation (TLCR) to allow depiction of a calcium imaging movie in a single image (Fig. 6D). Inter-B cell waves occurred at a frequency of 1.1 wave/min (n=17 waves, 8 slices, Fig. 6F) and were observed in 86% of the 3-6 min-long recordings (12/14 slices).

Inter-B cell calcium waves propagate on blood vessels and are gap junction-dependent

One characteristic of the inter-B calcium waves was the localization of calcium activity around blood vessels (Fig. 7A-C). TLCR illustrates the time and direction of travel as well as the en-passant engagement of several B cell processes along the blood vessel. time-lapse color representation (TLCR) also allows better appreciation that calcium waves travelled bidirectionally along the vessels. Calcium waves covered ~40 μm on blood vessels (n=29 waves, 11 slices, Fig. 7D).

We examined whether calcium waves were eliminated in the presence of meclofenamic acid (MFA, 100 μM) known to block gap junctions in B cells (Liu *et al*, 2006). Calcium wave frequency was significantly reduced in the presence of MFA, suggesting that MFA significantly blocked waves between B cells (Fisher's Exact test, $p \leq 0.01$, waves occurred in 1/5 slices in MFA versus 19/21 in control, Fig. 7E). P2Y receptors are expressed in SVZ cells and they contribute to wave propagation in other cell types including astrocytes (Fiacco and McCarthy, 2006; Lin *et al*, 2007; Wang and Bordey, 2008; Weissman *et al*, 2004). We thus tested the P2Y1 receptor blocker MRS 2179 (30 μM), and the broad spectrum P2 receptor blocker suramin (100 μM) that affect waves among astrocytes. Inter-B cell calcium waves persisted in the presence of the P2Y1 (MRS 2179, n=4/5 slices) and P2 receptor blockers (suramin, n=4/4 slices), respectively, Fig. 7E). Nevertheless, suramin and MRS 2179 significantly reduced calcium responses induced by UTP (100 μM , 30 s, n=3 slices) and the P2Y1 agonist MRS 2365 (100 nM, 10 s, n=3 slices) in B cells, respectively (data not shown). These data suggest that calcium waves among B cells are gap junction-dependent.

Discussion

Findings presented here demonstrate for the first time that B cells, including B1-B1 and B1-B2 cells form gap junction-mediated communication networks at the vascular interface in the SVZ.

Dye-coupled cells essentially include B cells based on their expression of GFP in *hGFAP*-GFP mice and ependymal cells. This network mediated through gap junctions involves 50-60 B cells in a $\sim 50 \times 320 \times 320 \mu\text{m}^3$ volume. In fact, 50-80 μm can cover the entire distance from the striatum to the lateral wall in our slices. Ependymal cells identified based on their cuboidal morphology and the presence of moving cilia on the phase-contrast image also participated in the gap junction network.

Coupled B cells but not E cells displayed active communication through calcium waves. The lack of calcium waves from B to E cells remains unclear. Between B cells, the waves involved both B1-B1 and B1-B2 cell propagation. Calcium waves did not involve ependymal cells even though these cells were dye-coupled to B1 cells. Calcium waves essentially involved 5-7 cells in a single optical section while coupling involved 50-60 B cells with the dye used in 3-dimension. It is expected that calcium waves would involve 15-21 cells in a 3-dimensional space, which is still lower than the dye-coupling spread. One explanation of such discrepancy is that the calcium spread may involve mechanisms not only based on calcium diffusion. Consistent with the presence of dye coupling, connexin 43 expression in B cells, these waves were dependent on the gap junction blocker MFA but not P2Y1 receptor activation as shown in other astroglial preparations (Fiacco and McCarthy, 2006; Wang and Bordey, 2008; Weissman *et al*, 2004). However, additional experiments using selective connexin 43 blockers or genetic manipulations would be required to conclude that these waves are mediated by connexin 43 gap junctions because MFA is not a selective blocker of gap junctions or connexin 43 (e.g. (Anderson *et al*, 2009)). These waves occurred spontaneously as previously shown in embryonic radial glia (Weissman *et al*, 2004). It remains to be examined in future studies whether other factors such as ambient GABA, prostaglandin E2 from the choroid plexus or locally, or even various neuronal inputs in the SVZ trigger or regulate these waves (Bolteus and Bordey, 2004; Liu *et al*, 2005; Platel *et al*, 2010b; Dave *et al*, 2010). Based on the preferential Fluo-4 loading of B cells, it is not possible to examine whether neuroblasts were activated “en passant”. Nevertheless, it is highly conceivable that inter-B cell calcium waves activate neuroblasts “en passant” through the calcium-dependent release of diffusible signals such as glutamate (Platel *et al*, 2010a).

One unexpected characteristic of the calcium waves is their propagation on blood vessels. Other groups have reported the presence of a large blood vessel network in the SVZ and examined the structural relationships between B1 cells and blood vessels in the SVZ (Mirzadeh *et al*, 2008; Shen *et al*, 2008; Snapyan *et al*, 2009; Tavazoie *et al*, 2008). Here, we reveal dynamic, intercellular calcium signaling at the B cell-vascular interface. On blood vessels, calcium waves may travel in astrocytic endfeet, pericytes (for capillaries), smooth muscle cells (for arterioles) or endothelial cells. In some instances, a process between the astrocytic endfeet and the vessel was visible on the phase-contrast image. This process was not loaded with the calcium indicator and was likely from a pericyte suggesting that the calcium wave propagated in the B cell endfeet. In addition, B cell processes were activated en-passant during the wave propagation suggesting that such calcium waves essentially involved B cells, i.e. GFAP⁺ cells. Such calcium waves between B cells travelling on blood vessels may have major functional implications. They could regulate glucose and metabolite uptake for transfer through the B cell network and control the release of active signals onto smooth muscle cells or endothelial cell, thus regulating the permeability of the blood brain barrier in the neurogenic niche (for review see (Wang and Bordey, 2008)). Finally, the restriction of the waves to a relatively small group (5-7 in a single optical section, expected 15-21 in 3-dimension) of B cells suggests the existence of functional microdomains close to blood vessels in the SVZ. These microdomains could be activated independently in terms of calcium activity and perhaps leading to synchronized proliferation and migration.

Collectively, this study provides evidence that in the neurogenic postnatal SVZ NPCs and niche astrocytes form communication networks that have a privileged location at the vasculature interface. This finding challenges previous concept that views B1 cells as an individual unit distinct from B2 cells. In addition, B2 cells may not only play a mere structural role separating the SVZ from the striatum, but may also actively shape the behavior of NPCs through functional coupling and calcium waves.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. B1 and B2 cells of the SVZ express connexin 43

(**A and C**) Confocal photographs of connexin 43 immunostaining (red) and GFP fluorescence (green) in coronal sections from *hGFAP-GFP* mice. The dotted lines delineate the SVZ. The arrow and arrowheads point to B1 and B2 cells, respectively, that both express connexin 43. Intense connexin 43 expression is visible in ependymal cells lining the lateral ventricle (LV). Connexin 43 is also enriched in a zone separating the SVZ and the striatum. (**B and C**) Zooms of the images in the white squares shown in (A and C). Scale bars: 50 μm (A and C) and 30 μm (B and D). Scale bars: 70 μm (A and C) and 50 μm (B and D)

Figure 2. Gap junctional coupling between B1-B1 and B1-B2 cells in the SVZ
(A and B) Images of coronal views of Alexa 568 (red), DIC, and overlay with GFP from *hGFAP*-GFP (green) at different depth inside the slice. **(C)** Box plot of the % of Alexa fluo 568⁺ cells that are GFP⁺ in sections from *hGFAP*-GFP mice. **(D)** Diagram illustrating the slice orientation. **(E)** Confocal 3-D perspective of Alexa fluor 568-coupled cells shown in (A). The white arrows point to B1 cells, the arrowheads to B2 cells and the blue arrow to ependymal cells. **(F)** Box plots of the # of coupled B cells using the Alexa fluor 568 dye. **(G)** Diagram illustrating the 3-D perspective of Alexa 568-coupled cells shown in (E). **(H)** Coronal view of 3-D perspective and top-down projection of Alexa fluor 568-coupled GFP⁺ cells shown in (B). The top-down view illustrates the location of presumed B2 cells inside the tissue while the somata of B1 cells are located close to the ventricle. **(I)** 3-D diagram illustrating the coronal, en-face, and top-down views of the coupled cells shown in A, E, and H. Box: SEM and median, open circle: mean. Scale bars: 20 μm (A-H).

Figure 3. Slow-cycling B1 cells with projections on capillaries display dye coupling to B2 cells
(A) Diagrams illustrating time points and corresponding locations of SVZ-born cells following electroporation at P1. At >3-4 weeks post-electroporation, slow-cycling neural progenitor cells persist in the SVZ while neuroblasts have integrated in the granule cell layer (GCL) of the olfactory bulb (OB). **(B)** Photograph of GFP fluorescence from a CAG-GFP plasmid and Texas-Red dextran (TR-dextran) fluorescence in capillaries prior to patch clamp recording of the B1 cell marked with the arrow in an acute sagittal slice from a 3 week-old animal electroporated at P1. **(C)** Photograph of the same cells as in (B) following patch clamp recording of the B1 cell filled with the Alexa fluor 488 dye. The recorded cell is dye-coupled to surrounding cells (diffuse green fluorescence). **(D)** Photograph of GFP or RFP fluorescence pseudo-colored white (from a CAG-GFP or -RFP plasmid) and Alexa fluor 633 dextran fluorescence prior to patch clamp recording of the B1 cell marked with the arrow in an acute sagittal slice from a 3 week-old animal electroporated at P1. **(E)** Photograph of the same cells as in (D) following patch clamp recording of the B1 cell filled with the Alexa fluor 488 dye. The recorded cell is dye-coupled to surrounding B1 cells including deeper cells in the tissue, presumably B2 cells. **(F)** Diagram illustrating the red fluorescence from CAG-RFP, the green fluorescence from the Alexa 488 dye and the red-green overlay (yellow) of the recorded cell. **(G)** Zoom of the image shown in the white square in (E).

Figure 4. En-face view of Alexa 488-coupled B1 cells

(A) Montage of en-face views of Alexa 488 (green) and DIC overlay at different depth inside the slice. The white arrow points to a presumed B1 cell, which was dye filled during patch clamp recording. (B) Confocal en-face 3-D perspective of Alexa fluor 488-coupled cells shown in (A). The coronal (left) and top-down (bottom) projections illustrate that the recorded cell and some of the dye coupled cells have their somata located along the lateral ventricle (dotted line). (C) Box plots of the # of coupled B and B1 cells using the Alexa fluor 488 dye in an en-face view section. (D) Confocal image of highly coupled B1 cells in the 3-D en-face perspective using intensity color representation. Intensity color scale bar: 0 to 255 (saturation). The arrow points to the recorded cell. Box: SEM and median, open circle: mean. Scale bars: 50 μm (A) and 40 μm (B).

Figure 5. B1-B1 calcium waves

(A) Composite photograph of an en-face view of Fluo-4 and TR-dextran fluorescence overlaid with DIC. (B) 3-D en-face perspective of Fluo-4 and TR-dextran from the composite slice shown in (A). The coronal and top-down projections illustrate the depth of the Fluo-4 loaded cells (B1 cells along the ventricular surface) and the red fluorescent capillaries. The ependymal cells were not loaded with Fluo-4. The boxed region is spatially and temporally expanded in (C). (C) Fluo-4 fluorescence at four time-points illustrating a calcium wave that propagates between B1 cells close to a capillary. (D) Calcium activity graph of the region of interests (ROI) 1 to 4 shown on the image in (C). (E and F) Box plot of the speed of the wave (E) and the number (#) of cells participating in a wave (F). Scale bars: 30 μm (A and B), 20 μm (C).

Figure 6. B1-B2 calcium waves

(**A and B**) Confocal photographs of an en-face view of Fluo-4 and TR-dextran (A) and TR-dextran overlaid with the DIC image (B). (**C**) Fluo-4 fluorescence at several time-points illustrating a calcium wave that propagates from B1 to B2 cells contacting a capillary. (**D**) TLCR of the calcium wave shown in (C). (**E**) Calcium activity graph of the ROI 1 to 5 shown on the image in (C). (**F**) Box plots of the number (#) of waves per min. Scale bars: 40 μm (A and B) and 20 μm (C and D).

Figure 7. B cell-calcium wave propagate on capillaries and are gap junction-dependent
(A) Fluo-4 fluorescence at several time-points illustrating a calcium wave that propagates on a capillary and involved calcium activity in B cells along the capillary. **(B)** TLCR of the wave shown in (A). **(C)** TLCR of another calcium wave propagating on a capillary. **(D)** Box plots of the distance travelled on blood vessels. **(E)** Bar graphs of the % of slices displaying calcium waves under control conditions and in the presence of MFA, MRS2179, and suramin. Scale bars: 30 μm (A-C).