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Neural Cell Activation by Phenolic Compounds from the Siberian Larch (*Larix sibirica*)

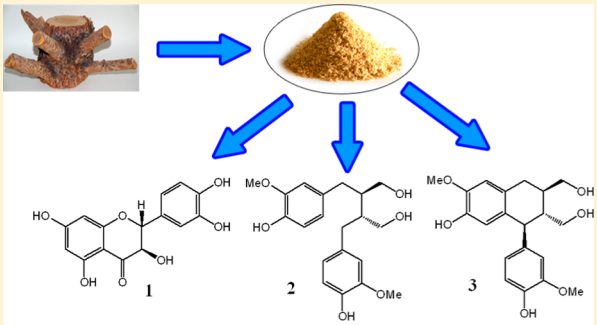
Gabriele Loers,[†] Dmitry V. Yashunsky,[‡] Nikolay E. Nifantiev,*[,‡] and Melitta Schachner*[,§]

[†]Institut für Biosynthese Neuraler Strukturen, Zentrum für Molekulare Neurobiologie Hamburg, Universitätsklinikum Hamburg-Eppendorf, Falkenried 94, 20251 Hamburg, Germany

[‡]Laboratory of Glycoconjugate Chemistry, N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prospect 47, 119991 Moscow, Russian Federation

[§]Center for Neuroscience, Shantou University Medical College, 22 Xin Ling Road, Shantou, Guangdong 515041, People's Republic of China

ABSTRACT: Small organic phenolic compounds from natural sources have attracted increasing attention due to their potential to ameliorate the serious consequences of acute and chronic traumata of the mammalian nervous system. In this contribution, it is reported that phenols from the knot zones of Siberian larch (*Larix sibirica*) wood, namely, the antioxidant flavonoid (+)-dihydroquercetin (**1**) and the lignans (−)-secoisolariciresinol (**2**) and (+)-isolariciresinol (**3**), affect migration and outgrowth of neurites/processes from cultured neurons and glial cells of embryonic and early postnatal mice. Compounds **1**−**3**, which were available in preparative amounts, enhanced neurite outgrowth from cerebellar granule neurons, dorsal root ganglion neurons, and motoneurons, as well as process formation of Schwann cells in a dose-dependent manner in the low nanomolar range. Migration of cultured astrocytes was inhibited by **1**−**3**, and migration of neurons out of cerebellar explants was enhanced by **1**. These observations provide evidence for the neuroactive features of these phenolic compounds in enhancing the beneficial properties of neurons and reducing the inhibitory properties of activated astrocytes in an in vitro setting and encourage the further investigation of these effects in vivo, in animal models of acute and chronic neurological diseases.



Neural cell activating phenols from Siberian larch

Natural phenol compounds have attracted attention because of their antioxidant, immunoadjuvant, and anti-inflammatory effects, as well as a variety of antitumor activities related to antiestrogenic action.[1−5] Also in the few past years, certain phenols have been identified as potential neuroprotective compounds, pointing to one of the greater challenges in drug discovery today. These compounds scavenge free radicals and change transcription of genes leading to protection of neurons or neuroblastoma cells in vitro against oxidative stress or glutamate-induced toxicity.[6−8] The compound 7,8-dihydroxyflavone inhibits the death of hippocampal neurons at concentrations as low as 10−250 nM by inducing dimerization and autophosphorylation of the tyrosine kinase receptor B in vivo.[9] However, only limited information is available on the effects of phenolic substances on glial cells other than activation of antioxidant defense mechanisms. Zhang et al. showed that the natural stilbenoid resveratrol promotes the release of neurotrophic factors from astroglia, and De Nicoló et al. reported that intraperitoneal injections of a mixture of olive oil phenols containing hydroxytyrosol as the main component enhanced levels of nerve growth factor and brain-derived neurotrophic factor in brain regions implicated in learning and memory.[10,11] The growth arrest of C6 rat glioma cells was observed in the presence of resveratrol and quercetin in the micromolar range, but the growth of astrocytes appeared not to be affected.[12] In addition to their antioxidant activity and their modulatory effect on neurotrophic factors, for some phenols such as quercetin, dieckol, and resveratrol and its hydroxylated derivative, piceatannol, the ability to influence the migration of non-neural cells has been reported.[9,13−17] In contrast, the influence of phenols on neurite outgrowth and migration, process formation of Schwann cells, and migration of activated astrocytes has rarely been observed or has not been investigated.

In the present study, neural cell activation by the flavonoid (+)-dihydroquercetin (**1**) and two lignans, (−)-secoisolariciresinol (**2**) (an isomer of (+)-secoisolariciresinol from flaxseeds[18]) and (+)-isolariciresinol (**3**) (also known as cyclolariciresinol according to the IUPAC nomenclature of lignans[19]), is described. All three compounds were extracted from the knot wood zones of Siberian larch [*Larix sibirica* Ledeb. (Pinaceae)]. Among these natural products, compounds **1** and **2** are of particular interest because of their high

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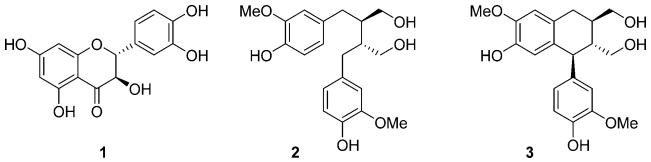
antioxidant activity.[20] Flavonoid **1** is currently used as a component in the production of more than 200 biologically active food additives, cosmetics, and other products. In addition to its antioxidant activity, lignan **2** exhibits also a considerable potential for the pharmaceutical, veterinary, and food industries, because the intestinal microflora can metabolize it into enterolactone,[21,22] a well-known mimetic of the mammalian estrogen 17β-estradiol. Lignan **2** has been isolated from different wood parts previously,[23,24] but only recently it became available in preparative amounts by isolation from the knot regions of larch wood.[25] The carbocyclic lignan **3** is a minor component of the extracts from Siberian larch knotwood but can be prepared chemically from 7-hydroxymatairesinol,[26] the dominant lignan in the knot wood of Norway spruce, which is currently produced in industrial amounts.[24]



There is a need to discover new compounds that counteract neural degeneration, for instance, in Alzheimer's and Parkinson's diseases and after acute injury, such as spinal cord injury or stroke. In these devastating afflictions, neurons not only suffer from oxidative stress but also from the presence of inhibitory or toxic molecules, such as amyloid beta, neurofibrillary tangles, myelin debris, loss of neurotrophic and glial support, excitotoxicity, and ischemia, to name just some of the types of cognate damage. Neurons are important targets for treatment of acute nervous system injuries and neurodegenerative diseases and also in the context of inflammation as a focus in multiple sclerosis and amyotrophic lateral sclerosis. Compounds that ameliorate the consequences of neuronal loss are therefore much needed as a complement to presently available but limited possibilities for cure.

The aim of this study was therefore to investigate whether compounds **1**–**3** not only exhibit neuroprotective effects through scavenging of free radicals but also stimulate other neuronal functions such as migration and neurite outgrowth. In addition, the effects of these compounds on glial cells were considered worthy of investigation, since these cells are essential in many different aspects in their positive and negative influences on neurons.

RESULTS AND DISCUSSION

Phenols **1**–**3** stimulated neurite outgrowth of primary cerebellar neurons from early postnatal mice in a concentration-dependent manner (Figure 1). These compounds were active in a range from 100 pM to 1 μM, and all three phenols tested enhanced neurite outgrowth in a comparable manner. Maximal stimulation of neurite outgrowth by 1.6-fold compared to the DMSO vehicle control was achieved at a 100 nM concentration. The application of higher concentrations of phenols (1 μM) did not further enhance neurite outgrowth but also had no adverse effect on the viability of cerebellar neurons. In contrast to cerebellar neurons, motoneurons showed enhanced neurite outgrowth in the range from 1 to 200 nM concentration of **1**–**3**, but at higher concentrations (500 nM) neurite outgrowth was reduced to control levels (Figure 2). Of note, compounds **1**–**3** stimulated neurite outgrowth of

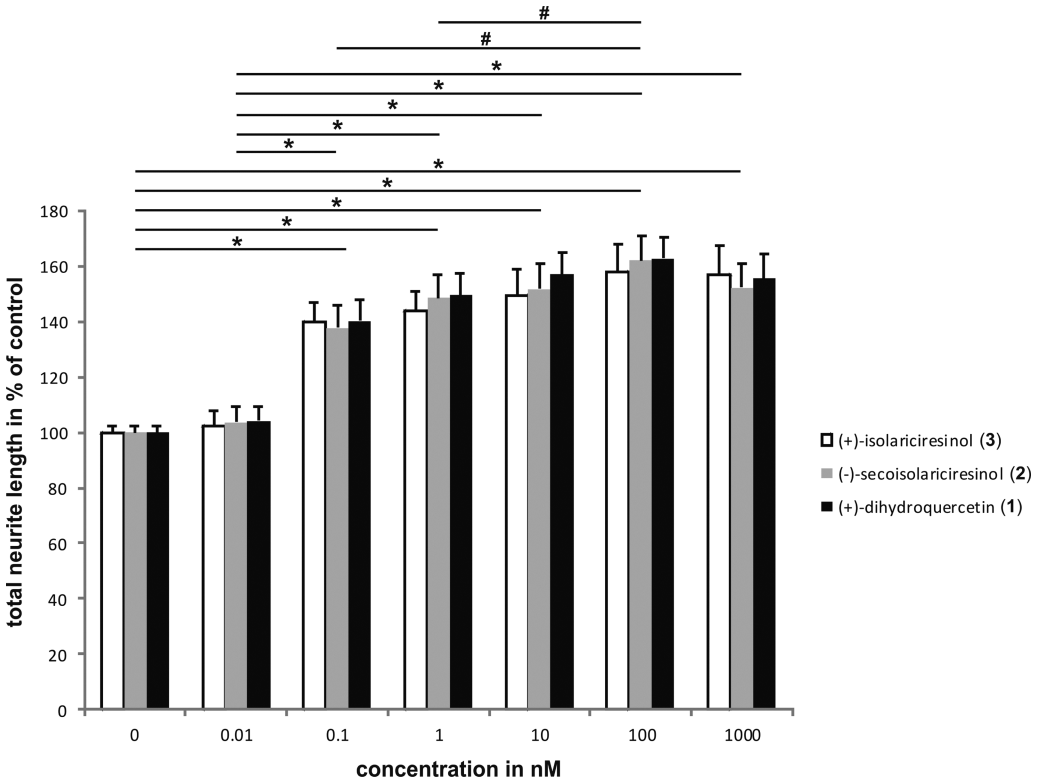


Figure 1. Phenols **1**–**3** stimulate neurite outgrowth of cerebellar granule neurons. Cerebellar neurons were isolated, seeded onto PLL substrate, and treated with the compounds at the indicated concentrations. Neurite outgrowth was determined after 24 h in vitro. Mean values ± SEM are shown ($^{\#}p < 0.05$, $^{*}p < 0.001$).

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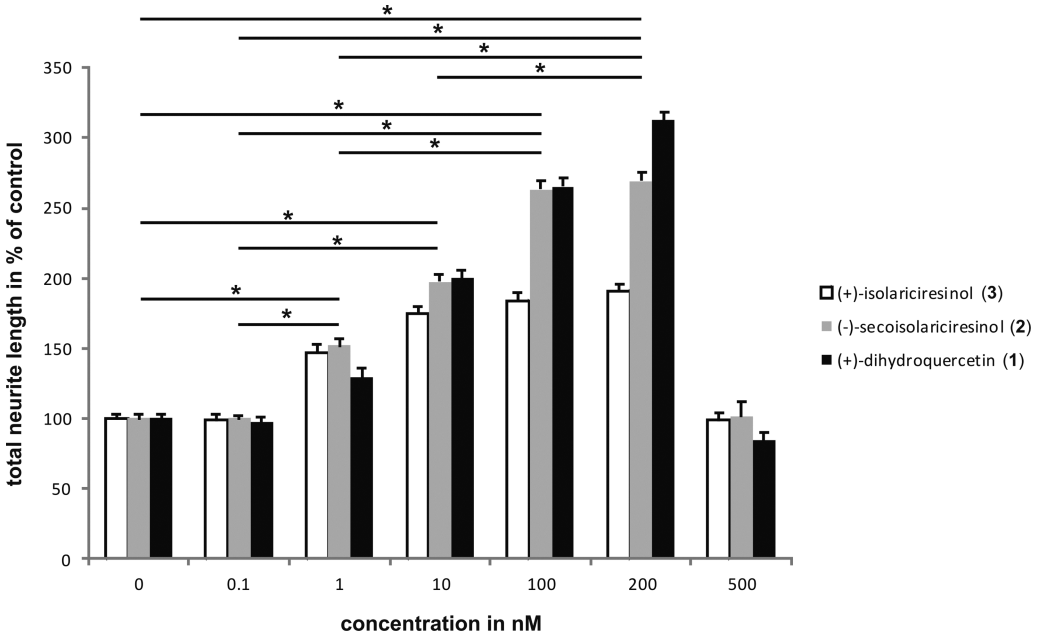

Figure 2. Phenols **1**–**3** stimulate neurite outgrowth of motoneurons. Motoneurons were isolated, seeded onto PLO substrate, and treated with compounds at the indicated concentrations. Neurite outgrowth was determined after 24 h in vitro. Mean values ± SEM are shown (*p < 0.001).

motoneurons to different degrees: maximal neurite outgrowth was stimulated 1.9-fold (compared to the DMSO vehicle control) with (+)-isolariciresinol (**3**), 2.7-fold with (−)-secoisolariciresinol (**2**), and 3.1-fold with (+)-dihydroquercetin (**1**). In cultures of dorsal root ganglion neurons the three phenols stimulated neurite outgrowth by a factor of 1.7 (Figure 3). These results show that especially (+)-dihydroquercetin (**1**) and (−)-secoisolariciresinol (**2**) enhance neurite outgrowth more actively than the previously described cardanol and curcuminoids, which are only active in the micromolar range and achieved maximal stimulation of neurite outgrowth by 2-fold. For instance, Tobinaga et al.[27] showed that cardanol

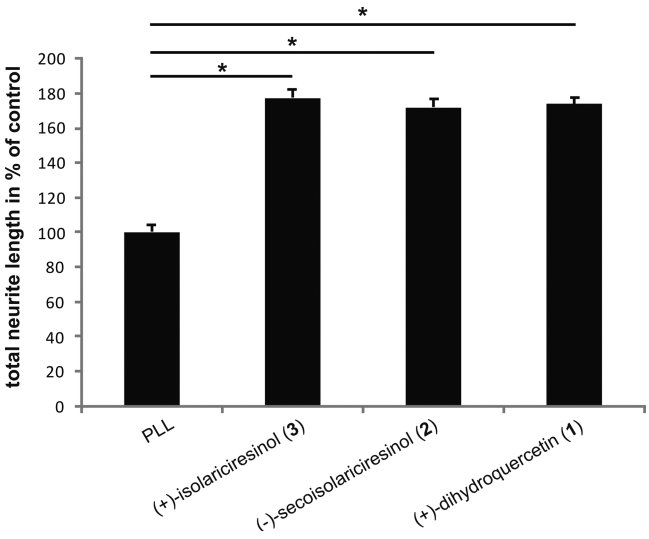

Figure 3. Phenols **1**–**3** stimulate neurite outgrowth of dorsal root ganglion neurons. Dorsal root ganglion neurons were isolated, seeded onto PLL substrate, and treated with compounds at a 100 nM concentration. Neurite outgrowth was determined after 24 h in vitro. Mean values ± SEM are shown [*p < 0.001 difference from control (PLL)].

(ginkgol) extracted from *Ginkgo biloba* leaves enhanced the growth of NSC-34 immortalized motoneuron-like cells (1.4-fold) and improved working memory-related learning in rats when administered chronically. Moreover, certain curcuminoids were shown to promote neurite outgrowth in PC12 cells at micromolar concentrations, and the total neurite length was increased by 1.4- to 2.2-fold.[28]

In previous studies, opposite effects of plant phenols on the migration of neurons were reported. Despite its neuroprotective effect in the neuronal cell culture and in a Parkinson's disease model,[29,30] (−)-epigallocatechin-3-*O*-gallate from green tea inhibited the migration of neurons from neurospheres.[31] Treatment of mice with olive oil phenols has led to elevated levels of neurotrophic factors in the hippocampus and olfactory bulbs, which enhanced neuronal migration and survival in vivo.[11] On the basis of these findings, phenols **1**–**3** were investigated for stimulation not only of neurite outgrowth but also of neuronal migration. Cerebellar explants were maintained in culture and monitored for migration of cells out of these explants (Figure 4). Lignans **2** and **3** enhanced the total number of migrating neurons by 1.2-fold, while flavonoid **1** enhanced the total number of migrating neurons by 1.4-fold. In comparison, in the DMSO vehicle control-treated explants, 72% of all cells migrated 1–150 μm, 25% of cells migrated 151–300 μm, and only 3% of cells migrated farther than 301 μm away from the explant edge. After treatment with (+)-isolariciresinol (**3**), 68% of all cells migrated 1–150 μm, 24% migrated 151–300 μm, and 8% migrated more than 301 μm. Treatment with (−)-secoisolariciresinol (**2**) led to a similar result with 65% of all cells migrating 1–150 μm, 27% of all cells migrating 151–300 μm, and 8% of all cells migrating more than 301 μm. After treatment with (+)-dihydroquercetin (**1**), only 59% of all cells stayed close to the explant border at a distance of 1–150 μm, 29% of all cells migrated 151–300 μm, and 12% of all cells migrated farther than 301 μm (Figure 4). These results show that the Siberian larch phenols investigated not only stimulate neurite outgrowth, but also support neuronal migration, with (+)-dihy-

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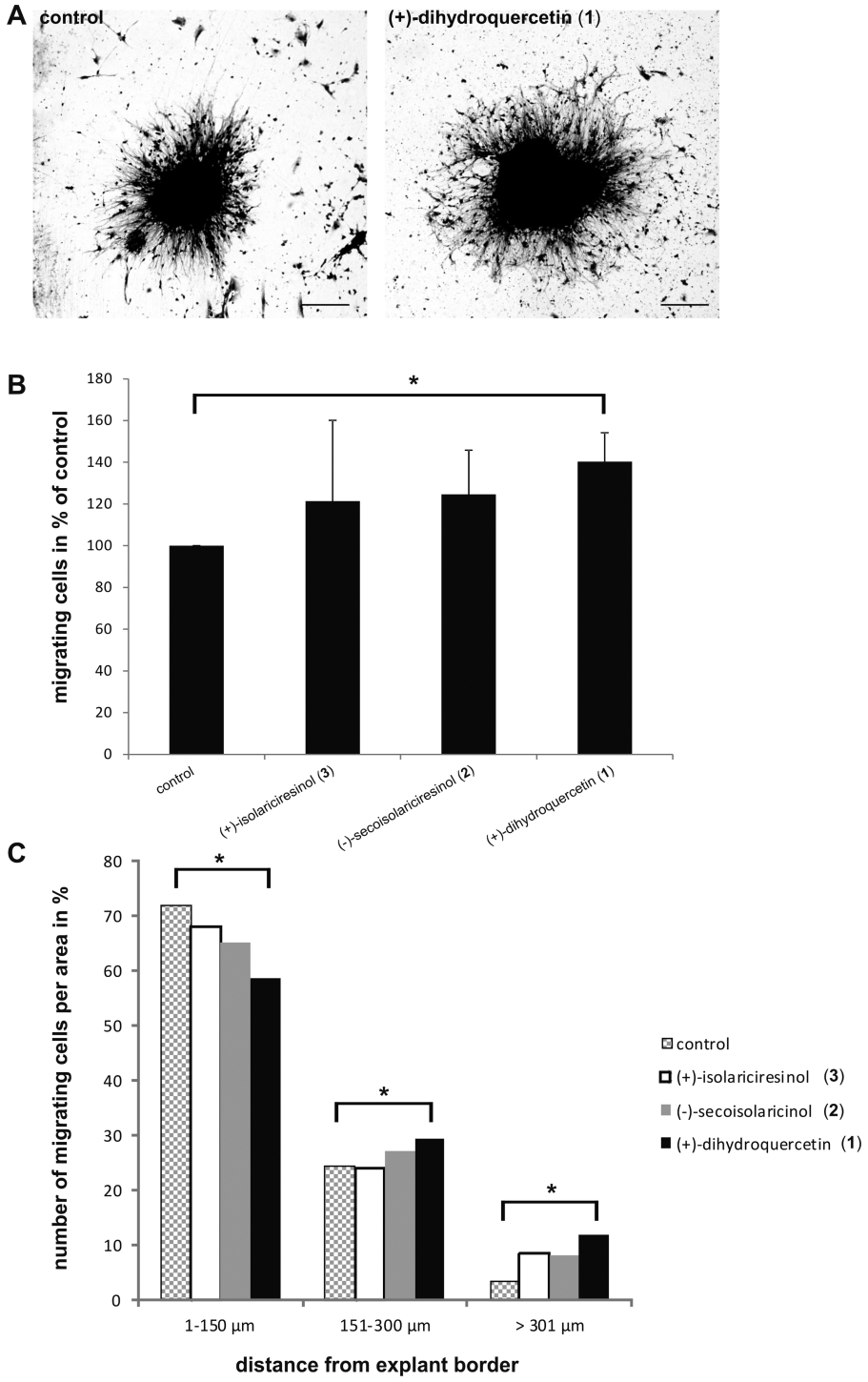

Figure 4. (+)-Dihydroquercetin (**1**) enhances the migration of neurons. Cerebellar explants were isolated, seeded onto coverslips coated with PLL, and treated with the test compounds (100 nM). Neuronal migration was determined after 48 h in vitro. (A) Representative images of an explant maintained in the presence of DMSO control (left) or (+)-dihydroquercetin (right). The scale bars depict 100 μm. (B) The total number of migrated cells per explant was counted and is shown in % of control. (C) The number of cells at a distance of 0−150, 151−300, and >301 μm from the explant border was determined and is shown as % of the total number of cells that had migrated. Mean values ± SEM are shown (*p < 0.001 difference from control).

droquercetin (**1**) being slightly more active than the two lignans (**2** and **3**) evaluated.

The modulatory effects of phenols on astrocytes and microglia, which induce neuroprotection by release of neurotrophic factors, up-regulation of the antioxidant defense, and inhibition of release of pro-inflammatory cytokines,[10,32,33] have attracted increasing attention in recent years. However, their effect on oligodendrocytes or Schwann cells has so far not been a focus of investigation. An extract from *Codonopsis pilosula* (also known as "Dang Shen" or "poor man's ginseng"), containing high amounts of phenolic glycosides, phenolic acids, and sterols, induced proliferation and migration of RSC96

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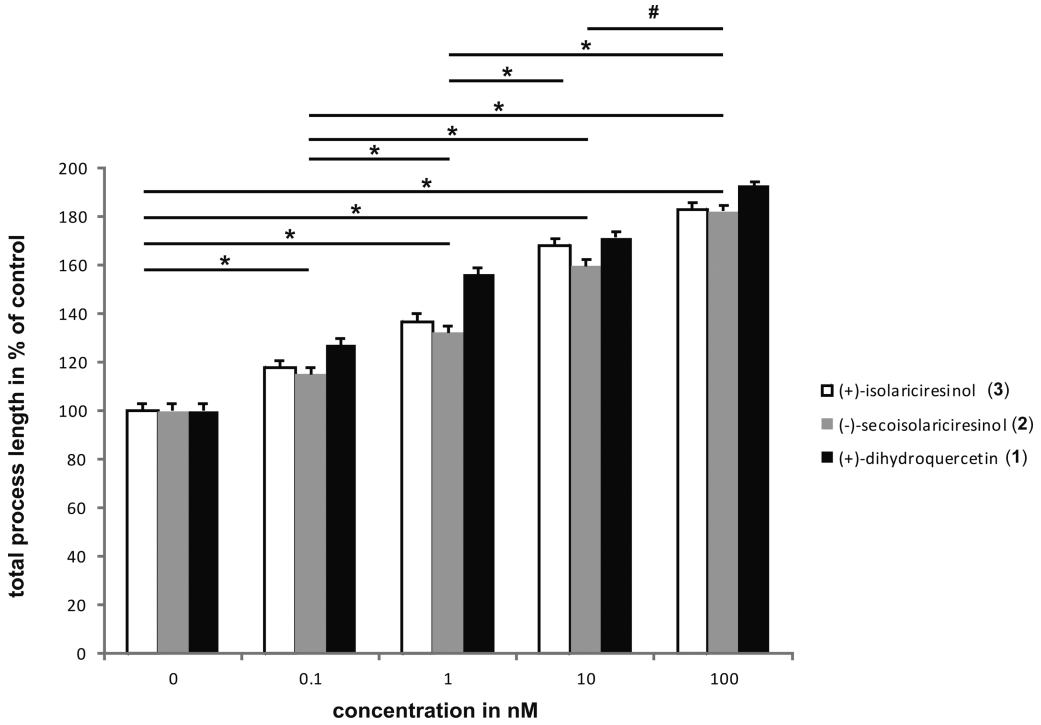

Figure 5. Phenols **1**–**3** enhance Schwann cell process formation. Schwann cells were isolated, seeded on PLL substrate, and treated with compounds at the indicated concentrations. After 24 h, the process formation was determined. Mean values ± SEM are shown ($^{\#}p < 0.05$, $^{*}p < 0.001$).

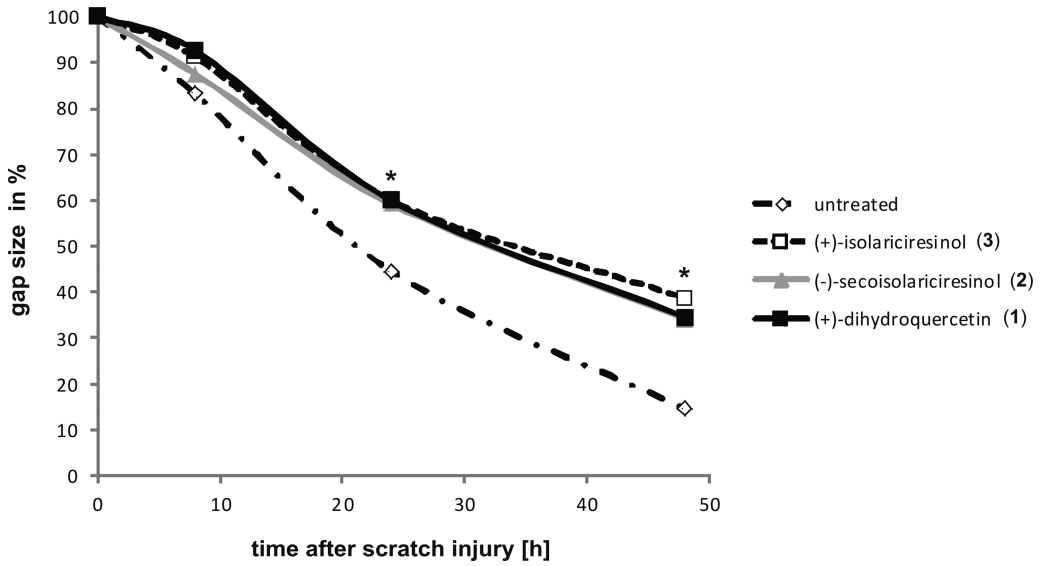

Figure 6. Phenols **1**–**3** reduce migration of astrocytes after scratch injury. A confluent monolayer of astrocytes was injured by scratching, resulting in a cell-free cleft (gap) approximately 850 μm wide, and compounds were added at 100 nM concentration. Directly after injury (0 h) and 8, 24, and 48 h after injury photographs were taken, and the size of the cell free cleft was determined. Mean values are shown ($^{*}p < 0.001$).

Schwann cells,[34] and quercetin was shown to enhance outgrowth and survival of cultured Schwann cells at 0.3–33 μM concentrations.[35] To analyze whether phenols **1**–**3** affect not only neuronal properties but also glial properties, they were analyzed for their effect on the process formation and process elongation of Schwann cells, which are the myelin-forming cells of the peripheral nervous system. Phenols **1**–**3** stimulated Schwann cell process formation/elongation in a concentration-dependent manner between concentrations of 100 pM and 100 nM (Figure 5). A maximal 1.8-fold enhancement in process length was observed after treatment with (−)-secoisolariciresinol (**2**) and (+)-isolariciresinol (**3**), and a 1.9-fold enhancement in process length was observed after treatment with (+)-dihydroquercetin (**1**). Higher concentrations of phenols **1**–**3** (up to 1 μM) did not enhance the process formation/elongation further but also had no adverse effects on Schwann cell viability (data not shown). This result shows that compounds **1**–**3** are active at lower concentrations than quercetin, raising the possibility that they prevent axonal and myelin degeneration and support the synthesis and maintenance of peripheral nervous system myelin by enhancing

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Schwann cell process of outgrowth and survival after acute and chronic injury.

Glial cells play important roles in injury and diseases of the nervous system; for example, after spinal cord injury the development of the glial scar contributes to the inhibition of axonal regrowth across the lesion site, thereby preventing successful functional recovery. In neurodegenerative diseases, inflammatory processes play an important role in the pathogenesis of the disease, and inflammatory components related to neuroinflammation in Alzheimer's disease include brain cells such as microglia and astrocytes.[36,37] Astrocytes in Alzheimer's disease form a corona at the perimeter of the neuritic halo that surrounds a dense core of an amyloid beta deposit, and activated astrocytic processes cover and interdigitate neurites in a manner reminiscent of glial scarring.[38,39] Plaque-associated astrocytes deposit proteoglycans and can thereby create a barrier preventing microglial attack that could clear the amyloid beta deposit.[39]

On the basis of these reported observations, phenols **1**−**3** were investigated for migration modulation of activated astrocytes after scratch injury. Astrocytes under control conditions (untreated or treated with vehicle control) migrated more quickly and farther than astrocytes treated with these phenols (Figure 6). After 48 h, control astrocytes had migrated by 700 μm, closing the cell-free gap to 85%, whereas astrocytes treated with phenols **1**−**3** had migrated only 500−550 μm, leaving a cell-free area of 35−40% of the original size. There was no significant difference in the speed of migration or distance moved between astrocytes treated with the three phenol compounds (Figure 6). Comparable effects on migration of astrocytes after scratch injury were observed after treatment with a combination of resveratrol and Temozolomide (a chemotherapeutic agent) and resveratrol alone, showing only a very small effect on astrocytic migration.[40] These results show that treatment with phenols **1**−**3** slows down and reduces astrocyte migration after injury, which is a beneficial feature in that it is likely to reduce glial scar formation that is generally considered to reduce axonal regrowth after spinal cord injury and to prevent or even only reduce the astrocyte corona around the amyloid beta plaque and to help clearance of amyloid beta by microglia in Alzheimer's disease.

The present study has demonstrated that, in addition to their known antioxidant actions on a variety of non-neural cell types, the phenols investigated also beneficially affect neuronal and glial functions, such as neuritogenesis and process formation by glial cells, as well as neuronal and glial cell migration. It cannot be said, however, whether the observed effects are due to a direct influence on the cellular parameters studied or, e.g., if the ability of the compounds to enhance neurite outgrowth and neuronal migration are due indirectly to an enhanced viability of cells. However, the fact that astrocytes are inhibited in their migration by the phenolic compounds studied points to the possibility that molecular mechanisms different from those of neurons are involved in the behavior of astrocytes. Despite of these insights, the data of the present study clearly demonstrate the considerable therapeutic potential of the studied phenols, which now need to be analyzed in vivo for the more easily available compounds **1** and **2** in animal models of acute injury and of neurodegenerative diseases.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined with the Kofler apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-360 polarimeter at 18−22 °C in the solvents specified. NMR spectra were recorded on a Bruker AMX-400 instrument. HRMS (ESI) spectra were obtained on a MicrOTOF II (Bruker Daltonics) instrument. An Axiovert 135 microscope equipped with an AxioVision camera and AxioVision 4.7 software (Carl Zeiss) was used to determine process length and migration of cells.

Extraction and Isolation. Extraction and purification of the tested phenols **1**−**3** were performed following previously elaborated and established approaches.[25] A glass reactor was charged with 1.0 kg of a ground wood material (larch knots crushed using a cutting mill to sawdust size 2 mm), and 5 L of 70% aqueous isopropyl alcohol was added. The mixture was stirred at room temperature for 24 h. The solution was separated from the wood particles by conventional filtration. The extraction of the same wood material was repeated with 3 L of 70% aqueous isopropyl alcohol by using the same process parameters. The extracts were combined and evaporated under reduced pressure on a rotary evaporator to give 83 g of the crude extract. This material was separated by flash column chromatography on silica gel 60 (40−63 μm, Merck) by elution with a mixture of ethyl acetate and methyl *tert*-butyl ether (0 → 50%) followed by crystallization of the target products from water to yield 3.8 g of (+)-dihydroquercetin (**1**): mp 222−224 °C (dec) (lit.[40] mp 218−220 °C); $[\alpha]_D$ +21 (*c* 1.0, MeOH); HRESIMS *m*/*z* 327.0483 $[M + Na]^+$, calcd for $C_{15}H_{12}O_7Na$ 327.0481; and 6.3 g of (−)-secoisolariciresinol (**2**): mp 112−113 °C (lit.[41] mp 111−112 °C); $[\alpha]_D$ −31 (*c* 1.0, MeOH); HRESIMS *m*/*z* 385.1626 $[M + Na]^+$, calcd for $C_{20}H_{26}O_{20}Na$ 385.1627; and 0.57 g of (+)-isolariciresinol (**3**): mp 150−152 °C (lit.[26] mp151−153 °C); $[\alpha]_D$ +54 (*c* 0.8, Me_2CO); HRESIMS *m*/*z* 383.1465 $[M + Na]^+$, calcd for $C_{22}H_{24}O_6Na$ 383.1462. NMR data for compounds **1**−**3** were in accordance with those puplished.[26,40,41] The purities of crystalline compounds **1**−**3** were estimated to be higher than 99% as shown by both C18 RP-HPLC and spectroscopic analyses and the same as their optical purities (higher than 99%), which were estimated by HPLC on a chiral column (Kromasil 3-CelluCoat RP, 4.6 × 150 mm).

Mice. C57BL/6J mice of either sex were used for the cell culture experiments. Mice were kept under standard laboratory conditions with food and water supply ad libitum and with an artificial 12 h light/dark cycle. All experiments were conducted in accordance with the German and European Community laws on protection of experimental animals, and all procedures used were approved by the responsible committee of the State of Hamburg (allowance numbers for organ removal from mice: Org 535 Morph and Org 679 Morph).

Preparation and Treatment of Dissociated Primary Neurons and Schwann Cells and of Cerebellar Explants. Motoneurons were prepared from spinal cords of mice at embryonic day 14.5, seeded at a density of 1.25×10^4 cells per well in 48-well plates coated with poly-L-ornithine (PLO), and cultured under serum-free conditions as previously described.[42] Cerebellar granule neurons were prepared from cerebella of 6- to 8-day-old mice, seeded at a density of 2.5×10^4 cells per well in 48-well plates in a defined serum-free medium on poly-L-lysine (PLL) substrate, and cultured in a defined serum-free medium as previously described.[42] Schwann cells and dorsal root ganglion neurons were prepared from dorsal root ganglia of 6- to 8-day-old mice, seeded at a density of 1.25×10^4 cells per well in 48-well plates coated with PLL, and cultured under serum free conditions as previously described.[42] Cerebellar explants were prepared from cerebella of 5- to 6-day-old mice and cultured as previously described.[43] The compounds were dissolved in 10% DMSO and added to the cells at the indicated concentrations 1 h (dissociated cells) or 16 h (explants) after seeding. The final concentration of DMSO in the culture medium was 0.1%. This DMSO amount was added to the cells/explants as formulation control. Dissociated cells were fixed after 24 h in vitro, and explants were fixed after 48 h in

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vitro. Fixed cells and explants were stained with 1% toluidine blue, 1% methylene blue in 1% borax.

Assessment of Neurite Outgrowth, Neuronal Migration, and Schwann Cell Process Elongation. Neurite length, neuronal migration, and Schwann cell process length were measured as previously described,[42,43] using an Axiovert 135 microscope equipped with the AxioVision 4.7 software (Carl Zeiss). To assess neurite outgrowth and Schwann cell process elongation, all treatments were performed in duplicates and at least 100 cells were counted for each treatment. The results from 3 independent experiments were averaged. For neuronal migration, treatments were performed in duplicates and at least 14 explants were analyzed per treatment. The results from four independent experiments were averaged.

Astrocyte Scratch Injury Assay. Astrocyte-enriched cultures obtained from whole brains of 1- to 2-day-old mice were prepared as described previously[44,45] and cultured for at least 1 week at 37 °C and 5% CO_2 with DMEM medium supplemented with 10% fetal bovine serum on PLL-coated 6-well plates until they had reached confluence. The scratch injury assay was performed as described by Etienne-Manneville[46] with slight modification. Briefly, the confluent astrocyte monolayer was scratched twice from the left to right wall and from the upper to lower wall of the well with a sterile yellow 100 μL plastic tip, resulting in a cell-free cleft with a width of approximately 850 μm. Phenols were added at 100 nM concentrations, and the plates were immediately examined under an Axiovert 135 microscope with the 5× objective. Photographs were taken with an AxioVision camera and the Rel. 4.7 software (Carl Zeiss) (time point zero with maximal scratch size). The plates were then placed back into the incubation chamber (37 °C, 5% CO_2) and 8, 24, and 48 h later photographs of the scratch were taken with an AxioVision camera at the 5× magnification. The size of the scratch at each time point was determined using the AxioVision software. All treatments were performed in duplicates, and at least 10 pictures were taken for each scratch injury time point and concentration. Results were confirmed in three independent experiments.

Statistical Analysis. Unpaired, two-tailed *t* test assuming homogeneity of the variances was performed with the Excel software.[47] An analysis of the variance was performed by the ANOVA posthoc test.

AUTHOR INFORMATION

Corresponding Authors

*Tel and Fax: +7 499 1358784. E-mail: nen@ioc.ac.ru.
*Tel and Fax: +86 (0)754 8890 0276. E-mail: schachner@stu.edu.cn.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Nagini, S.; Murugan, R. S. In *Tea in Health and Disease Prevention*; Preedy, V., Ed.; Academic Press: New York, 2013; Chapter 62, pp 737−750.
(2) Rodrigo, R.; Gil, D.; Miranda-Merchak, A.; Kalantzidis, G. In *Advances in Clinical Chemistry*; Makowski, G. S., Ed.; Elsevier: Amsterdam, 2012; Vol. *58*, Chapter 8, pp 225−254.
(3) Menaa, F.; Badole, S. L.; Menaa, B.; Menaa, A.; Bodhankar, S. L. In *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases*; Watson, R. R., Preedy, V. R., Ed.; Academic Press: New York, 2013; Chapter 32, pp 421−430.
(4) Schauss, A. G. In *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases*; Watson, R. R., Preedy, V. R., Ed.; Academic Press: New York, 2013; Chapter 28, pp 379−392.
(5) Eklund, P. C.; Långvik, O. K.; Wärnå, J. P.; Salmi, T. O.; Willför, S. M.; Sjöholm, R. E. *Org. Biomol. Chem.* **2005**, *3*, 3336−3347.
(6) Basli, A.; Soulet, S.; Chaher, N.; Mérillon, J. M.; Chibane, M.; Monti, J. P.; Richard, T. *Oxid. Med. Cell. Longevity* **2012**, *2012*, 805762.
(7) Campos-Esparza, M. del R.; Torres-Ramos, M. A. *Cent. Nerv. Syst. Agents Med. Chem.* **2010**, *10*, 269−277.
(8) Lin, B. *Mini Rev. Med. Chem.* **2011**, *11*, 1222−1238.
(9) Jiang, Y. L.; Liu, Z. P. *Curr. Med. Chem.* **2011**, *18*, 808−829.
(10) Zhang, F.; Lu, Y. F.; Wu, Q.; Liu, J.; Shi, J. S. *Exp. Biol. Med.* **2012**, *237*, 943−948.
(11) De Nicoló, S.; Tarani, L.; Ceccanti, M.; Maldini, M.; Natella, F.; Vania, A.; Chaldakov, G. N.; Fiore, M. *Nutrition* **2013**, *29*, 681−687.
(12) Zamin, L. L.; Filippi-Chiela, E. C.; Dillenburg-Pilla, P.; Horn, F.; Salbego, C.; Lenz, G. *Cancer Sci.* **2009**, *100*, 1655−1662.
(13) Cicha, I.; Regler, M.; Urschel, K.; Goppelt-Struebe, M.; Daniel, W. G.; Garlichs, C. D. *J. Atheroscler. Thromb.* **2011**, *18*, 1031−1042.
(14) Kwon, G. T.; Jung, J. I.; Song, H. R.; Woo, E. Y.; Jun, J. G.; Kim, J. K.; Her, S.; Park, J. H. *J. Nutr. Biochem.* **2012**, *23*, 228−238.
(15) Park, S. J.; Jeon, Y. J. *Mol. Cells* **2013**, *33*, 141−149.
(16) San Miguel, S. M.; Opperman, L. A.; Allen, E. P.; Zielinski, J.; Svoboda, K. K. *Arch. Oral Biol.* **2011**, *56*, 812−822.
(17) Tang, S. N.; Singh, C.; Nall, D.; Meeker, D.; Shankar, S.; Srivastava, R. K. *J. Mol. Signaling* **2010**, *5*, 14.
(18) Sicilia, T.; Niemeyer, H. B.; Honig, D. M.; Metzler, M. *J. Agric. Food Chem.* **2003**, *51*, 1181−1188.
(19) Moss, G. P. *Pure Appl. Chem.* **2000**, *72*, 1493−1523.
(20) Willför, S.; Ahotupa, M. O.; Hemming, J. E.; Reunanen, M. H. T.; Eklund, P. C.; Sjoholm, R. E.; Eckerman, C. S. E.; Pohjamo, S. P.; Holmbom, B. R. *J. Agric. Food. Chem.* **2003**, *51*, 7600−7606.
(21) Heinonen, S.; Nurmi, T.; Liukkonen, K.; Poutanen, K.; Wähälä, K.; Deyama, T.; Nishibe, S.; Adlercreutz, H. *J. Agric. Food Chem.* **2001**, *49*, 3178−3186.
(22) Saarinen, N. M.; Smeds, A.; Mäkelä, S. I.; Ämmälä, J.; Hakala, K.; Pihlava, J.-M.; Ryhänene, E.-L.; Sjöholm, R.; Santti, R. *J. Chromatogr.* **2002**, *777*, 311−319.
(23) Smeds, A. I.; Češková, I.; Eklund, P. C.; Willför, S. M. *Holzforschung* **2012**, *66*, 553−567.
(24) Holmbom, B.; Eckerman, C.; Eklund, P.; Hemming, J.; Nisula, L.; Reunanen, M.; Sjöholm, R.; Sundberg, F.; Sundberg, K.; Willför, S. *Phytochem. Rev.* **2003**, *2*, 331−340.
(25) Nifantiev, N. E.; Yashunsky, D. V.; Menshov, V. M.; Tsvetkov, Y. E.; Tsvetkov, D. E. EP 2143435 B1, 2007.
(26) Eklund, R.; Sillanpää, R.; Sjöholm, R. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1906−1910.
(27) Tobinaga, S.; Hashimoto, M.; Utsunomiya, I.; Taguchi, K.; Nakamura, M.; Tsunematsu, T. *Biol. Pharm. Bull.* **2012**, *35*, 127−129.
(28) Liao, K. K.; Wu, M. J.; Chen, P. Y.; Huang, S. W.; Chiu, S. J.; Ho, C. T.; Yen, J. H. *J. Agric. Food Chem.* **2012**, *60*, 433−443.
(29) Levites, Y.; Amit, T.; Youdim, M. B.; Mandel, S. *J. Biol. Chem.* **2002**, *277*, 30574−30580.
(30) Levites, Y.; Amit, T.; Mandel, S.; Youdim, M. B. *FASEB J.* **2003**, *17*, 952−954.
(31) Chen, C. N.; Liang, C. M.; Lai, J. R.; Tsai, Y. J.; Tsay, J. S.; Lin, J. K. *J. Agric. Food Chem.* **2003**, *51*, 7495−7503.
(32) Erlank, H.; Elmann, A.; Kohen, R.; Kanner, J. *Free Radical Biol. Med.* **2011**, *51*, 2319−2327.
(33) Magrone, T.; Marzulli, G.; Jirillo, E. *Curr. Pharm. Des.* **2012**, *18*, 34−42.
(34) Chen, H. T.; Tsai, Y. L.; Chen, Y. S.; Jong, G. P.; Chen, W. K.; Wang, H. L.; Tsai, F. J.; Tsai, C. H.; Lai, T. Y.; Tzang, B. S.; Huang, C. Y.; Lu, C. Y. *Am. J. Chin. Med.* **2012**, *38*, 359−372.
(35) Wang, W.; Huang, C. Y.; Tsai, F. J.; Tsai, C. C.; Yao, C. H.; Chen, Y. S. *Int. J. Artif. Organs* **2011**, *34*, 1095−1105.
(36) Rubio-Perez, J. M.; Morillas-Ruiz, J. M. *Sci. World J.* **2012**, DOI: 10.1100/2012/756357.

(37) Tuppo, E. E.; Arias, H. R. *Int. J. Biochem. Cell Biol.* **2005**, *37*, 289−305.
(38) Mrak, R. E.; Sheng, J. G.; Griffin, W. S. *J. Neuropathol. Exp. Neurol.* **1996**, *55*, 273−279.
(39) DeWitt, D. A.; Perry, G.; Cohen, M.; Doller, C.; Silver, J. *Exp. Neurol.* **1998**, *149*, 329−340.
(40) Nonaka, G.; Goto, Y.; Kinjo, J.; Nohara, T.; Nishioka, I. *Chem. Pharm. Bull.* **1987**, *35*, 1105−1108.
(41) Tominaga, S.; Sugahara, T.; Nishimoto, S.; Yamawaki, M.; Nakashima, Y.; Kishida, T.; Maruyama, M.; Yamauchi, S.; Akiyama, K. *Biosci. Biotechnol. Biochem.* **2009**, *73*, 35−39.
(42) Bushman, J.; Mishra, B.; Ezra, M.; Gul, S.; Schulze, C.; Chaudhury, S.; Ripoll, D. R.; Wallqvist, A.; Kohn, J.; Schachner, M.; Loers, G. *Neuropharmacology* **2014**, *79*, 456−466.
(43) Jakovcevski, I.; Siering, J.; Hargus, G.; Karl, N.; Hoelters, L.; Djogo, N.; Yin, S.; Zecevic, N.; Schachner, M.; Irintchev, A. *J. Comp. Neurol.* **2009**, *513*, 496−510.
(44) Won, C. L.; Oh, Y. S. *Brain Res.* **2000**, *887*, 250−258.
(45) Kleene, R.; Loers, G.; Langer, J.; Frobert, Y.; Buck, F.; Schachner, M. *J. Neurosci.* **2007**, *27*, 12331−12340.
(46) Etienne-Manneville, S. *Methods Enzymol.* **2006**, *406*, 565−578.
(47) Dytham, C. *Choosing and Using Statistics: A Biologist's Guide*; Wiley-Blackwell: Oxford, U.K., 2011.