THE ROYAL SOCIETY

Prospects for the clinical application of neural transplantation with the use of conditionally immortalized neuroepithelial stem cells

Jeffrey A. Gray[1,2*], **Helen Hodges**[1,2] **and John Sinden**[2]

[1]*Department of Psychology, and* [2]*ReNeuron Ltd, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, UK*

Although neural transplantation has made a relatively successful transition from the animal laboratory to human neurosurgery for the treatment of Parkinson's disease, the use of human embryonic brain tissue as the source of transplants raises difficult ethical and practical problems. These are likely to impede the widespread use of this otherwise promising therapy across the range of types of brain damage to which the results of animal experiments suggest its potential applicability. Various alternative approaches are reviewed briefly, aimed at developing sources of tissue for transplantation that can be maintained *in vitro* until needed, so obviating the requirement for fresh embryonic tissue at each occasion of surgery. Particularly promising are conditionally immortalized neuroepithelial stem cell lines in which the immortalizing gene is downregulated upon transplantation into a host brain. We describe experiments from our laboratory with the use of cells of this kind, the multipotent MHP clonal cell lines, derived from the developing hippocampus of a transgenic mouse harbouring a temperature-sensitive oncogene. Implanted into the hippocampus of rats and marmosets with damage to the CA1 cell field, the MHP36 line gave rise to healthy surviving grafts and to essentially complete recovery of cognitive function. Post-mortem study of the implanted rat brains indicated that MHP36 cells migrate to the region of damage, adopt both neuronal (pyramidal) and glial phenotypes *in vivo*, and reconstitute the normal laminated appearance of the CA1 cell field. We have previously shown that, when primary differentiated foetal tissue is used as the source of grafts in rats with CA1 damage, there is a stringent requirement for replacement with homotypic CA1 cells. We interpret our results as showing that the MHP36 cell line responds to putative signals associated with damage to the hippocampus and takes up a phenotype appropriate for the repair of this damage; they therefore open the way to the development of a novel strategy with widespread applicability to the treatment of the diseased or damaged human brain.

Keywords: neural transplantation; neuroepithelial stem cells; conditional immortalization; hippocampus; cognitive function

1. INTRODUCTION

Attempts to transplant neural tissue into the brain go back to the last century (Thompson 1890; Forssman 1898), followed by a steady but slow stream of further experiments throughout the present century (see table 1). However, the modern era of intense investigation of such grafting has a very short history, starting with the seminal experiments of Stenevi *et al*. (1976), Lund & Hauschka (1976) and Perlow *et al*. (1979). The recent work has been characterized by an intimate mixture of scientific and clinical concerns. Methodologically, transplantation of neural tissue into the living brain has provided a powerful tool for probing a variety of issues central to developmental neurobiology, as well as a novel approach to questions about the behavioural functions of the tissue so transplanted. At the same time, however, much of the motivation underlying such experiments has come from the hope that they would eventually give rise to a viable strategy for the treatment of human brain damage and disease. To some extent these hopes have already been realized.

This evolution has gone furthest with Parkinson's disease (Annett 1994; Björklund *et al*. 1994*a*; Brundin *et al*. 1994; Lindvall 1994). The major proximal cause of the disturbances of motor function (bradykinesia, rigidity, tremor and postural instability) that characterize this disease lies in degeneration of the dopaminergic cells that project, by way of the nigrostriatal pathway, from the substantia nigra in the midbrain to the caudate-putamen in the basal ganglia. It is possible to make an 'animal model' of Parkinson's disease by destroying these cells or their axons in the nigrostriatal pathway, using catecholamine-specific neurotoxins such as 6-hydroxydopamine in rodents, or 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine in monkeys. A large number of experiments with animals prepared in this manner have demonstrated motoric dysfunctions that can be alleviated temporarily by pharmacological agents, for example (–)-dihydroxyphenylalanine (L-DOPA), that are also effective (although to a limited degree) in the clinic. These dysfunctions can also be reversed permanently by transplantation into the caudate-putamen of suspensions of

*Author for correspondence.

Received 21 April 1998 *Accepted* 10 June 1998

Table 1. *Milestones in the development of neural transplantation*

(Adapted with permission from Cassel (1998).)

authors and year	experimental or clinical development
Thompson (1890)	first attempt to graft adult neuronal tissue into the adult brain
Forssman (1898)	first publication on neurotrophic effects of adult neural tissue grafts
Del Conte (1907)	first attempt to graft non-neuronal embryonic tissue into the adult brain
Ranson (1909)	first graft of spinal ganglia into the brain
Tello (1911)	first graft of peripheral nerve into the brain
Dunn (1917)	first demonstration of survival of embryonic neurons after intracerebral grafting
Shirai (1921)	first demonstration of the brain as an immunologically privileged site for implantation of living material
Faldino (1924)	first graft of brain tissue into the anterior chamber of the eye
Le Gros Clark (1940)	first successful intraventricular graft of foetal neuronal tissue in a neonatal host brain
Woolsey *et al*. (1944)	first unsuccessful attempt at medullary grafting in humans
Greene & Arnold (1945)	first graft of human cerebral tissue into an animal
Flerko & Szentagothai (1957)	first intraventricular graft of endocrine tissue
Halasz *et al*. (1962)	first intracerebral graft of endocrine tissue followed by functional improvement
Olson & Malmfors (1970)	first histological characterization of grafted embryonic neuronal tissue using histofluorescence, histochemistry and autoradiography
Hoffer *et al*. (1974)	first electrophysiological demonstration of conservation of functional specificity in neurons transplanted into the anterior chamber of the eye
Stenevi *et al*. (1976)	first systematic study of conditions favourable to the survival and development of brain tissue grafts
Lund & Hauschka (1976)	first demonstration of the existence of synapses in a graft of brain tissue
Perlow *et al*. (1979)	first demonstration of behavioural recovery due to a graft of dopaminergic cells in an animal model of Parkinson's disease (6-hydroxydopamine lesion of the nigrostriatal pathway)
Backlund *et al*. 1982	first unsuccessful attempt to graft catecholaminergic cells into the brain in two Parkinsonian patients (published in 1985)
Madrazo *et al*. 1986	first successful attempt to graft catecholaminergic cells into the brain of Parkinsonian patients (published in 1987)

partly dissociated cells derived from the region in the foetal rat midbrain that contains dopaminergic nigral cells. On the basis of results such as these, the technique of embryonic transplantation has been successfully taken to the clinic, especially in Sweden (Lindvall 1994): there are now several hundred patients with Parkinson's disease who have received intra-striatal transplants of human foetal nigral tissue, a significant number of whom have shown substantial (although far from total) clinical improvement. However, despite this real, albeit partial, therapeutic success, the prospects for the wider clinical application of embryonic neural transplantation are not good. The method is dogged by too many serious problems, of both an ethical and a practical nature, often in complex interaction with one another.

The ethical issues surrounding the general use of human embryonic tissue in medicine are well known. It is surely no accident that the main centre of human neurosurgery with the use of embryonic transplants has so far been Sweden, known for its pragmatic approach to such issues. Conversely, the United States, where the use of human embryonic tissue arouses particularly fierce passions, has been uncharacteristically tardy not only in clinical work but even in experimental studies in this field. Perhaps also attributable to these differences in national ethical climate, it is in the USA that the use of xenografts, in particular of porcine origin, has gone furthest in the treatment of brain disease, for patients with both Huntington's disease and epilepsy (Deacon *et al*. 1997). However, this strategy also has its attendant problems, especially with regard to the risk of transmission of infective agents novel to the human species (Butler 1998). In the UK, trials of human embryonic neural transplantation are currently in the planning stage in two centres, King's College Hospital, London, and Cambridge, for the treatment of patients with Huntington's disease and Parkinson's disease. Stringent ethical guidelines will be in force (as they have been in Sweden), ensuring for example that individuals responsible for the decision to abort are completely separate from those responsible for the decision to proceed with neurosurgery with the use of embryonic material. However, even if one is convinced that, given such safeguards, it is ethical to conduct such surgery, the limits to its applicability remain sharp.

If primary embryonic tissue is used as the source of neural transplants (and this has been the main source so far), it is vital to take the tissue from the embryonic brain at a time at which the requisite cells have already differentiated into their final phenotype but have not yet sprouted axons: take the tissue too early, and it is ineffective; take it too late, and the cells die. For Parkinson's disease, the age of the donor embryos has varied from 6 to 19 weeks, with most falling between about weeks 8–12 (table 1 in Lindvall (1994)). This is a period during which abortions are legal and relatively frequent, so (if the general ethical issues are resolved) the required tissue can be dissected from the embryonic brain and used either as tissue fragments or dissociated to prepare a cell suspension that is finally injected into the brain of the recipient patient. However, given the small number of dopaminergic cells in each embryonic

substantia nigra and their fragility during the handling period between dissection and transplantation, several embryos (up to eight have been used (Widner *et al*. 1992)) are required for each transplanted patient. There are other conditions, however, that have been shown in experiments with animals to be suitable for transplantation therapy but for which the requisite tissue does not reach the relevant time window until much later in pregnancy. A good example, considered in more detail below, is damage to the hippocampus, a region that has a critical role in many forms of cognitive function. This does not reach the appropriate stage of development until the late-embryonic–early-foetal stage of pregnancy, at about 15 weeks of gestation (Arnold and Trojanowski 1996), a stage at which the use of foetal tissue becomes ethically much more problematic. This combination of ethical and practical constraints therefore makes it unlikely that foetal neural transplants will ever be widely used to treat conditions that involve hippocampal damage, as after anoxic insult or in certain forms of epilepsy.

Even in conditions under which the requisite tissue becomes available within the time during which abortions are legal and frequent, there remain serious problems of supply and demand. As noted above, the application of neural transplantation as a treatment for Parkinson's disease grew naturally out of laboratory demonstrations that, in relevant animal models, such transplants give rise to recovery of function. Similar demonstrations have now been made for a number of other such animal models, including for Huntington's disease, in which striatal cells degenerate (Björklund *et al*. 1994*b*), anoxic damage to the hippocampus after global cerebral ischaemia (Hodges *et al*. 1994), anoxic damage to the striatum and cortex after stroke (Hodges *et al*. 1994) and damage to the ascending cholinergic innervation of the hippocampus and neocortex, which is severely compromised in Alzheimer's disease (Sinden *et al*. 1994). There is little reason to suppose that the list will stop there, because most attempts so far to restore function by neural transplants in animals have proved, to varying degrees, to be successful. It is likely that demand will grow for each of these lines of research to follow the same path to the clinic as did the work relating to Parkinson's disease. Now, as noted above, for each patient with Parkinson's disease given neural transplants, tissue is required from four to eight human embryos (Lindvall 1994; Widner *et al*. 1992). If neural transplantation is indeed extended to the large number of other conditions under which it might be effective, the requirement for embryonic tissue will become very large indeed. Even supposing that the existing supply of aborted tissue could keep up with this increased demand, it is likely that eventually developments of this kind would conflict with pressures to decrease the number of abortions, for example by improved contraception.

As well as this difficult mix of ethical and logistical problems, there are two important limitations of a scientific nature that prevent a major clinical extension of embryonic neural transplantation. First, if the requisite cells are to be taken at the right time from the developing brain, the developmental timetable for the cells concerned must be known in some detail. For many types of human brain tissue, such knowledge is as yet unavailable, and the experimental programme required to generate it is both substantial and beset with technical difficulties. Indeed, for the most part, the developmental programme of the human brain is inferred from relevant knowledge concerning the primate brain, and this can give rise to serious inaccuracies. Second, embryonic neural transplantation has so far been limited to cases in which the damaged tissue requiring replacement is homogeneous, for example dopaminergic cells in Parkinson's disease, and striatal cells whose principal neurotransmitter is γ-aminobutyric acid (GABA) in Huntington's disease. This therapeutic aim allows one to seek a corresponding population of cells from the embryonic brain (although these will inevitably form part of a tissue containing many other cell types), which in turn implies a single time window at which the relevant population reaches the critical stage of development. However, in many forms of brain damage or disease, a great variety of cell types is compromised. After prolonged alcohol intake, for example, there is a loss of cholinergic, noradrenergic and serotonergic cells among others (Arendt *et al*. 1989). It might be possible to use neural transplantation to treat these more complex types of brain damage, but the logistics become ever more difficult, requiring for example tissue from different sets of embryos at different gestational ages for each cell type required. Although there have been a few reports of the use of multiple sources of tissue for transplantation in experimental studies with animals (see, for example, Cassel *et al*. 1993), the gaps in our knowledge in this area are large. We know little or nothing, for example, of the likely interactions, positive or negative, between different cell types injected simultaneously or successively into a host brain. Multiple injections of primary embryonic tissue also increase the risk of mechanical damage to the host brain capable of adding to the deleterious effects of disease; such adverse effects have been shown in the rat (Cassel *et al*. 1990).

2. ALTERNATIVES TO PRIMARY EMBRYONIC TISSUE

Not surprisingly, given these potentially severe restrictions upon the eventual clinical use of primary embryonic transplants in the treatment of human brain disease, there have been many efforts to devise alternative sources of tissue for implantation. Much of this research has focused on the use of neuroepithelial stem and progenitor cells. Stem cells in the central nervous system have the potential to develop into neurons, astrocytes and oligodendrocytes; the term 'progenitor' refers to cells with a more restricted lineage potential than that of stem cells, whereas a 'precursor' is any cell type earlier in the developmental pathway that later leads to another (McKay 1997). McKay (1997) has recently reviewed the neurobiology of these cells; Martinez-Serrano & Björklund (1997) have reviewed the use of stem cells immortalized and developed into clonal cell lines in attempts to repair damage in the brain; we shall here bring out only a few salient points documented in those reviews.

From the clinical point of view, a central aim of our own research, described below, is to generate immortalized cell lines that combine two properties.

First, they should be capable of being maintained *in vitro* indefinitely, so that they are readily available for implantation into a host brain as and when required. One

strategy for achieving this is to render neuroepithelial stem cells immortal by the introduction of extra genetic information coding for oncogenes, and to bring the oncogene under the control of manipulatable factors (the resulting cell line being 'conditionally immortalized'). An example of such an oncogene that will be central to experiments described more fully below is the temperature-sensitive (ts) mutated allele (A58) of the simian vius 40 (SV40) large-T antigen (T-ag). This is stably expressed in cells cultured at temperatures below mammalian body temperature (for example 33 °C) but is downregulated at normal body—or brain—temperature (37–39 °C). Cells into which this oncogene has been introduced might therefore be maintained and expanded *in vitro* at the permissive temperature of 33 °C; on implantation into a host brain, however, they would cease dividing and enter the pathway to differentiation. Other oncogenes that have been used in a similar way include different isoforms of *myc*, *neu* and *p53* (Martinez-Serrano & Björklund 1997). Importantly, neither *in vitro* nor *in vivo* (after transplantation into a host brain) has there been any evidence so far of transformation of cells treated in this way.

Second, the cell lines so generated should be able to repair the damage present in the host brain. Because the range of both cell types and brain regions affected by brain disease or damage is large, the cell lines must correspondingly be applicable to a wide range of tasks of repair. Clearly, this might require the generation of many different types of cell line. However, the multipotentiality of neuroepithelial stem cells raises the possibility that a relatively small number of cell lines would be capable of repairing many different kinds of brain damage. We devote the rest of this paper to an exploration of this possibility, including a brief account of experiments from our own laboratory that suggest its feasibility.

A number of rodent neuroepithelial stem or progenitor cell populations have been immortalized, mostly with the tsA58 SV40 T-ag oncogene, and the resulting cell lines have been explored for their potential in populating and repairing the brain (Ryder *et al*. 1990; Renfranz *et al*. 1991; Snyder *et al*. 1992; White & Whittemore 1992; Whittemore & White 1993; Cattaneo *et al*. 1994; White *et al*. 1994; Anton *et al*. 1994). They have proved to be readily expandable in culture; amenable to transfection with reporter (for example, *lacZ* or *luciferase*) or other genes, these then being stably expressed; and capable of being selected as single clones. Multipotentiality has been demonstrated both *in vitro* and, importantly, *in vivo*.

Much of the work *in vivo* has been performed in the developing brain, starting with demonstrations that, after implantation into neonatal rats and mice respectively, a tsA58-immortalized hippocampal cell line (Renfranz *et al*. 1991) and a v-*myc*-immortalized cerebellar line (Snyder *et al*. 1992) each differentiated into both neurons and glia. Furthermore, the neuronal differentiation seen in such experiments takes place in a site-specific manner: that is, the implanted cells take on the phenotypic characteristics of host neurons that are developing at the time and place of implantation, indicating the existence of local signals capable of specifying the final cell type. Thus in the Renfranz *et al*. (1991) experiment, immortalized hippocampal cells transplanted into the developing cerebellum were seen to differentiate into typical cerebellar neurons. This plasticity is probably not due to the process of immortalization itself, because both primary striatal cells grafted into developing cerebral cortex (Fishell 1995) and primary cerebellar cells grafted into developing hippocampus (Vicario-Abejon *et al*. 1995) came to show features characteristic of the region into which they had been implanted. In these experiments transplantation was made into the postnatal brain. Implantation of genetically labelled primary striatal cells into the ventricles of the embryonic mouse brain, by injection via the uterine wall, further showed that these cells migrate to several different sites in the host brain and take up different characteristics depending on their eventual home. Thus the same population of striatal precursors migrated out to give rise to cortical, thalamic and tectal neurons (Campbell *et al*. 1995; Brustle *et al*. 1995).

The ability of neuroepithelial stem cells to migrate to multiple brain regions and to take up multiple phenotypes encourages hopes that these properties can be used for repair of the damaged brain. However, in the experiments reviewed so far, the sites in the developing brain at which the implanted cells differentiated into their final phenotypes were those in which neurogenesis was current at the time; and the observed migration of the implanted cells formed part of current migratory streams from the ventricles to the still forming brain mantle. The environmental signals that drive these responses in the implanted cells are presumably signals that occur as part of the normal process of development. It must be doubtful, therefore, that such signals should be still generated in the adult brain, or that they would be able to direct migration and/or neurogenesis in such a manner as to provide a means of repairing damage to the adult brain. However, as we shall see, there is evidence that processes of this kind can indeed occur.

Immortalized neural progenitors survive well in the adult host brain, without evidence of tumour formation or gross disruption of host brain tissue. However, most of the cell lines immortalized with the tsA58 oncogene, as well as the v-*myc*-immortalized cerebellar line Cl7-2, have differentiated, after implantation into the adult brain, predominantly into glia (Cattaneo *et al*. 1994; Martinez-Serrano *et al*. 1995*a*,*b*; Snyder & Macklis 1996; Lundberg *et al*. 1997). An exception is the tsA58-immortalized cell line RN33B, derived from the raphe, which differentiates mainly into neurons in both developing and adult brain, though some glia are also seen (Shihabuddin *et al*. 1995, 1996; Lundberg *et al*. 1996*a*,*b*). A migratory capacity has also been observed after transplantation of tsA58-immortalized cell lines into the adult striatum (reviewed by Martinez-Serrano & Björklund 1997). These workers report that such cells 'undergo, on average, 2–3 cell divisions over the first five days after transplantation and that they actively migrate out from the transplantation site during this time, for a distance of about 1–1.5 mm, to become fully integrated into the host tissue' (Martinez-Serrano & Björklund 1997, p. 531). They also note that the cell numbers and distribution of the tsA58-immortalized hippocampal HiB5 cell line reached their final state at two weeks after implantation and remained stable thereafter for at least six months. This is consistent with downregulation of the temperature-sensitive oncogene, as observed *in vitro* when the temperature is raised to 39 °C;

we have directly confirmed this downregulation in post-mortem material (Sinden *et al*. 1997) (see below). There was no evidence in any of these experiments of migration of the implanted cells specifically to sites of experimentally induced brain damage, including to the excitotoxic- or 6-hydroxydopamine-lesioned striatum (Lundberg & Björklund 1996; Lundberg *et al*. 1996*a*,*b*), excitotoxically lesioned cortex (Snyder 1994; Snyder & Macklis 1996) or the excitotoxic- or colchicine-lesioned hippocampal formation (Shihabuddin *et al*. 1996). A recent paper from Snyder's group, however, reports good survival and neuronal differentiation after implantation of the cerebellar-derived Cl7-2 line in the photolytically but not excitotoxically lesioned cortex (Snyder *et al*. 1997), raising the possibility that this line is responsive to specific signals associated with apoptotic cell loss (induced by photolysis) but not with necrotic cell loss (induced by excitotoxins). With this exception, the general impression from these experiments is that the transplanted cells tended to avoid areas of damage. Shihabuddin *et al*. (1996), for example, report that the raphe-derived cell line RN33B differentiated into hippocampal CA3 or dentate granule cells when implanted into intact CA3 or dentate gyrus, but underwent only partial neuronal differentiation after implantation into lesioned CA3 (induced by kainate injection) or dentate gyrus (induced by colchicine injection). Those authors therefore suggest that, at least in the RN33B cell line, direct cell-to-cell interaction through surface molecules on intact host neurons is required for differentiation to proceed along lines appropriate to the site of implantation in the adult brain.

Conditionally immortalized neuroepithelial stem cell lines can be subjected to further genetic engineering before implantation. This approach, providing as it does a useful tool for the delivery of desired substances to a host brain, has been exploited in a number of experiments. The feasibility of the technique has been demonstrated by the expression of reporter genes observed after implantation into both neonatal and adult brain (Renfranz *et al*. 1991; Eaton & Whittemore 1996). Control of expression of the reporter gene has also been observed. Thus Corti *et al*. (1996) brought the *luciferase* gene under the control of a tet-regulatable promoter and were able to decrease its expression by the systemic administration of oxytetracycline. In a more substantive experiment, Snyder *et al*. (1995) were able to correct a genetic model of a lysosomal storage disease, mucopolysaccharidosis type VII, with widely disseminated neuronal and glial degeneration, by injecting into the cerebral ventricles of newborn mice, homozygous for the deleted gene, the cerebellar line Cl7-2 modified to express the human gene coding for the missing enzyme, β-glucuronidase. The implanted cells migrated and were incorporated widely in the host brain; successful reversal of the disease was maintained for up to eight months. In the adult animal, attention has focused on the use of immortalized cell lines to deliver trophic factors to the host brain. Thus implants of the hippocampal HiB5 line, engineered to express nerve growth factor (NGF), in the region of the cholinergic cell bodies of the nucleus basalis were shown to induce hypertrophy of these cells and sprouting of axons towards the source of the NGF; injected into the medial septal area, the NGF-secreting cells blocked the degeneration of septal cholinergic cells normally seen after fimbria–fornix section; and, injected into either of these regions in aged rats, these cells reversed or even prevented the development of experimentally assessed cognitive impairment (Martinez-Serrano & Björklund 1997).

It is clear from these and other experiments reviewed by Martinez-Serrano & Björklund (1997) that immortalized neuroepithelial stem cells, genetically engineered to deliver therapeutic substances to the brain, provide a promising avenue for future human neurosurgery. Our own approach, however, has been different. We have asked whether the damaged adult brain might produce signals analogous to those that direct site-specific differentiation in the normal developing brain, with the capacity to bias implanted stem cells to adopt a phenotype appropriate to the damage. We have not, however, sought directly for such signals. We have instead adopted a functional approach: we suppose that any such signal-directed repair of the damaged brain should manifest itself as a recovery of the behavioural performance otherwise impaired by the damage.

These experiments have focused on the four-vessel occlusion (4VO) model of transient cerebral ischaemia, as occurs in heart attack or coronary artery occlusion. In this method the rat's vertebral arteries are permanently coagulated under halothane anaesthesia and on the next day the carotids are briefly ligated (for 10–20 min). As in a substantial proportion of human beings who recover from heart attack (Grubb *et al*. 1996), this manipulation leads to enduring cognitive deficits. Also as in the human case (Squire *et al*. 1990; Rempel-Clower *et al*. 1996), it causes damage to the hippocampal formation and especially to the CAl pyramidal cells (reviewed by Hodges *et al*. 1997). In some well-studied patients the damage, as assessed by both structural magnetic resonance imaging and post-mortem neuropathological examination (Squire *et al*. 1990; Rempel-Clower *et al*. 1996), is largely limited to CAl cells and the cells of the hilus of the dentate gyrus, yet this is associated nonetheless with a profound, enduring and disabling anterograde amnesia. In the rat, appropriate setting of the period of carotid ligation can similarly give rise to damage largely limited to CAl and hilar cells. Moreover, although additional damage is present in other regions (including the striatum and cingulate cortex (Nunn & Jarrard 1994)), transplants of primary foetal hippocampal tissue into the CAl region are able to reverse 4VO-induced cognitive deficits almost in their entirety (Netto *et al*. 1993; Hodges *et al*. 1996). These observations give reason to believe that transplants aimed at the human hippocampal formation might provide a successful treatment for the cognitive impairment consequent on transient cerebral ischaemia. Because such patients are often relatively young and, apart from their major cognitive impairment, otherwise in good health, they provide a clinical target for transplantation therapy that is in many ways more appropriate than the neurodegenerative diseases of old age more often envisaged in this context. However, as already noted, the prospects of applying conventional primary embryonic transplants to this condition are poor, because hippocampal tissue reaches an appropriate stage of development only in the late embryonic stage of pregnancy.

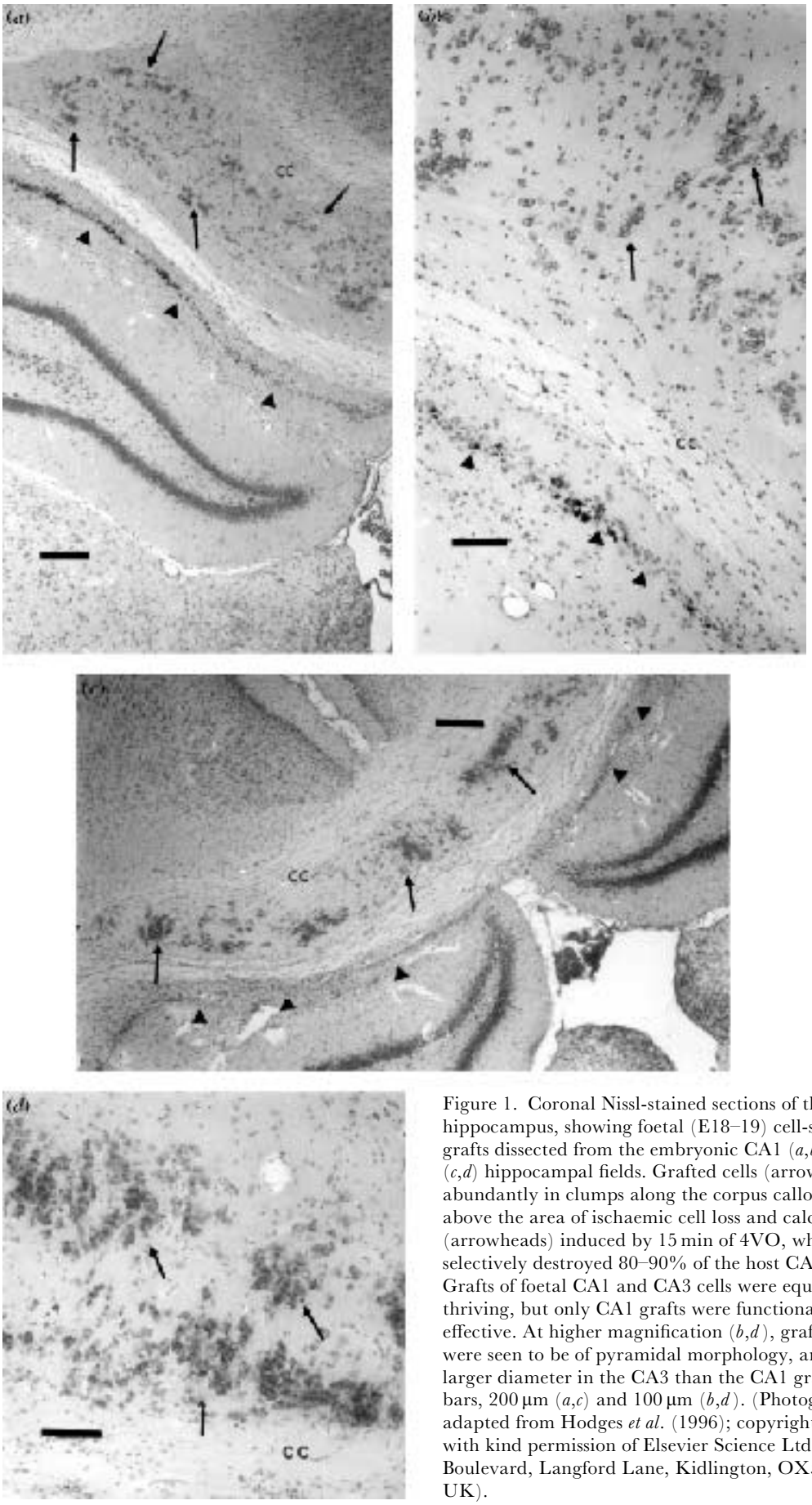

Figure 1. Coronal Nissl-stained sections of the hippocampus, showing foetal (E18–19) cell-suspension grafts dissected from the embryonic CA1 (*a*,*b*) or CA3 (*c*,*d*) hippocampal fields. Grafted cells (arrows) grew abundantly in clumps along the corpus callosum (CC) above the area of ischaemic cell loss and calcification (arrowheads) induced by 15 min of 4VO, which selectively destroyed 80–90% of the host CA1 cells. Grafts of foetal CA1 and CA3 cells were equally thriving, but only CA1 grafts were functionally effective. At higher magnification (*b*,*d*), grafted cells were seen to be of pyramidal morphology, and of larger diameter in the CA3 than the CA1 grafts. Scale bars, 200 μm (*a*,*c*) and 100 μm (*b*,*d*). (Photographs adapted from Hodges *et al.* (1996); copyright 1996, with kind permission of Elsevier Science Ltd, The Boulevard, Langford Lane, Kidlington, OX5 1GB, UK).

The experiments (Netto *et al*. 1993; Hodges *et al*. 1996) in which we injected suspensions of primary foetal cells into the damaged rat hippocampus demonstrated that, for recovery of cognitive function to be observed, there is a rather stringent requirement that the implanted cells be homotypic to the CA1 pyramidal cells that constitute the primary population damaged by 4VO. Cell suspensions derived from non-hippocampal, cholinergic-rich tissue (basal forebrain at embryonic day 16 (E16)), from E18–19 or postnatal (P) day 1–2 dentate gyrus or from the E18–19 CA3 cell field all failed to ameliorate the cognitive deficits observed in two tests of spatial learning and memory: the Morris water maze and the three-door runway. All of these cell suspensions except those derived from the P1–2 dentate gyrus produced substantial surviving grafts as observed in post-mortem histology. In contrast, cell suspensions derived from the E18–19 CA1 cell field gave rise to nearly total recovery of cognitive function over a period lasting from three to nine months after implantation, the maximum time for which the animals' progress was followed. The difference between the effects obtained with grafts derived from the CA1 and CA3 cell fields is particularly striking. In both cases the post-mortem material showed large and healthy surviving grafts located, somewhat ectopically, in the same regions, usually in white matter just above the site of damage to the host CA1 pyramidal cell layer, and containing mature pyramidal cells of appropriate morphology (figure 1). Both CA1 and CA3 pyramidal cells are excitatory and glutamatergic. Evidently, therefore, recovery of function after transplantation in the 4VO-damaged hippocampus requires more than just the presence of cells that secrete the right neurotransmitter in the right general location. This situation is very different, it should be noted, from that which is obtained in regard to transplantation-induced recovery of function after damage to either the dopaminergic or the cholinergic system. In these cases (Björklund *et al*. 1994*a*; Sinden *et al*. 1994, 1995), recovery of behavioural function is observed after the implantation of cells derived from a variety of tissue types, provided that they secrete transmitters of the appropriate type. Furthermore, in these cases the site of implantation is distal to the site of damage, in terminal regions of the projection of which the cell bodies of origin have been damaged, i.e. in the striatum after damage to dopaminergic cells of the substantia nigra or in the neocortex or hippocampus after damage to cholinergic cell bodies in the nucleus basalis or the medial septal area.

The stringent requirement, when using primary foetal cell transplants, of homotypic cell replacement for restoration of cognitive function in the 4VO model made this an ideal test bed with which to pose this question: would conditionally immortalized neuroepithelial stem cells respond to signals of damage in the adult brain by taking up a phenotype appropriate to the nature of the damage and so give rise to recovery of function? In seeking to answer this question we made use of a transgenic mouse harbouring the tsA58 SV40 T-ag oncogene under the control of the interferon-inducible H-2K^{b} promoter (Jat *et al*. 1991). With the use of this mouse, one can take undifferentiated cells from any tissue and grow them in culture at the permissive temperature of 33 °C. Taking advantage of this possibility we dissected the hippocampal proliferative-zone Anlage at E14, i.e. just before the time at which these cells migrate away from the ventricular zone to form the hippocampus (Sinden *et al*. 1997). In an initial experiment we studied the effects of transplants of an expanded population of cells taken from this region, labelled before implantation by two days of incubation with [^{3}H]thymidine, comparing them with E19 primary foetal CA1 cells. As in our earlier experiments, 4VO increased the time taken by rats to swim to the hidden platform in the water maze; this impairment was fully reversed by implants of foetal CA1 cell suspensions. The expanded E14 H-2K^{b}-tsA58 neuroepithelial cell transplant was as effective in reversing this deficit as the primary foetal CA1 cell suspension. In addition, the post-mortem material showed different patterns of engraftment in the two cases. Unlike the primary foetal grafts, the cell population transplant did not aggregate at the site of implantation; rather, cells seemed to have migrated extensively throughout both grey and white matter regions in the cortex and hippocampus.

Although these results were encouraging, they did not test the hypothesis that neuroepithelial stem cells might respond to damage encountered in a host brain by adopting a phenotype appropriate to that damage, because the population of implanted cells might simply have contained precursors already fated to become CA1 pyramidal cells. We therefore derived a number of clonal lines from the H-2K^{b}-tsA58 E14 hippocampal cell population. Single-cell suspensions were plated on fibronectin-coated dishes grown in serum-free defined media, supplemented with basic fibroblast growth factor (FGF2; 10 ng ml^{-1}) in permissive culture, i.e. at 33 °C with added γ-interferon (Kershaw *et al*. 1994). After a number of passages the population was transduced with a pPGKβ-geo plasmid (Friedrich & Soriano 1991), conferring both the *lac-Z* reporter gene and neomycin-resistance selectable marker genes. Out of 32 clones that proliferated and formed cell lines, nine were retained for futher study. Like other cell lines directly derived from the H-2K^{b}-tsA58 mouse (Noble *et al*. 1995), these were all shown to be conditionally immortal. At two to six days *in vitro* under permissive culture conditions they showed 1000–2000-fold proliferation in serum-free media without added FGF, with a rapid decrease in numbers when temperature was raised to 39 °C in the absence of interferon. One of these 'Maudsley hippocampal' (MHP) lines, MHP36, was extensively characterized further and used in transplantation studies (Sinden *et al*. 1997). In culture this cell line was highly responsive to FGF2, substantially increasing in number under both permissive and non-permissive conditions (figure 2*a*,*b*).

In permissive culture the MHP36 line ubiquitously expressed immunoreactivity to the SV40 T antigen and to the neuroepithelial stem cell marker nestin (Lendahl *et al*. 1990); labelling with bromodeoxyuridine (BrdU) confirmed the presence of dividing cells; no cells were observed that were positive for either the neuronal marker PGP 9.5 (Wilson *et al*. 1988) or the astroglial marker glial fibrillary acidic protein (GFAP). After a switch to non-permissive conditions in serum-free medium, and only in the presence of one of the following agents, retinoic acid, forskolin or dibutyril

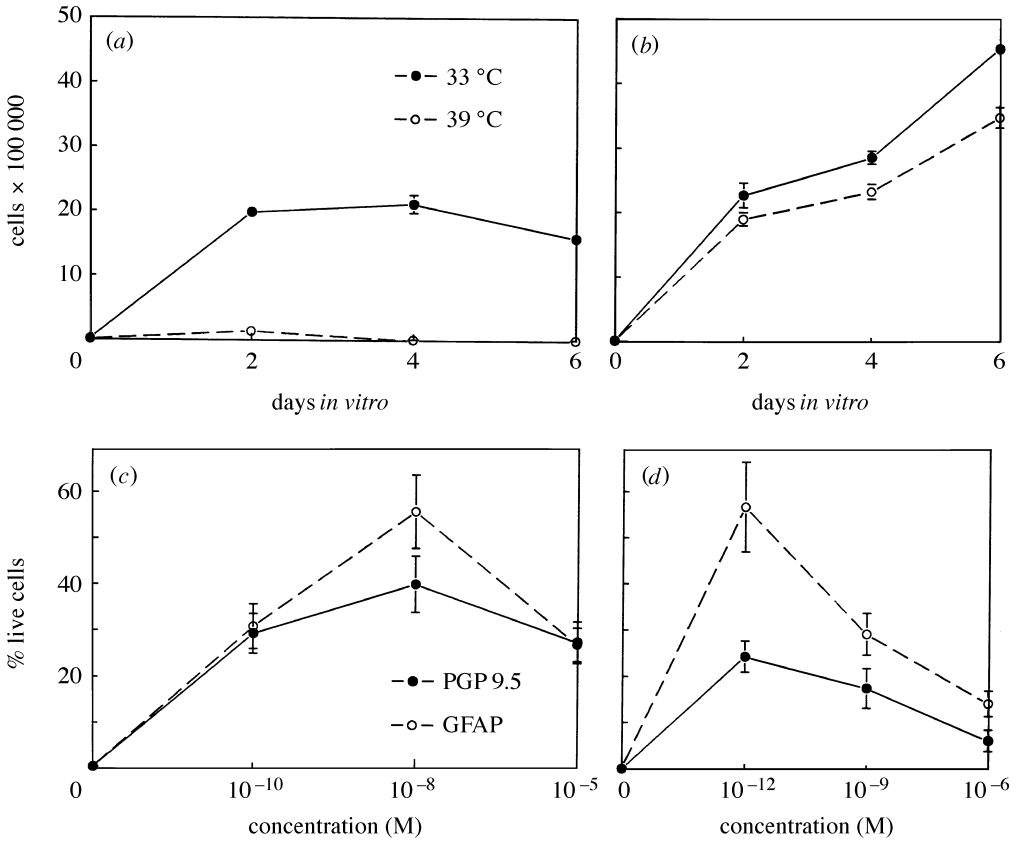

Figure 2. Growth and differentiation of MHP36 cells *in vitro*. (*a*,*b*) Approximately 10^4 cells were plated in fibronectin-coated 96-well plates at day 0. Cell counts at both 33 and 39 °C culture in serum-free medium (SFM) plus γ-interferon (*a*) and in the same medium with the addition of 10 ng ml^{-1} FGF2 (*b*) were measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assays and compared against standard cell number curves. Results are means ± s.e.m. for eight wells. MHP36 cells showed continued survival in SFM only at the permissive temperature for the tsA58 oncogene, FGF2 enhanced proliferation at both permissive and non-permissive temperatures. These results suggest that MHP36 is a normally quiescent, but FGF2-responsive, stem cell line. (*c*,*d*) Bipotential differentiation of MHP36 cells at 2DIV in non-permissive culture conditions (SFM at 39 °C) in the presence of different concentrations of the differentiating agents forskolin (*c*) and retinoic acid (*d*). Cells were plated on fibronectin-coated four-chamber slides at a density of 5×10^4. Results are percentages (means ± s.e.m.) of GFAP- and PGP 9.5-positive cells, compared with the number of live cells determined by morphological criteria, in ten randomly chosen fields set by a ×40 objective on a Leica fluorescence microscope. In the absence of a differentiating agent, no GFAP- or PGP 9.5-positive cells were found, indicating that inductive signalling was required to induce both neuronal and astroglial differentiation.

cAMP, could the cultures be maintained for at least a further 14 days *in vitro*. Both SV40 T antigen and nestin expression were strongly downregulated, staining with BrdU was greatly decreased, and cells positive for either PGP 9.5 or GFAP could now be detected. Many of the PGP 9.5-positive cells had long neuritic processes. Depending on the conditions of differentiation (for example, with retinoic acid or forskolin), the proportions of cells showing neuronal or glial markers, respectively, varied somewhat, but both types of cell were always observed (figure 2*c*,*d*).

These observations *in vitro* indicated that the MHP36 line is a multipotent neuroepithelial stem cell line and therefore a suitable tool with which to test our primary hypothesis. Accordingly, we implanted this cell line into rats that, two to three weeks previously, had been subjected to 4VO, with the expected consequent damage to the hippocampal CA1 pyramidal cell layer. We employed the same stereotaxic coordinates for injection as in the earlier experiments with primary foetal grafts, i.e. bilaterally just above the alveus directly over the region of maximal CA1 cell loss, not into the CA1 cell layer itself. Behavioural testing began three or five months (in two separate experiments) after the implants of MHP36 cells. In both cases, essentially complete recovery of function (as compared with intact controls) was observed in the Morris water maze (Sinden *et al*. 1997). Histological analysis post-mortem, with the use of either 5-bromo-4-chloroindol-3-yl β-D-galactopyranoside (X-Gal) histochemistry or an anti-β-Gal antibody to detect the *lac-Z* reporter gene, revealed a remarkable capacity of the injected cells to migrate to, and partly reconstitute the gross morphology of, the damaged host CA1 cell layer. The resulting appearance of the brain (figure 3*a*), with in particular a normal laminar pattern in the CA1 cell field, is quite different from that seen with either primary foetal grafts (figure 1) or the expanded population of E14 H-2K^b-tsA58 neuroepithelial cells. Double labelling for PGP 9.5 or GFAP showed further that some of the MHP36 cells had acquired a neuronal phenotype and others an astroglial phenotype (figure 3*b*,*c*). In most cells expressing the *lac-Z* gene, however, we failed to detect

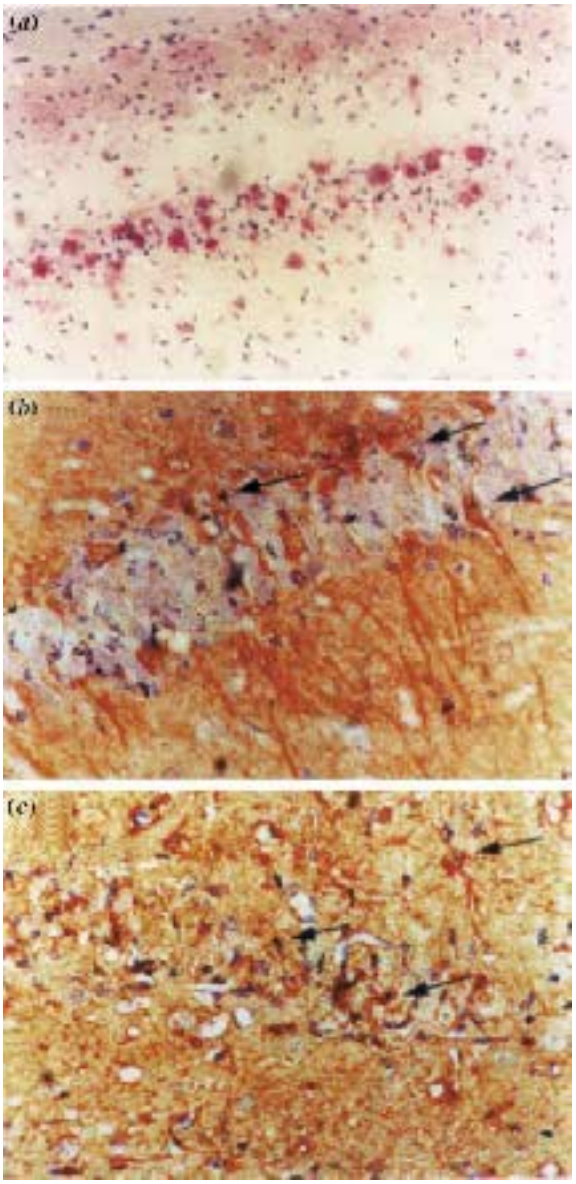

Figure 3. (*a*) Selective engraftment of dorsal hippocampal CA1 by the MHP36 cell line after 4VO ischaemia. At four weeks after grafting, graft cells were largely aggregated in the CA1 area of ischaemic hippocampus, as shown by anti-β-Gal immunohistochemistry (violet chromogen); haematoxylin counterstain. (*b*,*c*) Expression of neuronal and astroglial differentiation in coronal sections of 4VO ischaemic lesion rats with grafts of MHP36 cells. (*b*) At four weeks after grafting, tritiated thymidine ([^{3}H]Thy) autoradiography (silver grain accumulation over cell nuclei) and anti-PGP 9.5 immunohistochemistry (brown chromogen) revealed a number of graft-derived neurons (arrows) with morphologies and polarities indicative of pyramidal cells within graft aggregates in the lesioned CA1 layer. (*c*) At four weeks after grafting, [^{3}H]Thy silver grains were seen over anti-GFAP-positive astrocytes (arrows) within and near CA1 graft cell aggregations. Magnification ×100.

either marker (Sinden *et al*. 1997), although this does not preclude the possibility that these unidentified cells express markers for other cell types, such as oligodendrocytes, or other glial or neuronal cell types not expressing GFAP or PGP 9.5.

In further experiments we have followed the time-course of migration of the implanted cells towards the damaged CA1 cell region. In the first week after injection, the cells remain in a cloud around the injection site. By about four to six weeks they are largely concentrated in the hippocampus but not yet aligned along the CA1 layer. At three months the CA1 layer is essentially reconstituted. Notably, a number of labelled implanted cells are clearly observed in the hilus of the dentate gyrus, which is the second main area of damage seen in the hippocampal formation after 4VO. The MHP36 PGP 9.5-positive cells observed in the CA1 layer are clearly pyramidal in morphology. In some animals, cells were inadvertently injected into the dentate gyrus; in these, a few implanted cells were observed with the appearance of granule cells. It is likely that this outcome was dependent on the damage caused in the dentate gyrus by the needle insertion, because we did not otherwise see cells take up granular morphology or a location in the dentate gyrus.

These experiments seem to warrant a number of conclusions that are potentially of great importance, both scientifically and clinically.

First, confirming the earlier work briefly reviewed above, neuroepithelial stem cells are multipotent both *in vitro* and *in vivo*.

Second, such cells can be rendered conditionally immortal and maintained through multiple passages *in vitro* (currently, the MHP36 line is at passage 60), but still retain their capacity to differentiate on transition to non-permissive conditions favourable for differentiation.

Third, also confirming earlier work, the tsA58 SV40 T-ag oncogene is downregulated within one week after transplantation of MHP36 cells into a host brain; we have seen no sign of emergence of tumours for up to 12 months after transplantation.

Fourth, on implantation into a damaged adult brain, MHP36 cells preferentially migrate towards a region of damage and take up both a location and a morphology that are appropriate to the lost host cell type. This had not previously been observed in an adult host brain, although similar results have now been reported by Snyder *et al*. (1997). It is not at present clear why the MHP36 cell line targets damage in this way, whereas other cell lines (see above) have not generally shown this property.

Fifth, the implanted MHP36 cells gave rise to a recovery of cognitive function that was essentially complete, and as good as that seen after the transplantation of primary foetal cell suspensions. The study by Sinden *et al*. (1997) was the first report of behavioural recovery after implantation of neuroepithelial stem cells into a damaged adult brain. This might simply be because this was the first time that a comparable experiment had been performed, because we are unaware of any others. Because the model that we employed—4VO-induced hippocampal damage—is one in which there is a stringent requirement, when primary foetal tissue is used as the source of implanted material, for homotypic cell replacement (Netto *et al*. 1993; Hodges *et al*. 1996) (see above), this result strongly implies that the MHP36 cells responded to signals indicative of CA1 cell loss by taking up a phenotype capable of replacing the lost host cells with ones sufficiently like them to be able to restore function.

As a possible objection to the last of these conclusions it might be argued that the implanted cells that restored

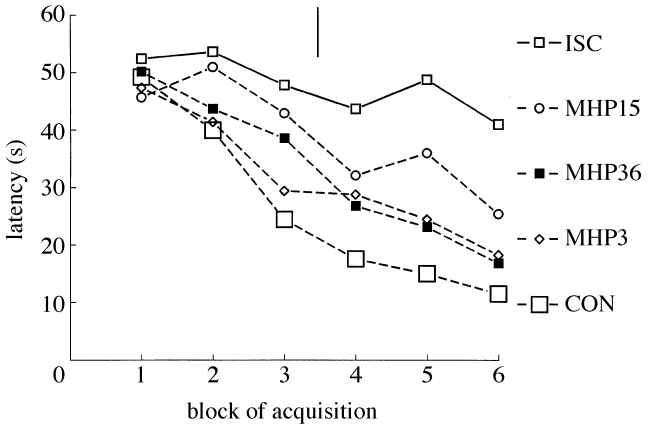

Figure 4. Performance of 15 min 4VO ischaemic (ISC) rats with three different clonal neuroepithelial cell-line grafts (MHP3, MHP15 and MHP36) on the acquisition of a submerged platform location in the water maze. The mean time taken to find the platform is expressed as a function of two-day (four-trial) blocks of training; means ± overall s.e.m., $n = 9$–12. The control group (CON), the MHP36 group and the MHP3 groups did not significantly differ in their rates of learning. The performance of the MHP15 group was intermediate between that of the control group and the ischaemic group with no cell transplant (ISC), differing significantly from both.

cognitive function did so because they were already fated to become CA1 pyramidal cells. This possibility can be discounted on several grounds: (i) the MHP36 cells do not seem to be unduly restricted in their lineage, because both neuronal and glial phenotypes emerge from the same clone; (ii) even within the neuronal lineage we have observed granule cell morphology in the case of MHP36 cells injected into the dentate gyrus; and (iii) in unpublished experiments we have studied two further clonal lines, MHP3 and MHP15, derived in the same manner as the MHP36 line, and both gave rise to some recovery of function in the 4VO model; the chances that we could have in all three cases picked out clones that happened already to be fated to become CA1 pyramidal cells are slight. However, the degree of behavioural recovery in the water maze after grafts of MHP36, MHP3 and MHP15 cells did differ (figure 4), with MHP36 and MHP3 cell lines producing equivalent high levels of behavioural recovery, whereas MHP15 cell grafts led to only marginal improvement. Interestingly, MHP3, like MHP36, is highly FGF2-responsive, whereas MHP15 is not. However, further research is required to determine whether this difference in the functional efficacy of the cell-line grafts is due to the extent of engraftment or to biological differences between the cell lines themselves.

Given the evidence (reviewed above) that the differentiation of implanted stem cells in the developing brain is influenced by ongoing neurogenesis, we must also consider the possibility that differentiation of the implanted MHP cell lines was influenced by continuing neurogenesis in the host brain. In this respect, it is indeed true that neurogenesis continues in the adult hippocampal formation. However, the cells that undergo this continuing neurogenesis are not the pyramidal cells of the hippocampus proper but the granule cells of the dentate gyrus (Kempermann *et al.* 1997). Thus if MHP36 cells implanted into the adult brain were simply to take part in a continuing process of neurogenesis, we should have observed them taking up a granule cell phenotype in the dentate gyrus. However, as noted above, this occurred only when, inadvertently, we damaged the dentate gyrus by inserting a needle there; we did not observe the implanted cells in the undamaged dentate.

3. CLINICAL PROSPECTS

The direct use of conditionally immortal neuroepithelial stem cells to repair brain damage potentially offers considerable advantages over the approach, largely adopted so far (Martinez-Serrano & Björklund 1997) (see above), of using these cells as a device to deliver other substances. In the latter case, for each such substance delivered, one will need a separately engineered cell line. In contrast, the results reported by Sinden *et al.* (1997) and reviewed briefly above raise the possibility that one cell line will be able to repair a variety of different types of brain damage. Much work remains to be done to determine, first, whether this is indeed true; and, second, if so, what are the nature of the limits that determine how broad a range of applicability that any given cell line might have. Current knowledge of the number of different cell lineages present in the central nervous system, or of the factors (such as spatial, temporal, genetic or environmental induction) that differentiate these lineages, is still too sketchy for one to answer such questions *a priori*. (Indeed, if we had settled for an answer *a priori* to the major question posed so far in these experiments, we should not have thought them worth conducting in the first place.) It will therefore be necessary to investigate the effects of the existing H-2K^b-tsA58 cell lines, derived as they are from the hippocampal proliferative zone, after implantation into other damaged regions of the brain: how will they fare when they encounter other pyramidal cell damage (for example in the cerebellum or the neocortex) or damage to other hippocampal cell types (for example GABAergic interneurons) or damage that both is in other regions of the brain and affects cell types other than pyramidal (for example GABAergic neurons in the striatum)? When a region or type of cell loss is encountered in which the existing cell lines fail to work (as must surely turn out to be so), can cell lines be derived in a similar manner but from other developing regions of the brain (for example the striatum or mesencephalon) that will repair the damage? In this respect, the availability of the H-2K^b-tsA58 mouse (Jat *et al.* 1991; Noble *et al.* 1995) offers major advantages as a tool for the rapid generation of comparable cell lines from other brain regions.

The experiments considered so far have all been conducted with rodents. Can the results be generalized to human beings? As a first step towards answering this question, we have begun a series of studies with a monkey from the New World, the common marmoset. In these experiments we have adopted procedures developed by Ridley *et al.* (1995, 1997) in which the CA1 field is lesioned by local injection of the excitotoxin *N*-methyl-D-aspartate (NMDA), and learning and memory are assessed in the Wisconsin General Test Apparatus. After

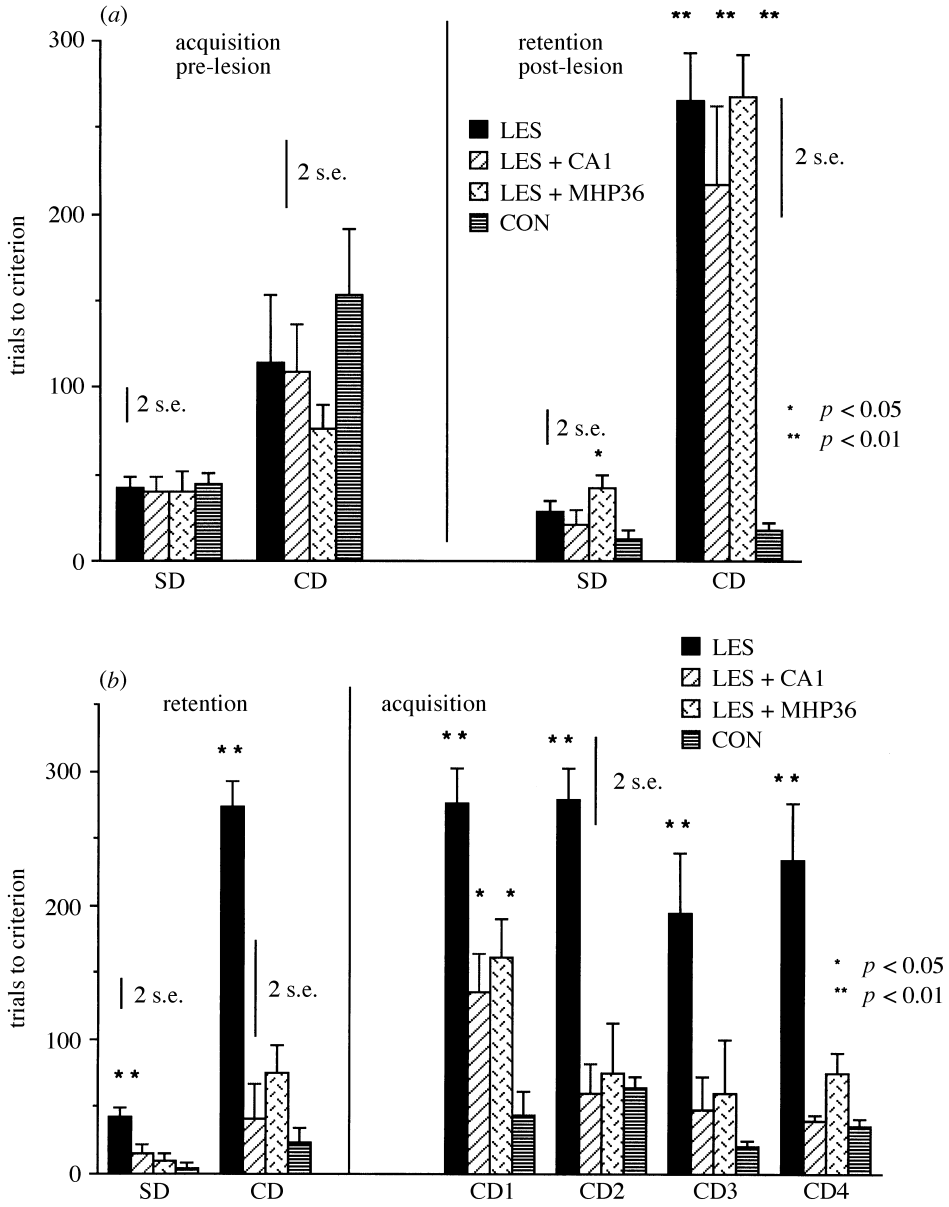

Figure 5. Mean number of trials to criterion (27 out of 30 correct, to a maximum of 300 trials) in the acquisition and retention of simple (SD) and conditional (CD) discriminations, in control (CON) and lesioned (LES) marmosets and lesioned monkeys with grafts of foetal CA1 cells (LES + CA1) or conditionally immortalized MHP36 cells (LES + MHP36). Before surgery (*a*), all animals learned the discriminations in comparable numbers of trials. After lesioning with NMDA along an angled trajectory through the CA1 field, marmosets were profoundly impaired in their ability to recall the conditional but not the simple discriminations; this deficit was as great in animals that subsequently received grafts as in the lesion-only group (*a*). After transplantation (*b*), both groups of grafted marmosets recalled conditional discriminations learned before lesioning as readily as controls, whereas lesioned animals remained impaired. In learning four new conditional tasks, grafted marmosets were initially impaired relative to controls, although they required significantly fewer trials than the lesion group. Thereafter, the grafted groups learned the discriminations as rapidly as controls; this robust improvement was equivalent in animals with foetal homografts and with grafts of non-foetal mouse-derived cells. Lesioned animals continued to show a substantial deficit throughout testing.

this lesion the monkey has no difficulty in retaining simple discriminations learned before surgery nor in learning new simple discriminations, but has profound difficulty in either retaining or learning new conditional discriminations. (In a simple discrimination, the animal must choose for example one object out of two to obtain a reward; there is an invariant association between the positive object and the reward. In a conditional discrimination, the animal is for example faced with two identical objects, and for some pairs the rule is to respond to the one on the left whereas for others it is to respond to the one on the right; here, then, the rule is relational.) This pattern of impairment bears many similarities to the cognitive deficits seen in human beings after hippocampal damage (Eichenbaum *et al*. 1994).

Ridley *et al*. (1997) showed that it is possible to ameliorate these effects of CA1 damage by the implantation of suspensions of primary cells taken from the E95 marmoset hippocampal CA1 field, a finding that we have confirmed (figure 5). Given these results with foetal

grafts, we were in a position to determine whether similar effects might be obtained with conditionally immortalized neuroepithelial stem cells. As far as we are aware, there are as yet no cell lines of this kind of primate origin. However, there are previous examples in which grafted neural tissue has succeeded in producing a recovery of function despite wide species differences (Wictorin *et al*. 1990, 1992), and indeed such xenografts (porcine) are already in use for the treatment of patients with Huntington's disease (Deacon *et al*. 1997). We therefore employed the same mouse-derived MHP36 cells as in the experiments on the 4VO rat. Unlike the experiments with rats, in which immunosuppression was applied to the hosts for only two weeks from the time of transplantation, the marmosets were administered cyclosporin cremaphor EL at a dose of 10 mg kg^{-1} intramuscularly five times a week until they were killed. The experiment was identical to that with the use of marmoset foetal grafts, but used the MHP36 cell line. The results obtained with the two types of transplanted tissue were very similar: like the foetal grafts, the MHP36 cells, implanted into the damaged marmoset CA1 region, restored the animal's capacity both to retrieve the previously learned conditional discrimination rule and to acquire new conditional discriminations (figure 5). In the marmoset experiment, as in the rat experiments, the MHP36 cells, identified by β-Gal immunohistochemistry, reconstructed the damaged CA1 field in a species-specific manner. In the rat material, the cells were densely aligned in the CA1 field, whereas in the marmosets they were evenly distributed across the broader, more sparsely populated, field; this is characteristic of the primate brain. Experimental details and full results of this study are to found in Virley *et al*. 1999.

These experiments with marmosets show that the transplantation of conditionally immortalized neuroepithelial stem cells can be extended from rodents to primates. However, further major questions will arise before one can confidently extend further the applicability of the approach to human beings. The transgenic route for derivation of conditionally immortal cell lines is clearly not available at the human level. Unless one follows the xenograft route (which poses its own non-trivial problems, both ethical and practical (Butler 1998)) it will therefore be necessary to derive the necessary cell lines by transfection of human embryonic brain cells with the tsA58 or other oncogenes *in vitro*. Returning to the ethical issues with which this article commenced, we conclude that it will therefore not be possible to avoid altogether the use of human embryonic material. However, this use should be limited to the relatively small number of occasions on which a particular cell line is derived. Once derived, the evidence so far suggests that it will be possible to maintain the cell line for long periods. From the technical point of view, however, owing to widespread variation in the human genetic background, immortalization of human cells might turn out to pose greater problems than are encountered in the rodent. Nevertheless, human embryonic brain cell lines have recently been generated by infection with a retroviral vector in which the expression of v-*myc* is regulated by tetracycline (Sah *et al*. 1997). In the presence of tetracycline v-*myc* is turned off, and in some of the lines this results in neuronal and glial differentiation. Cultures of human neuroepithelial progenitor cells can therefore be generated and retrovirally infected with oncogenes.

If we suppose that these problems can indeed be overcome and human cell lines are created sharing those properties of the MHP36 cell line that enable this to repair brain damage, might it nonetheless be true that something about the human brain would prevent the application of stem cell transplants in the clinic? This seems unlikely, given that the transplantation of primary embryonic tissue has already made the transition successfully from the animal laboratory to human neurosurgery, at least for Parkinson's disease (Lindvall 1994). As in that case, it will be possible, before taking human cell lines to the clinic, to study their effects in relevant animal models (Wictorin *et al*. 1992). Indeed, given the use of immortalizing genes, it will be essential in animal experiments either to verify that no tumours are formed from immortalized human cell lines or to devise strategies that can minimize or cope with any risk of tumorigenicity so identified. This can perhaps be achieved by addition of 'suicide' genes to the implanted cells that will inducibly kill revertants that do not stop dividing on inactivation of the oncogene, or by excision of the oncogene before implantation, using techniques for site-specific recombination (Westerman & Leboulch 1996).

There does not seem to be any reason in principle why these various problems cannot all be overcome. We are therefore cautiously optimistic that the transplantation of conditionally immortalized human neuroepithelial stem cell lines will prove eventually to be applicable to human neurosurgery across a wide range of conditions that, at present, are otherwise beyond treatment.

We thank David Virley for his collaboration in the marmoset studies and for allowing us to describe this as yet unpublished work.

REFERENCES

Annett, L. E. 1994 Functional studies of neural grafts in Parkinsonian primates. In *Functional neural transplantation* (ed. S. B. Dunnett & A. Björklund), pp. 71–102. New York: Raven Press.

Anton, R., Kordower, J. H., Maidment, N. T., Manaster, J. S., Kane, D. J., Rabizadeh, S., Schueller, S. B., Yang, J., Rabizadeh, S., Edwards, R. H., Markham, C. H. & Bredesen, D. E. 1994 Neural-targeted gene therapy for rodent and primate Hemiparkinsonism. *Exp. Neurol.* **127**, 207–218.

Arendt, T., Allen, Y., Marchbanks, R. M., Schugens, M. M., Sinden, J. D., Lantos, P. L. & Gray, J. A. 1989 Cholinergic system and memory in the rat: effects of chronic ethanol, embryonic basal forebrain brain transplants and excitotoxic lesions of cholinergic basal forebrain projection system. *Neuroscience* **32**, 195–201.

Arnold, S. E. & Trojanoowski, J. K. 1996 Human fetal hippocampal development. 1. Cytoarchitecture, myeloarchitecture and neuronal morphological features. *J. Comp. Neurol.* **367**, 274–292.

Backlund, E. O., Granberg, P. O., Hamberger, B., Sedvall, G., Seiger, A. & Olson, L. 1985 Transplantation of adrenal medullary tissue to striatum in Parkinsonism. In *Neural grafting in the mammalian CNS* (ed. A. Björklund & U. Stenevi), pp. 551–558. Amsterdam: Elsevier.

Björklund, A., Dunnett, S. B. & Nikkhah, G. 1994*a* Nigral transplants in the rat Parkinson model: functional limitations and strategies to enhance nigrostriatal reconstruction. In *Functional neural transplantation* (ed. S. B. Dunnett & A. Björklund), pp. 47–69. New York: Raven Press.

Björklund, A., Campbell, C., Sirinathsinghji, D., Fricker, R. A. & Dunnett, S. B. 1994*b* Functional capacity of striatal transplants in the rat Huntington model. In *Functional neural transplantation* (ed. S. B. Dunnett & A. Björklund), pp. 157–195. New York: Raven Press.

Brundin, P., Duan, W.-M. & Sauer, H. 1994 Functional effects of mesencephalic dopamine neurons and adrenal chromaffin cells grafted to the rodent striatum. In *Functional neural transplantation* (ed. S. B. Dunnett & A. Björklund), pp. 9–46. New York: Raven Press.

Brustle, O., Maskos, U. & McKay, R. 1995 Host-guided migration allows targetted introduction of neurons into the embryonic brain. *Neuron* **15**, 1275–1285.

Butler, D. 1998 Last chance to stop and think on risks of xenotransplants. *Nature* **391**, 320–325.

Campbell, K., Olsson, M. & Björklund, A. 1995 Regional incorporation and site-specific differentiation of striatal precursors transplanted to the embryonic forebrain ventricle. *Neuron* **15**, 1259–1273.

Cassel, J. C. 1998 Greffes de cellules nerveuses dans l'hippocampe du rat: caractérisation neurochimique et comportementale des effets. Habilitation à Diriger des Recherches, Université Louis Pasteur, Strasbourg.

Cassel, J. C., Kelche, C., Hornsperger, J. M., Jackisch, R., Hertting, G. & Will, B. 1990 Graft induced learning impairment despite graft-enhanced cholinergic functions in the hippocampus of rats with septohippocampal lesions. *Brain Res.* **534**, 295–298.

Cassel, J. C., Neufang, B., Kelche, C., Will, B., Hertting, G. & Jackisch, R. 1993 Effects of grafts containing cholinergic and/or serotonergic neurons on cholinergic, serotonergic and noradrenergic markers in the denervated rat hippocampus. *Brain Res.* **604**, 53–63.

Cattaneo, E., Magrazzi, L., Butti, G., Santi, L., Giavazzi, A. & Pezzotta, S. 1994 A short term analysis of the behaviour of conditionally-immortalized neuronal progenitors and primary neuroepithelial cells implanted into the foetal rat brain. *Devl Brain Res.* **83**, 197–208.

Corti, O., Horellou, P., Colin, P., Cattaneo, E. & Mallet, J. 1996 Intracerebral tetracycline-dependent regulation of gene expression in grafts of neural precursors. *NeuroReport* **7**, 1655–1659.

Deacon, T., Schumacher, J., Dinsmore, J., Thomas, C., Palmer, P., Kott, S., Edge, A., Penney, D., Kassissieh, S., Dempsey, P. & Isacson, O. 1997 Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. *Nature Med.* **3**, 350–353.

Del Conte, G. 1907 Einpflanzungen von embryonalem Gewebe ins Gehirn. *Beiträge zur Patologie und Anatomie* **42**, 193–202.

Dunn, E. H. 1917 Primary and secondary findings in a series of attempts to transplant cerebral cortex in the albino rat. *J. Comp. Neurol.* **27**, 567–574.

Eaton, M. J. & Whittemore, S. R. 1996 Autocrine BDNF secretion enhances the survival and serotonergic differentiation of raphe neuronal precursor cells grafted into the adult rat CNS. *Exp. Neurol.* **140**, 105–114.

Eichenbaum, H., Otto, T. & Cohen, N. J. 1994 Two distinctions of hippocampal-dependent memory processing. *Behav. Brain Sci.* **17**, 449–472.

Faldino, G. 1924 Sullo sviluppo dei tessuti embrionali omoplastici innestati nella camera anteriore dell'occhio del cognilio. *Arc. Scienze Biol.* **5**, 328–346.

Fishell, G. 1995 Striatal precursors adopt cortical identities in response to local cues. *Development* **121**, 803–812.

Flerko, B. & Szentagothai, J. 1957 Oestrogen sensitive nervous structures in the hypothalamus. *Acta Endocrinol.* **26**, 121–127.

Forssman, J. 1898 Über die Ursachen, welche die Wachstumsrichtung der peripheren Nerven fasern bei der Regeneration bestimmen. Thesis, Lund University, pp. 1–11. Jena: Gustav Fischer.

Friedrich, G. & Soriano, P. 1991 Promoter traps in embryonic stem cells—a genetic screen to identify and mutate developmental genes in mice. *Genes Dev.* **5**, 1513–1523.

Greene, H. S. N. & Arnold, H. 1945 The homologous and heterologous transplantation of brain and brain tumors. *J. Neurosurg.* **2**, 315–331.

Grubb, N. R., O'Carroll, R., Cobbe, S. M., Sirel, J. & Fox, K. A. A. 1996 Chronic memory impairment after cardiac arrest outside hospital. *Br. Med. J.* **313**, 143–146.

Halasz, B., Pupp, L. & Uhlarik, S. 1962 Hypophysiotrophic area in the hypothalamus. *J. Endocrinol.* **25**, 147–159.

Hodges, H., Sinden, J. D., Meldrum, B. S. & Gray, J. A. 1994 Cerebral transplantation in animal models of ischemia. In *Functional neural transplantation* (ed. S. B. Dunnett & A. Björklund), pp. 347–387. New York: Raven Press.

Hodges, H., Sowinski, P., Fleming, P., Kershaw, T. R., Sinden, J. D., Meldrum, B. S. & Gray, J. A. 1996 Contrasting effects of foetal CA1 and CA3 hippocampal grafts on deficits on spatial learning and working memory induced by global cerebral ischaemia in the rat. *Neuroscience* **72**, 959–988.

Hodges, H., Nelson, A., Virley, D., Kershaw, T. R. & Sinden, J. D. 1997 Cognitive deficits induced by global cerebral ischaemia: prospects for transplant therapy. *Pharmacol. Biochem. Behav.* **56**, 763–780.

Hoffer, B., Seiger, A., Ljungberg, T. & Olson, L. 1974 Electrophysiological and cytological studies of brain homografts in the anterior chamber of the eye: maturation of cerebellar cortex in oculo. *Brain Res.* **79**, 165–184.

Jat, P. S., Noble, M. D., Ataliotis, P., Tanaka, Y., Yannoutsos, N., Larsen, L. & Kioussis, D. 1991 Direct derivation of conditionally immortal cell lines from an H-2K^b-tsA58 transgenic mouse. *Proc. Natl Acad. Sci. USA* **88**, 5096–5100.

Kershaw, T. R., Rashid-Doubell, F. & Sinden, J. D. 1994 Immunocharacterisation of H-2K^b-tsA58 transgenic mouse hippocampal neuroepithelial cells. *NeuroReport* **5**, 2197–2200.

Le Gros Clark, W. E. 1940 Neuronal differentiation in implanted foetal cortical tissue *J. Neurol. Psychiat.* **3**, 263–284.

Lendahl, U., Zimmerman, L. B. & McKay, R. D. G. 1990 CNS stem-cells express a new class of intermediate filament protein. *Cell* **60**, 585–595.

Lindvall, O. 1994 Neural transplantation in Parkinson's disease. In *Functional neural transplantation* (ed. S. B. Dunnett & A. Björklund), pp. 103–137. New York: Raven Press.

Lund, R. D. & Hauschka, S. D. 1976 Transplanted neural tissue develops connections with host brain. *Science* **193**, 582–584.

Lundberg, C. & Björklund, A. 1996 Host regulation of glial markers in intrastriatal grafts of conditionally immortalised neural stem cell lines. *NeuroReport* **7**, 847–852.

Lundberg, C., Field, P. M., Ajayi, Y. O., Raisman, G. & Björklund, A. 1996*a* Conditionally immortalized neural progenitor-cell lines integrate and differentiate after grafting to the adult rat striatum—a combined autoradiographic and electron-microscopic study. *Brain Res.* **737**, 295–300.

Lundberg, C., Winkler, C., Whittemore, S. R. & Björklund, A. 1996*b* Conditionally immortalized neural progenitor-cell lines integrate and differentiate after grafting to the striatum, exhibit site-specific neuronal differentiation and establish connections with the host globus-pallidus. *Neurobiol. Dis.* **3**, 33–50.

Lundberg, C., Martinez-Serrano, A., Cattaneo, E., McKay, R. D. G. & Björklund, A. 1997 Survival, integration and differentiation of neural stem cell lines after transplantation to the adult rat striatum. *Expl Neurol.* **145**, 342–360.

McKay, R. 1997 Stem cells in the central nervous system. *Science* **276**, 66–71.

Madrazo, I., Drucker-Colin, R., Diaz-Simental, V., Martinez-Marta, J., Torres, C. & Becerril, J. J. 1987 Open microsurgical autograft of adrenal medulla to the right caudate nucleus in Parkinson's disease: a report of two cases. *New Engl. J. Med.* **326**, 831–834.

Martinez-Serrano, A. & Björklund, A. 1997 Immortalized neural progenitor cells for CNS gene transfer and repair. *Trends Neurosci.* **20**, 530–538.

Martinez-Serrano, A., Fischer, W. & Björklund, A. 1995*a* Reversal of age-dependent cognitive impairments and chlolinergic neuron atrophy by NGF-secreting neural progenitors grafted to the basal forebrain. *Neuron* **15**, 473–484.

Martinez-Serrano, A., Lundberg, C., Horellou, P., Fischer, W., Bentlage, C., Campbell, K., McKay, R. D., Mallet, J. & Björklund, A. 1995*b* CNS-derived neural progenitor cells for gene transfer of nerve growth factor to the rat brain: complete rescue of axomised cholinergic neurons after transplantation into the septum. *J. Neurosci.* **15**, 5668–5680.

Netto, C. A., Hodges, H., Sinden, J. D., LePeillet, E., Kershaw, T., Sowinski, P., Meldrum, B. S. & Gray, J. A. 1993. Effects of foetal hippocampal field grafts on ischaemic-induced deficts of spatial navigation in the water maze. *Neuroscience* **54**, 69–92.

Noble, M., Groves, A. K., Ataliotis, P., Ikram, Z. & Jat, P. S. 1995 The H-2Kb-tsA58 transgenic mouse—a new tool for the rapid generation of novel cell-lines. *Transgen. Res.* **4**, 215–225.

Nunn, J. A. & Jarrard, L. E. 1994 Silver impregnation reveals neuronal damage in cingulate cortex following 4VO ischaemia in the rat. *NeuroReport* **5**, 2363–2365.

Olson, L. & Malmfors, T. 1970 Growth characteristics of adrenergic nerves in the adult rat. Fluorescence, histochemical and ^{3}H-noradrenaline uptake studies using tissue transplantation to the anterior chamber of the eye. *Acta Physiol. Scand. Suppl.* **348**, 1–112.

Perlow, M. J., Freed, W. J., Hoffer, B. J., Seiger, A., Olson, L. & Wyatt, R. J. 1979 Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. *Science* **204**, 643–647.

Ranson, S. W. 1909 Transplantation of the spinal ganglion into the brain. *Q. Bull. Northwest Univ. Med. Sch.* **11**, 176–178.

Rempel-Clower, N. L., Zola, S. M., Squire, L. R. & Amaral, D. G. 1996 Three cases of enduring memory impairment after bilateral damage limited to the hippocampal formation. *J. Neurosci.* **16**, 5233–5255.

Renfranz, P. J., Cunningham, M. G. & McKay, R. D. G. 1991 Region-specific differentiation of the hippocampal cell line HiB5 upon implantation into the developing mammalian brain. *Cell* **66**, 713–729.

Ridley, R. M., Timothy, C. J., Maclean, C. J. & Baker, H. F. 1995 Conditional learning and memory impairments following neurotoxic lesion of the CA1 field of the hippocampus. *Neuroscience* **67**, 263–275.

Ridley, R. M., Pearson, C., Kershaw, T. R., Hodges, H., Maclean, C. J., Hoyle, C. & Baker, H. F. 1997 Learning impairment induced by lesion of the CA1 field field of the primate hippocampus: attempts to ameliorate the impairment by transplantation of fetal CA1 tissue. *Expl Brain Res.* **115**, 83–94.

Ryder, E. F., Snyder, E. Y. & Cepko, C. L. 1990 Establishment and characterisation of multipotent neural cell lines using retrovirus vector-mediated oncogene transfer. *J. Neurobiol.* **21**, 356–375.

Sah, D. W. Y., Ray, J. & Gage, F. H. 1997 Bipotent progenitor cell lines from the human CNS. *Nature Biotechnol.* **15,** 574–580.

Shihabuddin, L. S., Hertz, J. A., Holets, V. R. & Whittemore, S. R. 1995 The adult CNS retains the potential to direct region-specific differentiation of a transplanted neuronal precursor cell-line. *J. Neurosci.* **15**, 6666–6678.

Shihabuddin, L. S., Holets, V. R. & Whittemore, S. R. 1996 Selective hippocampal lesions differentially affect the phenotypic fate of transplanted neuronal precursor cells. *Expl Neurol.* **139**, 61–72.

Shirai, Y. 1921 Transplantation of rat sarcoma in adult heterogeneous animals. *Jap. Med. Wld* **1**, 14–15.

Sinden, J. D., Gray, J. A. & Hodges, H. 1994 Cholinergic grafts and cognitive function. In *Functional neural transplantation* (ed. S. B. Dunnett & A. Björklund), pp. 253–295. New York: Raven Press.

Sinden, J. D., Hodges, H. & Gray, J. A. 1995 Neural transplantation and recovery of cognitive function. *Behav. Brain Sci.* **18**, 10–35.

Sinden, J. D., Rashid-Doubell, F., Kershaw, T. R., Nelson, A., Chadwick, A., Jat, P. S., Noble, M. D., Hodges, H. & Gray, J. A. 1997 Recovery of spatial learning by grafts of a conditionally immortalized hippocampal neuroepithelial cell line into the ischaemia-lesioned hippocampus. *Neuroscience* **81**, 599–608.

Snyder, E. Y. 1994 Grafting immortalized neurons to the CNS. *Curr. Opin. Neurobiol.* **4**, 742–751.

Snyder, E. Y. & Macklis, J. D. 1996 Multipotent neural progenitor or stem-like cells may be uniquely suited for therapy for some neurodegenerative conditions. *Clin. Neurosci.* **3**, 310–316.

Snyder, E. Y., Deitcher, D. L., Walsh, C., Arnold-Aldea, S., Hartwieg, E. A. & Cepko, C. L. 1992 Multipotent neural cell lines can engraft and participate in development of mouse cerebellum. *Cell* **68**, 33–51.

Snyder, E. Y., Taylor, R. M. & Wolfe, J. H. 1995 Neural progenitor cell engraftment corrects lysosomal storage throughout the MPS-VII mouse brain. *Nature* **374**, 367–370.

Snyder, E. Y., Yoon, C., Flax, J. D. & Macklis, J. D. 1997 Multipotent neural precursors can differentiate toward replacement of neurons undergoing targeted apoptotic degeneration in adult mouse neocortex. *Proc. Natl Acad. Sci. USA* **94**, 11 663–11 668.

Squire, L. R., Amaral, D. G. & Press, G. A. 1990 Magnetic resonance imaging of the hippocampal formation and mammillary nuclei distinguish medial temporal lobe and diencephalic amnesia. *J. Neurosci.* **10**, 3110–3117.

Stenevi, U., Björklund, A. & Svengaard, N. A. 1976*a* Transplantation of central and peripheral monoamine neurons to the adult rat brain: techniques and conditions for survival. *Brain Res.* **114**, 1–20.

Stenevi, U., Kromer, L. F., Gage, F. H. & Björklund, A. 1976*b* Transplantation of central and peripheral monoamine neurons to the adult rat brain: techniques and conditions for survival. *Brain Res.* **114**, 1–20.

Tello, F. 1911 La influencia del neurotropismo en la regeneración de los centros nerviosos. *Trab. Lab. Invest. Biol. Univ. Madrid* **9**, 123–159.

Thompson, W. G. 1890 Successful brain grafting. *NY Med. J.* **51**, 701–702.

Vicario-Abejon, C., Cunningham M. G. & McKay, R. D. 1995 Cerebellar precursors transplanted to the neonatal dentate gyrus express features characteristic of hippocampal neurons. *J. Neurosci.* **15**, 6351–6363.

Virley, D., Ridley, R. M., Sinden, J. D., Kershaw, T. R., Harland, S., Rashid, T., French, S., Sowinski, P., Gray, J. A. Lantos, P. L. & Hodges, H. H. 1999 Primary CA1 and conditionally immortal MHP36 cell grafts restore conditional

discrimination learning and recall in marmosets after excitotoxic lesions of the hippocampal CA1 field. *Brain*. (In the press.)

Westerman, K. A. & Leboulch, P. 1996 Reversible immortalization of mammalian cells mediated by retroviral transfer and site-specific recombination. *Proc. Natl Acad. Sci. USA* **93**, 8971–8976.

White, L. A. & Whittemore, S. R. 1992 Immortalisation of raphe neurons: an approach to neuronal function *in vitro* and *in vivo*. *J. Chem. Neuroanat.* **5**, 327–330.

White, L. A., Eaton, M. J., Castro, M. C., Klose, K. J., Globus, M. Y., Shaw, G. & Whittemore, S. R. 1994 Distinct regulatory pathways control neurofilament expression and neurotransmitter synthesis in immortalised serotonergic neurons. *J. Neurosci*. **14**, 6744–6753.

Whittemore, S. R. & White, L. A. 1993 Target regulation of neuronal differentiation in a temperature-sensitive cell line derived from medullary raphe. *Brain Res.* **615**, 27–40.

Wictorin, K., Brundin, P., Gustavii, B., Lindvall, O. & Björklund, A. 1990 Reformation of long axon pathways in adult rat central nervous system by human forebrain neuroblasts. *Nature* **347**, 556–558.

Wictorin, K., Brundin, P., Sauer, H., Lindvall, O. & Björklund, A. 1992 Long distance directed axonal growth from human dopaminergic mesencephalic neuroblasts implanted along the nigrostriatal pathway in 6-hydroxydopamine lesioned adult rats. *J. Comp. Neurol.* **323**, 475–494.

Widner, H., Tetrud, J., Rehncrona, S., Snow, B., Brundin, P., Gustavii, B., Björklund, A., Lindvall, O. & Langston, J. W. 1992 Bilateral fetal mesencephalic grafting in two patients with Parkinsonism induced by 1-methyl-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *New Engl. J. Med.* **327**, 1556–1563.

Wilson, P. O. G., Barber, P. C., Hamid, Q. A., Power, B. F., Dhillon, A. P., Rode, J., Day, I. N. M., Thompson, R. J. & Polak, J. M. 1988 The immunolocalization of protein gene-product 9.5 using rabbit polyclonal and mouse monoclonal-antibodies. *Br. J. Exp. Pathol.* **69**, 91–104.

Woolsey, D., Minckler, J., Rezende, N. & Klemme, R. 1944 Human spinal cord transplant. *Expl Med. Surg.* **2**, 93–102.