Exofocal Dopaminergic Degeneration as Antidepressant Target in Mouse Model of Poststroke Depression


Background: Although poststroke depression (PSD) is a frequent chronic complication of stroke with high relevance for outcome and survival, underlying pathomechanisms remain inadequately understood. This may be because suitable animal models are largely lacking and existing models are poorly characterized.

Methods: Male 129/SV mice were subjected to 30-min middle cerebral artery occlusion (MCAo)/reperfusion and serial magnetic resonance imaging scans. A subset of animals received selective serotonin reuptake inhibitor citalopram starting 7 days after MCAo. Behavioral assessment was performed at 14 weeks. To identify biological correlates of PSD, we quantified corticosterone levels in serum and brain-derived neurotrophic factor levels in brain. The integrity of the mesolimbic dopaminergic system was assessed using tyrosine hydroxylase and dynorphin in situ hybridizations as well as dopamine transporter autoradiography.

Results: Left, but not right, MCAo, elicited anhedonia and increased anxiety and despair. This depression-like syndrome was associated with alterations in the mesolimbic reward system. MCAo resulted in delayed degeneration of dopaminergic neurons in ipsilateral midbrain, which was accompanied by reduced dopamine concentrations and decreased levels of dopamine transporter density along with increased brain-derived neurotrophic factor protein levels in ischemic striatum and increased dynorphin messenger RNA expression in nucleus accumbens. Chronic antidepressant treatment initiated as late as 7 days after stroke reversed the behavioral phenotype, prevented degeneration of dopaminergic midbrain neurons, and attenuated striatal atrophy at 4 months.

Conclusions: Our results highlight the importance of the dopaminergic system for the development of PSD. Prevention of secondary neurodegeneration by antidepressants may provide a novel target for subacute stroke therapy.

Key Words: BDNF, depression, dopamine, mesolimbic reward system, serotonin reuptake inhibition, stroke

A significant percentage of stroke patients develop depression during the first year after the event. Psychological distress linked to physical disability may promote the development of poststroke depression (PSD). However, the higher prevalence of mood symptoms in stroke survivors compared with orthopedic patients with the same degree of functional disability,

From the Klinik und Poliklinik für Neurologie (GK, MB, VP, KG, SJ, ME); Center for Stroke Research Berlin (GK, MB, VP, KG, SJ, ME); Institut für Pharmakologie und Toxikologie (BK, RSo, HH); Klinik für Psychiatrie und Psychotherapie (RH); Klinik und Hochschulambulanz für Psychiatrie und Psychotherapie (GK, IK, IH, JH-R), Charité-Universitätsmedizin Berlin; Experimental and Clinical Research Center (GK), Max-Delbrück Center and Charité Medical Faculty, Berlin; Behavioral Biology (PG), Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany; Stroke and Neurovascular Regulation Laboratory (CW), Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts; Department of Psychiatry (GJ), Ruhr-Universität Bochum, Bochum, Germany; and Center for Behavioral Brain Sciences (RSt) Research Group for Molecular and Systemic Neuropharmacology, Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany.

Authors GK and MB contributed equally to this work.

Address correspondence to Matthias Endres, M.D., Klinik und Poliklinik für Neurologie and Center for Stroke Research Berlin, Charité-Universitätsmedizin Berlin, Campus Mitte, Charitéplatz 1, D-10117 Berlin, Germany; E-mail: matthias.endres@charite.de.

Received Jun 7, 2011; revised Feb 1, 2012; accepted Feb 16, 2012.
beginning of experiments. Transient brain ischemia was induced as described previously (8).

Behavioral Testing

Behavioral analyses were performed during the dark phase of a 12:12 hour light–dark cycle. Tests were ordered to proceed from less stress producing to more invasive. Details of most procedures have been described previously (9,10). Additionally, we used sucrose consumption during a period of 24 hours as a hedonic measure (11). Novelty-suppressed feeding was tested after 48 hours of food deprivation. Chow was placed in the center of an open field (50 × 50 × 40 cm). Animals were placed individually in the open field, and the latency to feed was recorded. If the animal had not begun feeding within 6 min, it was assigned a latency score of 6 min.

Magnetic Resonance Imaging

T2-weighted images at 7 T (Pharmascan; Bruker Biospin, Ettlingen, Germany) were obtained using a fat suppressed two-dimensional turbo spin-echo sequence (repetition time: 5109 msec; echo time: 65.2 msec). A 2 × 2 cm field of view, a 128 × 128 matrix, an in-plane resolution of 156 µm, and a slice thickness of .4 mm with no interslice distance were realized (8).

Corticosterone Measurements

Serum levels of corticosterone were analyzed by a commercial radioimmunoassay according to the manufacturer’s instructions (ICN Biomedicals, Eschwege, Germany).

Immunohistochemistry

Brains were perfusion fixed with 4% paraformaldehyde and cut into 40-µm sections. Primary antibodies were applied in the following concentrations: anti-NeuN (mouse, 1:100; Millipore Bioscience Research Reagents, Darmstadt, Germany), anti-tyrosine hydroxylase (TH; rabbit, 1:250; Abcam, Cambridge, United Kingdom), anti-microtubule associated protein-2 (mouse, 1:200; Sigma, Taufkirchen, Germany), and anti-Iba1 (rabbit, 1:500; Wako, Neuss, Germany). Immunohistochemistry followed the peroxidase method with biotinylated secondary antibodies (all 1:500; Jackson Immunoresearch Laboratories, West Grove, Pennsylvania), ABC Elite reagent (Vector Laboratories, Burlingame, California), and diaminobenzidine (Sigma) as chromogen.

Radioactive In Situ Hybridization

Generation of riboprobes, hybridization and washing procedures, as well as detection of hybridization signals were performed as described using well characterized probes for pre-prodynorphin and TH (12–14).

Quantitative Analysis of Dynorphin messenger RNA (mRNA) Expression

The expression level of dynorphin mRNA was determined as described previously (12) in the nucleus accumbens (NAc) core and shell using NIH Image. For calibration, density measurements of the 14C standard were plotted against the tissue radioactivity equivalent (17). Brain-derived neurotrophic factor (BDNF) was measured using a commercial enzyme-linked immunosorbent assay kit in principle according to the manufacturer’s instructions (Promega, Mannheim, Germany) but adapted to a fluorometric technique (9,18).

Statistics

Experiments were performed in a blinded fashion. Values are given as means ± SEM. Comparisons were performed by analysis of variance with level of significance set at .05 and two-tailed p values. Post hoc testing was performed where appropriate.

Results

Left, but Not Right, Middle Cerebral Artery Occlusion Leads to Chronic ‘Depression-Like’ Behavior, Which Is Reversed by Serotonin Reuptake Inhibition

Mice were either subjected to mild transient brain ischemia by 30-min filamentous occlusion of the left (unless otherwise indicated) middle cerebral artery followed by reperfusion (MCAo) or given a sham operation (sham). Daily intraperitoneal treatment with SSRI citalopram (13 mg/kg body weight) or vehicle was begun at 7 days after MCAo/sham operation and continued until sacrifice (Figure 1A). The dose of citalopram used here roughly equates to a high dose prescribed in the clinical setting for the treatment of depression or obsessive-compulsive disorder and is also in keeping with precedence set by numerous previous studies in mice (19–22).

Spontaneous locomotion was examined at 14 weeks to gauge the potential effects of basal activity on the behavioral endpoints tested subsequently. Animals were monitored for spontaneous activity in individual cages for 8 hours (overnight) as described. Total time in motion did not differ significantly across groups (Figure 1B). The distance moved was highest in vehicle-treated MCAo animals (two-way analysis of variance for interaction between treatment and intervention; p = .02; Figure 1C).

The elevated plus maze is widely used to assess anxiety-like behaviors. Time spent and distance traveled in the open arms of the maze were reduced in vehicle-treated MCAo animals compared with sham controls. Citalopram treatment of MCAo animals completely reversed this “anxious” behavioral phenotype (Figure 1D, E). In the Pursolt forced-swim test, latency to float (i.e., the time to “give up”) and the total time floating serve as measures of despair. MCAo resulted in significantly reduced latency to float and significantly increased total time floating within vehicle-treated mice.
Citalopram treatment prevented this despair-related phenotype associated with MCAo (Figure 1F, G).

Reduced sucrose intake in rodents is frequently used as an index of anhedonia (11). Whereas MCAo resulted in a significant reduction in the consumption of a 1% sucrose solution within vehicle-treated animals, treatment of MCAo mice with the SSRI increased sucrose consumption to the levels observed in sham animals (Figure 1H).

The novelty-suppressed feeding paradigm is based on an approach–avoidance conflict between the fear of moving into the center of a brightly illuminated arena and the drive to ingest food (23). MCAo resulted in a significant increase in latency to feed in vehicle-treated animals. Again, treatment produced a significant decrease in latency to feed in MCAo animals (Figure 1I).

Corticosterone levels measured by radioimmunoassay did not differ significantly between experimental groups. All groups showed about fourfold higher corticosterone concentrations during the dark compared with the light phase (Figure S1A, B in Supplement 1).

Lateralization of the behavioral response to ischemia has previously been reported in rodents (24). We therefore investigated the behavioral effects of right MCAo (rMCAo) in a different set of animals. In contrast to left MCAo, rMCAo did not induce a despair-related phenotype in the Porsolt forced swim test in our long-term survival paradigm (latency to float [sec]; sham vehicle: 44.3 ± 3.5; sham citalopram: 48.4 ± 7.7; rMCAo vehicle: 52.3 ± 7.4; rMCAo citalopram: 69.4 ± 9.9; total time floating [sec]; sham vehicle: 91 ± 10.6; sham citalopram: 67.5 ± 10.7; rMCAo vehicle: 84.1 ± 17.9; rMCAo citalopram: 54.3 ± 12.1; n = 8–10 animals per group). Similarly, rMCAo did not result in reduced sucrose consumption (not shown).

Next, to corroborate the differential behavioral effects of right versus left-sided lesions, we directly compared, in a single experiment, mice that had been subjected to rMCAo, left (l)MCAo, or sham operation, respectively (n = 6–9 animals per group). Compared with the experiments described earlier in which all mice received daily intraperitoneal injections of either saline or citalopram, none of the mice investigated here received any injections. The analysis of spontaneous locomotion at 14 weeks yielded a significant increase in the distance moved (cm) in rMCAo animals relative to both sham and lMCAo mice (sham: 2701 ± 516; lMCAo: 3194 ± 250; rMCAo: 5926 ± 1164). However, time in motion (min) did not differ significantly across groups (sham: 22.7 ± 1.7; lMCAo: 23.2 ± 4.3; rMCAo: 24.5 ± 2.0). Time spent and distance traveled in the open arms of the elevated plus maze were significantly reduced in lMCAo compared with sham-operated mice. In contrast, rMCAo mice and sham mice did not differ significantly in these measures (time in the open arms [sec]: sham 37.0 ± 6.7; lMCAo 15.4 ± 2.2; rMCAo 24.8 ± 5.4; distance traveled in the open arms [cm]: sham 127.4 ± 28.1; lMCAo 44.9 ± 7.0; rMCAo 80.1 ± 19.5). Similarly, in the Porsolt forced swim test, lMCAo mice demonstrated significantly increased immobile time and reduced latency to float (total time floating [sec]; sham 79.5 ± 8.7; lMCAo 107.2 ± 7.9; rMCAo 90.4 ± 8.0; latency to float [sec]; sham 88.0 ± 8.8; lMCAo 57.4 ± 4.1; rMCAo 73.4 ± 15.4). Furthermore, lMCAo mice, but not rMCAo mice, again displayed a significantly increased latency to feed [sec] in the novelty-suppressed feeding paradigm (sham: 160.4 ± 39.6; lMCAo 299.4 ± 38.2; rMCAo 206.8 ± 37.0). Finally, lMCAo mice demonstrated anhedonic behavior with a significant decrease in the consumption [in ml/kg body weight] of a 1% sucrose solution relative to both rMCAo mice as well as to sham-operated mice (sham: 153.4 ± 10.2; lMCAo: 119.0 ± 5.2; rMCAo 160.9 ± 9.6).

Taken together with the results of the two experiments reported earlier, our findings indicate that the long-term behavioral consequences of mild brain ischemia dissociate between right- and left-sided lesions. The depressive-like syndrome with increased anxiety, despair-like behaviors and anhedonia at 14 weeks after 30 min MCAo/reperfusion is specific for left-sided lesions.

www.sobp.org/journal
BDNF protein concentrations in brain (www.sobp.org/journal lacetic acid, homovanillic acid (HVA), and 5-hydroxyindoleacetic and serotonin (5-HT), monoamine metabolites dihydroxyphenyl-

Despair-Related and Anhedonic Behavior

Striatal Dopamine Levels—Correlation with Measures of Serotonin Reuptake Inhibition Attenuates Chronic Loss of hemispheric differences in hippocampal BDNF were apparent (Fig-

Figure 2. Brain-derived neurotrophic factor (BDNF) measurements. (A–D) BDNF protein concentrations in brain (n = 9–10 animals per group). # p < .05 within the same treatment condition. MCAo, middle cerebral artery occlusion.

lesions. By contrast, right-sided lesions may induce increased locomotor activity (10,24).

Increased BDNF Protein Levels in Ischemic Striatum

BDNF levels were determined in striatum (including ventral striatum) and hippocampus using enzyme-linked immunosorbent assay at 16 weeks (Figure 2A-D). Across groups, we observed a significant inducing effect of MCAo on BDNF concentrations in striatum. By contrast, hippocampal BDNF levels did not differ significantly across experimental groups. However, pronounced inter-hemispheric differences in hippocampal BDNF were apparent (Figure 2C, D).

Serotonin Reuptake Inhibition Attenuates Chronic Loss of Striatal Dopamine Levels—Correlation with Measures of Despair-Related and Anhedonic Behavior

Levels of monoamine dopamine (DA), noradrenaline (NA), and serotonin (5-HT), monoamine metabolites dihydroxyphenylacetic acid, homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) and of amino acids glutamate, γ-aminobutyric acid, and taurine were determined in striatum (including ventral striatum), hippocampus, hypothalamus, amygdala and frontal and parietal cortices (Table S1 in Supplement 1). In line with previous reports on the effects of SSRIs on the use of 5-HT, the 5-HIAA/5-HT ratio was significantly reduced by citalopram in most brain regions (25).

Dopamine levels in left striatum were significantly reduced in vehicle-treated MCAo animals (Figure 3A). Surprisingly, sub-acute SSRI treatment led to a significant attenuation of this effect of MCAo on DA levels. Across experimental groups, striatal DA levels showed significant correlations with despair-like behaviors in the Porsolt forced swim test (latency to float: Figure 3B; vehicle-treated animals only: R = .53, p < .05; total time floating: R = -.35, p < .05; vehicle-treated animals only: R = -.46, p < .05). Furthermore, striatal DA levels showed a significant inverse correlation with latency to feed in the novelty-suppressed feeding paradigm (R = -.46, p < .01; vehicle-treated animals only: R = -.51, p < .05; n = 6–10 animals per group). HVA constitutes the major end-product of DA metabolism. Its concentrations in ischemic striatum were significantly decreased (>30%) in vehicle-treated MCAo compared with vehicle-treated sham animals. In contrast, HVA levels did not differ significantly between cita-

Delayed Citalopram Treatment Attenuates Striatal Dopamine Loss by Protecting Against Secondary Exofocal Degeneration of Dopaminergic Neurons

In our well-characterized MCAo model, the primary lesion is located in lateral striatum and typically spares overlying cortex and adjacent brain structures (Figure 4A). Using 7-Tesla magnetic resonance imaging, we followed lesion maturation over time. In line with the neurochemical data described earlier, we observed a secondary exofocal hyperintensity (Figure 4B) at 7 days (i.e., the time point when antidepressant treatment was initiated), which was associated with microglia activation in midbrain (Figure S2D–F in Supplement 1). This secondary midbrain hyperinten-
sity evolved during the first week after MCAo and was not present at the initial scan. In line with reduced DA levels in ischemic striatum in vehicle-treated mice (discussed earlier), further histological evaluation of the midbrain after completion of behavioral testing yielded a pronounced unilateral loss of TH-immuno-positive neurons in ipsilateral substantia nigra and ventral tegmental area (Figure 4C, D). NeuN-immunostaining confirmed neuronal loss rather than mere downregulation of tyrosine hydroxylase in ipsilateral midbrain at 16 weeks (Figure S2D–F in Supplement 1). The number of neurons showing TH mRNA expression (Figure 4E, F) was quantified in three representative coronal midbrain sections from approximately bregma −3.7 mm to bregma −4.6 mm. Data for TH mRNA expression in midbrain and for DA transporter (DAT) density in striatum are given as the ratios of ipsilateral over contralateral measurements, speci-

Figure 3. Citalopram attenuates the decrease in striatal dopamine (DA) levels after middle cerebral artery occlusion (MCAo). (A) DA levels in left striatum (n = 9–11 animals per group). # p < .05 for the effect of intervention (Tukey’s post hoc test; MCAo vs. sham) in vehicle-treated animals. * p < .05 for the effect of treatment (Tukey’s post hoc test; citalopram vs. vehicle) in MCAo animals. (B) Significant positive correlation between DA levels in left striatum and latency to float in Porsolt’s forced swim test.
tively (n = 5–9 animals per group). Relative to vehicle treatment, citalopram significantly increased the percentage of TH mRNA-expressing cells in ipsilateral midbrain (89% ± 3% [MCAo citalopram] vs. 56% ± 4% [MCAo vehicle], p < .01). Correspondingly, DAT density in left striatum as assessed by autoradiography was reduced after MCAo (Figure 4G, H). Again, this effect of MCAo was significantly attenuated by citalopram treatment (DAT density as percent of contralateral striatum: 78 ± 6 [MCAo citalopram] vs. 60 ± 5 [MCAo vehicle], p < .05). Finally, the size of the primary lesion was significantly reduced (−40%) in citalopram-treated MCAo animals (Figure S2 in Supplement 1).

Citalopram Attenuates Increased Dynorphin mRNA Expression in Ipsilateral NAc

The neurochemistry of the NAc and its relevance to depression is a topic of ongoing research (26,27). The dynorphin/κ-opioid receptor (KOR) system modulates the mesolimbic dopaminergic pathway and is implicated in the development of depressive-like behaviors (28–31). Using in situ hybridization, we here examined mRNA expression of dynorphin, the κ receptor preferring endogenous opioid (Figure 5). We observed increased dynorphin mRNA levels in ipsilateral NAc in MCAo animals. Again, SSRI treatment resulted in a significant reduction of ipsilateral dynorphin levels in NAc in MCAo animals.

Discussion

At least one-third of patients develop depression at some time after the onset of stroke. According to a recent meta-analysis, depressive symptoms even tend to increase in the long-term phase of recovery (32). The neurobiology of PSD remains poorly understood. Research on this topic has been impeded by the paucity of available animal models. Here, we investigated the behavioral consequences of mild brain ischemia at a chronic time point in mice. Similar to a number of clinical studies reporting beneficial effects of long-term antidepressant treatment on various aspects of stroke outcome (33–36), in the study reported here, we also investigated the consequences of citalopram treatment over a period of several months. Our data suggest that it is possible to recreate core features of depression after brain ischemia in mice. With testing beginning at 14 weeks, MCAo mice displayed both increased anxiety in the elevated plus maze and despair in Porsolt’s forced swim test. Furthermore, latency to feed was significantly increased in MCAo animals. Spontaneous locomotion was not reduced in MCAo animals, thereby ruling out a stroke-induced decrease in basal activity as a confounding factor. In addition, anhedonia as assessed here by sucrose consumption is not sensitive to motor impairments. Antidepressant treatment with citalopram reversed the depressive-like behavioral alterations induced by 30 min MCAo/reperfusion, confirming the predictive validity of our mouse model for PSD.

www.sobp.org/journal
In humans, a possible association between lesion characteristics and the risk for PSD remains a matter of ongoing debate. In particular, left frontal and left basal ganglia lesions have been linked to the occurrence of major PSD (37–43) (but see also Carson et al. [44]). Similarly, emotional lateralization following ischemic brain injury has previously been described in rodents (24). Furthermore, some studies in humans also found that severity of depressive symptoms after stroke is related to lesion volume (41,45,46). Acknowledging the limitations of an experimental study, our data fit well with these earlier reports.

It is only in recent years that interest in the role of the brain’s reward regions in depression has grown. Dopaminergic mechanisms are implicated in the therapeutic actions of certain antidepressants. Conversely, depression is the most frequent neuropsychiatric complication in Parkinson’s disease and is aggravated during “off” periods of motor fluctuations (47). In the same vein, unilateral stereotaxic lesions of dopaminergic neurons either in the left substantia nigra or in the left ventral tegmental area have been shown to produce depressive-like behaviors in rodents (48). Also, antianhedonic and antidepressant effects of NAc deep brain stimulation have recently been reported (49).

Here, we show that following transient mild brain ischemia, delayed neurodegeneration of TH+ neurons in midbrain results in reduced DA and its metabolite dihydroxyphenylacetic acid concentrations in striatum. Degeneration of ipsilateral midbrain neurons likely reflects a combination of retrograde and transsynaptic degenerations in reciprocally innervated systems (50). Our finding is in line with earlier histopathologic and neuroimaging studies including studies in monkeys and humans that also reported degenerative changes in ipsilateral midbrain subsequent to striatal infarction (51–53). In line with our finding of increased 5-HT levels in ischemic striatum (Table S1 in Supplement 1), a number of studies also described serotonergic sprouting in midbrain and striatum following dopaminergic lesions (54–56). Because citalopram treatment further increased striatal 5-HT content, it is tempting to speculate that serotonergic sprouting after MCAo may serve as a neuroprotective mechanism that can be harnessed by antidepressant treatment. Furthermore, our data suggest that the effects of citalopram in our model are primarily mediated by its effects on TH+ neurons in midbrain and, consequently, on striatal DA levels.

Figure 5. Delayed citalopram treatment attenuates increase in dynorphin messenger (m)RNA expression in ipsilateral nucleus accumbens (NAc) after middle cerebral artery occlusion (MCAo). Dynorphin mRNA expression was examined in the NAc by in situ hybridization. (A–D) False color images of autoradiograms at striatal level. aca, anterior commissure. Inset (C) shows the radiolabeled standard with tissue equivalents (nanoCuries per gram tissue). Note increased dynorphin expression in NAc on the side ipsilateral (ipsi) to MCAo (arrow). This MCAo-induced increase in dynorphin mRNA expression is attenuated by citalopram treatment (D, a–d). High-power darkfield images show ipsilateral NAc after coating with nuclear emulsion. (E) Quantitative analysis of dynorphin mRNA levels in NAc (n = 5–6 animals per group). On the ipsilateral side (ipsi), MCAo causes increase in dynorphin expression in vehicle-treated group (v) compared with the vehicle/sham group (v) and compared with the citalopram/MCAo group (c) (*p < .05). On the contralateral side (contra), dynorphin mRNA expression is not significantly altered.
tion, a recent study in rats also did not find an effect of chronic antidepressant treatment on striatal BDNF content (62). However, it is still interesting to note that, contrary to the situation in hippocampus, increased BDNF signaling specifically in ventral striatum has been linked to depressive-like behaviors (28,63). Similarly, numerous studies have implicated α-opioid signaling in the NAc in the pathophysiology of depression. For example, stressors such as immobilization or inescapable shock increase dynorphin expression in NAc (31) and administration of KOR agonists into the NAc causes conditioned place aversion (64). In contrast, intracerebroventricular injection of a KOR antagonist has been shown to decrease immobility in the Porsolt forced swim test (65). Furthermore, intra-accumbal injection of a KOR antagonist produces antidepressant-like effects in the learned-helplessness paradigm (31). Our finding that citalopram attenuates the increase in dynorphin mRNA expression in ipsilateral NAc after MCAo/repairfusion is in good agreement with these reports. However, the precise mechanisms underlying increased dynorphin levels after MCAo as well as the effects of citalopram in NAc remain to be elucidated further.

Neuroprotective effects of enhancing serotonergic neurotransmission have been described by some groups (66–69) but refuted by others (70–73). In line with the latter reports, we have also not been able to demonstrate neuroprotective effects of SSRI treatment in our MCAo model when it was given acutely or as a preventive treatment. However, to the best of our knowledge, this is the first experimental report of a neuroprotective strategy that affords long-term protection/regeneration that can be initiated as late as 1 week postevent. Our results provide a conceptual framework for the clinical finding that early antidepressant pharmacotherapy after stroke significantly reduces disability, promotes motor recovery (35) and may even increase patients’ survival (74).

Delayed-onset citalopram treatment not only attenuated remote neuronal damage in midbrain but also reduced the size of the primary ischemic lesion. Multiple mechanisms may have contributed to these beneficial effects of chronic SSRI treatment. These may include direct modulation of ion currents and attenuation of excitotoxicity (75–77). However, because treatment was initiated at a subacute time point and continued for many weeks, complex interactions between neurotrophic, immunomodulatory and pro-regenerative effects of the antidepressant may have played an even larger role. Of special note, dopaminergic midbrain neurons project topographically organized to the subventricular zone where they promote proliferation of neural progenitors (78).

In summary, our study provides new insight into the pathogenesis of depression after stroke. We present a novel mouse model for PSD which highlights the potential etiological significance of alterations of the mesolimbic dopaminergic system. Neuroprotection against secondary neuronal injury may also underlie the clinical benefits of antidepressant therapy after stroke regarding both functional recovery and survival. Lastly, exofacial neuronal cell death may emerge as a novel treatment target at late time points for stroke.

This work was supported by individual grants by the Deutsche Forschungsgemeinschaft (to RH, RSt, ME) as well as through Sonderforschungsbereich TR 43 and Cluster of Excellence 257 (NeuroCure), VolkswagenStiftung (Lichtenberg Program to ME), the Bundesministe- rium für Bildung und Forschung (Center for Stroke Research Berlin), and the European Union’s Seventh Framework Program (Grant No. FP7/2008-2013) under grant Agreement Nos. 201024 and 202213 (European Stroke Network).

ME received grant support from AstraZeneca and Sanofi; participated in advisory board meetings of Boehringer Ingelheim, Bristol Myers Squibb, MSD, Pfizer, Sanofi; and received honoraria from Novartis, Pfizer, Bayer Healthcare, AstraZeneca, Boehringer Ingelheim, Desitin, MSD, Sanofi, Berlin-Chemie, Trommsdorff, GlaxoSmithKline, Bristol-Myers-Squibb, Eisai, Ever, and Takeda. GJ reports research grant support from AstraZeneca, Bristol-Meyers Squibb, Eli Lilly, and Janssen and has received honoraria from or is on the advisory board for AstraZeneca, Bristol-Meyers Squibb, Eli Lilly, Janssen, Pfizer, Lundbeck, and Servier. IH acted as consultant to GE Healthcare, AstraZeneca, Novartis, and Bayer Healthcare. RH reports research grant support from Lundbeck, Merz, and Novartis as well as honoraria from Lundbeck, Merz, Novartis, Janssen, Pfizer, Eli Lilly, and Bristol-Myers Squibb. PG received research grants from Pfizer and Schwabe. The other authors reported no biomi- dical financial interests or potential conflicts of interest.

Supplementary material cited in this article is available online.


www.sobp.org/journal


68. Salazar-Colocho P, Del Rio J, Frechilla D (2008): Neuroprotective effects of serotonin 5-HT1a receptor activation against ischemic cell damage in gerbil hippocampus: Involvement of NMDA receptor NR1 subunit and BDNF. *Brain Res* 1199:159–166.


