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Citation
Borgmann-Winter, K, S L Willard, D Sinclair, N Mirza, B Turetsky, S Berretta, and C-G Hahn. 2015. “Translational potential of olfactory mucosa for the study of neuropsychiatric illness.” Translational Psychiatry 5 (3): e527. doi:10.1038/tp.2014.141. http://dx.doi.org/10.1038/tp.2014.141.

Published Version
doi:10.1038/tp.2014.141

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REVIEW
Translational potential of olfactory mucosa for the study of neuropsychiatric illness

K Borgmann-Winter1,2, SL Willard3, D Sinclair1, N Mirza5, B Turetsky1, S Berretta4,5 and C-G Hahn1

The olfactory mucosa (OM) is a unique source of regenerative neural tissue that is readily obtainable from living human subjects and thus affords opportunities for the study of psychiatric illnesses. OM tissues can be used, either as ex vivo OM tissue or in vitro OM-derived neural cells, to explore parameters that have been difficult to assess in the brain of living individuals with psychiatric illness. As OM tissues are distinct from brain tissues, an understanding of the neurobiology of the OM is needed to relate findings in these tissues to those of the brain as well as to design and interpret ex vivo or in vitro OM studies. To that end, we discuss the molecular, cellular and functional characteristics of cell types within the olfactory mucosa, describe the organization of the OM and highlight its role in the olfactory neurocircuitry. In addition, we discuss various approaches to in vitro culture of OM-derived cells and their characterization, focusing on the extent to which they reflect the in vivo neurobiology of the OM. Finally, we review studies of ex vivo OM tissues and in vitro OM-derived cells from individuals with psychiatric, neurodegenerative and neurodevelopmental disorders. In particular, we discuss the concordance of this work with postmortem brain studies and highlight possible future approaches, which may offer distinct strengths in comparison to in vitro paradigms based on genomic reprogramming.

Translational Psychiatry (2015) 5, e527; doi:10.1038/tp.2014.141; published online 17 March 2015

INTRODUCTION
A critical component of neuropsychiatric research is the delineation of neurobiological abnormalities in patients’ brains. Although decades of postmortem studies have yielded vital insights, the lack of access to living patients’ brain tissues has long been a major hurdle in the field. Recently, several in vitro paradigms have emerged, such as induced pluripotent stem cell (iPSC)1 and induced neuronal2 cell technologies, which offer unique and unprecedented opportunities to reprogram patients’ cells into developing neurons and glial cells.

This review focuses on another paradigm with a similar purpose and with distinct strengths; the olfactory mucosa (OM) tissue approach. The OM harbors neurons and glial cells residing in the nasal cavity and is readily accessible via biopsy. Neural tissues without genomic reprogramming can be captured via olfactory biopsy. OM tissues offer ex vivo and in vitro neuronal cells that may more closely reflect in vivo neural characteristics of the donors. OM cells also have regenerative potential, which permits them to propagate in vitro, yielding neuronal or glial cells in sufficient quantity for various assays.

Another unique advantage of this paradigm arises from the fact that the OM is the peripheral component of the olfactory circuitry, connecting olfactory sensory neurons (OSNs) to the olfactory bulb, the neurons of which then connect to the olfactory cortex. As such, neurobiological alterations in patients’ limbic regions may affect or be reflected in the olfactory circuitry, including neural cells in the OM. Indeed, Alzheimer’s disease,3,4 schizophrenia1–9 and mood and anxiety disorders (review10) are associated with olfactory dysfunction, and OM derived from these patients exhibit cellular and molecular alterations.11–20

Recently, a growing number of groups have examined OM biopsies in these disorders11,12,17,21–23 and others in which olfactory dysfunction is yet to be characterized as a key phenotype14,24–26. An important question is to what degree and/or in what ways these OM-derived cells reflect the pathophysiological mechanisms in patients’ brains. Given that the OM is a specialized neural tissue containing a regenerative neuroepithelium whose identity is distinct from brain cells, alterations in the OM cells can best be appreciated in the context of the neurobiological characteristics specific to the OM tissue. The goal of this paper, therefore, is to consider how the OM paradigm can contribute to pathophysiological understanding of neuropsychiatric illnesses by reviewing the neurobiological context in which these OM cells arise in vivo and in vitro.

CYTOARCHITECTURE AND MOLECULAR CHARACTERISTICS OF THE OM
A distinctive characteristic of the OM is that it contains a regenerative neuroepithelium. In this neural tissue, cells continually slough off and are replenished by newborn cells including neurons and other cell types. Multipotent cells of the OM must continuously produce replacement OSNs and other cell types in the adult mammalian OM.27,28 This continual process of renewal from multipotent cells to differentiated neurons offers a unique
Figure 1. Cytoarchitecture and molecular characteristics of the olfactory mucosa (OM). The multilaminar organization of the rodent OM is depicted on the left, and core molecular markers that define and distinguish mammalian OM cell types in vivo are listed on the right. Most of the studies identifying these markers have been reviewed elsewhere.27,33 Markers that distinguish lamina propria mesenchymal stem cells (LP-MSCs) have not been studied to the extent of markers for other OM cell types, but see Tome et al.37 Lindsay et al.38 and Delorme et al.39 for MSC-specific cluster of differentiation (CD) expression in these cells. GBC, globose basal cell; HBC, horizontal basal cell; INP, intermediate neural progenitor; OEC, olfactory ensheathing cell; OSN, olfactory sensory neuron; SUS, sustentacular cell.

The current knowledge of the cytoarchitecture and molecular profiles of the OM stems largely from rodent studies. The human olfactory neuroepithelium (ON) is thought to recapitulate histologic and molecular characteristics of the rodent counterpart, though the precise degree of similarity remains under investigation. The ON comprises a pseudostratified epithelium in adult mammals, comprising three primary layers; a basal layer, an intermediate region and an apical layer (as depicted in Figure 1). The apical layer is exposed to the nasal cavity and contains glial-like supporting cells called sustentacular cells. The intermediate region contains layers of immature and mature OSNs and exhibits a distinct laminar organization in the rodent ON (Figure 1), with the immature OSN layer residing closer to the basal lamina. In the human ON, however, immature and mature OSNs are more dispersed throughout, suggesting a less pronounced laminar organization of OSNs than observed in the rodent ON.30,31

Deep to the intermediate region lies the basal layer that contains globose and horizontal basal cells (GBCs and HBCs, respectively), and is separated from the underlying LP by the basal lamina. As shown in the mouse ON, GBCs and HBCs are the stem cells that initiate the ON lineage by proliferating into transit-amplifying progenitors that give rise to immediate neuronal precursors (INPs), which differentiate into immature and ultimately mature OSNs.32 Several rodent studies have delineated the expression of transcription factors in ON basal cells to define the lineage of ON cells.32,54 These defining factors and associated cell types are listed in Figure 1. Depending upon the niche, both HBCs and GBCs comprise stem cell populations in the rodent ON that can be both neuro-competent and multipotent.35,40

In the mouse ON, the basal layer exhibits a distinct organization. HBCs form a single layer next to the basal lamina, exhibit a flattened morphology and express cytokeratin-5 and -14, whereas GBCs exhibit a round morphology, do not express cytokeratins and reside in a single layer superficial to the HBCs (Figure 1).41,42

Similarities between rodent and human ON basal cells have been observed,30,31,43 yet the laminar organization and morphologies of the different basal cell types (flattened versus rounded) appear to be less clearly defined in human ON.30,31 In addition, molecular expression patterns of these basal cells may differ between species, as robust expression of the low affinity nerve growth factor p75NGFR has been observed in first- and second-layer basal cells in human ON, but not in adult rodent ON.13,30,44,45 These basal cells, whether analogous to rodent HBCs, GBCs or both, are thought to comprise the presumptive neural precursor population in human ON.

Juxtaposed to the basal cell layer, separated by the basal lamina, is the LP. Rodent studies have shown this to be a neural crest-derived ectomesenchymal tissue, which contains multipotent mesenchymal stem cells (LP-MSCs), olfactory ensheathing cells (OECs), axons originating from the OSNs and the acynus of Bowman glands.37,46,47 Studies of LP-MSCs in rodents and humans have shown that these cells are able to generate multiple cell phenotypes, including, but not limited to, OECs and neural lineages, both during development and in response to regenerative cues.35,37,39,46,47,48 The glial potential of OECs has been extensively studied in the context of spinal cord transplantation and has been reviewed elsewhere.49,50 OECs represent a highly specialized, likely heterogeneous glial cell population that share morphological and neurochemical features with Schwann cells and astrocytes, although they differ from both. Their neurochemical composition highlights their functional heterogeneity, as they express sets of proteins shared with astrocytes (for example, glial fibrillary acidic protein (GFAP) and s100B), oligodendrocytes and oligodendrocyte precursors (O4, NG2), CNS radial glia (basic lipid binding protein, BLBP), as well as developmentally important proteins CD 44, β1 integrin, P200 and Notch 3.36

USE OF EX VIVO OLFACTORY TISSUE IN THE STUDY OF NEUROPSYCHIATRIC AND NEURODEVELOPMENTAL DISEASES

As a regenerative neuroepithelium containing a range of morphologically and molecularly distinct neuronal and glial cells, the ex vivo ON represents a useful tool for examining cell type-specific biological changes in neuropsychiatric illness. Compared with all other in vitro neuronal models or blood cell studies, this cell type-specific resolution is a unique feature of this paradigm.
As such, the ON has been utilized as a platform for histologic assessment, investigation of intracellular signaling and gene expression profiling.

Using a histologic approach to study neuronal differentiation, Arnold et al. conducted immunohistochemical assessment of the ON using markers for specific stages of neuronal differentiation in ex vivo tissue from schizophrenic patients and controls. In this study, basal cells, immature and mature neurons were marked with antibodies for p75NTR, GAP-43 and OMP, and densities of immunoreactivity for these markers were used as indices for specific stages of differentiation. Compared with controls, patients exhibited decreased density of p75-labeled basal cells, a higher density of GAP-43-labeled and immature OSNs and an increased ratio of immature to OMP-labeled mature OSNs. Together these findings led to the postulate that neuronal lineage may be disrupted in the ON and by extension in the CNS of patients with schizophrenia.

In a similar paradigm, Pantazopoulos et al. examined chondroitin sulfate proteoglycans, prominent components of the extracellular matrix, in ex vivo ON from schizophrenic patients compared with controls. These extracellular matrix proteins are critical for cellular differentiation and migration, and are postulated as a possible mechanism for the altered neuronal lineage as observed in by Arnold et al. In that study, the decreased chondroitin sulfate proteoglycan density reported in mature OSNs in schizophrenia is consistent with previous findings of reduced proteoglycans in multiple brain regions of postmortem brains of schizophrenic patients. Thus, some of the neurobiological characteristics observed in the ON can be extrapolated to those of the brain.

Histologic changes in the ON that are specific to particular neuropsychiatric illnesses may hold promise as potential biomarkers. In the postmortem ON of patients with Alzheimer disease, Arnold et al. found higher frequency and abundance of amyloid-β and paired helical filament-tau pathologies in ON derived from Alzheimer patients compared with controls, essentially mirroring previous observations in postmortem brains of patients (reviewed in Hardy and Selkoe). If these and other findings described above are extended to ON biopsy tissues of living patients, and are correlated with clinical severity or diagnostic subtypes, they could serve as cellular biomarkers of psychiatric illnesses.

The ON can be utilized for gene expression profiling of neural cells with cell type-specific resolution. McCurdy et al. used microarray analyses in ex vivo tissues to demonstrate altered expression of genes relating to cell cycle and neurogenesis in schizophrenic patients, findings that may be consistent with neurodevelopmental dysregulation. More recently, Mor et al. used laser capture microdissection in ex vivo tissues to isolate mature OSNs, and observed increased miRNA expression of MiR-382 in schizophrenic patients versus controls. Notably, similar dysregulation in MiR-382 has been observed in the postmortem dorsolateral prefrontal cortex, again supporting the notion that certain aspects of brain pathology may be represented in the ON. Ex vivo ON tissues can also be examined for their electrophysiological properties. OSNs can be dissociated from biosynthetic tissue, readily identified by their morphology and examined using calcium imaging, voltage sensitive dye imaging and other electrophysiological measures. Indeed, Hahn et al. assessed odorant induced calcium signaling in OSNs derived from medication-free bipolar disorder patients and their matched controls, and demonstrated decreased intracellular calcium signaling in bipolar disorder patients. Given that dysregulated calcium signaling has been consistently reported in bipolar disorder patients in peripheral blood and brain, ON derived from bipolar patients may be useful for furthering understanding of the molecular pathologies of the disorder.

Ex vivo ON tissues also provide a unique paradigm for the study of neurodevelopmental disorders, given the lifelong regeneration of ON and the presence of OSNs at all the stages of development. One such developmental disorder where ex vivo ON has been studied is Rett syndrome, which results in profound intellectual disability and has been associated with deficits in neuronal maturation. Ronnett et al. reported fewer mature OSNs, increased immature OSNs and abnormal morphology of mature OSNs in Rett syndrome patients of various ages compared with age-matched controls. These observations support the notion that ON is a viable tool for studying neurodevelopmental aspects of diseases characterized by abnormal neuronal development.

**IN VITRO USE OF CULTURED OM-DERIVED CELLS IN THE STUDY OF NEUROPSYCHIATRIC ILLNESSES**

A second approach to the use of the OM in psychiatric research, which complements the study of ex vivo ON tissues, is the in vitro culture of OM-derived cells. Due to the regenerative nature of the OM, OM-derived cells can be readily propagated and are increasingly yielding insights into the cellular and molecular underpinnings of neuropsychiatric illness.

Properties of adult human OM-derived cells in vitro

When developing in vitro models for neuropsychiatric illnesses, it is critical to consider the specific characteristics of cell types arising in culture, which are determined by the tissue compartment from which they originate and conditions in which they are grown. In diverse variations of these factors, multiple approaches to the culture of OM-derived cells have been developed including the organotypic explants, dissociated cultures and neurospheres (see Box 1 for specifics of culture paradigms). Common to all these paradigms is that they seek to utilize regenerative characteristics of the ON and its underlying LP as neural tissues, within which progenitors differentiate into neurons, sustentacular cells and OECs.

The experimental utility of cultured OM cells as a model for neuropsychiatric illness is, in part, related to the extent to which cultured cells recapitulate the characteristics of in vivo OM cells or of donors’ neurobiological makeup. To define neuronal precursors, immature neurons or OSNs, a rapidly increasing database on the expression of molecular markers in cell types of each differentiation stage can be used as a guide. As described in Figure 1, human and rodent ON cells at different stages of neuronal differentiation in vivo are characterized by expression of molecular markers such as Cytokeratin-5, Sox2, Pax6, Ascl1, Neurog1, NeuroD1, Gap43 and OMP. Neuronal precursors can be defined as proliferative cells whose progeny ultimately become neuronal cells. Immature neurons and OSNs do not share this proliferative capacity, and OSNs are further defined functionally by their capacity to respond to odors.

Numerous markers have been used to characterize OM-derived cells in vitro (Figure 2). While some markers, such as NeuroD1 and NCAM, are selectively expressed in neuronal cells at varying stages of differentiation in vivo, the selectivity or appropriateness of other markers is less clear. For example, β-tubulin III and vimentin are expressed not only in olfactory neurons in vivo, but also in other cell types, thus limiting their utility as sole markers for neurons in vitro despite their widespread expression. While nestin has been recognized as a neural stem cell/progenitor marker in the mammalian brain, its expression in adult human ON in vivo has been demonstrated exclusively in sustentacular cells as opposed to basal cells of neural lineage. LP-MSCs, however, do express nestin in vitro, suggesting that the use of nestin immunoreactivity as a stem cell marker applies specifically to LP-derived neural stem cells.

Adult human OM-derived cells, originating from non-dissociated biopsy tissue and cultured in a monolayer in serum-containing media, are capable of expressing mRNA transcripts or...
Human OM is harvested from the nasal septum or turbinates typically by biopsy, or also by exfoliation. Cells can be subsequently propagated from the olfactory neuroepithelium (ON), its underlying lamina propria (LP) or from tissue containing both regions. Protocols have been established to enhance the neural stem cell properties of OM cells through neurosphere and monolayer culture.2

(A) Organotypic-monolayer cultures: Non-dissociated adult human ON biopsy tissues, containing a portion of underlying LP, can be utilized for propagating OM cells in monolayer cultures in serum-containing media on uncoated or fibronectin-coated plates or on a Matrigel substrate. In cultures on fibronectin-coated plates, epithelial cells first grow out from biopsy tissue to form sheets, over which grow round, more rapidly proliferating cells after 24–48 h.2,6,30

(B) Neurosphere cultures: Multiple groups have reported that cells collected from the human ON itself (with or without some underlying LP) can be cultured in neurospheres from dissociated biopsy tissue. In serum-containing media, when adult human ON tissue (without underlying LP) is first dissociated, cells with the bipolar morphology of neurons, along with presumptive olfactory ensheathing cells, are present.61 Such cells soon disappear in serum-containing media, and are replaced by a proliferating population, which form spheres and can be cultured for extended periods. Alternatively, adult human OM-derived cells can be dissociated and propagated on poly-l-lysine-coated plates in defined medium with EGF and FGF2 to form ‘primary neurospheres’, before being plated onto uncoated or fibronectin-coated plates and maintained in serum-containing medium in monolayer culture.16–57

Additional approaches that have been employed in rodent studies include culture of adult rat ON-derived cells on a feeder layer of newborn rat glial cells, and co-culture of ON-derived cells with the bipolar morphology of neurons, along with presumptive olfactory ensheathing cells, are present.61 Such cells soon disappear in serum-containing media, and are replaced by a proliferating population, which form spheres and can be cultured for extended periods. Alternatively, adult human OM-derived cells can be dissociated and propagated on poly-l-lysine-coated plates in defined medium with EGF and FGF2 to form ‘primary neurospheres’, before being plated onto uncoated or fibronectin-coated plates and maintained in serum-containing medium in monolayer culture.16–57

In vitro culture paradigms of adult human olfactory mucosa-derived cells

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In vitro culture of OM-derived cells from rodents and fetal humans

The OM paradigm has been employed using in vitro cells from rodents, shedding light on the stem cell potential of OM-derived cells. For example, proliferation of neuronal precursors and successful genesis of mature OMP-positive OSNs from adult rat ON-derived cells in culture has been reported.68 When adult rodent ON-derived cells are co-cultured with a 3T3 cell feeder layer at the air–liquid culture interface, cells with the molecular phenotypes of GBCs, HBCs, sustentacular cells and neurons can be generated which can successfully re-integrate into the ON after transplantation.69

Monolayer culture of rat p75NGFR-expressing OM-derived cells, which were purified by fluorescence-activated cell sorting, has been used for characterization of putative OECs in vitro.77 Cells from the mouse olfactory LP, when cultured in a monolayer, demonstrate the same OEC phenotype and cell-type-specific marker expression as human LP-derived cells.65 When cultured in spheres, mouse olfactory LP-derived cells have the capacity to integrate into lesioned hippocampus in vivo, differentiate into neurons and restore learning and memory.78

Numerous studies have used OM-derived cells from pre-natal/neonatal rodents and fetal humans, in part because such cells can be propagated more readily. This is observed under conditions in which more than 5% of OM cells in culture respond to helioin, a ligand for the OR3A1 odorant receptor, and up to 10% of OMP-positive cells responding to a mixture of other culture cells may be of a neural lineage. To date, although multiple OM-derived cell culture paradigms have been used in various studies, the relative expression of cell type-specific markers by cells cultured in these paradigms has not been directly compared. When indirectly compared, some similarities between paradigms exist in the expression of multiple markers, such as NCAM and nestin (Figure 2), suggesting a degree of consistency among paradigms employing serum-containing media. However, differences also exist, such as in β-tubulin III, which is expressed in 35% of ON-derived cells in the study of Matigian et al.,6 in the majority of cells in multiple other studies and in ~100% of ON-derived cells in the study of Benitez-King et al.25 Overall, further work to delineate the degree of consistency between culture paradigms, including characterization of cellular heterogeneity and marker expression, is needed.

The adult human olfactory LP alone can also be used to culture progenitors of ectomesenchymal origin, which are particularly suitable for the in vitro propagation of presumptive OECs.38,75 When cultured in serum-free media supplemented with NT3, almost all adult human LP-derived proliferating cells express OEC markers S100 and GFAP, ~80% express p75NGFR, whereas none express HNK1 (a marker for Schwann cells). These cells hold promise for autologous transplantation in the treatment of spinal cord injury.90,96

The properties of OM-derived cells, such as their proliferation, morphology and expression of cell type-specific markers, can be heavily influenced by the in vitro environment, particularly culture medium. The properties of OM-derived cells, such as their proliferation, morphology and expression of cell type-specific markers, can be heavily influenced by the in vitro environment, particularly culture medium. Table 1. In culture, BDNF (brain-derived neurotrophic factor), NT3 and NT4 promote ON-derived cell survival, FGFR2, FGFR8, EGF and TGF-α (transforming growth factor-alpha) have been used to stimulate progenitor proliferation, and serum deprivation, DB-cAMP, IGF-1 (insulin-like growth factor-1), retinoic acid, forskolin and sonic hedgehog have been used to drive human ON culture cells toward a
neuronal phenotype. Proliferation of human LP-derived cells with an OEC phenotype is promoted by NT3, BDNF or NGF. Human LP-derived cells can be driven toward a neuronal phenotype with a cocktail of retinoic acid, forskolin and sonic hedgehog, or dexamethasone, insulin, 3-isobutyl-1-methylxanthine, indomethacin and ethanol, and toward a osteoblast phenotype with a cocktail of dexamethasone, L-ascorbic acid-2-phosphate, and β-glycerophosphate or NaH₂PO₄. Other additional factors, many of which are relevant to the control of neurogenesis and differentiation in vivo, have also been shown to modulate OM-derived cell proliferation, morphology and expression of cell type-specific markers in vitro (Table 1).

THE USE OF OM-DERIVED CELLS IN PSYCHIATRY AND NEUROSCIENCE RESEARCH
Historically, the tools to study neural function and mechanisms of drug action in humans at a cellular level have proved elusive. OM-derived cells have emerged as a promising platform for such investigations in a range of neuropsychiatric, neurodegenerative and developmental disorders.

The ability to investigate functional cellular characteristics represents a distinctive opportunity afforded by the OM-derived cell paradigm. The processes of cell proliferation, adhesion, motility, metabolism and apoptosis have been investigated by Mackay-Sim and colleagues, who showed that ON-derived cells from schizophrenia patients (but not bipolar disorder patients), when cultured in a monolayer from non-dissociated biopsy tissue, proliferate more rapidly than ON-derived cells from healthy controls. This abnormal adhesion was accompanied by increased cell motility, decreased expression of cell type-specific markers such as p75NGFR, Vimentin; and, markers whose expression increase when cells are cultured in base (unsupplemented defined) media rather than serum-containing media; and, mesenchymal stem cell cluster of differentiation (MSC-CD) markers, used to confirm cell type, vary among studies. CK, cytokeratin; INP, intermediate neural progenitor; LP, lamina propria (indicates cultures derived solely from LP, without ON); LP-MSC, lamina propria mesenchymal stem cell; ND, not detected; NTR, neurotransmitter receptor; OEC, olfactory ensheathing cell; ON, olfactory neuroepithelium; OSN, olfactory sensory neuron.

Table 1.

Figure 2. Expression of putative molecular markers of stem cells, neural progenitors, neurons and glia by various olfactory mucosa (OM) cell types, both in adult OM in vivo and in cells generated from adult OM tissues in vitro. Studies of OM explant tissues or dissociated cells immediately ex vivo are not included. Percentage values are approximate, and refer to the proportion of cells in culture which express each marker. Values are only shown for markers displaying consistency between studies, and only for cells grown in serum-containing media. * studies using neonatally-derived rodent or fetally-derived human ON culture cells; † markers whose significance in OM culture cells is uncertain due to a discordance between human and rodent (for example, p75NGFR), low/uncertain levels of expression in adult ON in vivo (NF-M/H, Nestin, NeuN, MAP2) or a possible lack of cell type specificity (p75NGFR, Vimentin); ‡, markers whose expression increase when cells are cultured in base (unsupplemented defined) media rather than serum-containing media; ^, mesenchymal stem cell cluster of differentiation (MSC-CD) markers, used to confirm cell type, vary among studies. CK, cytokeratin; INP, intermediate neural progenitor; LP, lamina propria (indicates cultures derived solely from LP, without ON); LP-MSC, lamina propria mesenchymal stem cell; ND, not detected; NTR, neurotransmitter receptor; OEC, olfactory ensheathing cell; ON, olfactory neuroepithelium; OSN, olfactory sensory neuron.
Table 1. *In vivo* and *in vitro* factors influencing the proliferation, differentiation and survival of cells within, or derived from, the olfactory mucosa (OM)

| Factor      | Cell type impacted in vivo | Effect observed in vivo | Effect on OM-, ON- or LP-derived cells in vitro |
|-------------|----------------------------|-------------------------|-----------------------------------------------|
| TGF-α       | Stem cell                  | Stimulates proliferation and progression of rodent HBCs*99          | Stimulates ON-derived cell (HBC) proliferation*93 |
| EGF         | Stem cell                  | Stimulates proliferation of rodent HBCs*99                          | Stimulates ON-derived cell (HBC) proliferation*93 |
| ΔNp63 (p63) | Stem cell                  | Required for rodent HBC genesis and differentiation*100             | —                                              |
| Sox2        | Stem cell                  | Increases Ascl1 expression and differentiation of rodent GBC/HBCs into neural progenitors*94 | —                                              |
| Meis1       | Stem cell                  | Suppresses Ascl1 expression and stem cell differentiation*94        | —                                              |
| Foxg1       | Stem cell                  | Promotes differentiation of rodent Sox2-positive stem cells into neural progenitors*101 | —                                              |
| Retinoic acid| Stem cell                  | Stimulates basal cell proliferation and/or differentiation after lesion*102 | Promotes ON-derived cell (HBC) differentiation*94 and LP-derived neuronal differentiation*57 |
| Uncx        | Early neural progenitor     | Promotes proliferation of neural progenitors and survival of OSNs*103 | —                                              |
| FGF2        | Early neural progenitor     | Enhances neural progenitor proliferation*104                        | Stimulates OM-derived cell proliferation*72,83,90,105 |
| FGF8        | Early neural progenitor     | Promotes neurogenesis and progenitor proliferation*106              | —                                              |
| Ascl1       | Early neural progenitor     | Required for maturation of early neural progenitors into INPs*107,108 | —                                              |
| Six1        | Early neural progenitor     | Required for maturation of early neural progenitors into INPs*109    | —                                              |
| Ngn1        | Early neural progenitor     | Required for maturation of neural progenitors into INPs*108          | —                                              |
| BMP2,4,7    | Early neural progenitor, immature OSN | At lower concentrations, promotes survival of newly formed OSNs (BMP4 only),*110 at higher concentrations inhibit neurogenesis by targeting Ascl1 for proteolysis*111 | —                                              |
| FST         | Early neural progenitor     | Promotes progenitor proliferation by antagonizing GDF11 and ACTβB*112,113 | —                                              |
| ACTβB (activin) | Early neural progenitor     | Inhibits progenitor proliferation by promoting proteolysis*115       | —                                              |
| Hes1        | Early neural progenitor     | Suppresses Ascl1-mediated neural progenitor maturation*114,115      | —                                              |
| Hes5 (with Hes1) | Early neural progenitor     | Suppresses Ngn1-mediated neural progenitor maturation*114,115      | —                                              |
| MMP2, MT1-MMP | Early neural progenitor, INP | Downregulated following bulbectomy in rodent GBCs*116               | —                                              |
| TIMP        | Early neural progenitor, INP | Uregulated following bulbectomy in rodent GBCs*116                  | —                                              |
| GDF11       | INP                        | Inhibits proliferation of INPs by inducing reversible cell cycle arrest*112,113 | —                                              |
| Runx1       | INP                        | Stimulates INP proliferation*117                                     | Stimulates ON-derived cell proliferation*117    |
| NPY         | INP                        | Promotes, and is required for, INP proliferation and maturation*118-121 | Increases OM-derived cell proliferation via the Y1 receptor and PKC-dependent ERK1/2 phosphorylation*110,128 |
| Peptide YY  | INP                        | Regulates INP differentiation*118,119                               | Increases OM-derived cell proliferation*118,119 |
| Atf5        | Immature OSN               | Promotes survival and maturation of immature OSNs*122               | —                                              |
| Emx12       | Immature OSN               | Promotes survival and maturation of immature OSNs*123               | —                                              |
| Lhx2        | Immature OSN               | Promotes survival and maturation of immature OSNs*124,125           | —                                              |
| ZFP423/OAZ  | Immature OSN               | Inhibits maturation of immature OSNs*126                            | —                                              |
| LIF1        | Immature OSN               | Promotes survival and inhibits maturation of immature OSNs*127,128  | —                                              |
| BDNF        | Multiple cell types        | May provide trophic support to maturing ON cells*130-132            | —                                              |
| NT3,4       | Multiple cell types        | May provide trophic support to maturing ON cells*131                | —                                              |
| NGF         | Multiple cell types        | Expressed in neuronal ON layers, may provide trophic support to maturing ON cells*131 | —                                              |
| PACAP       | —                          | —                                                                    | —                                              |
| KGF-1       | —                          | —                                                                    | —                                              |
| Nitric oxide| —                          | —                                                                    | —                                              |
| Serum deprivation | —                          | —                                                                    | —                                              |

*References: 1-130*
Parkinson’s disease. Increased cell death has been observed in ON-derived cells from bipolar disorder patients (but not schizophrenia patients), relative to healthy controls, although a subsequent study reported increased caspase-3/7 activity in ON-derived cells from schizophrenia patients, suggesting increased apoptotic activity.

Cellular processes governing microtubule formation and cytoskeletal structure have also been investigated in OM-derived cells from individuals with psychiatric illness. Dynamic structural processes have been monitored across time in ON-derived cells, and increased microtubule stability in ON-derived cells from individuals with schizophrenia than in ON-derived cells from controls has been reported. In addition, in a paradigm employing exfoliation and monolayer culture, ON-derived cells from schizophrenia patients were described by Benitez-King and colleagues to display abnormal microtubule organization, whereas ON-derived cells from bipolar disorder patients displayed decreased microtubule length and cytoplasmic β-tubulin III protein expression. Decreased L-type voltage-activated calcium currents in the ON-derived cells from individuals with schizophrenia were also reported in this study. Overall, these studies suggest the potential utility of OM-derived cells for the investigation of cellular function in neuropsychiatric disorders. The reported findings in OM-derived cells implicate processes and pathways, which have been independently implicated in schizophrenia and bipolar disorder by postmortem studies, such as in neurogenesis, neuronal migration, and apoptosis. However, they also hold the promise of revealing aspects of neural cell function in psychiatric illness that are difficult to investigate using traditional approaches.

The OM-derived cell culture approach also provides a powerful platform to explore mechanisms of drug action, and to target pharmacologically the molecular pathways of interest in psychiatric disorders. Studies have been performed investigating the effects of pharmacological agents on intracellular signaling, molecular expression and cell function in ON-derived cells derived from schizophrenia, bipolar disorder and major depressive disorder patients. Feron et al. demonstrated that dopamine increases apoptosis in control ON-derived cells, but decreases apoptosis in schizophrenia cells. Cellular effects of lithium, a first-line bipolar disorder treatment, have also been explored in ON-derived cells, revealing amelioration by lithium of ionic stress-induced apoptosis and TRPM2 overexpression. In major depressive disorder, there is evidence that ON-derived cells display decreased nuclear translocation of the glucocorticoid receptor, GR, in response to glucocorticoids relative to controls. This diminished receptor translocation was accompanied by greater cytoplasmic association of GR with the immunophilin FKBP51. These studies suggest the potential utility of ON-derived cells for understanding current treatments and developing novel pharmacological agents in psychiatry.

The experimental potential of OM-derived cells from psychiatric patients has also been highlighted by studies of subcellular molecular abnormalities. As in work with ex vivo ON tissue, many molecular and cellular studies using ON-derived cells have demonstrated a concordance with postmortem brain studies. ON-derived cells from Alzheimer’s patients exhibited increased amyloid precursor protein levels, along with other protein alterations, consistent with changes in multiple brain regions in the illness. Dysregulation of gene expression in multiple cellular pathways, including cell cycle regulation, oxidative stress response, focal adhesion and axon guidance have been reported in ON-derived cells from schizophrenia patients, some of which may be mediated by altered histone H3 lysine 4 trimethylation. These findings are consistent with postmortem brain studies in schizophrenia showing aberrant histone H3 lysine 4 trimethylation within the GAD1 gene and dysregulated expression of immune genes. Protein changes in oxidative stress and cell adhesion pathways have also been recently reported in a preliminary human iPS cell study. As described earlier, increased expression of miR382 has been reported in ON-derived cells from individuals with schizophrenia, mirroring findings in ex vivo microdissected ON tissue and postmortem prefrontal cortex. In ON-derived cells from bipolar disorder patients, gene expression changes within the phosphatidylinositol signaling pathway have been reported. In Alzheimer’s and Parkinson’s diseases, OM-derived cells have also demonstrated altered gene and protein expression profiles, implicating mitochondrial function, oxidative stress and xenobiotic metabolism in these illnesses. Finally, OM-derived cells have also yielded insights into molecular pathologies in developmental disorders. In an early study interpreted as suggesting blood–brain concordance of FMRP deficits in fragile X syndrome, decreased FMRP protein was observed in ON-derived cells from affected individuals. LP-MSCs from individuals with familial dysautonomia, which is caused by mutations within the IKBKBAP gene, display lower IKBKBAP mRNA expression, decreased IKAP/hELP1 protein, altered IKAP/hELP1 subcellular localization and dysregulation of genes involved in cell migration and cytoskeletal reorganization. Overall, these studies indicate the potential for OM-derived cells to shed light on molecular abnormalities associated with brain disorders, and the concordance of many OM-derived cell studies with postmortem findings highlights the potential for OM-derived cells to reflect pathophysiological mechanisms in the brain.

CONCLUSIONS
Advantages and challenges for olfactory paradigms in the study of neuropsychiatric illnesses
Olfactory mucosal biopsy studies afford a unique opportunity in neuropsychiatric research, offering ex vivo and in vitro neural cells from living individuals, which harbor biological characteristics that may be more relevant to neuropsychiatric disease than blood cells or skin fibroblasts. Figure 3 provides a summary of the previous and potential uses of OM-derived cells in psychiatric and neuroscience research, as described throughout this review.

The OM model has a number of strengths. First, ex vivo OM tissues were previously exposed to the in vivo neurohormonal...
milieu and contain neurobiological signatures of the in vivo condition. As such, they can serve as a point of reference for in vivo and in vitro OM findings, bridging the gap between these two approaches. Second, OM biopsies, though limited by the quantity of tissue available, can be safely obtained from the same subjects multiple times (monthly, up to three consecutive times in our human subjects protocols) and can be fitted with longitudinal clinical study designs. In this approach, OM biopsies are obtained in specific phases of the illness, while patients’ clinical conditions are carefully characterized. Interpretation of neurobiological parameters in the context of clinical changes is a rare opportunity in neuropsychiatric research. Examples of such use include but are not limited to the collection and examination of OM biopsies from (1) individuals at risk for schizophrenia and unaffected relatives before and after the onset of illness, (2) mood disorder patients before, during and after disease episode (such as in depressive disorders or bipolar disorder), (3) any neurodevelopmental or psychiatric disease before, during or after treatment. Finally, one promising aspect of using ex vivo tissues in psychiatric illness involves their relation with the broader olfactory neurocircuitry: olfactory mucosa → olfactory bulb → olfactory cortex. Disease-associated alterations within the ON may not occur in isolation, and may reflect brain abnormalities within the broader olfactory neurocircuitry. Indeed, numerous structural and functional decrements associated with neuropsychiatric diseases are reported in many of these brain regions. Olfactory circuitry can now be interrogated by psychophysical testing, electroolfactogram and evoked potentials. Combining these measures with molecular and cellular measures derived from OM biopsy tissues can help us to link molecules to cellular changes and then to circuit activity.

An important consideration in using ex vivo tissues is that the human ON is patchy and thus some biopsy tissues may not contain OSNs. Approximately 70 to 80% of ON biopsy tissues obtained from nasal septum or middle turbinate yield olfactory receptor neurons when dissociated immediately from the biopsy (unpublished observations). This is an important consideration, however, particularly for histologic examination of OE tissues. As the neuroepithelial patches are intermixed with respiratory epithelium, quantitative assessment of protein immunoreactivity or mRNA needs to be conducted by delimiting neuroepithelial versus respiratory patches in histologic sections. Under in vitro conditions, however, such limitations do not appear to figure greatly into the overall cellular composition of resultant culture cells. Nonetheless, quantitative assessment of cell type-specific proteins, whether neuronal, glial or epithelial, should be a guide for including cell lines for between-group comparison studies.

As noted in the preceding sections, the in vitro ON paradigm is particularly informative in studies of active cellular processes and pathways, where pharmacologic manipulations are to be tested or where larger numbers of cells from an individual are required. Importantly, as these cells are of neuronal and glial lineages they better represent the molecular dysregulations of neuropsychiatric disease than those of other cell types used in in vitro studies, such as blood cells. Indeed gene expression profiles of cells derived
from OM biopsy more closely resemble brain tissue, stem cells and neurons derived from stem cells than blood cells (see Horiiuchi et al.150 for a comparison of gene expression profiles of brain tissue, OM cells, stem cells and iPSCs). While iPSC and induced neuronal cell paradigms offer unparalleled opportunities to generate differentiated neurons in vitro from living individuals, there has been considerably less study of the capacity of OM cells to generate differentiated neurons. Nevertheless, OM cells have distinct advantages (Table 2). The timeline from OM biopsy to availability of proliferated precursor cells is ~4 weeks, whereas iPSC reprogramming protocol timelines are considerably longer, varying on the basis of source cells and methods. In addition, OM cells are readily proliferative and can be rapidly expanded over multiple passages. Thus OM studies have lower costs and require less time and labor (Table 2).

Most importantly, OM tissues are obtained as neural tissues (Table 2) and thus they do not require genomic reprogramming as with iPSC or induced neuronal cells. A challenge in the iPSC and induced neuronal paradigms is to determine to what degree OM-derived neurons or glia is not entirely clear at environment. To what degree OM-derived cells assume characteristics of their molecular phenotypes and the characteristics of differentiated neurons or glia is not entirely clear at environment. To what degree OM-derived cells assume character-

| Characteristic                        | Adult human OM-derived cells | Adult human iPSC cells | Adult human iN cells |
|--------------------------------------|------------------------------|------------------------|----------------------|
| Ease of collection                   | Moderate                     | High                   | High                 |
| Current cost to prepare cells for assay | Low–moderate                 | High                   | Moderate             |
| Extent of in vitro modification      | Little modification: only the influence of in vitro conditions | Substantial modification: reprogramming for pluripotency plus the influence of in vitro conditions | Substantial modification: reprogramming for somatic conversion plus the influence of in vitro conditions |
| Availability of ex vivo neural reference tissue | Yes                          | No                     | No                   |
| Proliferative capacity of cells used for assays | Moderate–high               | Moderate               | None                 |
| Capacity to be driven to neuronal differentiation | Varying between protocols. | Robust, high efficiency | Robust, moderate efficiency |
| Cellular phenotype commonly used in assays | Proliferative putative neural progenitor cell | Differentiated neuron | Differentiated neuron |
| Action potential generation         | To be further demonstrated15 | Yes                    | Yes                  |

Detailed discussion of iPSC and iN cell paradigms is beyond the scope of this review, but these paradigms have been reviewed extensively elsewhere.1,2 See text for details and references for OM-derived cell studies. *Collection of OM tissues is performed by a qualified otorhinolaryngologist using local anesthetic. An important brain region and neural circuits in psychiatric illness. This will involve the examination of olfactory-derived tissues and function at multiple levels of the olfactory circuit, as well as direct parallels between OM findings and brain pathology.

**CONFLICT OF INTEREST**
The authors declare no conflict of interest.

**ACKNOWLEDGMENTS**
This work was supported by funding from K23MH079498 (KB-W), MH059852 and MH080193 (C-GH), T32MH019112-24 (SLW) and funding from the NHMRC (Australia) CJ Martin Fellowship APP1072878 (DS).

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