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LETTER TO THE EDITOR

Quantitative evaluation of the human subventricular zone

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Sir, In a recent publication in *Brain,* van den Berge *et al.* (2011) examined the brains of 10 controls, 10 patients with Parkinson's disease, and five cases with incidental Lewy body disease and found no difference in the number of proliferating cells in the subventricular zone, thereby casting doubt on previously obtained evidence suggesting that central dopamine depletion impairs adult neuronal precursor cell proliferation. However, in this recent article the anatomical definition of the region of interest, the sampling procedure, some of the immunostaining procedures and the quantification methods were of limited precision and as such no firm conclusions can be drawn from this study about the dopamine regulation of subventricular zone neurogenesis in the human brain.

The subventricular zone around the lateral ventricles in the adult mammalian brain harbours a specialized type of $GFAP^+$ astrocytes, so-called B cells, which act as neural stem cells with the potential to self-renew and to give rise to astrocytes, oligodendrocytes and neurons (Doetsch *et al.,* 1997). B cells generate frequently dividing transit-amplifying C cells. Division of C cells gives rise to A cells, a restricted type of neural precursor cells that can migrate to the olfactory bulb, where they mature into functional interneurons. The potential for neurogenesis is also conserved in the adult human subventricular zone (Sanai *et al*., 2004), and as such this population of cells may be a potential resource for endogenous brain repair in brain disorders.

To date, a comprehensive series of *in vitro* and *in vivo* experiments in adult rodents and non-human primates conducted by various independent research groups has provided compelling evidence that activation of dopamine receptors, mainly D2-type receptors, increases the proliferation of neural precursor cells, mainly C cells, in the subventricular zone (Baker *et al.,* 2004; Coronas *et al*., 2004; Höglinger *et al*., 2004; Van Kampen *et al*., 2004; Freundlieb *et al*., 2006; Winner *et al*., 2006, 2009; Yang *et al*., 2008; O'Keeffe *et al*., 2009*a, b*).

With regard to human patients with Parkinson's disease, where there is a profound cerebral dopamine depletion, a putative dysfunction of adult neurogenesis has been proposed as a pathological substrate for non-motor symptoms (e.g. hyposmia) or as a factor aggravating neurodegeneration via a limited capacity of the brain to repair itself via neurogenesis. Inversely, stimulation of adult neurogenesis has been proposed as a novel therapeutic approach to counteract the neurodegenerative process (Arias-Carrion *et al*., 2007). In order to evaluate such concepts, it is essential to

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know whether or not the dopaminergic regulation of adult neurogenesis described in animals is also conserved in humans.

This question had been addressed in three previous papers. First, Höglinger *et al.* (2004) found a significantly reduced number of cells expressing the 'proliferating cells nuclear antigen' (PCNA) in the subventricular zone of human patients with Parkinson's disease compared with controls matched for age, gender and time from death to tissue fixation ($n = 4$ per group). In agreement with this, O'Keeffe *et al.* (2009*b*) found a significantly reduced number of C cells, identified by their expression of the epidermal growth factor (EGF)-receptor, in the subventricular zone of patients with Parkinson's disease compared with controls matched for age and gender ($n = 6$ per group). Finally, O'Sullivan *et al.* (2011) studied the subventricular zone of patients with Parkinson's disease ($n = 32$) and found the expression of Musashi1, a marker of neural stem and progenitor cells, was negatively associated with disease duration and positively correlated with the cumulative lifetime levodopa dose (O'Sullivan *et al*., 2011). Together, these data provide evidence that the dopaminergic modulation of precursor proliferation in the subventricular zone may be conserved in adult humans and is reduced in Parkinson's disease.

In contrast, in their recent paper, van den Berge *et al.* (2011) compared the subventricular zone of patients with Parkinson's disease and controls matched for age and gender ($n = 10$ per group) and found no differences in the expression of the S-phase marker PCNA and of the G2/M-phase marker phosphohistone H3. To exclude a possible bias through dopaminergic pharmacotherapy, the authors evaluated the subventricular zone of five cases with incidental Lewy body disease, and again found no difference to controls. They also analysed the subventricular zone of mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and found no differences in these markers. Based on these results and additional *in vitro* work, the authors concluded that the hypothesis that dopamine stimulates proliferation of human neural precursor cells has to be reconsidered. However, the data of van den Berge *et al.* (2011) should be interpreted with caution.

The human subventricular zone is separated medially by a small gap from the ependymal cell layer of the lateral ventricle; the lateral margin of the human subventricular zone is defined by a ribbon of specialized astrocytes (Sanai *et al*., 2004). $PCNA^+$ cells of the subventricular zone are typically located between the gap and the astrocytic ribbon. While the subventricular zone comprises only a few cell layers in the central part, the majority of subventricular zone cells lie in the dorsal and ventral parts (Fig. 1). The variability of the human subventricular zone in the rostro-caudal extension is recognized (Bernier *et al*., 2000), yet ill-defined at present. Given this heterogeneous composition, precise anatomical matching is an essential prerequisite to avoid high sampling errors and to obtain qualitatively and quantitatively comparable data from cases and controls. The identification of the region of interest is ideally done on large tissue sections containing the entire lateral ventricle. To ensure identical anatomical levels in all subjects of our initial study (Höglinger *et al*., 2004), we analysed the subventricular zone in the defined limits from the corpus callosum to the vena thalamostriata on coronal sections taken at the level of the anterior commissure (Fig. 1). We counted cells within the entire region of interest; however, a systematic random sampling procedure with parameters obtained by considerations of estimation precision according to the principles of stereology might also have been applied.

In contrast, van den Berge *et al.* (2011) used paraffin-embedded striatal tissue blocks without detailed information about the rostro-caudal and dorso-ventral localizations and analysed five images taken at random (not systematic random) locations of the subventricular zone. Consistently, the authors provide illustrations of the subventricular zone, intended to demonstrate the high variability of their observations, which are derived either from the central subventricular zone [Fig. 2D–F in van den Berge *et al.* (2011)] or from the very dorsal or ventral subventricular zone, as identified by the triangular shape of the subventricular zone at this site [Fig. 2A–C in van den Berge *et al.* (2011)]. Thus, the highly variable shape of the subventricular zone observed by van den Berge *et al.* (2011) reflects typical intraindividual anatomical differences, rather than interindividual differences.

Furthermore, van den Berge *et al.* (2011) have drawn an area of interest around the area of staining in the subventricular zone, measured therein the PCNA-staining intensity, and calculated the average percentage of the subventricular zone area that was occupied by PCNA staining (Fig. 2A), in an attempt to depict changes in cell number on the assumption of stable subventricular zone size and stable staining intensity per cell (Fig. 2B). However, all factors leading to reduced (Fig. 2C) or increased (Fig. 2D) staining intensity per cell at unchanged cell numbers erroneously leads to reduced or increased results with this method. In addition, the thickness and thereby the area of the subventricular zone is likely to increase proportionally to the cell number therein, any increase (Fig. 2E) or reduction (Fig. 2F) in the subventricular zone cell numbers with stable staining intensity per cell is unlikely to be depicted with this method. Furthermore, van den Berge *et al.* (2011) have not shown a linear relationship between staining intensity and concentration of antigen (PCNA or phosphohistone H3). Due to complex treatment of the tissue (size of the tissue blocks influencing degree of fixation, antigen retrieval procedure, concentration of antigen, enzymatic peroxidase reaction), it is unlikely that the immunohistochemical procedures resulted in quantitative data. Thus, cell counts, standardized or not to the invariable subventricular zone length rather than to the variable subventricular zone area, as done in previous work (Höglinger *et al*., 2004; O'Keeffe *et al*., 2009*a*), are essential to provide reliable estimates of cell proliferation in the subventricular zone.

When studying the anatomically comparable pictures presented in Fig. 2D and F in van den Berge *et al.* (2011), the patient with Parkinson's disease appears to have ~30% less $PCNA^+$ cells in the subventricular zone compared with the control case, which is consistent with our previous results (Höglinger *et al.,* 2004). Since the persons with incidental Lewy body disease in the van den Berge *et al.* (2011) study had no significant reduction in the striatal tyrosine hydroxylase $(TH)^+$ (dopaminergic) innervation, the unaltered number of $PCNA^+$ cells in the subventricular zone is in line with our predictions.

With regards to the mouse work, we note several technical problems that may have contributed to the apparent discrepancy with previously published results of a reduced proliferation in the dopaminergic denervated subventricular zone. First, although the

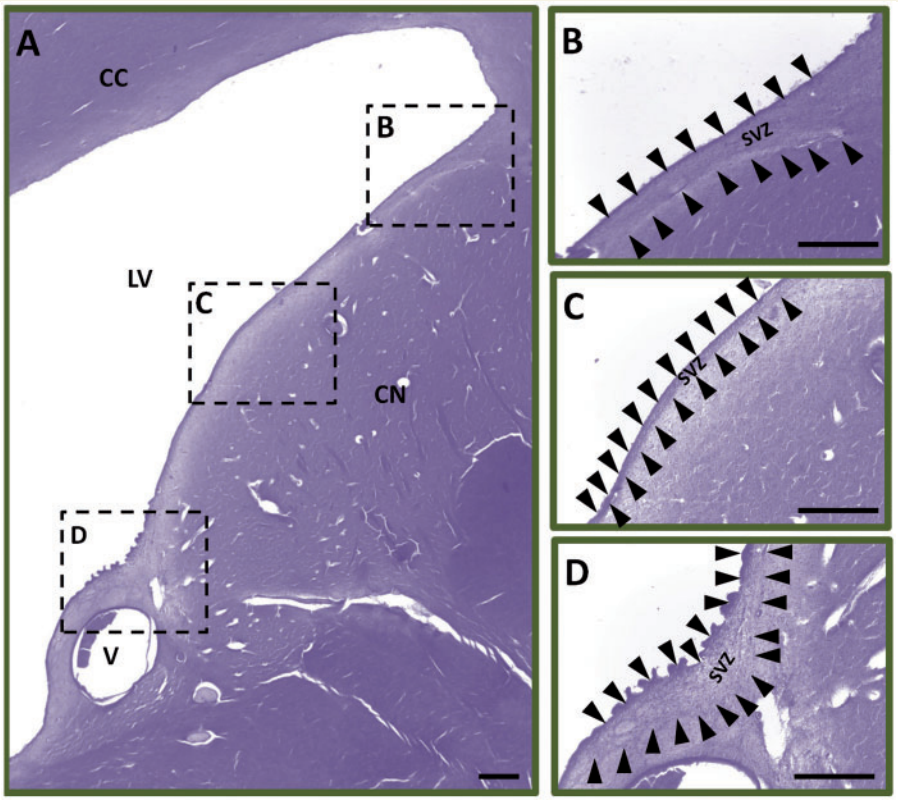

Figure 1 Anatomical extension of the human subventricular zone (SVZ). (**A**) Macroscopic overview of a cresyl violet-stained coronal section of an adult human brain corpus taken at the antero-posterior level of the anterior commissure. (**B–D**) Microscopic details from the boxed areas in **A** showing the subventricular zone at different dorso-ventral locations. CC = corpus callosum; LV = lateral ventricle; CN = caudate nucleus; V = vena thalamostriata. Scale bars = 500 μm.

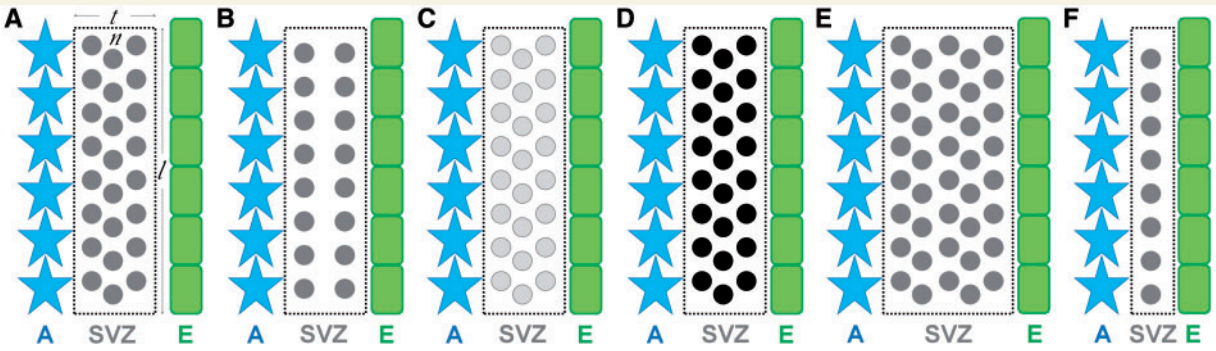

Figure 2 Quantitative evaluation of the human subventricular zone (SVZ). (**A**) The subventricular zone is separated medially by small gap from the ependymal cell layer (**E**) of the lateral ventricle. The lateral margin of the human SVZ is defined by a ribbon of specialized astrocytes (**A**). The area between **A** and **E** has a given length *l* and a variable thickness *t*, depending on the number *n* of $PCNA^+$ cells present therein. A variety of changes to the PCNA-immunoreactive area may occur, some of which are shown in **B–F**. (**B**) Reduced cell number, no change in area and staining intensity per cell. (**C**) No change in cell number and area, but reduced staining intensity per cell. (**D**) No change in cell number and area, but increased staining intensity per cell. (**E**) Increased cell number, but no change in area and staining intensity per cell. (**F**) Reduced cell number, but no change in area and staining intensity per cell. Measuring the staining intensity and calculating the percentage of SVZ area that is occupied by staining, as done by van den Berge *et al.* (2011), would only correctly depict the real changes in cell numbers in condition (**C**), whereas counts of absolute cell numbers yield an unbiased measure.

MPTP model used is known to denervate the subventricular zone, the small decrease in TH-staining intensity in the striatum suggests that either the background staining is high or the lesions may not have been complete [Fig. 5A, B and D in van den Berge *et al.* (2011)]. Without analysis of TH-positive terminals at higher magnification it is unclear whether the subventricular zone was indeed denervated. Secondly, van den Berge *et al.* (2011) use PCNA immunostaining, which is less reliable compared to the more

standard labelling with bromodeoxyuridine (BrdU). Moreover, their immunostaining procedure includes antigen retrieval, which has a chance of introducing non-specific binding sites. The lack of a negative control for the primary antibody such as purified IgG of the same species is therefore of concern. We also suggest that even if the stainings were to be acceptable, the use of the improper quantification method outlined above instead of methodologically sound unbiased stereological counting methods in the subventricular zone of mice makes the data unreliable. Changes as large as 30% can be missed without appropriate counting methodology.

The fact that van den Berge *et al.* (2011) were able to grow and differentiate subventricular zone cells from Parkinson's disease brains is not unexpected and does not really address the question being posed. No previous study has claimed that there is a total loss of these cells, just that their proliferation is influenced by the local dopaminergic innervation. Thus, it would have been interesting to study their dopamine receptor profile and response to dopaminergic treatment *in vitro*. It remains unclear why the authors used a foetal immortalized cell line and a normal foetal cell line to study the effects of dopamine [Fig. 6 in van den Berge *et al.*, (2011)], and not the neural progenitors they obtained from the patients with Parkinson's disease and controls, which would have been more appropriate to address the core hypothesis of the work. The authors also do not describe the use of ascorbic acid to stabilize dopamine against oxidative degradation during the 5-day treatment period and it remains unclear whether dopamine was supplemented over the 5-day period and if so how often. This is a critical question as the authors show in Fig. 6C that dopamine levels are down to almost half by 8 h even in the absence of cells. In addition, it therefore remains unclear how the authors expect to depict an increase in proliferation with their BrdU-incorporation assay, where already at baseline conditions 100% of the cells incorporate BrdU (Fig. 6G). Thus, the negative finding is not really surprising for a variety of methodological reasons.

In summary, we conclude that the data presented by van den Berge *et al.* (2011), while interesting, do not fundamentally require us to revise the hypothesis of a dopaminergic control of adult subventricular zone precursor cell proliferation.

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References

Arias-Carrion O, Freundlieb N, Oertel WH, Hoglinger GU. Adult neurogenesis and Parkinson's disease. CNS Neurol Disord Drug Targets 2007; 6: 326–35.

Baker SA, Baker KA, Hagg T. Dopaminergic nigrostriatal projections regulate neural precursor proliferation in the adult mouse subventricular zone. Eur J Neurosci 2004; 20: 575–9.

Bernier PJ, Vinet J, Cossette M, Parent A. Characterization of the subventricular zone of the adult human brain: evidence for the involvement of Bcl-2. Neurosci Res 2000; 37: 67–78.

Coronas V, Bantubungi K, Fombonne J, Krantic S, Schiffmann SN, Roger M. Dopamine D3 receptor stimulation promotes the proliferation of cells derived from the post-natal subventricular zone. J Neurochem 2004; 91: 1292–301.

Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. J Neurosci 1997; 17: 5046–61.

Freundlieb N, Francois C, Tande D, Oertel WH, Hirsch EC, Hoglinger GU. Dopaminergic substantia nigra neurons project topographically organized to the subventricular zone and stimulate precursor cell proliferation in aged primates. J Neurosci 2006; 26: 2321–5.

Höglinger GU, Rizk P, Muriel MP, Duyckaerts C, Oertel WH, Caille I, et al. Dopamine depletion impairs precursor cell proliferation in Parkinson disease. Nat Neurosci 2004; 7: 726–35.

O'Keeffe GC, Barker RA, Caldwell MA. Dopaminergic modulation of neurogenesis in the subventricular zone of the adult brain. Cell Cycle 2009a; 8: 2888–94.

O'Keeffe GC, Tyers P, Aarsland D, Dalley JW, Barker RA, Caldwell MA. Dopamine-induced proliferation of adult neural precursor cells in the mammalian subventricular zone is mediated through EGF. Proc. Natl Acad. Sci. USA 2009b; 106: 8754–9.

O'Sullivan SS, Johnson M, Williams DR, Revesz T, Holton JL, Lees AJ, et al. The effect of drug treatment on neurogenesis in Parkinson's disease. Mov Disord 2011; 26: 45–50.

Sanai N, Tramontin AD, Quinones-Hinojosa A, Barbaro NM, Gupta N, Kunwar S, et al. Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. Nature 2004; 427: 740–4.

van den Berge SA, van Strien ME, Korecka JA, Dijkstra AA, Sluijs JA, Kooijman L, et al. The proliferative capacity of the subventricular zone is maintained in the parkinsonian brain. Brain 2011; 134: 3249–63.

Van Kampen JM, Hagg T, Robertson HA. Induction of neurogenesis in the adult rat subventricular zone and neostriatum following dopamine D3 receptor stimulation. Euro J Neurosci 2004; 19: 2377–87.

Winner B, Geyer M, Couillard-Despres S, Aigner R, Bogdahn U, Aigner L, et al. Striatal deafferentation increases dopaminergic neurogenesis in the adult olfactory bulb. Exp Neurol 2006; 197: 113–21.

Winner B, Desplats P, Hagl C, Klucken J, Aigner R, Ploetz S, et al. Dopamine receptor activation promotes adult neurogenesis in an acute Parkinson model. Exp Neurol 2009; 219: 543–52.

Yang P, Arnold SA, Habas A, Hetman M, Hagg T. Ciliary neurotrophic factor mediates dopamine D2 receptor-induced CNS neurogenesis in adult mice. J Neurosci 2008; 28: 2231–41.