Yemisci, Gursoy-Ozdemir and Dalkara reply:

We thank our colleagues[1] for the opportunity to clarify the interpretation of some data in our recent paper[2].

First, we think that the sausage-like appearance of capillaries after ischemia is caused by pericyte contractions and not by decapitation or fixation artifacts. We consistently observed consecutive capillary constrictions separated by the distance between pericytes during intravital recordings (Fig. 6a–d in our paper). These constrictions, which started within the second hour of ischemia, were not artifacts, because they were reversed when reperfusion or peroxynitrite application was combined with *N*-tert-butyl-α-phenylnitrone or ebselen treatments, respectively. We agree that these observations strongly suggest, but do not directly prove, that the constrictions were caused by pericyte contractions. For this reason, we showed in isolated retinas that the capillary constrictions and pericyte contractions were spatially and temporally colocalized. Vates *et al.*[1] question these data on the grounds that retinal pericytes might not behave like cerebral pericytes. However, despite some differences, retinal and cerebral pericytes do share the same basic vasoregulatory characteristics and respond similarly to vasoactive stimuli[3]. We chose to use the retina because it could be examined without disrupting its integrity, as opposed to brain slices, which are subjected to additional injury by tissue sectioning.

If one enlarges Figure 6f, constrictions between the successive red blood cell rouleaus and tapering of the ends of each fluorescent dye column can be identified. The intermittently stacked pattern of erythrocyte rouleaus is hard to reconcile with end-feet or endothelial swelling. As discussed in the paper[2], this discontinuous pattern is more compatible with an actively constricting element rather than passive swelling of endothelia or end-feet, although their contribution to luminal narrowing cannot be ignored. We agree that future multiphoton microscopy studies should include direct demonstration of pericyte contraction in cortical microcirculation with selective *in vivo* markers of pericytes. However, one must be cautious, as these experiments may yield differing results depending on the experimental conditions. Indeed, we noticed during intravital experiments that microvascular responses crucially depended on systemic physiological parameters and window conditions in addition to the duration of ischemia. Very focal ischemic lesions produced by laser beams in multiphoton experiments may not represent the proximal middle cerebral artery ischemia model that we used. Arguing that nodal constrictions on capillaries in ischemic brain and red blood cell entrapments, which have been described by several groups over the past 40 years[4–8], are artifacts would require direct experimental evidence.

Second, we used the term 'pericyte-encircled lumens' when describing the pharmacological data illustrated in Figure 3 and carefully referred to these structures as microvessels, not as capillaries. As we had already documented the association between capillary constrictions and pericyte contraction in Figures 1 and 2, we focused on relative luminal changes in the pharmacological experiments illustrated in Figure 3. To avoid variability and selection bias in luminal diameter caused by sectioning of microvessels at various angles, we compared only the pericyte-encircled ovoid and circular microvessel cross sections between groups. Because of this measurement method, the average diameter seemed artificially larger than the capillary diameter, although most of these microvessels were capillaries. In all parts of the study, we paid attention to the size of the microvessel lumen, and the averages that we specified as the capillary diameter were around 6 μm. We did confirm the identity of the smooth muscle actin–labeled cells with NG2 and also with other markers such as claudin-5 (Fig. 4). When compared with images in the bottom of Figure 2, the NG2-labeled microvessels have the same shape as illustrated in Figure 3d,j. This appearance of pericytes on cross-sections is strikingly similar to their electron microscopic appearance on capillary cross-sections[9,10]. They were not perinuclearly stained cells, because none of these images had a nucleus in the unstained area corresponding to the lumen with nuclear stains.

Third, we did refer to arterioles several times by indicating in the introduction that "precapillary arterioles generally remain open," by stating in the results that "peroxynitrite induced a modest increase in arteriolar diameter from 27 ± 1 μm to 33 ± 1 μm" and by discussing potential mechanisms of different responses between capillaries and arterioles.

After initial enthusiasm in the 1970s about the no-reflow phenomenon—impaired reperfusion of the brain tissue due to microcirculatory failure after successful recirculation—the hypothesis lost its popularity largely as a result of claims that the observations might be histological artifacts[11]. These critiques unfortunately slowed down progress in this potentially important area in stroke. Recently, the Echoplanar Imaging Thrombolytic Evaluation (EPITHET) trial showed that tissue reperfusion predicts stroke outcome independently of recanalization[12]. Similarly, a recent report showed that complete reopening of an occluded artery with tissue plasminogen activator may not lead to adequate reperfusion of the ischemic tissue[13]. These results underscore the need for more research on the no-reflow phenomenon. We hope to see new studies rigorously testing our hypotheses in the near future and are happy to have already stimulated this discussion.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Being too inclusive about synuclein inclusions

To the Editor:

We read with interest the recent review by Obeso *et al.*[1], in which they discuss recent studies on the presence of α-synuclein inclusions in tissue transplanted to people with Parkinson's disease. Although the review cites our related study[2], we do not think that the work is correctly described; our work, which shows no α-synuclein pathology in the transplant, is listed by the authors together with other studies that show α-synuclein aggregates. The finding that 5–8% of transplanted neurons have Lewy bodies that the authors give as a generalization applies only to the findings of Kordower *et al.*[3] and not to ours.