Neurogenesis following Stroke Affecting the Adult Brain

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A bulk of experimental evidence supports the idea that the stroke-damaged adult brain makes an attempt to repair itself by producing new neurons also in areas where neurogenesis does not normally occur (e.g., the striatum and cerebral cortex). Knowledge about mechanisms regulating the different steps of neurogenesis after stroke is rapidly increasing but still incomplete. The functional consequences of stroke-induced neurogenesis and the level of integration of the new neurons into existing neural circuitries are poorly understood. To have a substantial impact on the recovery after stroke, this potential mechanism for self-repair needs to be enhanced, primarily by increasing the survival and differentiation of the generated neuroblasts. Moreover, for efficient repair, optimization of neurogenesis most likely needs to be combined with promotion of other endogenous neuroregenerative responses (e.g., protection and sprouting of remaining mature neurons, transplantation of neural stem/progenitor cells [NSPC]–derived neurons and glia cells, and modulation of inflammation).

Stroke is caused by occlusion of a cerebral artery, which gives rise to focal ischemia with irreversible injury in a core region and partially reversible damage in the surrounding penumbra zone. In another type of insult, abrupt and near-total interruption of cerebral blood flow as a consequence of cardiac arrest or coronary artery occlusion leads to global ischemia and selective death of certain vulnerable neuronal populations, such as the pyramidal neurons of hippocampal CA1. During the last decade, these ischemic insults have been reported to induce the formation of new neurons in the adult rodent brain from neural stem/progenitor cells (NSPCs) located in two regions: the subventricular zone (SVZ), lining the lateral

ventricle, and the subgranular zone (SGZ) in the dentate gyrus. Ischemia-induced neurogenesis is triggered both in areas where new neurons are normally formed, such as the dentate gyrus, and in areas that are nonneurogenic in the intact brain (e.g., the striatum).

In this review, we will summarize the current status of research on neurogenesis after stroke. We will also discuss the basic scientific problems that need to be addressed before this potential self-repair mechanism should be considered in a clinical-therapeutic perspective. Stroke is a leading cause of chronic disability in humans. No effective treatment to promote recovery in patients exists. Many different types of neurons and glial cells die in stroke. To repair

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the stroke-damaged brain may, therefore, seem unrealistic. However, even reestablishment of only a fraction of damaged neuronal circuitries could have important clinical implications. Here we will focus on the stroke-induced formation of new neurons in damaged areas where neurogenic mechanisms do not normally operate. The modulation of neurogenesis in the dentate gyrus by focal ischemic stroke will not be covered here.

ANIMAL MODELS OF STROKE

Experimental models, which mimic the conditions during ischemic stroke in humans, have been developed in animals. These models cause motor, sensory, and cognitive deficits similar to what is observed in stroke patients, and studies in postmortem specimens confirm the relevance of the animal models for the human condition (Leifer and Kowall 1993). In the most common model for neurogenesis research, stroke is induced by transient middle cerebral artery occlusion (MCAO) in rats and mice, which is accomplished by insertion of a filament through the internal carotid artery to the origin of the middle cerebral artery (MCA). Recirculation is restored by withdrawal of the filament. Depending on the duration of the occlusion, either only the dorsolateral part of the rat striatum will be damaged (30 min MCAO in rats) or the lesion will extend into the overlying parietal cortex (2 h MCAO). In another model of ischemic stroke, the MCA of spontaneously hypertensive rats is ligated with a thread distal to the origin of the striatal branches. In normal rats, the same procedure is combined with bilateral occlusion of the carotid arteries during about 1 h. Both methods lead to selective ischemic lesions of the cerebral cortex without damage to the striatum.

Also, other animal models of stroke are being used to study neurogenesis. Subjecting exposed crania of rats to light beam with simultaneous systemic infusion of photosensitizer induces photothrombotic stroke with region-at-risk cortical tissue (Gu et al. 1999; Keiner et al. 2009). Wiping the pia and attached blood vessels from the cortical surface causes perma-

nent devascularization and damage to the cerebral cortex (Gonzalez and Kolb 2003). Embolic ischemic lesions to the striatum and cerebral cortex are induced by placing a blood clot at the origin of the MCA (Zhang et al. 1997).

OCCURRENCE OF NEUROGENESIS IN ADULT RODENT STRIATUM AND CEREBRAL CORTEX AFTER STROKE

The most solid evidence for stroke-induced neurogenesis in areas of the adult brain where new neurons are not normally formed has been obtained in the striatum (Fig. 1A). The initial findings were, first, that cells coexpressing the thymidine analog BrdU, given intraperitoneally after stroke, and markers of immature (e.g., doublecortin [DCX], PSA-NCAM, Hu) and mature neurons (e.g., NeuN and DARPP-32) are detected in the damaged striatum (Arvidsson et al. 2002; Parent et al. 2002). After BrdU injections, given early or late after the insult, the colabeled cells are first DCX immunoreactive over 2–3 wk but then gradually lose this expression and become labeled with NeuN, consistent with a maturation process (Thored et al. 2006). Second, cells expressing neuroblast markers, such as DCX, are detected in the stroke-damaged striatum and coexpress transcription factors specific for developing striatal projection neurons (i.e., Pbx and Meis2) (Arvidsson et al. 2002). Third, injection of Cre-encoding plasmid into the lateral ventricles of transgenic mice carrying a floxed green fluorescent protein (GFP) gene, specifically labeling SVZ cells and their progeny, gives rise to GFP-labeled cells in the striatum coexpressing NeuN and having a mature neuronal morphology at 90 d after stroke (Yamashita et al. 2006). As described in detail below, a bulk of experimental data has now established that NSPCs in the SVZ give rise to neuroblasts, which migrate into the injured striatum and differentiate to mature neurons.

Recent evidence indicates that stroke, in addition to triggering striatal neurogenesis from NSPCs in SVZ, can also elicit a latent neurogenic program in striatal astrocytes in mice (Magnusson et al. 2014). Stroke was induced by MCAO, giving rise to a lesion primarily in

Figure 1. Schematic representation of stroke-induced neurogenesis in the striatum and cerebral cortex. (A) Neural stem or progenitor cells reside in the subventricular zone (SVZ) adjacent to the lateral ventricle (1). Focal ischemic insults, which lead to pronounced loss of striatal and cortical neurons, give rise to increased proliferation of progenitors (2). Neuroblasts formed after and also, to some extent, before the stroke migrate to the damaged part of the striatum (3), where they differentiate and express markers specific for mature striatal projection neurons (4). The migration of neuroblasts and formation of new neurons continues for 1 yr after the insult. (B) Focal ischemia-induced damage to the cortex triggers proliferation of SVZ progenitors (2). Neuroblasts migrate toward the cortical damage (3), and in the peri-infarct area start to express markers of mature neurons (4). Some experimental evidence suggests that, following stroke affecting the cerebral cortex, neurons are also produced from local cortical progenitors.

the striatum. Using genetic methods for fate mapping, astrocytes in this area were found to form immature neurons, which differentiated to mature neurons. In the healthy brain, Notch1 signaling was inhibiting the neurogenic program and prevented the astrocytes from generating neurons. Following a stroke, Notch1 signaling was suppressed and astrocytes could enter the neurogenic program. It remains to be shown whether the neurons derived from astrocytes are functional and to what extent they contribute to the spontaneous recovery after stroke.

The occurrence of neurogenesis in the cerebral cortex following stroke is less well established. In the initial studies describing stroke-induced striatal neurogenesis, no significant

numbers of new neurons in the ischemic cerebral cortex were detected (Zhang et al. 2001; Arvidsson et al. 2002; Parent et al. 2002). However, it seems that under certain circumstances, limited accumulation of new neurons may occur in the cerebral cortex after stroke (Fig. 1B). Several other studies have described the presence of new cells expressing markers of neuroblasts or mature neurons in the ischemia-damaged cerebral cortex (Jiang et al. 2001; Zhang et al. 2006a; Ziv et al. 2007). It has also been shown that cortical neurogenesis after stroke can be triggered (Kolb et al. 2007) or enhanced (Jiang et al. 2001; Taguchi et al. 2004; Zhang et al. 2006a; Wang et al. 2007; Ziv et al. 2007) by additional manipulation (e.g., growth factor infusion). Leker and

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coworkers (2007) reported cortical neurogenesis in the distal MCAO model, which causes selective cortical damage. When BrdU was injected during days 1–5 after the insult, the majority of BrdU⁺ cells in the peri-infarct cortex at 1 wk were expressing markers for NSPCs, such as Sox2 and nestin. However, at 3 mo after the stroke, ~12% of BrdU⁺ cells were also NeuN⁺, indicating maturation, at least partly, of NSPCs into neurons.

Do new cortical neurons generated after stroke originate in the SVZ? Jin and coworkers (Jin et al. 2003) reported migration of DCX⁺ neuroblasts from SVZ to the boundaries of the cortical lesion. However, whether these neuroblasts were formed in response to the ischemic insult was unclear (Jin et al. 2003). Following photothrombotic cortical stroke in mice, DCX⁺ cells, presumably neuroblasts, seemed to migrate during 1 yr from the SVZ within the corpus callosum to the lesion site but no mature neurons were formed (Osman et al. 2011). Another study (Kreuzberg et al. 2010) using transgenic mice, in which neuroblasts originating in SVZ expressed GFP, reported that neural progenitors migrated toward the cortical infarct after MCAO. Here they differentiated to mature neurons expressing the interneuron marker calretinin. Similarly, Saha et al. (2013) found that following aspiration of motor cortex in mice, neuroblasts originating in SVZ, transduced with a GFP-expressing lentiviral vector, migrated to the injured cortical tissue and differentiated to glial cells and, to a much lesser extent, mature neurons.

Cortical neurogenesis has also been reported in rats after transient global forebrain ischemia induced by bilateral common carotid artery occlusion (Ohira et al. 2010). Following the insult, progenitors located in the subpial region of the neocortex (layer 1) proliferated and then migrated into deeper cortical layers where they differentiated to GABAergic interneurons. Analysis of c-Fos expression after exposure to an enriched environment indicated that the new neurons were incorporated into cortical circuitry.

The larger variability in the magnitude of cortical as compared to striatal neurogenesis in different studies could be related to the stroke

model used, the pattern, location, extent and dynamics of the ischemic lesion, and the resulting differences in the cues responsible for migration and neuronal differentiation of the new cells. It is also important to emphasize that to firmly establish the occurrence of neurogenesis in different brain areas, especially in a pathological environment, a combination of currently available molecular and genetic approaches and their vigorous use are crucial (Breunig et al. 2007).

EVIDENCE FOR A NEUROGENIC RESPONSE AFTER STROKE IN HUMANS

The SVZ has been identified as the main source of neuroblasts that are generated after stroke and migrate toward the damaged area in rodents. It is well established also that the adult human brain contains NSPCs in the SVZ (Eriksson et al. 1998; Bernier et al. 2000; Sanai et al. 2004). However, whether neuroblasts from SVZ migrate through the rostral migratory stream to the olfactory bulb in adult humans similar to rodents (Curtis et al. 2007) or migration stops at 18 mo after birth (Sanai et al. 2011) is controversial. Although it seems possible that SVZ in humans has a latent potential for producing new neurons for repair, the evidence for neurogenesis in the brain of stroke patients is so far limited. Suggesting a neurogenic response, Macas et al. (2006) found increased number of cells expressing the proliferation marker Ki67 in the ipsilateral SVZ, and of neural progenitor cells in the parenchyma close to the ventricular wall. Clear signs of increased proliferative activity and numbers of neural progenitors in the ipsilateral SVZ were detected in a group of aged patients who died during the first 2 wk after stroke (Marti-Fabregas et al. 2010).

Some preliminary evidence suggests increased neurogenic activity in the human brain after stroke also in areas remote from SVZ in the ischemic penumbra surrounding cortical infarcts. The presumed new cells expressing neuronal markers, such as DCX, were preferentially localized in the vicinity of blood vessels (Jin et al. 2006). Minger et al. (2007) reported cells from an aged patient that expressed markers of

NSPCs both around and distant from the infarcted area as well as an increased number of NSPCs in the neurogenic region of the lateral ventricular wall. Finally, Nakayama et al. (2010) observed cells expressing nestin and musashi-1, markers associated with NSPCs, in the ischemic cerebral cortex of humans during the first month after embolic stroke, suggesting a regenerative response.

Whether stroke-induced neurogenesis is significant in the human cerebral cortex was addressed by measuring the level of ^{14}C for birth dating as has been done in healthy individuals and show an occurrence of hippocampal (Spalding et al. 2013) but an absence of cortical neurogenesis (Bhardwaj et al. 2006). However, although Huttner et al. (2014) found evidence for DNA fragmentation and repair in neurons in the penumbra after stroke, the patients' genomic DNA had a ^{14}C concentration corresponding to the time of birth of the individual. Thus, significant numbers of neurons are not regenerated in the adult human neocortex following stroke. Consistent with this finding, stroke-generated $\text{DCX}^+/\text{BrdU}^+$ cells migrating along the corpus callosum to the peri-infarct cortex were detected at 2 wk after distal MCAO in mice (Huttner et al. 2014). At 4 wk, no $\text{NeuN}^+/\text{BrdU}^+$ cells were found in the peri-infarct cortex. Thus, the new DCX^+ , presumed neuroblasts, observed in the peri-infarct cortex in the 2-wk group, had died after 4 wk and had not differentiated to mature neurons. Moreover, some studies in rats (Arvidsson et al. 2002; Parent et al. 2002) failed to detect significant numbers of new neurons in the injured cerebral cortex following MCAO despite the occurrence of robust stroke-induced striatal neurogenesis from NSPCs in SVZ. It remains to be analyzed, using the ^{14}C method for birth dating, whether the increased cell proliferation in SVZ after stroke also leads to striatal neurogenesis in humans. Interestingly, in a consecutive series of stroke cases, Delavaran et al. (2013) found that $\sim 50\%$ of patients had infarcts close to (≤ 2 mm) the SVZ, which may trigger a neurogenic response. The lack of neurogenesis in the human neocortex following stroke indicates that neuronal replacement strategies to promote

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functional recovery in this structure will require intracortical implantation of neuronal precursors. Alternatively, in the future, this may be possible with recruitment of SVZ-derived neuroblasts or direct in vivo reprogramming and differentiation to cortical neurons.

MECHANISMS INVOLVED IN STROKE-INDUCED NEUROGENESIS

Proliferation

Overall, cell proliferation in the ipsilateral SVZ is increased 1 to 2 wk after MCAO (Jin et al. 2001; Arvidsson et al. 2002; Zhang et al. 2004a,b), but has returned to baseline at 6 wk (Thored et al. 2006). However, the ipsilateral SVZ is expanded at 2, 6, and 16 wk after MCAO (Thored et al. 2006), and the number and size of neurospheres isolated from the ipsilateral SVZ are increased at 6 wk. Taken together, these findings provide evidence that stroke leads to long-term alterations in the stem-cell niche in the SVZ.

During embryonic development, the orientation of mitotic cleavage regulates neurogenesis. Zhang et al. (2004a) have shown that not only the number of mitotic cells increases but also the cleavage orientation in the SVZ changes from horizontal to vertical during the first week after stroke in the adult brain. Thus, stroke leads to a transient alteration in SVZ cell division from asymmetric to symmetric, thereby expanding the pool of progenitor cells. Stroke was also found to increase the proportion of dividing cells in the SVZ and reduce their cell-cycle length, largely as a result of a shortening of the G_1 phase (Zhang et al. 2006b). These actively proliferating cells were most likely progenitor cells.

Interestingly, Zhang et al. (2007a) found that the ependymal cells lining the lateral ventricular wall also proliferate acutely and transiently after stroke. The ependymal cells were transformed into radial glia cells, and were surrounded by type-C (progenitors) and type-A (neuroblasts) cells in the SVZ. In mice, ependymal cells proliferated and gave rise to neuroblasts and astrocytes after stroke caused by suppression of Notch signaling (Carlen et al. 2009). None of the stroke-generated neuroblasts

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derived from ependymal cells survived to become mature neurons. Ependymal cells delaminated from the ependymal layer and did not self-renew (Carlen et al. 2009). Another study could not demonstrate proliferating ependymal cells, and the ependymal layer remained intact after stroke in mice (Young et al. 2013). Using long-term fate mapping of FoxJ1-expressing cells in lateral ventricular wall of adult rats (including ependymal cells and a subset of astrocytes) by in vivo electroporation combined with piggyBac transposon, Devaraju et al. (2013) found that these cells give rise to neuroblasts and mature neurons in the olfactory bulb both in intact and stroke-damaged brain. However, Devaraju et al. (2013) failed to detect any significant contribution of these cells to striatal neurogenesis after stroke.

Several molecular mechanisms are involved in regulating NSPC proliferation after stroke. MicroRNAs (miRNAs) are small noncoding RNA molecules that regulate gene expression. Recent experimental evidence indicates an important role of miRNAs in mediating stroke-induced neurogenesis, suggesting novel therapeutic targets for improving cellular repair. Stroke alters the expression of a number of miRNAs in SVZ NSPCs of adult rats (Liu et al. 2011), including a reduction of miR-124a expression at 7 d after MCAO, concomitantly with a marked increase of NSPC proliferation. Interestingly, Liu et al. (2011) found that introduction of miR-124a inhibited ischemic NSPC proliferation and promoted neuronal differentiation in vitro by targeting Jagged-1, a ligand of Notch. Members of the miR17-92 cluster of miRNAs are up-regulated in the SVZ NSPCs at 7 d after MCAO in adult rats and mice (Liu et al. 2011, 2013). Overexpression of the miR17-92 cluster in SVZ of ischemic animals increased, whereas inhibition of individual members of the miR17-92 cluster suppressed cell proliferation (Liu et al. 2013). Activation of the sonic hedgehog signaling pathway up-regulated miR17-92 cluster expression.

Both Notch1 and the naturally occurring activator, Jagged1, are expressed in the adult SVZ (Stump et al. 2002). Reduced Jagged1/Notch1 signaling decreased SVZ cell proliferation in

mice (Nyfeler et al. 2005). Conversely, administration of Notch ligands increased the number of proliferating cells in SVZ and new cells expressing the immature neuronal marker Hu in the cerebral cortex of intact rats (Androutsellis-Theotokis et al. 2006). Also following cortical stroke, supplying Notch ligands stimulated cell proliferation in SVZ (Androutsellis-Theotokis et al. 2006). Studies in neurosphere cultures derived from adult rats provided further evidence that the Notch signaling pathway mediates SVZ progenitor proliferation (Wang et al. 2009a). Finally, rats subjected to MCAO showed increased levels of Notch1 and its downstream transcriptional targets, Hes1 and sonic hedgehog, in SVZ at 4 and 24 h (Wang et al. 2009b). The stroke-induced cell proliferation in the SVZ was markedly attenuated by inhibition of the Notch1 signaling pathway. Taken together, these findings suggest that Notch ligands may be useful to enhance NSPC expansion during the recovery phase after stroke.

The adaptor protein LNK is an intrinsic negative regulator of hematopoietic stem-cell proliferation and expansion (Takaki et al. 2000; Buza-Vidas et al. 2006). Recently, Ahlenius et al. (2012) demonstrated that LNK is expressed in NSPCs in the adult rodent and human SVZ. Deletion of *Lnk* caused increased NSPC proliferation, whereas overexpression decreased mitotic activity of these cells in vitro. *Lnk*^{-/-} mice exhibited increased NSPC proliferation after stroke, but not in intact brain or following status epilepticus. *Lnk* expression after stroke increased in SVZ through the transcription factors STAT1/3. LNK attenuated insulin-like growth factor (IGF)-1 signaling by inhibition of AKT phosphorylation, resulting in reduced NSPC proliferation. These findings identify LNK as a stroke-specific endogenous negative regulator of NSPC proliferation in SVZ.

Tumor necrosis factor receptor 1 (TNFR-1) is another negative regulator of stroke-induced SVZ NSPC proliferation (Iosif et al. 2008). The increased cell proliferation at 1 wk after stroke occurred concomitantly with elevated microglia numbers and TNF- α and TNFR-1 gene expression in SVZ of wild-type mice. TNFR-1 was expressed on sorted SVZ progenitor cells from

nestin-GFP reporter mice. In animals lacking TNFR-1, stroke-induced SVZ cell proliferation and neuroblast formation were enhanced. In contrast, deletion of TNFR-1 did not alter basal or status epilepticus-stimulated cell proliferation in SVZ. Addition of TNF- α reduced size and numbers of SVZ neurospheres through a TNFR-1-dependent mechanism without affecting cell survival.

Survival

About 80% of the stroke-generated striatal neurons die during the first 2 wk after their formation (Arvidsson et al. 2002). Caspases are probably involved in this neuronal loss, and administration of caspase inhibitors markedly promotes neuroblast survival in the damaged striatum (Thored et al. 2006). It is conceivable that the inflammatory changes accompanying the ischemic damage contribute to the poor survival of the new striatal neurons similar to what has been described for new hippocampal neurons in an inflammatory environment (Ekdahl et al. 2003; Monje et al. 2003). In agreement, Hoehn et al. (2005) found that administration of the nonsteroidal anti-inflammatory drug indomethacin increased the number of neuroblasts in the striatum after stroke. Also, Liu et al. (2007) reported that minocycline, administered during the 4 wk after transient MCAO in rats, reduced the number of activated microglia and increased the number of new, mature neurons in the dentate gyrus.

The role of inflammation for stroke-induced neurogenesis is, however, complex, and microglia can most likely also be beneficial (e.g., by producing factors that are important for migration of newly generated neuroblasts). Interestingly, Thored et al. (2009) showed that during the several months following stroke in rats, increased numbers of activated microglia are localized in the ipsilateral SVZ concomitant with the continuous production of new neuroblasts migrating into the striatum. The immune cells included both resident microglial cells and blood-borne central nervous system (CNS)-infiltrating macrophages. It is tempting to speculate that factors released from these microglia/

macrophages contribute to the maintenance of the neurogenic response. In line with this idea, this latter effect was, at least in part, attributed to the ability of a substantial proportion of these immune cells to express IGF-1 (Thored et al. 2009). Furthermore, chronically activated microglia are permissive to neuronal differentiation and survival in adult mouse SVZ cultures (Cacci et al. 2008), and microglia and microglia-conditioned media rescue the in vitro formation of neuroblasts from SVZ NSPCs, a property that is otherwise lost over the course of continued culture (Walton et al. 2006). Moreover, interleukin (IL)-15, which is expressed by NSPCs in SVZ and produced by activated microglia, has been reported to promote proliferation and self-renewal of SVZ NSPCs, maintaining them in an undifferentiated state (Gomez-Nicola et al. 2011). Finally, microglia activated by IL-4, which are reminiscent of alternatively activated macrophages, support neurogenesis (Butovsky et al. 2006).

In addition to innate immune cells, adaptive immune cells, in particular, T cells, are recruited within CNS injured areas, take part in the repair process, and might influence neurogenesis (Kokaia et al. 2012). Cytokines released by T-helper cells promote neurogenesis in vitro via direct effect on innate immune cells (Butovsky et al. 2006). Immune-deficient mice show impaired neurogenesis, whereas genetically modified CNS-specific T-helper cells promote hippocampal neurogenesis (Ziv et al. 2006; Wolf et al. 2009), possibly through a remote mechanism. Under pathological conditions, the interaction between lymphocytes and NSPCs is even more complex. For example, lymphocytes have been reported to be important in the abortive neuroregenerative response following stroke confined to the cerebral cortex in mice. Immunodeficient mice and mice selectively depleted of CD4⁺ T lymphocytes exhibit reduced apoptosis and enhanced proliferation of NSPCs following cortical stroke (Saino et al. 2010). Activated CD4⁺ T cells expressing glucocorticoid-induced TNFR have been reported to be responsible for the negative effect on NSPCs (Takata et al. 2012). This complexity reflects the dynamics of the immune response following injury

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and, as a consequence, the continued changes in the need for the repair; the same immune cells can be helpful or harmful at different stages in the response to injury.

Recruitment to Damaged Area

The new neuroblasts exhibit distinct migratory behaviors and can divide on their way through the striatum toward the damage (Zhang et al. 2007b). Radial glia, which are distributed both in the SVZ and the ischemic striatum after stroke, are probably involved in guiding the migrating neuroblasts, as evidenced by both in vivo and in vitro data (Zhang et al. 2007a). There is also a close association between the newly formed neurons and the vasculature when they migrate toward the damaged area. At 18 d following 30 min MCAO in mice, Yamashita et al. (2006) found chains of neuroblasts around endothelial cells, the neuroblasts being elongated parallel to the blood vessels and the chains oriented in the mediolateral direction. In another model, at 7 d after focal cortical stroke in mice (Ohab et al. 2006), neuroblasts were located in large numbers in physical proximity to endothelial cells in the peri-infarct cortex, where active vascular remodeling occurred. Thored et al. (2007) found that the newly formed neuroblasts were located in the vicinity of blood vessels throughout long-term neurogenesis (at least up to 16 wk after the insult). The majority of the neuroblasts migrated toward the ischemic damage through the striatal area, which, selectively, as compared to other striatal areas adjacent to SVZ, exhibited both a long-lasting increase of vessel density and, during the first 2 wk after the insult, endothelial cell proliferation, consistent with angiogenesis. The blood vessels probably play a role both for migration and survival and differentiation of the closely located neuroblasts by endothelial release of factors, such as stromal cell-derived factor 1 (SDF-1) and brain-derived neurotrophic factor (BDNF) (Louissaint et al. 2002; Ohab et al. 2006; Wang et al. 2006).

The close association between GFP-labeled neural progenitor cells/neuroblasts and the vasculature, both in the early phase when the

cells exit the SVZ and in the striatum, has been visualized in living slices of ischemic mouse brains using time-lapse imaging (Zhang et al. 2009; Kojima et al. 2010; Grade et al. 2013). Neuroblasts in the migrating chains were highly motile and extended or retracted their processes (Zhang et al. 2009). The interaction with blood vessels seemed to provide directional guidance for the progenitor cells (Kojima et al. 2010). The average speed of neuroblast migration in the ischemic striatum was estimated at 28.7 ± 1.0 (Zhang et al. 2009) or $33.1 \pm 1.6 \mu\text{m/h}$ (Kojima et al. 2010) with images acquired every 15 or 30 min, respectively. However, using images acquired every 30 sec (Grade et al. 2013), it was possible to distinguish resting and migratory phases. Interestingly, compared to neuroblasts migrating in the rostral migratory stream, neuroblasts in the ischemic striatum spent more time in the resting state, whereas the speed of migration was similar (ipsilateral rostral migratory stream (RMS): $204.9 \pm 3.9 \mu\text{m/h}$; striatum: $192.0 \pm 7.3 \mu\text{m/h}$).

The molecular mechanisms involved in directing the new neurons to the damaged area are only partly understood. The most well established one regulating migration is the chemokine SDF-1 and its cognate receptor CXCR4. Following stroke, SDF-1 is expressed in reactive astrocytes and activated microglia in the damaged area, and SDF-1 levels are increased in the stroke hemisphere (Robin et al. 2005). CXCR4 is expressed on the neural progenitors and stroke-generated neuroblasts (Robin et al. 2006). When blocking CXCR4, the migration of neuroblasts was markedly attenuated (Robin et al. 2006; Thored et al. 2006). Taken together, these data indicate that SDF-1/CXCR4 signaling regulates the directed migration of new striatal neurons toward the ischemic damage. The occurrence of this mechanism is another example of how the inflammation, which is associated with stroke, is a major regulator of striatal neurogenesis.

Another factor of importance for neuroblast migration after stroke is monocyte chemoattractant protein-1 (MCP-1) (Yan et al. 2007). MCP-1 is up-regulated in activated microglia and reactive astrocytes in the cortex and striatum, and the new neuroblasts express the MCP-1

receptor CCR2 after stroke. Arguing for involvement of this factor in the migration, transgenic mice lacking MCP-1 or CCR2 showed less recruitment of neuroblasts into the striatum after stroke (Yan et al. 2007). Similarly, osteopontin, which is up-regulated in microglia/macrophages following stroke, seems to promote neuroblast migration in ischemic striatum by acting on the integrin $\beta 1$ receptor on the progenitors themselves (Yan et al. 2009). Infusion of a neutralizing antibody against osteopontin into striatum markedly suppressed stroke-induced neuroblast migration in striatum.

An important question is how the newly formed neuroblasts can migrate through the ischemic and partially damaged striatum. Lee et al. (2006) reported that the extracellular protease matrix metalloproteinase 9 (MMP-9) is colocalized with the neuroblast marker DCX in the SVZ and striatum following stroke in mice. Administration of an MMP inhibitor markedly reduced neuroblast migration. These data indicate that extracellular proteolysis through the action of MMP is involved in neuroblast migration after stroke (Lee et al. 2006). Barkho et al. (2008) provided evidence that not only MMP-9 but also MMP-3 is expressed in migrating DCX⁺ neuroblasts in a mouse stroke model. Using an in vitro assay system and specific small interfering (si)RNAs, endogenous MMP-3 and MMP-9 were found to promote chemokine-induced migration.

Erythropoietin (EPO) administered to the stroke-damaged brain promotes neurogenesis (Table 1). However, endogenous EPO also seems to be involved in the regulation of neurogenesis after stroke. Conditional knockdown of the EPO receptor in mice subjected to cortical stroke leads to reduced SVZ cell proliferation and impaired continued migration of neuroblasts to peri-infarct cortex (Tsai et al. 2006).

The extension of the damage plays an important role for the degree of recruitment of new neuroblasts to the striatum after stroke. Following 30 min MCAO, which gave rise to a lesion restricted to the striatum, the number of new striatal neuroblasts was markedly fewer as compared to a 2 h insult, which caused more extensive striatal damage and, in addition, inju-

ry to the overlying parietal cortex (Thored et al. 2006). The number of neuroblasts correlated significantly to the volume of striatal injury. Thus, the extent of the ischemic lesion influences the degree of activation of the molecular and cellular mechanisms that promote recruitment of new striatal neurons after stroke.

Stroke mainly affects older people and it is, therefore, of major clinical importance whether new neurons can also be recruited to the ischemically damaged area in the aged brain. No differences between young (3 mo) and old (15 mo) rats were observed in number and distributional pattern of new neuroblasts and mature neurons in the damaged striatum after stroke (Darsalia et al. 2005). Thus, the stroke-damaged aged brain is also permissive for neuroblast migration, and this potential self-repair mechanism seems to operate similarly in young and old brains.

Differentiation

In most studies, NeuN or MAP-2 coexpression in BrdU-labeled cells has been taken as evidence for the formation of new mature neurons after stroke. Little is known about the specific phenotype of these neurons. Arvidsson et al. (2002) estimated that 42% of the mature, new BrdU⁺/NeuN⁺ neurons formed after stroke in the striatum expressed DARPP-32, which is a specific marker for striatal medium-sized spiny neurons. This is the phenotype of most of the neurons destroyed by the ischemic lesion. Similarly, Parent et al. (2002) reported that a majority of new neurons in the striatal peri-infarct area after stroke were DARPP-32⁺. Hou et al. (2008) reported that the stroke-induced striatal cells, labeled through retroviral transfection of SVZ cells, differentiated to GABAergic and cholinergic neurons. In another model of striatal injury (excitotoxic lesion), Collin et al. (2005) described the generation of cells expressing markers of interneurons (parvalbumin and neuropeptide Y). Such cells were not detected following MCAO in rats (Parent et al. 2002). In contrast, Teramoto et al. (2003) observed no BrdU⁺ cells expressing DARPP-32 but a substantial number of BrdU⁺ cells colabeled with

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Table 1. Examples of treatments modulating neurogenesis following focal ischemic stroke

Stroke model/duration	Species/strain	Treatment/compound	Effect	References
MCAO/2 h	Rat/Wistar	Intraventricular infusion/caspase inhibitor	Increased survival of neuroblasts (DCX ⁺ and Dcx ⁺ /BrdU ⁺) in striatum	Thored et al. 2006
MCAO/2 h	Rat/Wistar	Infusion/GDNF	Increased proliferation of progenitors (BrdU ⁺ and Ki67 ⁺) in SVZ and neuroblasts (DCX ⁺) in striatum	Kobayashi et al. 2006
MCAO/30 min	Rat/Wistar	Intranigral injection/rAAV-BDNF	Increased number of neuroblasts (Dcx ⁺) in striatum	Gustafsson et al. 2003
MCAO/2 h	Rat/Sprague–Dawley (SD)	Orally/indomethacin	Increased number of newborn (BrdU ⁺) cells coexpressing Dcx, nestin, GFAP, or NG2 in striatum and cortex	Hoehn et al. 2005
Embolic MCAO	Rat/Wistar	Orally/Tadalafil	Increased number of BrdU ⁺ cells in SVZ	Zhang et al. 2006a
MCAO/20 min	Mouse/129S6/SvEvTac	Intraventricular infusion/EGF	Increased number of neuroblasts (DCX ⁺) and newborn neurons (BrdU ⁺ /NeuN ⁺) in striatum	Teramoto et al. 2003
Embolic MCAO	Rat/Wistar	Intraperitoneally/EPO	Increased number of BrdU ⁺ , nestin ⁺ and DCX ⁺ cells in SVZ	Wang et al. 2004
Distal MCAO	Transgenic mice/C57B/6	Conditional brain-specific EPO receptor knock out	Decreased number of proliferating (BrdU ⁺) cells in SVZ; decreased number of neuroblasts (DCX ⁺) in cortex (impaired migration)	Tsai et al. 2006
Devascularization stroke	Rat/Long–Evans	Intraventricular infusion/EGF + EPO	Increased cell migration (β-gal-labeled) from SVZ to cortex	Kolb et al. 2006
MCAO/90 min	Rat/SD	Intraventricular infusion/HB-EGF	Increased proliferation (BrdU ⁺) in SVZ; decreased migration of neuroblasts (DCX ⁺) in striatum	Jin et al. 2004
Embolic MCAO/permanent	Rat/Wistar	Orally/Sildenafil	Increased number of proliferating (BrdU ⁺) in SVZ; increased number of neuroblasts (Tuj-1 ⁺) in the SVZ and striatum	Zhang et al. 2002

Continued

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Table 1. Continued

Stroke model/duration	Species/strain	Treatment/compound	Effect	References
MCAO/2 h	Rat/Wistar	Orally/statins	Increased number of BrdU ⁺ cells and BrdU ⁺ /Tuj-1 ⁺ neuroblasts in SVZ	Chen et al. 2003
MCAO/90 min	Rat/SD	Intraventricular infusion/VEGF	Increased survival of progenitors (BrdU ⁺) in SVZ	Sun et al. 2003
MCAO/permanent	Transgenic mice/ C57BL/6	VEGF overexpression	Increased number of BrdU ⁺ cells and BrdU ⁺ /DCX ⁺ neuroblasts in SVZ; increased number of BrdU ⁺ /NeuN ⁺ new neurons in cortex	Wang et al. 2007
MCAO/permanent	Rat/SD	Subcutaneously/down-regulator of T-cell activity (poly-YE)	Increased number of new (BrdU ⁺ /MAP2 ⁺) neurons in cortex	Ziv et al. 2007
Distal MCAO/permanent	Mouse/SCID	Intravenously/CD34 ⁺ cord blood cells	Increased number of PSA-NCAM ⁺ neuroblasts and BrdU ⁺ /NeuN ⁺ neurons in cortex	Taguchi et al. 2004
Embolic MCAO/permanent	Rat/Wistar	Intravenously/nitric oxide donor	Increased proliferation (BrdU ⁺) in SVZ	Zhang et al. 2001
Distal MCAO/permanent	Mouse	Subcutaneously/granulocyte colony-stimulating factor (G-CSF) and stem-cell factor	Increased number of SVZ progenitors (BrdU ⁺ /Musashi-1 ⁺)	Kawada et al. 2006
MCAO/90 min	Rat/Wistar	Intravenously/G-CSF	Increased number of DCX ⁺ neuroblasts in cortex	Schneider et al. 2005
Distal MCAO/permanent	Rat/ hypertensive	Intracerebral injection/AAV-FGF-2	Increased number of BrdU ⁺ cells in SVZ; increased number of BrdU ⁺ cells coexpressing Sox2 ⁺ , Pax6 ⁺ , Hu, and NeuN in peri-infarct cortex	Leker et al. 2007
MCAO/30 min	Rat/SD	Intraventricular infusion/Bcl-2 plasmid	Increased number of BrdU ⁺ cells coexpressing DCX and ChAT in striatum, and Tuj-1 ⁺ , MAP2 ⁺ , GAD67 ⁺ cells in striatum and frontal cortex	Zhang et al. 2006b
MCAO/90 min	Rat/SD	Exercise/running wheel	Increased number of Ki67 ⁺ and IGF-1 ⁺ cells	Zhang et al. 2013

Continued

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Table 1. *Continued*

Stroke model/duration	Species/strain	Treatment/compound	Effect	References
MCAO/permanent	Rat/SD	Subcutaneously/ estradiol	Increased number of BrdU ⁺ , nestin ⁺ , DCX ⁺ and BrdU ⁺ /DCX ⁺ cells in ipsilateral SVZ	Zheng et al. 2013
MCAO/permanent	Rats/Wistar	Intraperitoneally/ cerebrolysin	Increased number of BrdU ⁺ and DCX ⁺ cells in ipsilateral SVZ and striatal ischemic boundary zone	Zhang et al. 2010
MCAO/90 min	Rat/Wistar	Application on cortical surface/hepatocyte growth factor	Increased number of BrdU ⁺ and BrdU ⁺ / DCX ⁺ cells in ipsilateral SVZ	Shang et al. 2011
Photothrombotic stroke	Rat/Wistar	Intraperitoneally/ citicoline	Increased number of BrdU ⁺ and BrdU ⁺ / NeuN ⁺ cells in ipsilateral SVZ, dentate gyrus and peri-infarct cortical area	Diederich et al. 2012
Photothrombotic stroke	Rat/Wistar	Osmotic minipump into lesioned cortex/BDNF	Increased number of BrdU ⁺ and BrdU ⁺ / DCX ⁺ cells in perilesional cortical area	Keiner et al. 2009
MCAO/45 min	Mouse/ C57BL/6	Intraperitoneally/ cilostazol	Increased number of BrdU ⁺ and DCX ⁺ cells in SVZ and peri-infarct area	Tanaka et al. 2010
MCAO/90 min	Rat/SD	Enriched environment and retinoic acid diet	Increased number of DCX ⁺ cells in SVZ and BrdU ⁺ /NeuN ⁺ neurons in striatum	Plane et al. 2008
MCAO/permanent	Rat/SD	Functional electrical stimulation	Increased number of BrdU ⁺ /DCX ⁺ cells in SVZ	Liu et al. 2013

MCAO, Middle cerebral artery occlusion; GDNF, glial cell-derived neurotrophic factor; DCX, doublecortin; BDNF, brain-derived neurotrophic factor; EPO, erythropoietin; EGF, epidermal growth factor; SVZ, subventricular zone; GFAP, glial fibrillary acidic protein; VEGF, vascular endothelial growth factor; HB-EGF, heparin-binding EGF; FGF, fibroblast growth factor; AAV, adeno-associated virus; rAAV, recombinant AAV.

parvalbumin after stroke in mice, which had been infused with EGF. Similarly, Liu et al. (2009) found no new striatal cells, labeled with BrdU or a retroviral GFP reporter, expressing markers of medium-sized spiny neurons (DARPP-32 and calbindin). The SVZ-derived striatal cells differentiated exclusively into calretinin-expressing interneurons (Liu et al. 2009).

Regional heterogeneity of SVZ progenitors may be one reason for these discrepancies (Merkle et al. 2007). It is interesting to note, though, that under normal conditions in humans, calretinin-expressing interneurons but no DARPP-32⁺ neurons were formed in the striatum (Ernst et al. 2014). Whether this finding indicates that there is a difference between humans and

rodents in the subtype of neurons generated in SVZ following stroke remains to be elucidated.

Morphological and Functional Integration

When new striatal neurons are generated by adenoviral overexpression of BDNF and Noggin in the ventricular wall of intact animals, the new neurons establish efferent connections with neurons in the globus pallidus (Chmielnicki et al. 2004). Yamashita et al. (2006) have reported that the axons of the stroke-induced new striatal neurons contain abundant presynaptic vesicles and form synapses with neighboring cells, indicating some level of integration into existing neural circuitries. Recently, Sun et al. (2012) provided evidence that a population of the new striatal neurons reestablished the striatal projection to the substantia nigra in the stroke-injured brain of adult rats. BrdU and NeuN staining and intraventricular injection of a retroviral-GFP vector were used to label new neurons, which was combined with microinjection of the retrograde tracer Fluorogold (FG) into substantia nigra. Interestingly, FG⁺ new neurons (also BrdU⁺/NeuN⁺ and GFP⁺/NeuN⁺) were detected in the ipsilateral striatum at 12 wk but not 2 wk after MCAO. This finding indicates that the new striatal neurons can form long axons targeting the substantia nigra. Sun et al. (2012) also obtained immunohistochemical evidence for the expression of dopamine and glutamate receptors on the stroke-induced striatal neurons, raising the possibility that they may respond to cortical glutamatergic and nigral dopaminergic inputs similar to striatal neurons in intact brain.

A crucial question is whether the new cells develop into neurons with the functional properties, including synaptic connectivity, characteristic of those neurons that they should replace. Hou et al. (2008) observed that the stroke-induced striatal neurons exhibited a time-dependent increase in dendrite length and branch formation. The new neurons expressed phosphorylated synapsin I and showed typical synaptic structures comprising dendrites and spines. Importantly, both the presumed GABAergic and cholinergic new striatal neu-

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rons, labeled with DiI, injected in the ventricle, and BrdU, fired action potentials and received excitatory and inhibitory synaptic inputs. This study provides the first experimental evidence that the ischemia-induced new striatal neurons can become functionally integrated into neural networks in the adult brain after stroke.

Another important issue is how the characteristics of the pathological environment influence the development of the functional synaptic connectivity of the new neurons. For the neurons generated after stroke, this is virtually unknown. When new hippocampal neurons are produced in an environment characterized by status epilepticus (Jakubs et al. 2006) or LPS-induced microglial activation (Jakubs et al. 2008), they develop increased inhibitory drive at their afferent synapses. Conversely, in a less severe seizure environment, without prominent microglial activation, this increase in inhibitory input is not observed (Wood et al. 2011). Thus, it seems that following insults to the adult brain, the pattern of synaptic alterations at afferent inputs to newly generated neurons, which could determine whether neurogenesis counteracts or exacerbates pathological effects, is dependent on the characteristics of the environment, including the cross talk with immune cells (Kokaia et al. 2012).

STRATEGIES TO STIMULATE ISCHEMIA-INDUCED NEUROGENESIS

It is important to emphasize that neurogenesis after stroke is not just one process but comprises several steps: (1) proliferation of NSPCs, (2) survival of immature or mature neurons, (3) migration of new neuroblasts to appropriate location, (4) differentiation of new neuroblasts to phenotype of neurons that need to be replaced, and (5) development of functional synaptic connectivity counteracting disease symptoms. Considering our own findings of long-term neurogenesis after stroke, one could argue that the main problem for effectiveness is not insufficient production of new neuroblasts. Therefore, the proliferation step may not be the most suitable target but the major emphasis should probably be put on approaches improv-

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ing the survival of newly formed neuroblasts and their differentiation to mature neurons.

Many approaches have been reported to promote neurogenesis after stroke (Table 1) and some of them have been associated with improved functional recovery. Of particular interest in a therapeutic perspective are those strategies that could be applicable also in a clinical setting. For example, intrastriatal infusion of glial cell-derived neurotrophic factor (GDNF) increased cell proliferation in ipsilateral SVZ and recruitment of new neuroblasts into the striatum after MCAO, and improved survival of new mature neurons (Kobayashi et al. 2006). Similar intrastriatal infusion of GDNF protein has been shown to induce sprouting of dopaminergic neurons in a Parkinson's patient (Love et al. 2005). Thus, administration of this factor may become of therapeutic value to promote neuroregenerative responses in stroke patients.

Recently, it was reported that transplantation of human embryonic stem-cell-derived NSPCs into the cortical infarct caused by distal MCAO in rats increased the number of DCX⁺ cells in SVZ (Jin et al. 2011), and that human fetal NSPCs implanted into the cortical peri-infarct area in rats with permanent MCAO promoted cell proliferation in SVZ (Zhang et al. 2011). Mine et al. (2013) showed that human fetal NSPCs implanted into the striatum after stroke increased the numbers of proliferating cells in SVZ and new SVZ-derived migrating neuroblasts and mature neurons in the striatum. This enhancement of endogenous neurogenesis was associated with improved behavioral recovery. These findings support the idea that in a future clinical setting, the most effective NSPC-based approach for cell therapy after stroke probably combines transplantation of NSPCs with stimulation of endogenous neurogenesis.

FUNCTIONAL CONSEQUENCES OF ISCHEMIA-INDUCED NEUROGENESIS

Although there is a bulk of indirect evidence suggesting an association between endogenous neurogenesis and stroke recovery, a direct causal link needs to be established (Lagace 2012). Spontaneous motor recovery after stroke in

rats affecting only the striatum occurred over several months concomitantly with striatal neurogenesis (Thored et al. 2006). Administration of molecules that promote neural proliferation in the SVZ and striatal neurogenesis after stroke (see Table 1) has been reported to be associated with improved functional outcome. Recent attempts to ablate DCX⁺ SVZ neuroblasts to determine the contribution of these cells to post-stroke behavioral recovery did not give clear results. Genetic ablation of newly formed neuroblasts in transgenic mice caused worsening of postischemic behavioral deficits and more extensive ischemic lesion as early as 24 h after MCAO, which makes it inconceivable that a lack of new mature neurons replacing the dead ones had contributed to the observed effect. It seems more likely that the newly generated neuroblast exerted a trophic action at this early time point, but the findings do not exclude the possibility that functional improvement may occur through neuronal replacement later after stroke (Jin et al. 2010). In a follow-up study, the effect on infarct volume was shown to be maintained at 12 wk after distal MCAO, whereas the behavioral deficits, which were greater in the mice subjected to neuroblast ablation during the first 8 wk, were completely reversed at 12 wk in parallel to restoration of neurogenesis (Wang et al. 2012). Taken together, these data support the idea that endogenous neurogenesis can induce improved outcome after stroke, although the mechanisms of action of the new neurons still are unclear.

Recent experimental evidence suggests that NSPCs in SVZ may also have a homeostatic regulatory role and protect striatal neurons from glutamate-induced excitotoxicity after stroke (Butti et al. 2012). Selective ablation of SVZ NSPCs in transgenic mice using ganciclovir increased the frequency and duration of glutamate-evoked spontaneous excitatory postsynaptic currents in striatal medium-sized spiny neurons in acute brain slices. Stroke in these animals caused increased mortality and larger lesion volume. The protective effect of SVZ NSPCs was probably a result of secretion of endocannabinoids, which were shown to reverse both the electrophysiological alterations and

mitigate the ischemic injury caused by NSPC ablation (Butti et al. 2012).

CONCLUDING REMARKS

The demonstration of a mechanism by which endogenous NSCPs generate new neurons migrating toward the ischemically damaged area after a stroke affecting the adult brain has raised a lot of interest in both the scientific and clinical community. It is now a major challenge to determine how the reservoirs of NSPCs located in the SVZ and SGZ could be efficiently recruited for repair of the stroke-damaged brain. However, many fundamental issues remain to be addressed regarding this potential self-repair mechanism. Most important, we need more solid data demonstrating the role of endogenous neurogenesis for functional restoration after stroke. Also, we need to develop strategies to optimize neurogenesis by generating sufficient numbers of new, functionally integrated neurons with the correct phenotype, which survive in the pathological environment.

Obviously, more knowledge about mechanisms of cell proliferation, differentiation, migration, survival, and functional integration in stroke-induced neurogenesis, as well as about the occurrence of this mechanism in patients, is needed. Also, what is the maximum level of improvement after stroke that may be achieved by neurogenesis? Because stroke-induced neurogenesis can continue for many months (Thored et al. 2006), various interventions to promote neurogenesis are not restricted to the acute postischemic period but can be applied over an extended time period. It seems unlikely that stimulation of neurogenesis from endogenous stem cells per se could be developed into a novel therapeutic approach and provide optimum repair of the stroke-damaged brain. Such a strategy probably needs to be combined with transplantation of NSPCs or their progeny in the vicinity to the damaged area. For efficient repair, it may also be necessary to provide the new cells, generated from endogenous and grafted stem cells, with a platform in the form of synthetic extracellular matrix so that they can reform appropriate brain structure.

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