of thrombotic stroke. Because the blood–brain barrier is compromised during stroke, tPA might gain access to the brain parenchyma, thus contributing to neuronal cell death. Second, inhibition of tPA activity in the adult mouse could protect against certain neuronal pathologies.

To distinguish between a developmental or an acute need for tPA, we delivered tPA protein bilaterally to the hippocampus of $tPA^{-/-}$ mice, then injected kainate (a glutamate analogue) on one side, and assessed the extent of neuronal death. We found that $tPA^{-/-}$ mice infused with buffer (control) did not exhibit significant neuronal degeneration after kainate injection (Fig. 1, top)[5]. In contrast, $tPA^{-/-}$ mice infused with tPA exhibited dramatic neurodegeneration in the kainate-injected hippocampus (Fig. 1, bottom). We detected proteolytic activity[1] equally over both sides of the hippocampus of the tPA-infused $tPA^{-/-}$ mice (verfying that tPA was delivered appropriately), whereas we observed no tPA activity in the control $tPA^{-/-}$ mice. Because we observed degeneration only in the kainate-injected side, tPA alone at this concentration is not sufficient to kill neurons. These results indicate that tPA is required for neuronal death in the adult animal at the time of excitotoxic insult.

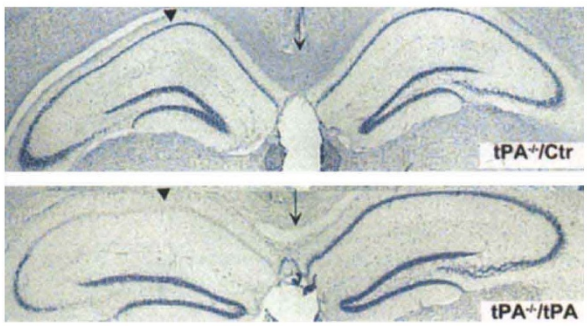

FIG. 1 Intra-hippocampal delivery of tPA restores susceptibility of hippocampal neurons to kainate injury in $tPA^{-/-}$ mice. Coronal sections through the hippocampus of $tPA^{-/-}$ mice stained with cresyl violet to reveal the status of neuronal cells. Mice were infused with PBS (top) or recombinant human tPA (bottom) for 2 days, injected unilaterally with kainate, and the infusion continued for 5 days further, at which point the mice were analysed. Percentage lengths of hippocampal subfields remaining intact after kainate infusion: 100% CA1, 91.2 ± 0.1% CA2/CA3 (top); 10.3 ± 4.4% CA1, 9.1 ± 5.2% CA2/CA3 (bottom). Arrowheads, site of injection; arrows, site of infusion. A micro-osmotic pump (Alzet Inc.) containing buffer (for control animals), or 100 μl human tPA (rtPA, 0.12 mg ml^{-1}), was placed subcutaneously in the back of anaesthetized mice, and a brain infusion cannula connected to the pump was positioned at coordinates: bregma −2.5 mm, medial–lateral −0.5 mm and dorsoventral 1.6 mm, for delivery near the midline. The pump was allowed to infuse (0.5 μl h^{-1}) for 2 days. Mice were then injected with kainate and analysed[4].

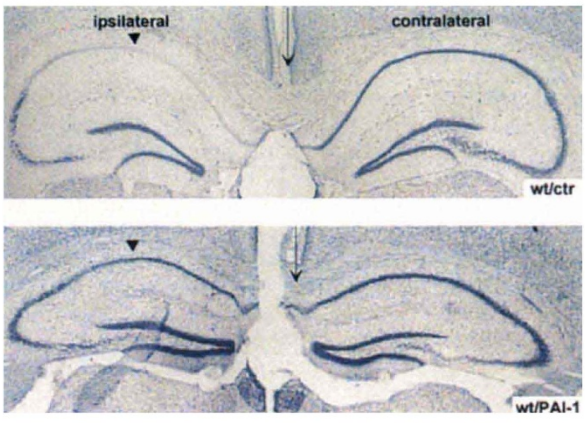

FIG. 2 Plasminogen activator inhibitor type-1 prevents kainate-induced neuronal degeneration. Cresyl violet-stained coronal sections through the hippocampus of wild-type mice. The mice were infused with buffer (top), or mouse PAI-1 (0.12 mg ml^{-1}, American Diagnostica; bottom) for 2 days, and kainate injected as in Fig. 1. Percentage lengths intact after kainate infusion: 6.3 ± 3.1% CA1, 18.1 ± 3.6% CA2/CA3 (top); 68.3 ± 7.4% CA1, 80.9 ± 4.1% CA2/CA3 (bottom). Arrowheads, site of injection; arrows, site of infusion.

As tPA is required to promote neuronal degeneration, we tested whether inhibition of its enzymatic activity might retard neuronal death. We infused mice with plasminogen activator inhibitor-1 (PAI-1; a serine-protease inhibitor that inhibits tPA[8]), injected them with kainate, and assessed neuronal survival in the hippocampus. Wild-type mice infused with buffer were sensitive to neuronal degeneration in the hippocampus (Fig. 2, top). In contrast, mice infused with PAI-1 were resistant to kainate-induced neuronal death (Fig. 2, bottom). The resistance of wild-type mice infused with PAI-1 was comparable to that observed with buffer-infused $tPA^{-/-}$ mice.

Various neuropathological conditions may involve excitotoxic damage to the brain[9]. The identification of extracellular proteases as contributing to the degeneration pathway provides an attractive target for therapeutic intervention. One concern of such therapy in humans would be that inhibition of tPA might lead to clotting disorders. However, $tPA^{-/-}$ mice do not exhibit spontaneous thromboses[4], indicating that blood homeostasis is possible in the absence of tPA. Therefore, it seems reasonable to investigate further the effectiveness of protease inhibitors for the therapy of excitotoxic-mediated brain disorders.

Thrombotic stroke is thought to involve an excitotoxic pathway[9], and tPA can promote neuronal death after excitotoxic insult. Recent studies report treatment of ischaemic stroke within three hours of onset with intravenous tPA[10,11]. Certainly the excitotoxic cell death caused by injections of kainate does not exactly mimic the damage observed in cerebral ischaemia. Nevertheless, given the possibility that administration of tPA to stroke patients might lead to increased protease levels in the brain parenchyma[12], it seems prudent to examine the potential risk that tPA may cause neuronal death in the already vulnerable tissue.

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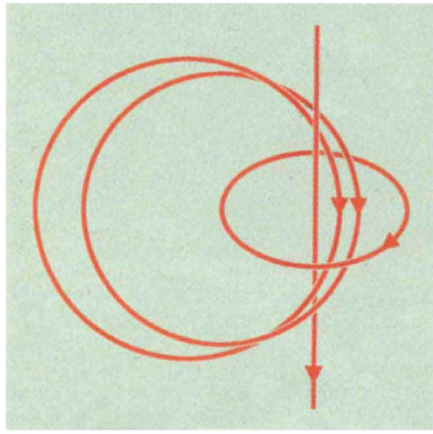
Correction



In the Scientific Correspondence "Is ball lightning an electromagnetic knot?" by A. F. Rañada and J. L. Trueba (*Nature* **383**, 32; 1996), Fig. 1, a schematic version of the magnetic lines in the case $n=1$, was printed incorrectly. The correct version is shown above. In Fig. 2*a*, the origin on the ordinate axis should have read '1'.