Anamnestic Recall of Stroke-Related Deficits
An Animal Model

Dannielle Zierath, BS; Jessica Hadwin, BS; Anna Savos, BS; Kelly T. Carter, BS; Allison Kunze; Kyra J. Becker, MD

Background and Purpose—Anamnestic recall of stroke-related deficits is a common clinical observation, especially during periods of systemic infection. The pathophysiology of this transient re-emergence of neurological dysfunction is unknown.

Methods—Male Lewis rats underwent 3 hours middle cerebral artery occlusion and were treated with lipopolysaccharide or saline at the time of reperfusion. The delayed-type hypersensitivity (DTH) response to myelin basic protein was examined 28 days after middle cerebral artery occlusion. Changes in behavioral outcomes were assessed after DTH testing and repeat administration of lipopolysaccharide or saline at 34 days. At the time of euthanasia (36 days), the immunologic response of splenocytes to myelin basic protein, neuron-specific enolase, and proteolipid protein was determined by enzyme-linked immunospot assay and the number of lymphocytes in the brain determined by immunocytochemistry.

Results—Animals treated with lipopolysaccharide at middle cerebral artery occlusion had a greater DTH response to myelin basic protein than animals treated with saline. Among those animals that had fully recovered on a given behavioral test before DTH testing, those treated with lipopolysaccharide at middle cerebral artery occlusion displayed more neurological deterioration after DTH testing and had more CD8+ lymphocytes within the ischemic core of the brain. Furthermore, the Th1 immune response to brain antigens in the spleen was more robust among those animals that deteriorated after DTH testing and there were more CD4+ lymphocytes in the penumbral region of animals with a Th1 response to myelin basic protein.

Conclusions—Our data suggest that an immune response to the brain contributes to the phenomenon of anamnestic recall of stroke-related deficits after an infection. The contribution of the immune response to this phenomenon deserves further investigation. (Stroke. 2010;41:2653-2660.)

Key Words: immunology ■ infectious disease ■ inflammation ■ lymphocytes ■ outcome ■ Th1

For many patients who have recovered from stroke, a stressor such as an infection can lead to transient reappearance of their initial stroke symptoms. Despite wide recognition of this phenomenon, which is often referred to as anamnestic recall, there is virtually nothing known about the pathophysiology of the transient re-emergence of stroke-related deficits. The term anamnestic means “able to recall to mind” and is used to describe a variety of biological phenomena. In the immunology literature, anamnestic recall refers to the immune response that occurs on re-encounter with an antigen to which it has already been “educated.” This “memory” response occurs in both the cellular and humoral arm of the immune system and results in a much greater response than occurred with the primary antigen exposure; it is the greater efficiency of this secondary immune response that forms the rationale for vaccinations.

Animals exposed to a systemic inflammatory stimulus at the time of stroke are at increased risk of developing a Th1-type immune response to central nervous system (CNS) antigens and this response is associated with worse outcome. We hypothesized that animals with a Th1 type immune response to CNS antigens would experience a reactivation of these CNS-specific lymphocytes when exposed to a systemic inflammatory stimulus at a point in time remote from the initial stroke (similar to that which occurs during an infection). Once activated, these lymphocytes could traffic into the brain, and an inflammatory response within the vulnerable parenchyma might precipitate a re-emergence of the initial stroke-related symptoms. To investigate whether the clinical syndrome of anamnestic recall in stroke could be immunologically mediated, we examined whether animals predisposed to a Th1 immune response by treatment with lipopolysaccharide (LPS) at stroke onset would experience neurological worsening when exposed to a delayed inflammatory stimulus and whether this worsening was related to the immune response to brain antigens.
Materials and Methods

Animals
Experiments were approved by the Institution’s Animal Care and Use Committee. Male Lewis rats (250 to 300 g) were used for all studies (Charles River).

Middle Cerebral Artery Occlusion
Anesthesia was induced with 5% and maintained with 1.5% isoflurane. After a midline neck incision, the right common carotid, external carotid, and pterygopalatine arteries were ligated. A monofilament suture (4-0) was inserted into the common carotid artery and advanced into the internal carotid artery to occlude the origin of the middle cerebral artery. Animals were maintained at normothermia during surgery and reperfused 3 hours after middle cerebral artery occlusion (MCAO). In sham-operated animals (N=4), the filament was inserted into the carotid but not advanced. Rectal temperature and body weight were assessed at set time intervals. Animals were euthanized 36 days after MCAO. See Figure 1 for details of the experimental paradigm.

Administration of Immunologically Active Substances
Active substances were treated with either LPS or saline at the time of reperfusion (3 hours after MCAO) and then randomly assigned to receive either LPS or saline again at 34 days after MCAO (“delayed” administration) resulting in 4 groups of animals (LPS/LPS, saline/LPS, LPS/saline/saline). LPS (from Escherichia coli, serotype 026:B6) was mixed at a concentration of 1 mg/mL in saline and given at a dose of 1 mg/kg intraperitoneally; saline was given at the volume of 1 mL/kg intraperitoneally. All sham-operated animals received LPS at stroke onset and again 34 days after MCAO.

Delayed-Type Hypersensitivity Response
Ear thickness was measured 28 days after MCAO using a micrometer. Myelin basic protein (MBP; Sigma; 50 μg in 0.05 mL saline) was then injected into the ear and the change in ear thickness measured 72 hours later.

Neurological Outcome
Neurological outcome was assessed at set time points. Tests included a modification of the Bederson scale (0=no deficit, 1=holds forepaw in flexed posture, 2=inaability to resist lateral push, 3=circling, 4=agitated circling, 5=stupor),7 the “sticky tape test,”8 performance on the Rotorod,9 and the foot fault test.10 The “sticky tape test” assesses sensorimotor function; the time the animal takes to attend to a piece of adhesive tape placed on the affected forelimb is recorded. The Rotorod assesses motor coordination and fatigue; animals were trained before surgery until they could remain on the rotating rod at 5 rpm for 100 seconds; after surgery, the longest time animals could remain on the Rotorod before falling (100 seconds maximum) was recorded (using the average of 3 trials). The foot fault test was done to test forelimb motor coordination; rats were placed on a wire grid for 3 minutes and the number of times the affected front paw slipped through the grid per total number of steps taken was recorded as a percentage.

Enzyme-Linked Immunospot Assay
Animals were euthanized 36 days after MCAO and mononuclear cells isolated from the spleen using previously described methods.2,11,12 Mononuclear cells were cultured (1×10⁵ cells/well) for 48 hours in 96-well plates (MultiScreen-IP; Millipore) in media alone or in media supplemented with MBP (50 μg/mL; Sigma), neuron-specific enolase (NSE; 10 μg/mL; Sigma), or proteolipid protein (PLP) 139-151 (10 μg/mL; ANASPEC). All experiments were performed in triplicate. The ratio of the relative increase in the number of cells secreting interferon-γ when cultured with antigen instead of media alone to that of the relative increase in the number of cells secreting transforming growth factor-β1 when cultured with antigen instead of media alone was used as an indicator of the Th1 response. Spots were counted by 2 independent investigators blinded to treatment status and aided by a semiautomated software system (Metamorph). Animals were considered to have a Th1 response to the given antigen if the ratio of interferon-γ-transforming growth factor-β1 response to that antigen was greater than the 75th percentile of sham-operated animals.

Immunocytochemistry
After removal of the spleens, animals were perfused with 4% paraformaldehyde in phosphate-buffered saline and the brains removed and stored in fixative overnight at 4°C. Cryoprotection was in 30% sucrose solutions for 24 to 48 hours before freezing in embedding compound at −80°C. Brains were then sectioned (20 μm) and stained for CD4, CD8, and CD45RA (present on B cells); slides were counterstained with cresyl violet. Antibodies were obtained from AbD Serotec (CD4 and CD8) and GenWay Biotech (CD45RA). The number of the cells labeled within 6 high-powered

---

Figure 1. Experimental design. IP indicates intraperitoneally.
fields (100×) in 8 distinct regions (4 in the infarcted hemisphere and 4 in the noninfarcted hemisphere) was determined in 3 different sections through the infarct (bregma 2.70 mm, −0.26 mm and −3.14 mm); individuals counting cells were masked to the treatment/outcome status.

Statistics

Parametric data are displayed as the mean±SEM and compared using the t test or analysis of variance. Nonparametric data are displayed as the median (interquartile range) and compared using the Mann–Whitney U test or the Kruskal-Wallis H test as appropriate. Categorical data were evaluated using the χ² test statistic. Significance was set at P<0.05.

Results

Neurological Outcome to Day 28

Mortality was identical (8 of 34 [24%]) among animals that received LPS at stroke onset and those that did not; there was no mortality among sham-operated animals. The body temperatures did not differ among animals treated with LPS or saline at any time point after MCAO (Figure 2A), but the amount of weight lost after MCAO was greater in LPS-treated animals (Figure 2B). The temperatures of LPS-treated animals undergoing MCAO were higher than that of LPS-treated animals undergoing sham surgery from 3 hours through 48 hours after stroke onset (data not shown). Weight loss in LPS-treated animals undergoing MCAO was greater than that of LPS-treated sham-operated animals throughout the duration of the study (data not shown).

Stroke severity, as assessed by the neurological score, was identical at 3 hours after MCAO in animals that went on to receive either LPS or saline at reperfusion; after reperfusion, the neurological score was higher among LPS-treated animals until 21 days after MCAO, at which time stroke severity was similar in both groups. At 28 days after MCAO, just before delayed-type hypersensitivity (DTH) testing, there were no differences in
the performances on any of the behavioral tests between LPS- and saline-treated animals. Furthermore, there was no difference in the proportion of LPS- and saline-treated animals that had fully recovered on any of these tasks up to this time point. LPS-treated sham-operated animals performed better on all behavioral tests throughout the duration of the study than animals treated with LPS and subjected to MCAO (data not shown). Similarly, more sham-operated animals had complete recovery on the behavioral tasks at this time point (3 of 4 on the tape test, 4 of 4 on the foot fault test, and 2 of 4 on the Rotorod).

**DTH Response**

At 72 hours after intradermal injection of MBP into the ear, animals treated with LPS at MCAO had more ear swelling than those treated with saline at MCAO; the increase in ear thickness was 0.048±0.008 mm in LPS-treated animals compared with 0.029±0.005 mm in saline-treated animals (P=0.004). In sham-operated animals, the increase was 0.038±0.013 mm, which is not significantly different than the ischemic animals.

Six days after DTH testing (and just before re-exposure to LPS or saline), 18 of 25 (72%) of animals treated with LPS at MCAO and 11 of 26 (42%) of animals treated with saline at stroke onset displayed worsening performance on the foot fault test (P=0.032). Those animals that worsened had a strong trend toward a more significant DTH response (0.045±0.008 mm versus 0.029±0.004 mm; P=0.084). The deterioration in performance on the sticky tape test and Rotorod during this same time period did not differ between LPS- and saline-treated animals. Among sham-operated animals, 1 of 4 (25%) worsened on the foot fault test after DTH.

### Table 1. Changes in Behavioral Outcome After DTH Testing and LPS Treatment

<table>
<thead>
<tr>
<th>Treatment at Stroke Onset</th>
<th>Days from MCAO</th>
<th>Change in Tape Test (Seconds), Median (IQR)</th>
<th>Change in Foot Fault (Percent Total Steps), Median (IQR)</th>
<th>Change in RR Performance (Percent of Baseline), Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35</td>
<td>+LPS (N=26) 3 (1 to 24) -LPS (N=26) 2 (0 to 6)</td>
<td>+LPS (N=26) 7 (0 to 16) -LPS (N=26) 1 (0 to 5)</td>
<td>+LPS (N=26) 15 (0 to 35) -LPS (N=26) 9 (0 to 33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>+LPS (N=26) 3 (0 to 4) -LPS (N=26) 2 (0 to 6)</td>
<td>+LPS (N=26) 10 (0 to 20) -LPS (N=26) 4 (0 to 8)</td>
<td>+LPS (N=26) 3 (0 to 4) -LPS (N=26) 2 (0 to 4)</td>
</tr>
</tbody>
</table>

### Table 2. Changes in Behavioral Outcome Among Those Animals Who Had Fully Recovered on the Given Test Before DTH Testing and Delayed LPS or Saline Treatment

<table>
<thead>
<tr>
<th>Treatment at Stroke Onset</th>
<th>Days from MCAO</th>
<th>Change in Tape Test (Seconds), Median (IQR)</th>
<th>Change in Foot Fault (Percent Total Steps), Median (IQR)</th>
<th>Change in RR Performance (Percent of Baseline), Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35</td>
<td>+LPS (N=25) 3 (1 to 31) -LPS (N=25) 1 (0 to 4)</td>
<td>+LPS (N=25) 8 (0 to 3) -LPS (N=25) 0 (0 to 3)</td>
<td>+LPS (N=25) 15 (0 to 31) -LPS (N=25) 9 (0 to 24)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>+LPS (N=25) 9 (0 to 30) -LPS (N=25) 1 (0 to 1)</td>
<td>+LPS (N=25) 13 (0 to 21) -LPS (N=25) 5 (0 to 11)</td>
<td>+LPS (N=25) 24 (0 to 39) -LPS (N=25) 12 (0 to 12)</td>
</tr>
</tbody>
</table>

IQR indicates interquartile range; RR, relative risk; NS, nonsignificant.
testing, which is less than that of ischemic animals treated with LPS ($P = 0.066$) and similar to that of animals treated with saline ($P = $nonsignificant) at stroke onset.

**Response to Delayed LPS Injection**

Animals treated with saline at stroke onset and LPS at 34 days had higher temperatures than animals in other treatment groups at 35 days (Figure 3); this difference was no longer seen at 48 hours after delayed LPS administration. Administration of LPS at 34 days led to rapid and significant weight loss, as evidenced in Figure 3. The increase in temperatures among sham-operated animals that received the second dose of LPS was similar to that of animals with stroke receiving a dose of LPS at this time point; ironically, the decrease in body weight after the second exposure to LPS was more in sham-operated animals ($P = 0.005$).

Behavioral changes after the second exposure to LPS (or saline) were dictated by treatment at the time of MCAO (LPS or saline); further results are therefore dichotomized by initial treatment. Table 1 depicts the changes in behavioral outcomes for each animal after DTH testing and delayed LPS or saline administration; absolute changes were computed by using the best performance to 34 days as the new “poststroke baseline.” Animals treated with LPS at stroke onset experienced a greater increase in the latency to respond to the sticky tape at the study completion; there was no apparent effect of delayed LPS administration on the sticky tape test (Table 1). Absolute changes in the percentage of foot faults and latency to fall from the Rotorod at study completion in comparison to the best performance on these tasks before 34 days were similar among treatment groups.

The proportion of animals that fully recovered on the sticky tape test, the foot fault test, and the Rotorod before DTH testing and delayed administration of LPS or saline was similar among treatment groups (data not shown). If one restricts analyses to only those animals who had fully recovered on each of the given tests by 34 days, LPS treatment at stroke onset was strongly associated with a decline in performance on the sticky tape test and the foot fault test after delayed administration of LPS or saline (Table 2).

**Immunologic Outcome**

There was no difference in the degree of the Th1 response to MBP, NSE, or PLP or the proportion of animals with a Th1 response to these antigens among any of the treatment groups (Figure 4). There was a strong correlation between the immune response to MBP and performance on the foot fault test; the more robust the Th1 response to MBP, the more foot faults made ($r^2 = 0.366$, $P = 0.008$). There was also an inverse relationship between the Th1 response to MBP and performance on Rotorod; the more robust the Th1 response, the shorter the latency to fall ($r^2 = -0.244$, $P = 0.084$). The relationships between the Th1 responses to NSE and PLP and Rotorod performance were more robust ($r^2 = -0.347$, $P = 0.013$ and $r^2 = -0.338$, $P = 0.015$, respectively). Furthermore, those animals that experienced complete recovery on the Rotorod had less robust responses to NSE (0.71 [0.52 to 1.34] versus 1.06 [0.83 to 1.45]; $P = 0.070$) and PLP (0.80 [0.61 to 1.14] versus 1.13 [0.88 to 1.58]; $P = 0.010$).

To address the question of an immunologic contribution to neurological worsening, analyses were restricted to animals that had fully recovered on the given behavioral measure before immunologic manipulations (DTH testing and delayed LPS or saline administration). For both the sticky tape test and the Rotorod, animals that demonstrated deterioration in
performance by the study’s end had more robust Th1 responses to both NSE and PLP (Figure 5A). Individual animal data for each antigen and the sticky tape test are also displayed graphically in Figure 5. Among animals that experienced a full recovery on the sticky tape by Day 28 after MCAO, a greater proportion of animals that worsened after the immunologic manipulations had evidence of a Th1 response to NSE (15 of 20 [75%] versus 2 of 10 [20%]; \( P = 0.004 \)) and PLP (13 of 20 [65%] versus 3 of 10 [30%]; \( P = 0.070 \)). Graphic data for the Rotorod are not shown, but among the animals that deteriorated after immunologic manipulations, the proportion of animals with a Th1 response to PLP was higher (7 of 13 [54%] versus 1 of 7 [14%]; \( P = 0.085 \)). There was no relationship between deteriorating performance on the foot fault test and the degree of the immune response to the brain antigens, but as for the other behavioral tasks, those that deteriorated after the immunologic manipulations were more likely to have a Th1 response to PLP (12 of 17 [71%] versus 0 of 8; \( P = 0.020 \)).

Immunocytochemistry

There were no differences in the total number of CD4\(^+\), CD8\(^+\), or CD45\(^+\) cells in the infarcted hemispheres (Regions 1 to 4) among the different treatment groups (Figure 6); virtually no lymphocytes were found in the nonischemic hemisphere (Regions 5 to 8). There was a general trend for more CD4\(^+\) cells to be found in the penumbral regions (Regions 1 and 3) and more CD8\(^+\) cells to be found within the cortical region of the infarct (Region 2). CD45\(^+\) cells were seen throughout these regions. Animals treated with LPS at stroke onset had higher numbers of CD8\(^+\) cells in Region 2 (\( P = 0.019 \); Figure 6B); delayed treatment with LPS (at Day 34) did not influence the number of CD8\(^+\) lymphocytes in the brain. For animals that experienced a full recovery on the foot fault test before immunologic manipulations and subsequently worsened, there were more CD4\(^+\) cells in the infarcted hemisphere (48 [32 to 65] versus 26 [20 to 35]; \( P = 0.027 \)), and this increase in CD4\(^+\) cells was primarily seen in Region 2 (\( P = 0.011 \)). No differences were seen in the numbers of cells in brain among those animals that experienced worsening on the sticky tape test or the Rotorod. Finally, animals with a Th1 response to MBP, NSE, or PLP tended to have more CD4\(^+\) cells in the penumbral regions; this reached significance for a Th1 response to MBP in Region 3 (15 [8 to 63] versus 8 [4 to 20]; \( P = 0.045 \)).

Discussion

In this study, we attempted to model anamnestic recall of stroke-related deficits after experimental MCAO. Based on prior data, we hypothesized that the transient clinical worsening seen in patients with stroke when infected at a remote time from the initial stroke could be due to a reactivation of a CNS autoimmune response. Specifically, we have demon-
strated that LPS exposure at the time of MCAO results in an increased likelihood of developing a Th1 response to brain antigens; it was thus anticipated that a second exposure to LPS (to mimic an infection) would result in reactivation of these cells and lead to clinical worsening as a result of the ensuing CNS-specific immune response. In actuality, we found that the second exposure to LPS was not as important as the first. The fact that animals treated with LPS at stroke onset and with saline at 34 days deteriorated similarly to those treated with LPS at both time points can probably be explained by the exposure to MBP during DTH testing with reactivation of the immune response after the antigenic challenge. Given that animals treated with LPS at MCAO demonstrated a significant response to intradermal MBP injection, indicating the presence of a memory response to this antigen, reactivation of the MBP-specific response during DTH testing may have precipitated the decline in neurological function.

Despite the DTH response to MBP among LPS-treated animals, we did not see an increase in the degree of the Th1 response to this antigen (or any of the other brain antigens) among LPS-treated animals at study completion (8 days after DTH testing). For those animals that had fully recovered on a given task before DTH testing (and delayed LPS or saline administration), the Th1 response to NSE and PLP was greater among those animals that experienced subsequent worsening on the sticky tape test and the Rotorod; similar trends were seen for the Th1 response to MBP. The reason that the Th1 response to MBP at study completion did not correlate as well with deteriorating performance as the other antigens is unclear but could be related to a fundamental change in the immune response after MBP exposure with either induction of Treg cells or apoptosis of autoreactive T cells.

Animals with Th1 response to MBP (as detected by enzyme-linked immunospot on splenocytes) had more CD4+ cells in the penumbral regions of brain (Region 3) and animals that experienced “anamnestic recall” on the foot fault test had more CD4+ cells in the ischemic hemisphere. These small differences in cell numbers, however, may not adequately represent the difference in the nature of the immune response. For instance, the CD4+ cells in the brains of animals with a Th1 response to brain antigens in the spleen may truly have been Th1 effector cells, whereas the CD4+ cells in the brains of animals without a Th1 response to brain antigens in the spleen may have been Treg cells. Further histological analyses for key effector cytokines as well as nonlymphocytic inflammatory cells (ie, neutrophils and monocytes) would provide key data regarding the nature of the immune response in brain. Alternatively, enzyme-linked immunospot analyses could be done to characterize the immune responses of lymphocytes extracted from the brain.

The phenomenon of anamnestic recall of stroke-related deficits is familiar to most clinicians who care for patients with stroke, although virtually nothing is known or written about it. The fact that this transient re-emergence of stroke-related symptoms occurs primarily in the setting of systemic infection must provide a clue to its pathophysiology. In this study, we show that among the animals most likely to have developed a Th1 immune response to brain antigens (ie, those treated with LPS at stroke onset), subsequent challenge with

Figure 6. Intraclass correlation coefficient was done in both the infarcted hemisphere (Regions 1 to 4) and the noninfarcted hemisphere (Regions 5 to 8) at predefined levels (A). The most abundant inflammatory cell infiltrates were seen in Region 2. Within Region 2, there were more CD8+ cells among animals treated with LPS at stroke onset (B). Examples of cells labeled for CD4, CD8, and CD45 in region 2 are seen in C. RX indicates treatment; NS, nonsignificant.
a brain antigen (ie, MBP) is associated with an “anamnestic recall” of initial stroke-related dysfunction. If during an infection there is an adequate inflammatory stimulus to activate systemic lymphocytes, these cells could cross an intact blood–brain barrier.5,18 Alternatively, a particularly robust inflammatory stimulus may compromise the blood–brain barrier allowing lymphocytes to encounter CNS antigens in either the brain or the periphery; these encounters could lead to lymphocyte reactivation.19,20 Both of the situations outlined could potentially lead to an inflammatory response in the brain with transient worsening of neurological function. Indeed, systemic inflammation is capable of reactive immunemediated lesions in the rat brain.21 Another clinically relevant scenario occurs in patients with recurrent strokes; ischemic death of brain tissue leads to antigen release into the peripheral circulation where lymphocytes previously “educated” to the antigen might reencounter and become reactivated to the antigen allowing for transit across an intact blood–brain barrier.5,22–25 In addition, the blood–brain barrier is compromised after an ischemic insult, which allows even quiescent lymphocytes access to the CNS for re-encounter to brain antigens.26,27 The ensuing immune response may help to explain why patients with recurrent stroke are more prone to dementia and have more neurological dysfunction than might be expected for the amount of injured brain.28,29

In summary, we have attempted to model the phenomenon of anamnestic recall of stroke-related deficits after experimental stroke. We had hypothesized that delayed exposure to an inflammatory stimulus (LPS) would lead to re-emergence of stroke-related deficits in animals most likely to have a Th1 response to brain antigens but found that it was the exposure to MBP during DTH testing that precipitated the decline in performance on the behavioral tasks. These observations, along with the fact that the Th1 responses to brain antigens were greatest in the animals that experienced deterioration in performance after DTH testing, suggest that the anamnestic recall of stroke-related deficits may have an immunologic basis.

Disclosures
None.

Sources of Funding
This work was supported by NINDS (R01 NS056457).

References
Anamnestic Recall of Stroke-Related Deficits: An Animal Model
Dannielle Zierath, Jessica Hadwin, Anna Savos, Kelly T. Carter, Allison Kunze and Kyra J. Becker

Stroke. 2010;41:2653-2660; originally published online October 14, 2010;
doi: 10.1161/STROKEAHA.110.592865
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/41/11/2653

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/