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ADULT OLFACTORY PRECURSOR CELL PROLIFERATION AND DIFFERENTIATION IS MEDIATED BY THE NEUROPEPTIDE Y SIGNALLING PATHWAY

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The identification of factors that promote neurogenesis within the olfactory neuroepithelium can provide clues to the process of mammalian nervous system repair. Neuropeptide Y (NPY) is expressed in neurons and supporting cells of the olfactory system. NPY regulates neuroproliferation of olfactory, hippocampal and sub-ventricular zone precursor cells via the Y1 and Y2 receptors. Another member of this family of peptides is peptide YY (PYY) that is also expressed in neurons, though to a lesser extent. *In vivo* analysis of the olfactory neuroepithelium was performed to quantify the numbers of olfactory receptor neurons in wildtype (WT), Y1, NPY, PYY and NPYPYY knockout ($Y1^{-/-}$, $NPY^{-/-}$, $PYY^{-/-}$ and $NPYPYY^{-/-}$) mice. Interestingly, the absence of NPY alone did not have the same effect on neuronal differentiation as the absence of both NPY and PYY. Further investigations of $NPYPYY^{-/-}$ and $PYY^{-/-}$ mice identified a significantly greater number of olfactory receptor neurons compared to WT, $Y1^{-/-}$ and $NPY^{-/-}$ mice ($p<0.0001$). Furthermore, $NPY^{-/-}$ mice had a significantly reduced number of mature olfactory receptor neurons ($p<0.05$). We have also examined the proliferation of olfactory neurospheres in primary olfactory precursor cell cultures isolated from WT, $Y1^{-/-}$, $NPY^{-/-}$, $NPYPYY^{-/-}$ and $PYY^{-/-}$ mice. The number of neurospheres that survive *in vitro* from $NPY^{-/-}$ are significantly reduced compared to WT controls at 3 weeks ($p<0.05$). Olfactory neurospheres from $NPYPYY^{-/-}$ and $PYY^{-/-}$ are significantly reduced compared to WT controls at 1, 2 and 3 weeks ($p<0.0001$). These results indicate an important role for the NPY signalling pathway in the proliferation and differentiation of adult olfactory precursor cells.

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COMMUNICATION BETWEEN TWO NEUROGENIC ZONES IN THE ADULT MOUSE NERVOUS SYSTEM

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There is ongoing neurogenesis in the subventricular zone of the adult brain which supplies interneurons to the olfactory bulb. There is also continuous neurogenesis in the olfactory epithelium supplying new olfactory sensory neurons whose axons terminate in the olfactory bulb. These axons synapse with tyrosine hydroxylase positive periglomerular neurons within the olfactory bulb, which are the product of subventricular zone neurogenesis. We hypothesise that focal denervation of the olfactory sensory neurons and thereby lesioning of the presynaptic input to the Type 1 neurons would result in their degeneration, and a subsequent upregulation of subventricular zone neurogenesis. Adult mice (n=26) were treated with methimazole causing the ablation of the olfactory epithelium, and the tissues examined at multiple time-points after treatment. The survival of the olfactory sensory neurons within the olfactory epithelium was assessed together with their terminals within glomeruli of the olfactory bulb. The loss of tyrosine hydroxylase periglomerular neurons was quantified. Cell proliferation in the subventricular zone was also quantified using an antibody against Ki67, a marker of proliferating cells, and EdU, a thymidine analogue to track cell proliferation. Methimazole treatment led to loss of olfactory sensory neurons in the olfactory epithelium, loss of their terminals in the glomeruli and loss of tyrosine hydroxylase positive periglomerular neurons in the olfactory bulb 14-18 days later ($p=0.05$). Cell proliferation in the subventricular zone was increased 14 days post methimazole treatment ($p=0.02$). The results are consistent with our hypothesis that neurogenesis in the brain has a common neurogenic axis with the olfactory neuroepithelium. We propose the presence of a signalling pathway between these two neurogenic zones, which remains to be elucidated.

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IMMUNOLOCALISATION OF BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) AND RECEPTOR TRKB IN THE HUMAN BRAINSTEM MEDULLA

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Brain-derived neurotrophic factor (BDNF) and its receptor TrkB are essential in promoting normal development of the central nervous system, with key roles in respiratory control, coordination of movement and balance, and feeding activities. Expression of these markers have not been previously studied in the human infant. This study provides a detailed account of the distribution and localisation of pro- and recombinant human- (rh) forms of BDNF, and of TrkB in the human infant brainstem medulla, with qualitative comparison to the expression in the human adult. It is hypothesised that all markers will be present in the studied nuclei and that the expression of BDNF and TrkB will be higher during development compared to adulthood. Using commercially available antibodies, we applied immunohistochemistry on formalin fixed and paraffin embedded human brainstem tissue [n=8 for infant, n=6 for adult], and qualitatively analysed the expression of proBDNF, rhBDNF and TrkB. Amongst the medulla nuclei studied, the highest expression of the markers was in the inferior olivary nucleus and arcuate nucleus. Lowest expression was in the nucleus of the solitary tract. Comparison between infants and adults showed higher expression in the infant brainstem nuclei of the hypoglossal, vestibular, and cuneate for all the studied markers. We conclude that BDNF and TrkB play important roles in development and control of respiration, movement, balance and feeding. Expression of the TrkB receptor is age-sensitive showing highest expression during early development.

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PERIPHERAL AND CENTRAL PROJECTIONS OF MID-SIZE SENSORY NEURONS CONTAINING CALCITONIN GENE-RELATED PEPTIDE BUT NOT SUBSTANCE P IN MICE

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Many small diameter sensory neurons in dorsal root ganglia (DRG) contain both calcitonin gene-related peptide (CGRP) and substance P (SP). These neurons generally have a nociceptive function. However, in DRG of mice, a population of mid-diameter neurons express CGRP but not SP. The projections and functions of these neurons are not known. Therefore, we have used multiple-labelling immunohistochemistry and axonal tracing with Neurobiotin in vitro to map the projections of these neurons from the cervical spinal cord to the forelimb. Mice (C57/Bl6) were anaesthetised with a lethal dose of inhaled isofluorane, prior to removal of the upper spinal cord, brachial plexus, dorsal root ganglia and skin of the fore paws. For pathway tracing (n=3), Neurobiotin was applied to the C7 ventral ramus and the brachial plexus-spinal cord was incubated for 4 hours in vitro. Neurobiotin was detected with streptavidin-Cy3 or -DTAF in spinal cord and DRG that were also labelled for CGRP and SP. Skin was labelled with antibodies to CGRP, SP and neuron specific enolase (NSE). In paw skin (n=3), varicose fibres containing CGRP but not SP were most prominent within dermal papillae of glabrous skin and around hair shafts in hairy skin. Within cervical spinal cord (n=4), fibres containing CGRP were prominent in the superficial dorsal horn (lamina I) and deeper dorsal horn (lamina IV). CGRP fibres lacking SP were most prominent in lateral areas of lamina I and in lamina IV. These data suggest that DRG neurons with CGRP but not SP have multiple somatotopic projections consistent with a polymodal mechanoceptor function.