Olfactory neurogenesis: a window on brain development

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Introduction

Freud and the nose

It is understandable that members of the public are surprised to learn that a biopsy taken from inside the nose may be able to provide clues into neurological and psychiatric disorders. Those with knowledge of the development of Freudian psychoanalysis may have a particular shudder of recognition at any project that links psychiatry with surgical procedures on the nose. Wilhelm Fliess (1858-1928) was a Berlin based Ear Nose and Throat specialist (oto-rhino-laryngologist), who was preoccupied with the influence of biological rhythms on health and disease. He also developed a bizarre theory linking the erectile tissue in the nose with sexuality. Sigmund Freud, the father of psychoanalysis, corresponded with Fliess over many years. They shared a delusion that surgery on the nose could interrupt 'reflex nasal neuroses', and thus be an effective treatment for various neurotic disorders thought to be connected to sexuality. The details of this theory do not warrant further scrutiny apart from one incidence that became pivotal in the development of psychoanalysis. Freud had sent one of his most celebrated patients (Emma Eckstein) to Fliess for the nasal surgery as a treatment for her 'psychological' condition. In an act of gross negligence, Fliess left a nasal pack *in situ* after the surgery and did not provide appropriate follow-up or after-care. After much suffering, the mistake was discovered by another surgeon, and the nasal pack removed. When the pack was removed, the patient had a life-threatening nasal bleed and Freud felt so sickened that he had to leave the room. Fortunately for all concerned, Emma Eckstein survived. Later, Freud wrote a glaringly self-serving analysis of a dream he had about this episode. It was this self-analysis that laid the foundations for his work on dream interpretation (Zucker & Wiegand, 1988).

It is not widely appreciated that an Ear Nose and Throat surgeon made such an important contribute to the 'psycho-analytic revolution' of psychiatry. But collaborations between Ear Nose and Throat surgeons and psychiatrists have recently made important contributions to our understanding of neuropsychiatric disorders. This chapter will outline a program of research that examined olfactory neuroepithelium in schizophrenia as an example of the heuristic value of this research. While the chapter will focus on schizophrenia, the techniques can be applied to many different brain disorders.

How can we study brain development in schizophrenia?

The neurodevelopmental hypothesis of schizophrenia proposes that genetic and epigenetic factors alter early brain development leaving the affected individual at increased risk of developing schizophrenia (McGrath & Murray, 2003; McGrath, Feron, Burne, *et al*, 2003). It has been suggested that disruptions in key events during brain development related to neuronal proliferation, migration, differentiation and cell death may underlie some of the subtle neuroanatomical and cytoarchitectural features associated with schizophrenia (Jones & Murray, 1991; Weinberger, 1995). However, researchers interested in linking altered neurodevelopment with neuropsychiatric disorders face several major hurdles. Firstly, many of the key processes underlying early brain development are not open to scrutiny *in vivo*. In addition, by the time schizophrenia becomes clinically apparent, most of the central processes in brain development are long finished. While neurogenesis in the adult human brain (Eriksson, Perfilieva, Bjork-Eriksson, *et al*, 1998) might theoretically be used to investigate fundamental aspects of the neurodevelopmental hypothesis, harvesting neural precursors from the human brain is ethically unjustifiable, dangerous, and technically difficult. Animal models (e.g. transgenic mice, early life brain lesions or exposure to infection, toxins etc) can provide an indirect window on what may be happening in neurodevelopmental disorders, however the validity of these models remains suboptimal

(Lipska & Weinberger, 2003). A model for brain development can be found in the human olfactory epithelium, the organ of the sense of smell, and this is the model discussed below. The human olfactory epithelium remains in a state of embryo-like development throughout adult life such that new neurons are being continually formed, a process called neurogenesis, under the influence of growth factors and other regulatory molecules that shape development of the embryonic brain. Of note, and also discussed below, is the continuing neurogenesis now recognised to play an important role in adult brain which continues to contribute to interneurons in the olfactory bulb in the adult brain. It is possible that the altered olfactory function in persons with schizophrenia may result from alterations in the continuing neurogenesis present in the olfactory epithelium and olfactory bulb.

The neurodevelopmental hypothesis of schizophrenia

The neurodevelopmental hypothesis of schizophrenia has been reviewed elsewhere but it is reconsidered again here because it is central to our thesis that olfactory neurogenesis provides a valid model for neurodevelopmental factors that may be operating in early brain development.

Developmental abnormalities

Various “Minor Physical Anomalies” have been noted in schizophrenia and other psychiatric disorders (Lane, Kinsella, Murphy, *et al*, 1997; McGrath, El-Saadi, Grim, *et al*, 2002). These developmental anomalies are subtle variations in soft-tissue, cartilaginous and bony structures that are the result of an uncertain mix of genetic and environmental factors that operate prenatally. Minor physical anomalies are of interest because they may represent persistent evidence of fetal maldevelopment, and may also serve as markers of early events influencing brain development (Lyon, Barr, Cannon, *et al*, 1989) and specific anomalies may provide clues to the timing of the disruption (e.g. the major features of the palate are essentially complete by 16-17 weeks). “Minor Physical Anomalies” occurring in schizophrenia and other developmental disorders include variations in the shape and proportions of the head, face, mouth, fingers, hands and toes and aberrant dermatoglyphics (Green, Satz & Christenson, 1994). For example, various dermatoglyphic measures are different in schizophrenia patients and well controls (Fananas, Moral & Bertranpetit, 1990; Mellor, 1992; Weinberger & Marengo, 2003), including affected and non-affected monozygotic twin pairs (Rosa, Fananas, Bracha, *et al*, 2000).

Early last century, Kraepelin observed that a high palate and low set ears were more common in patients with schizophrenia (Weinberger, 1995). A closer analysis revealed an overall narrowing and elongation of the mid- and lower face, a widening of the skull base and numerous minor physical anomalies in the eyes, ears and mouth in schizophrenia compared to well controls (Lane, Kinsella, Murphy, *et al*, 1997; McGrath, El-Saadi, Grim, *et al*, 2002) and the ratio of skull width/skull length was significantly larger in those individuals with psychotic disorders compared to the healthy controls (McGrath, El-Saadi, Grim, *et al*, 2002). The odds of having a psychotic disorder were increased in those with wider skull bases, lower facial heights, protruding ears, and shorter and wider palates. The relationship between facial features and psychosis is also indicated in Velocardiofacial Syndrome (VCFS), or 22q11 deletion syndrome, which is associated with an increased frequency of schizophrenia (Bassett, Hodgkinson, Chow, *et al*, 1998; Gothelf, Frisch, Munitz, *et al*, 1997) and bipolar mood disorder (Carlson, Papolos, Pandita, *et al*, 1997).

Recently, Waddington and colleagues have drawn attention to the close links between brain growth and development of the face, providing a developmental-progressive basis to the relationship between craniofacial dysmorphogenesis and schizophrenia (Waddington, Lane, Larkin, *et al*, 1999). Epigenetic exposures that can affect the bony structures of the face and cranium include prenatal viral exposures, obstetric complications (Sperber, 2001), protein malnutrition (Miller & German, 1999), maternal contact (Helm & German, 1996),

hyper-vitaminosis A (Thorogood, Smith, Nicol, *et al*, 1982), and low prenatal vitamin D (Engstrom, Linde & Thilander, 1982). The correlation between individual measures Minor Physical Anomalies and brain morphology is weak, with one study reporting a correlation between dermatoglyphic ridge count and ventricular volume as measured (van Os, Woodruff, Fananas, *et al*, 2000) while other studies reporting lack of association (McGrath, El-Saadi, Grim, *et al*, 2002; Rosa, Fananas, Bracha, *et al*, 2000).

Brain morphology in schizophrenia

Morphological differences in anatomical features of schizophrenic brain are well documented (Bogerts, 1993) and the most recent reviews of MRI studies collated the most replicated findings of altered global and regional neuroanatomy in schizophrenia (Lawrie & Abukmeil, 1998; Wright, Rabe-Hesketh, Woodruff, *et al*, 2000). Brains from schizophrenia patients have reductions in overall size, volume, weight and are shorter through the anterior-posterior axis, compared to brains from healthy controls. Other alterations in brain anatomy include increased ventricular size and reductions in the volume of limbic structures such as the hippocampus, amygdala, parahippocampus (Lawrie & Abukmeil, 1998; Wright, Rabe-Hesketh, Woodruff, *et al*, 2000) and olfactory bulb (Turetsky, Moberg, Yousem, *et al*, 2000). The problems experienced by schizophrenia patients in the higher integrative and associative brain functions, for example speech and thought disorders, hallucinations and poor motivation, are associated with these structural and functional deficits (Bogerts, 1993). Another interesting difference in the anatomy of the schizophrenic brain is the lack of normal asymmetric features (Bogerts, 1993; Lawrie & Abukmeil, 1998; Wright, Rabe-Hesketh, Woodruff, *et al*, 2000; Zaidel, 1999). The normal structural asymmetry includes a longer Sylvian fissure, and larger right frontal and temporal lobes, differences that are absent in schizophrenia patients. The brain volume differences in schizophrenia are not correlated with the duration of the illness (Lawrie & Abukmeil, 1998) and exist in first episode patients (Nopoulos, Torres, Flaum, *et al*, 1995). These findings suggest that the structural brain differences probably result from abnormalities in brain development associated with, and leading to, schizophrenia pathogenesis.

Olfactory epithelium as a model for neurodevelopment

Our favoured model for neurodevelopment is the adult olfactory epithelium, which provides access to developing neural tissue in living patients. The olfactory neuroepithelium is capable of regeneration and there is continual renewal of the sensory neurons (Graziadei & Monti Graziadei, 1978). As a model for neurodevelopment the olfactory neuroepithelium is pertinent because all the elements of neuronal cell lineage, migration and survival are regulated by the same growth factors that act on the developing brain (Mackay-Sim & Chuah, 2000). When olfactory mucosa is cultured in explanted slices of tissue, cells leave the explant and form a sheet of epithelioid cells around it. These cells are born in vitro and express markers of the olfactory basal cells, presumptive neuronal precursors, as well as markers of developing neurons (Caggiano, Kauer & Hunter, 1994; MacDonald, Murrell, Bartlett, *et al*, 1996; Newman, Feron & Mackay-Sim, 2000). Some neurons born in these cultures take a bipolar morphology and express proteins of mature olfactory sensory neurons (Féron, Perry, McGrath, *et al*, 1998; MacDonald, Murrell, Bartlett, *et al*, 1996; Murrell, Bushell, Livesey, *et al*, 1996). Some cells express markers of olfactory ensheathing cells, the glia of the olfactory nerve (MacDonald, Murrell, Bartlett, *et al*, 1996; Pixley, 1992). Like neurogenesis in the developing nervous system, neurogenesis in the adult olfactory neuroepithelium is tightly regulated (Mackay-Sim, 2003; Mackay-Sim & Chuah, 2000). It is similar to the developing nervous system in that there is an overproduction of immature neurons, limited neuronal survival dependent on factors from axonal target, neuronal survival dependent on activity, neuronal precursor proliferation and cell survival dependent

on thyroxine and on autocrine and paracrine growth factors and cytokines (Mackay-Sim, 2003; Mackay-Sim & Chuah, 2000).

We argue therefore that cultures of adult olfactory neuroepithelium provide a convenient and informative model for studying key features of neural development. In particular, properties of olfactory neuroepithelium cultures in individuals with psychotic disorders may have heuristic value with respect to unravelling functions related to both early brain development and even with respect to current brain function, given the continuing neurogenesis now known to occur in adult brain (see below).

Biopsy of the human olfactory epithelium

Based on mapping studies of human olfactory mucosa (Féron, Perry, McGrath, *et al*, 1998; Leopold, Hummel, Schwob, *et al*, 2000), we now have a clearer understanding of the distribution of the olfactory mucosa in the nose. Over the last 7 years, we have biopsied more than 60 persons under local anesthesia as participants in our studies of olfactory neurogenesis in psychosis. Additionally, we have biopsied more than 250 persons under general anesthesia who were undergoing surgery for septoplasty or turbinectomy. All samples were obtained under a protocol approved by the ethics committees of the hospital and university. We have taken biopsies from persons as old as 82 years and in all cases we have identified olfactory epithelium in the biopsy material. In all cases where we have tried, we have been able to grow this tissue in vitro to produce neuronal progenitors and neurons. It is notable that in adults the olfactory epithelium was found more anterior and more inferior than is generally recognised (Féron, Perry, McGrath, *et al*, 1998) an observation confirmed by electrophysiological analysis of odour-evoked potentials from the nasal cavity (Leopold, Hummel, Schwob, *et al*, 2000). In a recent study, we surveyed participants about their experiences after nasal biopsy under local anesthesia. Of the 30 individuals surveyed about one third of the subjects experienced mild, self-limiting bleeding and/or discomfort after the biopsy procedure. One subject has a post-operative nasal bleeding that required the insertion of a nasal pack overnight. We recommend that research teams undertaking these procedure ensure that participants are monitored after the procedure and that they have ready access to the research team in case of serious adverse events.

On gross inspection, the olfactory mucosa is not clearly demarcated from respiratory mucosa. There is a fine patchwork of intermingling microscopic areas of respiratory and olfactory mucosa. The ratio of respiratory to olfactory mucosa is highest in the superior nasal cavity, and lowest in the inferior regions. There is a concentration of olfactory mucosal zones in the front of the middle turbinate but the higher and further back the biopsy is taken, the more likely one will find larger areas of olfactory mucosa present (Perry, Mackay-Sim, Féron, *et al*, 2002). We recommend taking biopsy specimens on the most posterior areas of the septum and the superior turbinates only. Even in the dorsoposterior regions of the adult nasal cavity, where the probability of finding olfactory epithelium was highest, only 40% of the specimens contained olfactory epithelium exclusively. Considering the small surface area within each biopsy specimen (1 mm²), it is apparent that the olfactory and respiratory tissues are intimately dispersed in the adult nasal cavity. Comparison of fetal and adult tissues suggests that invasion of respiratory tissue into olfactory epithelium increases with age.

Neurogenesis in adult olfactory epithelium: a model for neurodevelopment in schizophrenia

Olfactory mucosa is the only part of the nervous system that is available via biopsy, yielding neurons, glia and neuronal progenitor cells, without leading to any sensory deficits (Féron, Perry, McGrath, *et al*, 1998). It is also available at autopsy and can produce viable cultures from people of all ages for at least a day postmortem (Murrell, Bushell, Livesey, *et*

al, 1996). This availability has been exploited to reveal some aspects of human diseases of aging and neurodevelopment. Neuroblast cell lines were derived from human olfactory epithelium (Wolozin, Sunderland, Zheng, *et al*, 1992) and shown to have altered processing of the amyloid precursor protein in Alzheimer's disease (Wolozin, Lesch, Lebovics, *et al*, 1993). More recently, histology of the olfactory epithelium revealed fewer olfactory neurons and more neuronal precursors in Rett's syndrome, a neurodevelopmental disorder leading to profound motor and cognitive deficits (Ronnett, Leopold, Cai, *et al*, 2003).

In schizophrenia, olfactory epithelium has also been exploited to reveal evidence in support of a neurodevelopmental aetiology for this disorder (Arnold, Han, Moberg, *et al*, 2001; Féron, Perry, Hirning, *et al*, 1999). Our group has demonstrated the utility of olfactory mucosal culture as a tool to explore the neurodevelopmental hypothesis of schizophrenia (Féron, Perry, Hirning, *et al*, 1999). We found that compared to healthy controls, slices of olfactory mucosa from patients with schizophrenia were less likely to attach to the culture dish (29.9 versus 73.5% in patients and controls respectively) and within the attached cultures, there was significantly more cell proliferation (as measured by mitotic activity) in the patient group versus the controls (0.69% versus 0.29% of cells were mitotic in patients and controls respectively). The reduction of attachment to the plastic culture wells in the schizophrenia group suggested decreased cellular adhesion, a finding in accordance with two studies that reported reduced adhesion in skin fibroblasts in schizophrenia compared to healthy controls (Mahadik, Mukherjee, Wakade, *et al*, 1994; Miyamae, Nakamura, Kashiwagi, *et al*, 1998). Altered cell proliferation in cultures olfactory epithelium in schizophrenia (Féron, Perry, Hirning, *et al*, 1999) is consistent with an altered trajectory of brain development in these patients. Histology of posmortem olfactory epithelium in aged humans with schizophrenia revealed greater proportions of immature neurons and mature neurons in this tissue when compared to the precursor cells, indicating dysregulated neurogenesis in this tissue in schizophrenia (Arnold, Han, Moberg, *et al*, 2001). It is interesting to note that Rett's syndrome and schizophrenia are both disorders of neurodevelopment but each appears to alter the ratios of neuronal precursors and neurons in characteristic ways. This suggests that the measurable dynamics of olfactory neurogenesis may be identifiable in different disorders, further illustrating the potential for this tissue as a tool for investigating the aetiologies of different neurodevelopmental disorders.

Neurogenesis in adult brain: role in schizophrenia ?

The long held dogma that the adult mammalian brain cannot generate new neurons was first questioned by evidence for mitotic cells in the forebrain subventricular zone of the adult rodent and mitotic precursor cells were shown to give rise to neurons in the dentate gyrus of the hippocampus and olfactory bulb (Altman, 1969; Altman & Das, 1965; Kaplan & Hinds, 1977). It was not until the early 1990's that new evidence (Reynolds & Weiss, 1992; Richards, Kilpatrick & Bartlett, 1992) confirmed the formation of new neurons in the adult brain, with general acceptance of this concept only in the last 10 years.

It is now well recognized that neurogenesis continually provides new neurons not just to the olfactory epithelium but also to the olfactory bulb. Within the olfactory bulb, neuronal precursors migrate from its core to the periphery where they differentiate into local interneurons, olfactory granule cells and periglomerular cells (Alvarez-Buylla & Garcia-Verdugo, 2002). Significantly, the majority of the granule cells are produced postnatally and newly generated interneurons are found during adulthood (Bayer, 1983; Kaplan & Hinds, 1977). Progenitors that give rise to olfactory interneurons reside in the anterior area of the subventricular zone (Luskin, 1993) and migrate tangentially along a restricted pathway named the rostral migratory stream (Doetsch & Alvarez-Buylla, 1996; Lois & Alvarez-Buylla, 1994; Luskin & Boone, 1994). Within this rostral migratory stream, neuroblasts are assembled in chains ensheathed by slowly dividing astrocytes (Garcia-Verdugo, Doetsch,

Wichterle, *et al*, 1998; Lois, Garcia-Verdugo & Alvarez-Buylla, 1996; Peretto, Merighi, Fasolo, *et al*, 1999; Rousselot, Lois & Alvarez-Buylla, 1995) Migrating and dividing cells express markers of immature neurons such as doublecortin and the polysialylated form of neural adhesion molecule (Bonfanti & Theodosis, 1994; Doetsch & Alvarez-Buylla, 1996; Gleeson, Lin, Flanagan, *et al*, 1999; Hu & Rutishauser, 1996). Once in the olfactory bulb, neuronal progenitors migrate along radial glia and differentiate into fully mature and electrophysiologically active interneurons (Carleton, Petreanu, Lansford, *et al*, 2003).

Forebrain neurogenesis in adulthood has been observed in every examined mammalian species and neural stem cells have been isolated from the human brain (Bernier, Vinet, Cossette, *et al*, 2000; Johansson, Svensson, Wallstedt, *et al*, 1999; Kukekov, Laywell, Suslov, *et al*, 1999; Nunes, Roy, Keyoung, *et al*, 2003; Pagano, Impagnatiello, Girelli, *et al*, 2000; Pincus, Harrison-Restelli, Barry, *et al*, 1997; Roy, Wang, Jiang, *et al*, 2000). Migration of neuron precursors along the rostral migratory stream is well documented in non human primates (Alvarez-Buylla & Garcia-Verdugo, 2002; Kornack & Rakic, 2001; Pencea, Bingaman, Freedman, *et al*, 2001) but, to date, not in humans. A recent study performed post mortem on nine human brain samples did not find any evidence of chains of migrating neurons in the olfactory peduncle or a structure similar to the rostral migratory stream of other mammals (Sanai, Tramontin, Quinones-Hinojosa, *et al*, 2004). However, it cannot be excluded that neuronal precursors migrate individually. Indeed, a previous report has described the presence of individual neural precursors in the adult human olfactory bulb (Liu & Martin, 2003). Furthermore, considering the relatively poor sense of smell of human species, it is likely that the replacement of bulbar interneurons, if it occurs, is a rare event that cannot be detected easily with the contemporary techniques.

Regulation of neurogenesis in adult brain

Many studies have indicated that hormones could be regulators of adult neurogenesis. Prolactin, an hormone that increases during the first half of pregnancy and postpartum, stimulates neurogenesis in the female subventricular zone contributing to new interneurons in the olfactory bulb (Shingo, Gregg, Enwere, *et al*, 2003). Erythropoietin infusion into the adult lateral ventricles decreases the numbers of neural stem cells in the subventricular zone, increases the number of newly generated cells migrating to the olfactory bulb, and increases the number of new olfactory bulb interneurons (Shingo, Sorokan, Shimazaki, *et al*, 2001). Progesterone reduces proliferative activity within the subependymal layer (Giachino, Galbiati, Fasolo, *et al*, 2003). Pituitary adenylate cyclase-activating polypeptide promotes neural stem cell proliferation in the subventricular zone as well as in the dentate gyrus of the hippocampus (Mercer, Ronnholm, Holmberg, *et al*, 2004).

Vitamins are also suspected to play a role in adult neurogenesis. Vitamin A deficiency increased cell proliferation in the rat olfactory epithelium (Asson-Batres, Zeng, Savchenko, *et al*, 2003). Vitamin E deficiency induced neural precursor proliferation and cell death in the rat dentate gyrus (Cecchini, Ciaroni, Ferri, *et al*, 2003; Ciaroni, Cecchini, Ferri, *et al*, 2002; Ciaroni, Cuppini, Cecchini, *et al*, 1999).

Several growth factors regulate adult neurogenesis in vitro. Epidermal growth factor or basic fibroblast growth factor induce survival and self renewal of neural stem cells from adult subventricular zone (Kuhn, Winkler, Kempermann, *et al*, 1997; Reynolds & Weiss, 1992)(for updated review (Galli, Gritti, Bonfanti, *et al*, 2003)). After removal of these factors, the progeny differentiate spontaneously into astrocytes, neurons and oligodendrocytes (Doetsch, Caille, Lim, *et al*, 1999; Kilpatrick & Bartlett, 1993; Morshead, Reynolds, Craig, *et al*, 1994; Vescovi, Reynolds, Fraser, *et al*, 1993). The growth factors, sonic hedgehog and fibroblast growth factor 8, then induce neuronal precursors to develop into dopaminergic neurons (Kim, Lee, Lee, *et al*, 2003).

In vivo, subventricular zone progenitors give rise to olfactory bulb interneurons and their destiny can be modified by growth factor administration. Intracerebroventricular

administration of epidermal growth factor and transforming growth factor α dramatically increases subventricular precursor proliferation (Craig, Tropepe, Morshead, *et al*, 1996) while basic fibroblast growth factor is less potent (Kuhn, Winkler, Kempermann, *et al*, 1997) and also has a proliferative effect after subcutaneous delivery (Wagner, Black & DiCicco-Bloom, 1999). Epidermal growth factor and basic fibroblast growth factor differ in molecular mechanism and induce different lineages: the former induces production of more astrocytes in the olfactory bulb and in striatum of infused animals whereas the latter induces more olfactory bulb interneurons (Craig, Tropepe, Morshead, *et al*, 1996; Kuhn, Winkler, Kempermann, *et al*, 1997).

In addition to these well studied ligands, other growth factors have emerged as potential regulators of bulbar neurogenesis. The ciliary neurotrophic factor supports neural stem cell renewal via Notch signaling (Chojnacki, Shimazaki, Gregg, *et al*, 2003; Hitoshi, Alexson, Tropepe, *et al*, 2002; Shimazaki, Shingo & Weiss, 2001) while Ephrin molecules, when infused in the lateral ventricle, increase cell proliferation and decrease neuroblast migration (Conover, Doetsch, Garcia-Verdugo, *et al*, 2000). A highly glycosylated molecule expressed on differentiating neurons during development, mCD24, decreases cell proliferation in the subventricular zone (Belvindrah, Rougon & Chazal, 2002). Furthermore, it has been demonstrated that neurogenesis and gliogenesis in the forebrain are regulated by two antagonistic growth factors. Bone Morphogenetic Proteins, expressed by subventricular cells, promote astroglial lineage whereas Noggin, produced by ependymal cells, antagonises this (Gross, Mehler, Mabie, *et al*, 1996; Lim, Tramontin, Trevejo, *et al*, 2000; Shou, Rim & Calof, 1999).

Several factors have been identified that regulate neuronal precursor migration. The polysialylated form of NCAM, expressed by olfactory interneuron precursors, is a key player during the rostral migration (Bonfanti & Theodosis, 1994; Chazal, Durbec, Jankovski, *et al*, 2000; Doetsch & Alvarez-Buylla, 1996; Lois, Garcia-Verdugo & Alvarez-Buylla, 1996; Rousselot, Lois & Alvarez-Buylla, 1995) and, not surprisingly, NCAM mutant mice display a decreased number of newly generated granule cells as well as an impaired odor discrimination (Gheusi, Cremer, McLean, *et al*, 2000). The protein Reelin, known for guiding and positioning neuronal precursors during development, acts during adulthood as a detachment signal for chain-migrating interneuron precursors in the olfactory bulb (Hack, Bancila, Loulier, *et al*, 2002). Moreover, two soluble proteins of the Slit family are considered to be prime candidates for guiding neuroblasts within the rostral migratory stream (Hu & Rutishauser, 1996; Mason, Ito & Corfas, 2001; Wu, Wong, Chen, *et al*, 1999).

Finally, the list of factors influencing olfactory bulb neurogenesis should include neurotransmitters. Glutamate and serotonin modulate adult neurogenesis within the hippocampus (Banasr, Hery, Printemps, *et al*, 2004; Bernabeu & Sharp, 2000; Brezun & Daszuta, 1999; Cameron, Hazel & McKay, 1998; Gould, 1999). Serotonin depletion increases cell proliferation and neurogenesis in the dentate gyrus and olfactory bulb (Banasr, Hery, Printemps, *et al*, 2004; Brezun & Daszuta, 1999) and destruction of the cholinergic input to the dentate gyrus and the olfactory bulb there are fewer granule cells and more apoptotic cells in the granule cell layers of these structures (Cooper-Kuhn, Winkler & Kuhn, 2004).

Neurogenesis in olfactory bulb affects olfactory function

Olfaction in schizophrenia is impaired. At present the locus of this olfactory dysfunction is not known. The observed changes in neurogenesis in the olfactory epithelium may contribute but it is possible that changes to the olfactory bulb or higher centres may contribute. Of relevance in this regard is a recent study of neurogenesis and olfactory function in aged mice (Enwere, Shingo, Gregg, *et al*, 2004). Two year old mice showed impairments in olfactory function, making discriminations between closely related odours. They also have less proliferation in the subventricular zone and fewer interneurons newly

contributing to the olfactory bulb, even though they have more interneurons in total. Of interest here was the reduced expression of the epidermal growth factor receptor in the aged mice compared to the younger controls. Independently of age, mice lacking the ligand for this receptor (transforming growth factor α) also showed reduced neurogenesis and a similar olfactory deficit (Enwere, Shingo, Gregg, *et al*, 2004). Another mutant mouse with reduced neurogenesis (leukemia inhibitory factor heterozygote) and reduced new olfactory bulb interneurons, also exhibited olfactory dysfunction independently of age (Enwere, Shingo, Gregg, *et al*, 2004). It was concluded that reduced neurogenesis in the olfactory bulb must contribute directly to reduced olfactory function. We speculate that neurogenesis may be impaired in the adult schizophrenic brain, specifically in the olfactory bulb and this may contribute to the olfactory dysfunction. There is no direct evidence for this although the olfactory bulbs are reported to be smaller in schizophrenia (Turetsky, Moberg, Yousem, *et al*, 2000) and neurogenesis in the nose is impaired (Arnold, Han, Moberg, *et al*, 2001; Féron, Perry, Hirning, *et al*, 1999). We have evidence that the rate of neurogenesis in the rodent olfactory epithelium may be related to the rate of neurogenesis in the olfactory bulb of the same animal (unpublished data). If this were true in humans the olfactory epithelium would indeed be a “window into the brain”.

Because neurotransmitters influence adult neurogenesis, it can be expected that a disease-induced neurotransmitter depletion or excess might impair neurogenesis in the patient's brain. Three recent studies of brains of individuals with neurodegenerative diseases (Parkinson, Huntington and Alzheimer) support this hypothesis, although the findings are not completely concordant. A reduced number of proliferating subependymal zone cells and neuronal precursors cells in the olfactory bulb and dentate gyrus was found in the brains of patients with Parkinson disease (Hoglinger, Rizk, Muriel, *et al*, 2004) while an increased cell proliferation and neurogenesis was observed in the subependymal layer of human Huntington's disease brains (Curtis, Penney, Pearson, *et al*, 2003). Consistent with the latter report but in contrast with animal models, another study showed that neurogenesis is increased in the hippocampus of individuals with Alzheimer's disease (Jin, Peel, Mao, *et al*, 2004). It is possible that the differences in neurogenesis implied by these observations may be explained by the nature of the neurotransmitter pathways disrupted in each disorder. More studies, using larger cohorts, need to be performed in order to clarify this issue. However, the data from the study by Hoglinger *et al* (Hoglinger, Rizk, Muriel, *et al*, 2004) raises the possibility that impairment of olfactory bulb neurogenesis, in addition to striatal depletion of dopamine, contributes to olfactory impairment in Parkinson disease.

Conclusions

The ability to examine *in vitro*, the dynamic process of neuronal birth, differentiation and death in neuroepithelium from individuals with psychosis provides a powerful tool for understanding neurobiological correlates of schizophrenia and bipolar disorder. It will also provide measurable neurobiological factors associated with affective and nonaffective psychoses that may serve as informative endophenotypes for future genetic studies (Gottesman & Gould, 2003). Additionally, there is growing interest in the possible links between neurogenesis and affective disorders. Medications widely used to treat psychosis and depression have recently been shown to promote neurogenesis and to be neuroprotective in the rat brain. These include lithium (Chen, Rajkowska, Du, *et al*, 2000), olanzapine and risperidone (Wakade, Mahadik, Waller, *et al*, 2002), fluoxetine, and tranylcypromine (Duman, Nakagawa & Malberg, 2001). While the evidence is far from complete, these curious properties of psychotropic medications have led some researchers to speculate that disrupted neurogenesis in the adult brain may be implicated in the pathogenesis of affective disorders (Duman, Nakagawa & Malberg, 2001; Manji, Moore & Chen, 2000). Conversely, the neuroprotective properties of compounds such as lithium have led to speculation that its therapeutic effect is related to its reduction of neuronal cell death in bipolar disorder (Manji

& Duman, 2001). The ability to examine mitosis and cell death in olfactory neuroepithelium cultures from individuals with affective disorders lends itself to exploring these hypotheses.

The observed differences in cell biology in the olfactory neuroepithelium in psychosis also lend themselves to deeper analysis using gene and protein expression arrays. Pertinent to this are recent attempts to investigate the genetic bases of neurogenesis, determination of cell fate and differentiation during development (Blackshaw & Livesey, 2002), procedures which could be applied to olfactory neuroepithelium cultures. With these technologies one could compare the differential expression of genes and proteins between control patient groups (Hakak, Walker, Li, *et al*, 2001; Mirnics, Middleton, Marquez, *et al*, 2000; Vawter, Barrett, Cheadle, *et al*, 2001) and one could investigate the effects of psychotropic agents on the expression of genes or proteins such as the impact of lithium on the anti-apoptotic protein Bcl-2 (Manji, Moore & Chen, 2000).

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