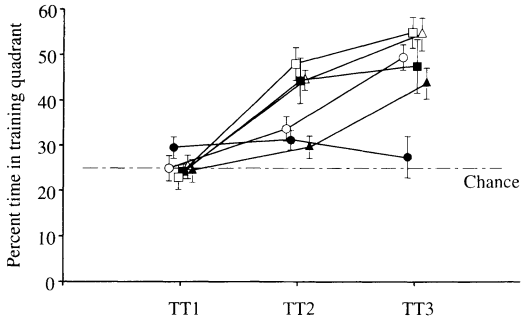

FIG. 4 Comparison of the percentage time in the upstairs training quadrant (± s.e.m.) in transfer tests 1, 2 and 3 by the three AP5 (filled symbols) and three aCSF (open symbols) groups given either no pretraining downstairs (circles, experiment 1), spatial pretraining (squares, experiment 2) or non-spatial pretraining (triangles, experiment 4) (chance = 25%). Note all groups started upstairs at chance levels of performance. As noted in the text, the ANOVA revealed a significant triple interaction between drug group, pretraining condition, and transfer test ($F = 4.23$, d.f. 2/64, $P < 0.025$). Non-pretrained AP5-treated rats showed no evidence of spatial learning at any point. AP5-treated rats given non-spatial pretraining showed little spatial learning at TT2 but substantially more than the non-pretrained AP5 group by TT3 ($P < 0.01$; planned F-test using error-mean square from ANOVA). AP5 rats given spatial pretraining learned equivalently to their aCSF controls ($P > 0.10$) and showed clearly better performance than the non-spatially pretrained AP5 group during both transfer test 2 ($P < 0.05$) and transfer test 3 ($P < 0.05$).

that may be more sensitive to disruption of LTP[23]. Other components of spatial learning could include learning to attend appropriately[24], to navigate in a spatially directed way[25], and/or to develop expectations about the reliable location of fixed objects in space[26]. Such learning cannot take place during non-spatial pretraining. If acquiring this information usually involved NMDA-receptor dependent synaptic plasticity, and was dependent upon the hippocampus for its expression (even if stored elsewhere), a dissociation between the effects of hippocampal lesions and AP5 upon subsequent spatial learning would result (experiments 2 and 3). This is precisely what we observed in animals given spatial pretraining. □

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Spatial learning without NMDA receptor-dependent long-term potentiation

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HIPPOCAMPAL lesions impair spatial learning in the watermaze[1]. Drugs that antagonize *N*-methyl-D-aspartate (NMDA)-receptor activity, which is required for long-term potentiation (LTP) at various hippocampal synapses[2–4], block LTP and impair watermaze learning[4–6]. This has led to the hypothesis that NMDA receptors, through their involvement in LTP, may be necessary for spatial[4–11] and other forms of learning[12,13]. We examined this hypothesis using NPC17742 (2*R*,4*R*,5*S*-2-amino-4,5-(1,2-cyclo hexyl)-7-phosphonoheptano acid), a potent and specific antagonist of NMDA receptors[14–17]. Here we report that NPC17742 completely blocked dentate gyrus LTP but did not prevent normal spatial learning in rats that had been made familiar with the general task requirements by non-spatial pretraining. Although these results do not rule out a contribution of NMDA-mediated dentate LTP to spatial learning, they indicate that this form of LTP is not required for normal spatial learning in the watermaze.

In experiments that involved a detailed behavioural analysis of watermaze learning in naive rats given NMDA antagonists, performance of the required behaviours was impaired by the sensorimotor disturbances that result from NMDA antagonism[18,19], such as thigmotaxic and slowed swimming, or swimming over or deflecting off the platform. Correlations between the sensorimotor disturbances and measures of maze acquisition were robust. Non-spatial pretraining[5,6] in the general task requirements eliminated acquisition deficits[18,19]. Therefore we used non-spatial pretraining to evaluate whether rats familiar with the task requirements could acquire spatial information when NMDA-dependent LTP was blocked. The same rats were used in both electrophysiological and behavioural testing to confirm that LTP was blocked by the same treatment as used for NMDA receptor antagonism during maze testing. Groups and procedures are summarized in Fig. 1.

We first evaluated the ability of NPC17742 (5.0 mg per kg body weight in dimethyl sulphoxide, intraperitoneal) to block dentate gyrus LTP. NPC17742, a potent and specific competitive NMDA-receptor antagonist[14–17], readily crosses the blood–brain barrier. The dose used was twice the 50% effective dose for blocking NMDA-induced seizures and had the expected effects on seizures, spontaneous behaviour, motor ability[14,19] and kindling (D.S. and D.P.C., manuscript submitted). NPC17742 completely blocked the induction of LTP (Fig. 2).

One subgroup ($N = 7$) of pretrained NPC-treated rats was trained in the watermaze, followed 1–2 weeks later by LTP without NPC17742. The other subgroup ($N = 7$) was tested similarly in reversed order. Three rats failed to complete this LTP test, leaving subgroups of 5 and 6 rats, which did not differ on any measure ($P > 0.05$) and were combined. Without NPC17742 the expected LTP occurred (Fig. 2), confirming that the experimental arrangements were capable of inducing and detecting LTP.

Five days after non-spatial pretraining, the pretrained NPC-treated rats received 5.0 mg per kg NPC17742, and 1 h later were trained in the hidden-platform task (10 trials; elapsed time 1 h) followed by a 1-min probe trial (hidden platform removed), followed by the visible-platform task (10 trials). Other groups also completed the watermaze task (Fig. 1).

The improvement in mean search time for the pretrained groups from pretraining days 1–4 indicated that they acquired

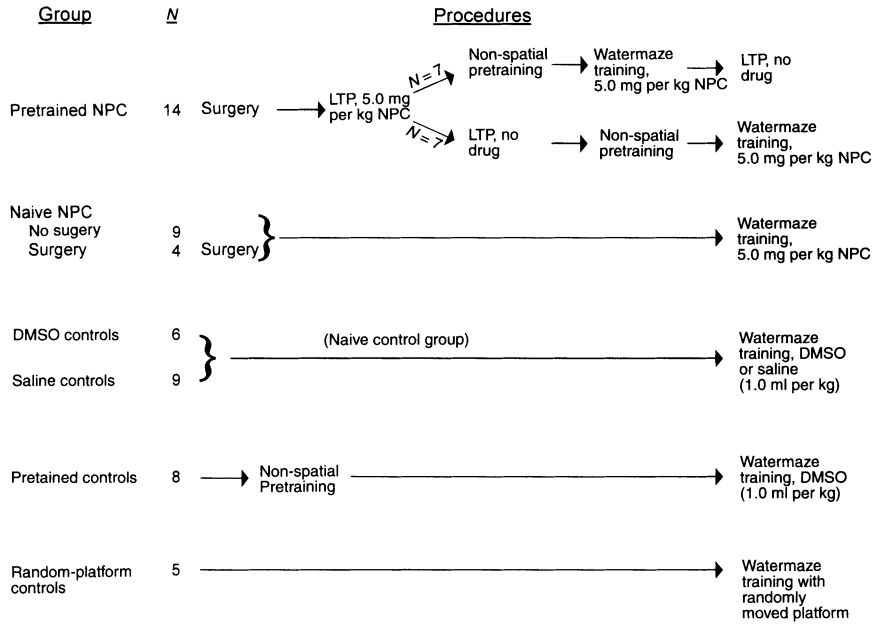

FIG. 1 Flow diagram indicating groups, procedures and treatments. *N* indicates number of subjects. Four naive NPC-treated rats with electrode implants to evaluate effects on maze learning did not differ from the unimplanted subgroup on any measure ($P > 0.05$). DMSO and saline controls did not differ on any measure ($P > 0.05$) and were combined into the naive control group. Surgery and LTP procedures were similar to those in earlier reports[24,28] and are described in Fig. 2. For watermaze training a white circular (1.5 m) pool with a hidden platform (15 × 15 cm) 1 cm below the water surface was used. Water temperature was 29 °C, and animals were placed under a heat lamp between trials. Swim paths were videotaped, digitized, and analysed using the Poly-Track system (San Diego Instruments). For visible-platform training trials the platform protruded 2.5 cm above the surface, was marked by a 15-cm high object, and was moved between trials. Non-spatial pretraining for those rats that received it was conducted on 4 consecutive days beginning 8 days before watermaze training began (3 trials per day, 4-h intertrial interval, 12 trials in total, no drug administered), with the hidden platform moved pseudorandomly to a new quadrant after every trial[5,6,18,19]. Black curtains around the pool eliminated distal cues. Rats swam until they found the hidden platform or until 120 s elapsed, at which time they were placed on the platform, where they remained for 30 s.

strategies appropriate for coping with the task, such as swimming away from the maze wall (pretrained NPC group, 44.0, 19.2 s; pretrained controls, 42.8, 19.0 s). The pretrained NPC group readily learned the location of the hidden platform, with search times similar to those of the naive and pretrained control groups (Fig. 3*a*). Analysis of behaviour in the pool revealed numerous sensorimotor disturbances in the naive NPC group, but few disturbances in the pretrained NPC group[19] (data not shown). Similar results occurred in the visible-platform task; the naive NPC group had longer search times than the pretrained NPC group (Fig. 3*a*), which is consistent with sensorimotor disturbances interfering with performance of this cue-mediated task[19,20]. Probe trial search time in the hidden-platform quadrant remained near chance (15 s) for the naive NPC and random-platform groups but above chance for the other groups (Fig. 3*b*), suggesting that they had learned the spatial location of the

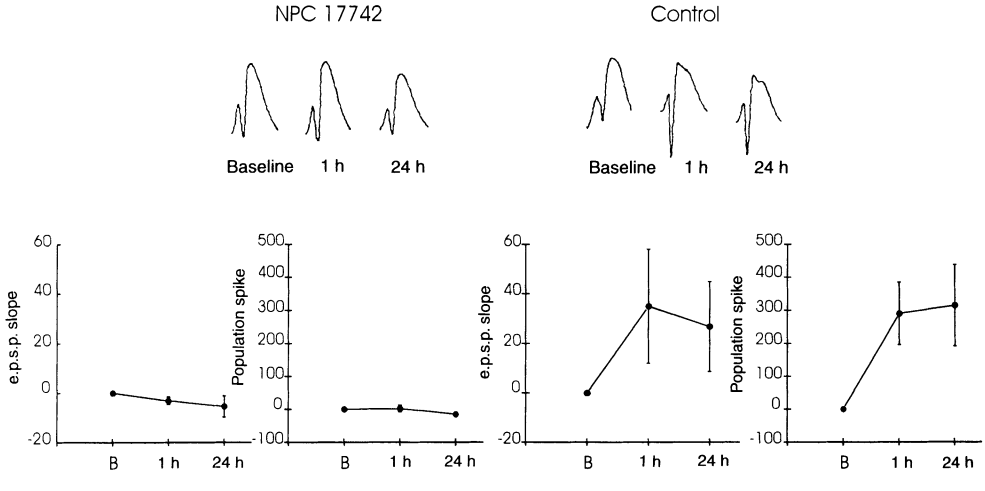

FIG. 2 Effects of NPC17742 on LTP (left), and normal LTP (no NPC17742, right) in the pretrained NPC group. Representative averages at baseline and at 1 and 24 h after high-frequency trains are from the same rat (top). Group mean (±s.e.m.) values (bottom) represent the baseline (B), and percentage change at 1 and 24 h. One-tailed non-parametric Wilcoxon signed-ranks tests were used to evalute the reliability of the changes because the data were not normally distributed, and we predicted that under normal conditions the measures would increase[24,28]. Baseline measures did not differ between the NPC17742 and no-treatment conditions ($P > 0.05$), indicating good stability of the experimental arrangements. The measures did not change ($P > 0.05$) under NPC17742 (left). The excitatory postsynaptic potential (e.p.s.p.) slope increase in the no-treatment condition (right) was reliable at 1 h ($P < 0.025$) and 24 h ($P < 0.05$); the population spike increase was reliable at 1 h ($P < 0.005$) and 24 h ($P < 0.005$). Male hooded rats anaesthetized with sodium pentobarbital (65 mg per kg) received implantation of chronic stimulating and recording electrodes (127 μm) into the perforant path and the ipsilateral dentate hilus to record evoked field potentials[24,28]. Single diphasic (0.1 ms per phase) test pulses were delivered only during behavioural immobility[28], a minimum of 10 s apart. Final positioning of the electrodes was determined by recordings from the hilar electrode. Responses were digitized, averaged (10 sweeps), and analysed for the rising phase of the field e.p.s.p. and area under the population spike. Rats studied exhibited a negative-going extracellular population spike to stimulation <100 μA; the response to a test pulse of 800 μA was 10.4 ± 1.3 mV (mean ± s.e.m.). Input/output determination was followed by NPC17742 and determination of baseline 1 h later. For LTP, 10 high-frequency trains (8 diphasic pulses, 0.1 ms per phase, 400 Hz, near-maximal input/output response) were applied to the perforant path. Rats were unanaesthetized throughout testing.

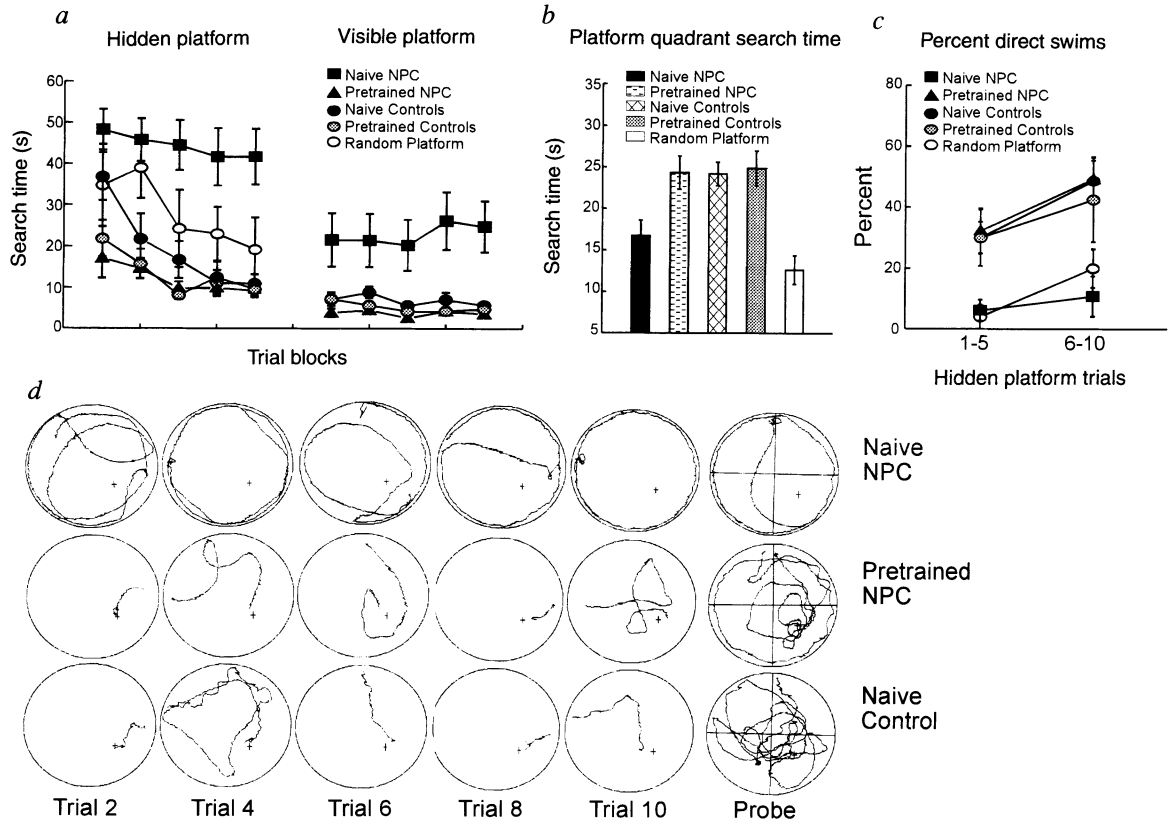

FIG. 3 Watermaze acquisition. *a*, Mean (±s.e.m.) search times (s) for hidden and visible platform tasks plotted as 2 trials per block. Repeated measures ANOVA of hidden-platform data yielded effects of group ($F(3,9)=18.1$, $P<0.0001$; naive NPC versus all other groups, $P<0.05$, Newman-Keuls), trials ($F(9,405)=11.8$, $P<0.0001$), and a group × trials interaction ($F(27,405)=1.5$, $P<0.05$). Separate repeated-measures ANOVAs of pretrained NPC, pretrained control, or naive control data yielded block effects, indicating comparable learning in these groups (pretrained NPC, $F(4,44)=3.1$, $P<0.03$, block 1 versus 3, 4 or 5, and block 2 versus 5, $P<0.05$; pretrained control, $F(4,24)=6.0$, $P<0.002$, block 1 versus 3, 4 or 5, and block 2 versus 3, $P<0.05$; naive control, $F(4,52)=9.5$, $P<0.001$, block 1 versus 2, 3, 4 or 5, and block 2 versus 5, $P<0.05$). Analysis of visible-platform data yielded an effect of group ($F(3,9)=7.6$, $P<0.001$; naive NPC versus all other groups, $P<0.05$; pretrained NPC versus naive control group, $P<0.05$). These findings were robust; for data in *a*–*c*, when the 3 pretrained NPC rats that did not complete the no-treatment LTP procedure and the 4 naive NPC rats with electrode implants were excluded from the analysis, the identical pattern of findings was obtained. *b*, Mean search time in the hidden-platform quadrant after training. Search time differed among the groups ($F(3,34)=4.4$, $P<0.008$; naive NPC versus all other groups, $P<0.05$). *c*, Mean percentage direct swims (a swim path that remained inside a band 18cm wide from the start to the hidden platform). Analysis as in *a* yielded effects of group ($F(3,1)=9.0$, $P<0.0001$; naive NPC versus all other groups, $P<0.05$) and trials ($F(1,45)=8.4$, $P<0.006$). *d*, Individual swim paths and probe trials for rats with the summed hidden-platform search time closest to their group mean. Cross indicates hidden-platform position. Naive NPC rats swam thigmotaxically and infrequently encountered the hidden platform. Pretrained NPC and naive control rats searched the inner areas of the pool, frequently encountering the hidden platform; having missed the platform they swam back to it (pretrained NPC, trials 6, 10; naive control, trial 4). Pretrained control rats swam similarly (not shown). Search time in the hidden-platform quadrant (probe trial) for these 3 rats was: naive NPC, 12.4 s; pretrained NPC, 24.0 s; naive control, 20.2 s.

hidden platform. The percentage of direct swims was similar in the pretrained NPC, pretrained control, and naive control groups (Fig. 3*c*), a further indication of spatial learning. Naive NPC rats swam thigmotaxically and seldom encountered the platform, whereas the other groups searched the inner area of the pool, frequently encountering the platform (Fig. 3*d*).

In two earlier studies involving non-spatially pretrained rats trained under NMDA antagonism with D(-)-2-amino-5-phosphonovaleric acid (AP5), the pretraining improved but did not completely normalize watermaze performance[5,6]. Our consistent finding in this and earlier research[18,19] has been that non-spatially pretrained rats can learn the watermaze as effectively as controls. Two differences in procedure, among others[18,19], might explain the different results. First, the substantially greater task difficulty and longer mean intertrial interval in the earlier studies[5,6] would be expected to prevent the AP5-treated rats from finding the hidden platform as effectively as controls within the limited number of trials allowed[21]. Second, our use of an acute drug administration and behavioural testing protocol avoided possible disruptive consequences on maze learning of the pathomorphological reaction that occurs in neocortex in response to chronic administration of NMDA antagonists[22,23].

The occurrence of robust spatial learning under the same NMDA antagonism that blocked LTP suggests that NMDA-mediated dentate-gyrus LTP is not required for spatial learning in this task, a conclusion consistent with reports that LTP saturation does not disrupt spatial learning[24–27]. The possibility that NMDA-mediated LTP makes a non-essential contribution to spatial learning cannot be excluded, but the demonstration of this will require additional research. □

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NT-4-mediated rescue of lateral geniculate neurons from effects of monocular deprivation

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ALTERING the balance of activity between the two eyes during the critical period for visual-system development profoundly affects competitive interactions among neurons in the lateral geniculate nucleus and primary visual cortex[1–6]. Neurons in the lateral geniculate nucleus that are deprived of activity by closing or silencing one eye atrophy as a result of competition with non-deprived neurons for some critical factor(s) presumed to be present in the cortex. Based on their actions in the developing visual system[7–12], neurotrophins are attractive candidates for such factors. We tested whether neurotrophins mediate intracortical competition of afferents from the lateral geniculate nucleus by using monocular deprivation and a new method for highly localized, *in vivo* delivery of neurotrophins. This method allowed unambiguous identification of neurons that were exposed to neurotrophin. Here we report that only one neurotrophin, the TrkB ligand NT-4, rescued neurons in the lateral geniculate nucleus from the dystrophic effects of monocular deprivation.

To overcome limitations of common methods for *in vivo* application of neurotrophins (such as osmotic minipumps and intracortical or intraventricular injection) we developed a new delivery system in which neurotrophins were bound to fluorescent latex microspheres. Coated microspheres had activity comparable to that of free neurotrophin, retained activity for at least four days at 37 °C (ref. 13), and remained competent to undergo retrograde axonal transport. To determine whether neurotrophins modulate the effects of monocular deprivation (MD) in the developing visual system, we closed one eye of each of 21 ferrets (postnatal day 38 to 42) and made injections of microspheres coated with nerve growth factor (NGF), NT-4, NT-3 or brain-derived neurotrophic factor (BDNF) into the contralateral primary visual cortex (V1; Fig. 1, top). The microspheres were taken up by nerve terminals and retrogradely transported[14]. Thus we could identify the projection neurons in the lateral geniculate nucleus (LGN) that were exposed to neurotrophin (Fig. 1, middle and bottom). Within individual animals, adjacent injections of control microspheres allowed comparison of these neurons with others that projected to V1

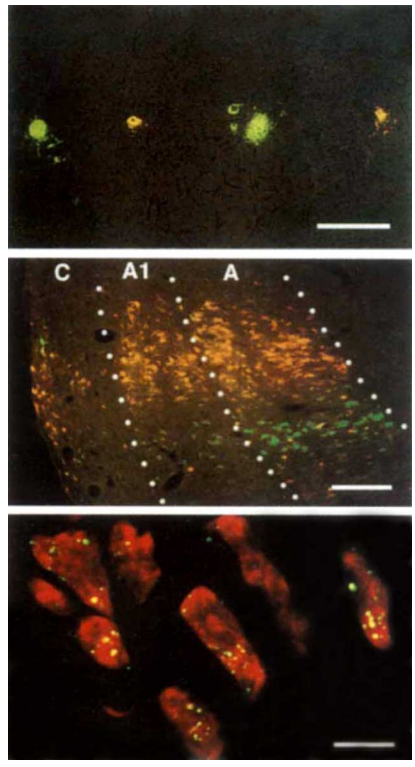

FIG. 1 *In vivo* delivery of neurotrophins. A horizontal section through area 17 of ferret cortex is shown containing four injection sites, two with red NT-4-coated microspheres (which appear orange in this double exposure) and two with green control microspheres (top; scale bar, 500 μm). Columns of retrogradely labelled neurons extend across the A and A1 layers of the LGN after injections in V1 (middle; scale bar, 200 μm); laminar borders are indicated by dotted lines (sublaminae are apparent within A and A1). Adjacent regions of red and green labelled cells projected to adjacent injection sites in the visual cortex. At higher magnification, green microspheres are apparent throughout the cytoplasm of several labelled neurons (bottom; scale bar, 10 μm, dual-channel confocal micrograph).

METHODS. Ferrets (P38–P42) were prepared for aseptic surgery as previously described[28]. A burr hole was opened on the right side to expose much of the length of area 17, which in the ferret lies at the posterior pole of the cortex[29]. The dura was incised and microspheres were pressure injected at 4–6 sites in V1 (in the area corresponding to central visual field), using a glass micropipette with a tip diameter of 20–30 μm. For each injection the pipette was advanced to a depth of 0.85 mm and approximately 200 nl of microspheres was then ejected as the pipette was withdrawn. Injections of neurotrophin-coated and control microspheres of different colours were interdigitated and separated by 0.75–1.0 mm (both immunocytochemical analysis with antibodies to NGF and injections of horseradish peroxidase-coated microspheres indicated that proteins do not diffuse from the microsphere injection site). The contralateral eye was then closed using standard techniques[1]. One litter of ferrets was used to test the effects of each neurotrophin; in half of the ferrets from each litter the neurotrophin was bound to red microspheres and the controls were green; in the remainder the colours were reversed. The ferrets were killed after four days by sodium pentobarbital overdose (100 mg kg^{-1}) and perfused with phosphate-buffered paraformaldehyde. The brain was removed, blocked, postfixed and cryoprotected. The right occipital cortex was dissected for separate sectioning before the block containing the right LGN was sectioned in the sagittal plane using a cryostat. Alternate sections (30 μm) of the LGN were mounted, counterstained with ethidium bromide, and coverslipped.