Communication via gap junctions underlies early functional and beneficial interactions between grafted neural stem cells and the host

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Contributed by Richard L. Sidman, January 5, 2010 (sent for review November 25, 2009)

How grafted neural stem cells (NSCs) and their progeny integrate into recipient brain tissue and functionally interact with host cells is as yet unanswered. We report that, in organotypic slice cultures analyzed by ratiometric time-lapse calcium imaging, current-clamp recordings, and dye-coupling methods, an early and essential way in which grafted murine or human NSCs integrate functionally into host neural circuitry and affect host cells is via gap-junctional coupling, even before electrophysiologically mature neuronal differentiation. The gap junctions, which are established rapidly, permit exogenous NSCs to influence directly host network activity, including synchronized calcium transients with host cells in fluctuating networks. The exogenous NSCs also protect host neurons from death and reduce such signs of secondary injury as reactive astrogliosis. To determine whether gap junctions between NSCs and host cells may also mediate neuroprotection in vivo, we examined NSC transplantation in two murine models characterized by degeneration of the same cell type (Purkinje neurons) from different etiologies, namely, the nervous and SC47 mutants. In both, gap junctions (containing connexin 43) formed between NSCs and host cells at risk, and were associated with rescue of neurons and behavior (when implantation was performed before overt neuron loss). Both in vitro and in vivo beneficial NSC effects were abrogated when gap junction formation or function was suppressed by pharmacologic and/or RNA-inhibition strategies, supporting the pivotal mediation by gap-junctional coupling of some modulatory, homeostatic, and protective actions on host systems as well as establishing a template for the subsequent development of electrochemical synaptic intercellular communication.

Results

Differentiation and Integration of Grafted NSCs. To examine the stages of exogenous NSC integration into host neural cytoarchitecture, we initially used a rodent striatal organotypic slice culture system (4) into which were grafted early passages of a stable clonal population of murine NSCs (mNSCs) (5). To track and record from individual cells in situ, a subclone was engineered to overexpress green fluorescent protein (GFP). Grafted mNSCs migrated throughout the organotypic cultures (OCs) (Fig. 1, Fig. S1, and Movie S1). Whereas at 7 days postimplantation, ~25% expressed nestin (a marker of the undifferentiated state) (Fig. S1) and ~20% expressed fibrous glial acidic protein (GFAP), an intermediate filament protein and an astroglial and progenitor cell marker (Fig. S1), by 28 days in vivo (DIV), few mNSC-derived cells were nestin+ (0.1%) or GFAP+ (0.6%) (Fig. S1 and Table S1). Expression of the early neuronal marker β-III tubulin (Tuj1) increased in these mNSCs from 39% (7 DIV) to 71% (28 DIV) (Fig. S1 and Table S1) and expression of the more mature neuronal marker MAP2b increased from 4 ± 1% (7 DIV) to 19 ± 4% (28 DIV). Of endogenous cells in the OCs, ~40% were Tuj1+ throughout the culture period (Fig. S1) and showed no change in resting membrane potential (Vm) or membrane resistance (Rm) over that time span. Grafted mNSCs, by contrast, displayed significant developmental differences in both Vm and Rm (Fig. S2). Although unengrafted mNSCs (n = 10) displayed no active membrane properties (Na+/K+ channel activity), by 28 DIV, 66% of mNSCs did (n = 32), indicative of differentiation toward a neuronal...
Grafted NSCs Establish Functional Gap Junctions with OC Cells. As the influence of mNSCs on Ca^{2+} waves greatly preceded development of mature neuronal properties and reciprocal electrochemical transynaptic signaling, some antecedent mechanism must have allowed earlier intercellular communication. We postulated that this mechanism might involve formation of gap junctions (6–8). Located on the plasma membrane and composed of connexin (Cx) proteins, gap junctions are clusters of channels connecting the interiors of coupled cells through which molecules < 1 kDa may be exchanged. Therefore, we examined expression of Cxs in NSCs and OCs, focusing on Cx26 (Fig. 1 I–J) and Cx43 (Fig. 2 and Fig. S2 L). Cxs prevalent during neural development (6–11).

Before grafting, ~50% of mNSCs expressed Cx43 and <10% expressed Cx26 (Fig. S3). Within 3 days of grafting, the proportion of mNSCs expressing Cx43 increased to 92 ± 1% (n = 60 OCs) (Fig. 2 A and B) and Cx26 to 24 ± 2% (Fig. 1 H–I) (n = 29 OCs). As early as 2–18 h after grafting, mNSCs started to form functional gap junctions with host cells via Cx43 (Fig. 2 D–L, and Fig. S4).

Interestingly, Cx expression in grafted mNSCs appeared to have a developmental dynamic that paralleled that of the maturing cell type identities of mNSC derivatives in the OCs. For example, when most of the NSC-derived cells (70 ± 2%; n = 214) were still at a NSC stage (nestin+), the majority of them were Cx43+ (92 ± 1%). Instead, by 7 DIV, when only 25% of the mNSC-derived cells were nestin+ and nearly 40% had become Tuj1+, Cx43 expression decreased to 35% of the mNSC-derived cells (n = 75) (Fig. 2 A and Table S1). By 28 DIV, when the mNSCs had predominantly begun to assume a neuronal phenotype, <5% (n = 44) of donor mNSC progeny expressed Cx43 (Fig. 2 and Movie 5) (although all surrounding host astrocytes did so; n = 62). Cx43 was commonly seen in host striatal OCs cells and colocalized with nestin or GFAP. Whereas Cx43 appeared to be most closely associated with immature NSCs and/or non-neuronal cells, Cx26, by contrast, appeared to be most closely associated with neurally differentiated mNSCs, their predominant fate by 28 DIV (Fig. 1 I–J): 98 ± 2% of mNSC-derived Tuj1+ cells (n = 54) and 96 ± 4% of host-derived Tuj1+ cells (n = 177) coexpressed Cx26. Cx26-containing gap junctions were observed between grafted mNSC and host neurons (n = 48), as well as between grafted mNSC themselves (n = 51) and between host neurons themselves (n = 189) (Fig. S1 and Movie S4). Hence, while Cx43 was prominent in the “early” stages of the engrafted OCs’ “development” and decreased with time, Cx26 expression increased.

The observations described above for murine NSCs applied as well to bFGF-propagated fetal human NSCs (hNSCs), i.e., NSCs from a different species, expanded and maintained under different conditions. hNSCs also expressed Cx43 upon initial contact with OC cells (Fig. 2 C and Movie S8), exhibited functional intercellular communication, and demonstrated a similar pattern of spontaneous and inducible Ca^{2+} flux (Fig. 2 G and H, Fig. S4, 2–18 h after grafting, mNSCs started to form functional gap junctions with host cells via Cx43 (Fig. 2 D–L, and Fig. S4).

![Fig. 1. Grafted mNSC and host cell interactions in fluctuating networks are blocked by inhibiting gap junction function. (A) Communication between grafted mNSCs (GFP+, green arrowhead and endogenous OC cells tracked by ratiometric [Ca^{2+}] imaging. (B) Typical [Ca^{2+}] response, after 14 DIV, of mNSC (no. 1) and endogenous OC cells (nos. 2–6) in A. Note that no spontaneous [Ca^{2+}] events are detected during control conditions (aCSF) or during ATP application. Glutamate causes an instantaneous rise in [Ca^{2+}] and induces temporary coupled oscillations in both mNSCs and OC cells. [Ca^{2+}] traces (gray box in A) are enlarged in C. The mNSC trace is green; overlapping events are marked with dotted lines I–VII. (D) Activity plot showing even variations in [Ca^{2+}] transients in all parts of the field (Movie S3). (E and F) mNSC (green cells in E and green arrowhead in F) and OC cells (white arrowheads in F) characterized by time lapse [Ca^{2+}], imaging (F). Curves in G depict the [Ca^{2+}] response of cells 1–3 in F. Overlapping events are marked with dotted lines I–VII. Glutamate causes an instantaneous rise in [Ca^{2+}] and induced temporary coupled oscillations in both mNSCs and OC cells. Addition of gap junction inhibitor CBX disrupts the synchronous communication and uncouples the successive low frequency fluctuations. (H and I) Intercellular gap-junctional contacts (here Cx26) between GFP+ (green) mNSCs themselves (red punctate immunopositivity in H); between mNSCs and OC cells; and between host OC cells themselves (white punctate immunopositivity in I) (Movie S4). White arrows in I mark gap-junctional plaques between Tuj1+ mNSC-derived cells and OC cells. (Bars: 20 μm, A, E, F, and I; 5 μm, H).](image_url)
and Movie S6 and S7). Ca\(^{2+}\) waves propagated between cells at 30 ± 6 \(\mu \text{m/s}\) (\(n = 6\) OCs), comparable to that observed in OC.

To confirm that gap junctions established between grafted and host cells were functional, standard dye transfer studies were performed (detailed in SI Text). Briefly, donor mNSCs or hNSCs were loaded with gap junction–permeant dyes (e.g., calcein or lucifer yellow), transfer of which to host OC cells was first documented and then suppressed by the pharmacologic gap junction blocking agents carbenoxolone (CBX; 100 \(\mu \text{M}\)) or 18-\(\alpha\)-glycyrrhetinic acid (C18-\(\alpha\)-GA; 50 \(\mu \text{M}\)) (Fig. 2 D–I and Fig. S4). Pharmacological inhibition of gap junctions not only blocked dye transfer from murine and human NSCs to host cells in vitro (Fig. 2 D–I and Fig. S4 G–L) but also inhibited coupled Ca\(^{2+}\) transients (Fig. 1 E–G, Fig. S4 D–F, and Movie S7), suggesting that gap-junctional coupling was integral to early intercellular cross-talk and coordination.

**Donor NSCs Improve Host Cell Health in OCs via Gap Junction Formation and Function.** Although slice cultures are essential for electrophysiological assessments of network functions, it is known that preparation of OCs is a traumatic perturbation involving axotomy with consequent cell death, reactive astrogliosis, and scarring—undesirable but necessary and accepted “collateral damage” (4). Unexpectedly, we observed that, in OCs in which donor NSCs had become integrated via gap junction formation, the baseline proportion of host OC cell death was reduced by 74% compared with OCs without grafted NSCs (0.58 ± 0.67% vs. 2.27 ± 0.58% in nongrafted OCs, \(n = 42\) and 41, respectively, \(P = 0.008\)) (Fig. 3 A–C). Accordingly, after 72 h, the overall survival of OCs grafted with mNSCs (\(n = 76\)) was significantly higher (\(P = 0.03\)) compared with control cultures (\(n = 72\)). Similarly, high GFAP expression in host cells, conventionally used to monitor the degree of astrogliotic reaction to the mechanical trauma of establishing the slice, was reduced in grafted slices at 7 and 14 DIV (\(P = 0.004\) and \(P = 0.008\), respectively) (Fig. 3 D–F and Fig. S1 D–F).

Pharmacological inhibition of gap junction function abrogated the beneficial impact of murine or human NSCs on host cells. For example, the proportion of GFAP+ host cells was 30 ± 6% following hNSC engraftment (\(n = 8\) OCs), but rose nearly 2-fold to 51 ± 4%, the degree of astrogliotic reaction seen in nongrafted OCs (Fig. 3 D–F and Fig. S1 F), if CBX (50 \(\mu \text{M}\)) was applied during the first 3 days postengraftment.

To control for off-target actions of the pharmacologic inhibitors, we complemented these gap junction function-blocking studies by suppressing gap junction formation through silencing Cx26 and Cx43 in mNSCs via RNA interference (RNAi) (Fig. 3 G–L, Fig. S5, and SI Text). We first established an optimal level of RNAi suppression needed to ablate mNSC viability without compromising their cell numbers or differentiation. Compared to mock-RNAi–transfected mNSCs (“Mock” and “Ctrl-RNAi”, respectively), Inhibition of Cx43 ranging from 30% to 70% blocked mNSC-mediated beneficial actions (e.g., the antigliotic actions) in a dose-dependent manner. Interestingly, Cx26 suppression alone had little impact on blunting this mNSC action (Fig. 3 I and Fig. S5D), suggesting that mNSC therapeutic actions are principally mediated by the developmentally earlier Cx43.
Gap junctions are necessary for beneficial effect of NSCs. (A–C) mNSCs (GFP*, green) reduce host OC cell death. The proportion of necrotic OC cells in nongrafted cultures (Non-graft.) (A) was decreased in grafted cultures (Graft.) (B) by 74% (C), indicated by a decrease in PI+ (red) cells (white arrowheads) in A and B. DAPI marks all nuclei blue. (D–F) Functional gap junction formation between hNSCs and OC cells (demonstrated transfer of calcein dye) is associated with a decrease in astrogliosis. (D) OC after engraftment with hNSCs, preloaded with calcein (green) and prelabeled with Dil (white). Note Dil’/calcein’ hNSCs (yellow arrowheads) and calcein’ (green) host cells (white arrowheads). (E and F) Host astrogliosis is reduced where gap junctions have been formed by hNSCs, an action blocked by CBX. (G) mNSCs, including those transfected with a scrambled nonfunctional siRNA (“Ctrl-RNAi”), grafted to OCs reduce GFAP expression in OC cells at 7 DIV. (H) Suppression of Cx43 in mNSCs by functional RNAi (Cx43i) blunted their ability to decrease OC gliosis in a dose-dependent manner, as quantified in I: Cx43i+ was more potent than Cx43i+. Suppression of Cx26 (Cx26i) had no impact. Control OCs (Ctrl) were grafted with mNSCs pre-treated with mitomycin. In “Mock” OCs, mNSCs were exposed to the same procedures as RNAi-transfected mNSCs but without siRNA being transfected. (J) Cx43 mRNA in mNSCs was reduced as shown at 2, 4, and 7 days posttransfection. (K) RNAi did not change mNSCs survival postgrafting (normalized to “Mock”; 100 ± 22%). “Ctrl-RNAi”: 116 ± 25% of mock; “Cx43i”: 112 ± 23% of mock. Similarly, their differentiation profile was unchanged (L); expression of nestin, GFAP, and Tuj1 by mNSCs transfected with Cx43i (black histograms) vs. Ctrl-RNAi (gray histograms). Only the mNSCs’ antgliotic effect was abrogated: Note that the suppression of Cx43i in J is correlated to the mNSC effect on gliosis in I. *P < 0.05; **P < 0.01; ***P < 0.001. (Bars: 100 μm; A–D, 20 μm; E–H).

Fig. 3. Gap junctions are necessary for beneficial effect of NSCs. (A–C) mNSCs (GFP*, green) reduce host OC cell death. The proportion of necrotic OC cells in nongrafted cultures (Non-graft.) (A) was decreased in grafted cultures (Graft.) (B) by 74% (C), indicated by a decrease in PI+ (red) cells (white arrowheads) in A and B. DAPI marks all nuclei blue. (D–F) Functional gap junction formation between hNSCs and OC cells (demonstrated transfer of calcein dye) is associated with a decrease in astrogliosis. (D) OC after engraftment with hNSCs, preloaded with calcein (green) and prelabeled with Dil (white). Note Dil’/calcein’ hNSCs (yellow arrowheads) and calcein’ (green) host cells (white arrowheads). (E and F) Host astrogliosis is reduced where gap junctions have been formed by hNSCs, an action blocked by CBX. (G) mNSCs, including those transfected with a scrambled nonfunctional siRNA (“Ctrl-RNAi”), grafted to OCs reduce GFAP expression in OC cells at 7 DIV. (H) Suppression of Cx43 in mNSCs by functional RNAi (Cx43i) blunted their ability to decrease OC gliosis in a dose-dependent manner, as quantified in I: Cx43i+ was more potent than Cx43i+. Suppression of Cx26 (Cx26i) had no impact. Control OCs (Ctrl) were grafted with mNSCs pre-treated with mitomycin. In “Mock” OCs, mNSCs were exposed to the same procedures as RNAi-transfected mNSCs but without siRNA being transfected. (J) Cx43 mRNA in mNSCs was reduced as shown at 2, 4, and 7 days posttransfection. (K) RNAi did not change mNSCs survival postgrafting (normalized to “Mock”; 100 ± 22%). “Ctrl-RNAi”: 116 ± 25% of mock; “Cx43i”: 112 ± 23% of mock. Similarly, their differentiation profile was unchanged (L); expression of nestin, GFAP, and Tuj1 by mNSCs transfected with Cx43i (black histograms) vs. Ctrl-RNAi (gray histograms). Only the mNSCs’ antgliotic effect was abrogated: Note that the suppression of Cx43i in J is correlated to the mNSC effect on gliosis in I. *P < 0.05; **P < 0.01; ***P < 0.001. (Bars: 100 μm; A–D, 20 μm; E–H).

In the second model, the nervous (nr) mutant mouse, the same PN cell type degenerates from a different etiology and at an earlier age and more rapid pace. In addition, the murine NSCs (C17.2) were prepared entirely differently as monolayers of clonal neural progenitors in which self-renewal was enhanced. The nr mice, most mNSCs degenerate by 22–35 days of age because of a series of dysregulated intracellular signaling pathways downstream of tissue plasminogen activator (tPA) (13). mNSCs transplanted at birth into the premorbid nr cerebellum can rescue most PNs and their projections from impending death in early adulthood (reflected morphologically and in preserved rotarod function) if, and only if, direct cell–cell contact is made between the NSC and the PN (1). Pathologically high levels of tPA within the PN are returned to normal following transplantation of, and direct contact with, wild-type NSCs; this normal tPA level, in turn, restores equipoise to downstream intracellular pathways regulating neurotrophic factor processing and mitochondrial function (1, 13). Interestingly, gliosis and inflammation are also decreased. Because neuronal rescue in the nr brain paralleled that in the SCA1 brain, and both recalled observations in the slice culture system, we again sought evidence for gap-junctonal coupling between engrafted NSCs and host PNs. Indeed, in the adult cerebellar cortex of mNSC-transplanted nr mice, precisely in the regions where exogenous mNSCs had integrated (typically in the internal granular layer [GL]) (Fig. 5 C and E) and were interfacing with numerous rescued host PN somata (Fig. 5 D and F) (Fig. 4 in ref. 1), Cx43 immunoreactivity was abundant on rescued PN somata which, in turn, were surrounded by mNSC-derived Cx43+ cell processes (Fig. 5 C–D), indicative of gap-junctonal coupling between the integrated mNSCs processes and the rescued endogenous PN somata, much as described above in the SCA1 mouse. Also as in the SCA1 mouse, the cerebellar cortices of adult wild-type mice and adult untransplanted nr mice evinced low or no expression of Cx43 adjacent to PN cell bodies (Fig. 5 A and B). Although some integrated mNSCs expressed Cx26 in their cell bodies, no Cx26 protein was detected in the mNSCs processes and the rescued endogenous PN somata, much as described above in the SCA1 mouse. Also as in the SCA1 mouse, the cerebellar cortices of adult wild-type mice and adult untransplanted nr mice evinced low or no expression of Cx43 adjacent to PN cell bodies (Fig. 5 A and B). Although some integrated mNSCs expressed Cx26 in their cell bodies, no Cx26 protein was detected in the mNSCs processes and the rescued endogenous PN somata, much as described above in the SCA1 mouse.
expression in mNSCs and the gap junctions that they enable with adjacent PNs appeared to play a critical, and possibly indispensable, role in mNSC-mediated rescue of impaired endogenous neurons.

Discussion

The critical questions posed by this study are by what mechanism exogenous NSCs (i) come to participate in the host nervous system’s physiological properties, and (ii) protect host neurons from impending cell death.

Gap Junctions Mediate Early Functional Integration.

Formation of functional gap junctions appears to be a very early step wherein grafted NSCs, their progeny, and host cells establish communication via Ca++ even before electrophysiological maturation has been consummated (Fig. 1A–G and Fig. S2). The differentiation of an NSC into a neuron during the first 4 weeks postgrafting is accompanied by a progressive decrease in resting membrane potential (consistent with an increase in Na+/K+ channels) (6), a decrease in Rm, and voltage-dependent spiking. However, the trains of repetitive action potentials characteristic of fully mature neurons are not yet evident by the end of that period, nor is synaptotagmin, which would indicate presence of the functional machinery for Ca++-dependent, fast synaptic vesicle exocytosis and neurotransmitter release underlying fast synaptic transmission. Similarly, strong synaptic input to the NSCs is not yet detected, a finding consistent with recent data indicating that newborn rodent neurons in vivo do not begin receiving synaptic input until 21–30 days after division, acquiring mature excitatory inputs only after 60 days (14). Nevertheless, connexin-

composed gap-junctional intercellular networks, which emerge rapidly following engraftment and develop in parallel with the maturation of NSCs into neurons, appear to be sufficient to begin establishing functional cross-talk, as suggested by their influence on host Ca++ waves and the downstream intercellular actions so induced. Time-lapse Ca++ imaging confirms direct communication similar to that seen during normal CNS development (8, 15). Intercellular spread of synchronized Ca++ waves after implantation of mouse or human NSCs (Fig. 1A–G, Fig. S2 F–K, and S4 D–F, Movies S3, S6, and S7, and Table S2) has a speed and frequency consistent with...
with the establishment of functional intercellular gap junctions (15, 16) and is blocked by suppression of gap junction function. Additional evidence that the gap-junctional couplings between NSC derivatives and host cells are functional is offered by the classical defining assay for transfer of gap junction–permeable dyes, egress blocked by gap-junction antagonists (Fig. 2 D–L and 3 D and E and Fig. S4 G–L). Such functional gap junctions can be established within 2–18 h after grafting. Thus, we propose that gap-junctional intercellular networks are an early form of communication that precedes and sets the stage for later electrochemical synapses and “traditional” electrophysiological communication (17) between grafted and host cells, mimicking the connexin-mediated interaction of endogenous progenitors with their neighboring cells during CNS development.

Gap Junctions Are Essential Participants in NSC Protection of Host Neurons in Vitro and in Vivo. Exogenous NSCs can protect or rescue endogenous neurons at risk for degeneration, as first shown after spinal cord injury and established in neurodegenerative conditions in mice (13) and monkeys (2). The mechanisms underlying these events have been only partially characterized. Although diffusible factors have typically been implicated (1–3), we have begun to recognize that direct contact of NSCs with host cells is an important underappreciated feature of NSC action (15). In OCs, connexin-associated gap junction formation and function was pivotal for ensuring host cell well-being (Fig. 3 and Fig. S1). In vivo, NSC-mediated rescue of imperiled host neurons did not occur when gap junction formation was suppressed (Fig. 5 G–J). Future studies will explore how early establishment of functional gap junctions mediates rescue. It is likely that such coupling permits direct transcellular delivery of factors that promote survival and/or neutralize pathologized molecules or processes. Alternatively, effective gap-junctional gating could prevent widespread passage of toxic molecules from cell-to-cell across an organ. Grafted NSCs may form gap-junctional couplings with reactive astrocytes, so that preservation of neurons could be a downstream effect of this interaction.

The role of astrocytes in early stages of neuroprotection is increasingly recognized, and initial NSC-astrocyte interactions could, in addition to subsequent NSC–neuron couplings, be important especially during early stages in donor-host interactions.

That gap junction formation plays a neuroprotective role in at least two neurodegeneration models characterized by different etiologies suggests that gap junction–mediated actions might intervene at final common death pathways. That these long-lasting translationally relevant actions are not restricted solely to murine NSCs in neurogenetic diseases is suggested by preliminary findings that gap junction–associated Cx43 expression appears also to characterize the beneficial influence of human NSCs (prepared as neurospheres) on host cervical spinal motor neuronal fibers in adult rats subjected to cervical spinal cord contusion whose respiratory and motor function improves after transplantation (Fig. S8). Therefore, we are led to conjecture that such NSC-associated gap junction–mediated improvement is not limited to a particular host species and age, NSC preparation or source, region of CNS insult, or initiating pathophysiological event, and hence may have broad applicability.

Although the present study focused on Ca2+ signaling, other signaling molecules—such as cAMP, ATP, and Ins[1,4,5]P3 (10), as well as other ions, amino acids, and even polypeptides and micro-RNAs—are also gap junction permeant and may influence Na+, Ca2+, and metabolic activity, dissipate K+ and neurotransmitter gradients, and directly control gene expression or antigen presentation (10, 11, 18). Although beyond the scope of this report, such hypotheses are now testable. (See also SI Text.)

In conclusion, this study suggests that one of the first, and possibly essential, ways in which grafted NSCs integrate functionally into host neural circuitry is via early functional gap-junctional coupling permitting transcellular delivery of homeostasis-modulating molecules as well as directly influencing host network coordinated activity via Ca2+ waves. Such actions, when recapitulated in pathological conditions, may benefit otherwise endangered neuronal populations. The molecular substrate for gap junction formation between NSCs and host cells appears to evolve in culture along a developmentally appropriate trajectory, Cx43 preceding Cx26 in the neuronal maturation of NSCs; interestingly, it is the earlier expressed, Cx43, that seems most pivotal in the NSC-mediated rescue actions.

Methods

Details of the experimental procedures and protocols are provided in SI Text. For mNSCs, two different types of isolation, propagation and maintenance techniques were used. NSCs were isolated from the SVZs of 4–8 week-old FVB/N mice transgenic for GFP transcribed from a ubiquitous promoter (12). A stable, well-characterized, clonal population of engraftable mNSCs (C17.2) was used (1, 3, 5).

“Primary” hNSCs from three different populations were assessed, all initially isolated from the telencephalic ventricular zone (VZ) of 13- to 11-week human fetal cadavers (2, 3) and SI Text.

Striatal and cerebellar organotypic cultures were obtained from P0–P3 and P9 rodents and cultured by the roller drum and Stoppini methods, respectively, as described in detail elsewhere (1) and in SI Text. Whole-cell patch-clamp recordings were performed from NSC-derived and host cells in the OCs after 7, 14, 21, and 28 DIV. (Ca2+), fluctuations were measured by time-lapse Ca2+ imaging in grafted and nongrafted OCs with Ca2+ indicator Fluo-4 and the ratiometric Ca2+ dye Fura-2 AM (Invitrogen) (SI Text). “Gliotic ratio” is the percentage of GFAP+ host cells in OCs divided by %GFAP+ host cells in the OCs. Ca2+ waves and nerve models and experimental procedures are detailed in SI Text and elsewhere (5, 12). RNA interference (RNAi) for Cx26 and Cx43 (10–100 nM) and RNAi controls; scrambled siRNA, GAPDH siRNA, and nontransfected cells are detailed in SI Text Data are presented as mean ± SEM.

ACKNOWLEDGMENTS. We thank Ruth Deflottson for technical assistance and Seiger and Wahlberg for providing human fetal tissue. Supported by the Swedish Research and Stockholm County Councils, KL, SFF, Freemason Child, M&M Wallenberg, Swedish Medical Association, Heart and Lung and European Science Foundations, NLM Family Fund, PALS, A-T Children’s Project.