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# Response to Comment on “Human Neuroblasts Migrate to the Olfactory Bulb via a Lateral Ventricular Extension”

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In contrast to a previous study of Sanai *et al.*, our study had the advantage of using serial sagittal sections of the human basal forebrain, combined with 5-bromo-2'-deoxyuridine labeling, rigorous magnetic resonance imaging, and polymerase chain reaction analysis. We believe these methods convincingly demonstrate the presence of a rostral migratory stream in the human brain that resembles that in other mammals.

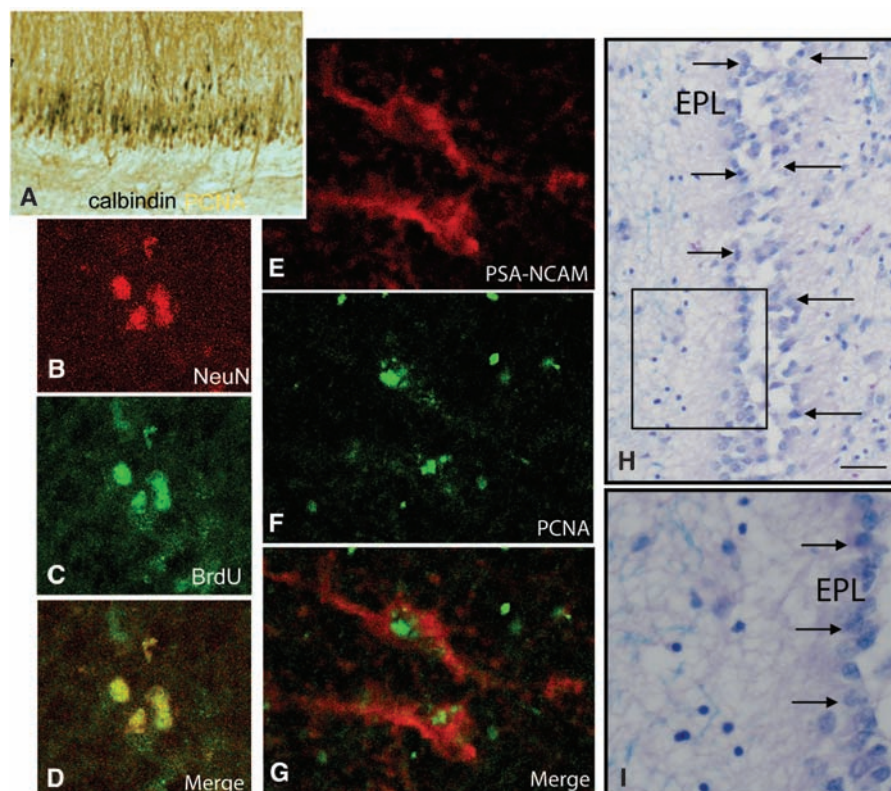
We recently identified a previously partly undefined structure in the human forebrain, which we named the ventriculo-olfactory neurogenic system (VONS) (1). We proposed the VONS to be defined as the subventricular zone (SVZ), the rostral migratory stream (RMS), and the olfactory tract and bulb. Sanai *et al.* (2) previously reported that an RMS did not exist in the human brain, but they missed vital clues in their study that pointed to the possibility of an RMS. We expand on this idea and on their questions and critiques (3) regarding our study below.

Concerning the questions of Sanai *et al.* (3) about the number of proliferating cell nuclear antigen (PCNA)-positive cells in the RMS, we have the following responses. First, not all of the RMS cells are dividing. Second, the images in figure 1 in our original study (1) were captured from 50- $\mu\text{m}$ -thick sections, so a tightly packed PCNA appearance exists. In thick sections from human hippocampus, another neurogenic region (Fig. 1A), PCNA-positive cells are also tightly packed, but clearly not all are PCNA-stained (evidenced by calbindin staining). Third, we have previously published results of the control experiments that show that PCNA detects proliferating cells (4) and have carefully evaluated PCNA and K<sub>i</sub>-67 as markers for proliferation, with similar results (5). Sanai and colleagues appear not to have used PCNA in their studies or to have experimental proof of what their cell cycle markers detected. Perhaps this is why their study did not reveal an RMS. In our RMS mapping studies, we used human brain cases that were neurologically normal

(all our cases died from heart or lung pathology), whereas Sanai *et al.* studied many cases that had brain tumors or vascular malformations that normally would have been treated with radiotherapy. It may therefore be possible that such treatment reduced the abundance of dividing cells and migrating neuroblasts in the specimens they used.

Cells positive for 5-bromo-2'-deoxyuridine (BrdU) and neuronal nuclei (NeuN) in the olfactory bulb were seen in the periglomerular layer of all examined BrdU cases. As stated in the supporting online material for (1), the cause of death in these cases was squamous cell carcinoma at the base of the tongue, and patients were not treated by radiotherapy. The ages of the patients were (B1) 57, (B2) 64, and (B3) 70 years of age, and the time between injection and mortem date was 274, 128, and 578 days, respectively. NeuN labeling was found in two forms: (i) with strong cytoplasmic labeling and (ii) with weaker nucleus-specific labeling (Fig. 1, B to D). Similar cytoplasmic labeling has previously been reported in the brains of sub-human species (6, 7).

In response to Sanai *et al.*'s comments about our histological data, we have shown the critical double-labeled cells, including PCNA/polysialylated-neural cell adhesion molecule (PSA-NCAM)-positive (Fig. 1, E to G) and PSA-NCAM/ $\beta$ III-tubulin and double cortin (DCX)-positive cells in the RMS, and these cells exist throughout the RMS. Migratory neuroblasts also



**Fig. 1.** (A) Demonstration of PCNA and calbindin staining in the human hippocampus where the PCNA-positive cells appear tightly packed but not all of the cells are PCNA positive. (B) NeuN labeling, (C) BrdU labeling, and (D) merged image of a newly born periglomerular neuron. Demonstration of (E) PSA-NCAM, (F) PCNA, and (G) the two colabeled in the same cell in the descending limb of the RMS, indicating the presence of proliferating migratory neuroblasts. (H) Demonstration of the ependymal-like lining of the VOE in the descending limb. The ependymal-like cells resemble those of the lateral ventricle and are morphologically clearly different from endothelial and smooth muscle cells. There is clear separation between the ependymal-like cells on the left and right side. Scale bar, 70  $\mu\text{m}$ . (I) Higher magnification of the ependymal-like cells of the descending limb.

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exist throughout the RMS (SVZ-OB), as evidenced in figures 3 and 4 in (1). We also note that the ultrastructure of the cells in figure 6E in (1) clearly shows that the progenitor cells have lax chromatin as compared with a neighboring cell that has dense heterochromatin, representing a mature cell. Furthermore, we showed PSA-NCAM-labeled cells using immunoelectron microscopy (PSA-NCAM is a reliable marker of migrating neuroblasts (8)).

As with PCNA, we can say confidently that  $\beta$ III-tubulin-positive (4) cells are specifically labeled and do not represent "indiscriminate labeling of white matter fibers." Sanai *et al.* suggest that chain migration is the only possible method for cell migration in the adult brain, but chain migration is a phenomenon mainly reported by Alvarez-Buylla and colleagues (9) with little evidence for it as the sole migratory mechanism in the human brain. We therefore believe that rejecting the presence of cell migration in the human brain on these narrowly defined criteria would be premature and counterproductive (9).

Sanai *et al.* claim that the VONS/RMS is identical to the region they studied in 2004 (2). However, as stated in the materials and methods section of their 2004 study, they used coronal sections of the human brain, as is clearly demonstrated by the red ring around the brain region they describe [figure 4A in (2)]. They claim that figure 4B in their study is identical to the image shown in figure 1A in (1), but their figure is in fact more akin to the first section of the descending limb seen in coronal section and is very similar to that displayed by Bernier *et al.* [figure 6A in (10)] and by Weickert *et al.* [figure 4C in (11)] where the RMS curves beneath the caudate nucleus and where the terminus of the RMS is not present. Because figure 4B in (2) is reportedly coronally cut [as are the sections in (10) and (11)], it appears quite different in shape

from what we show in sagittal section. We therefore disagree with the authors' retrospective interpretation of this structure where the anatomy is wholly unlabeled/unnamed and resembles that of previous studies (10, 11). Hence, we are confident that we have provided the first definitive description and illustration of the RMS in the human brain. However, what is critical to us is the demonstration of an equivalent migratory stream in the human brain as is seen in the rodent brain. To that end, we reexamined the ventral olfactory extension (VOE) in serial sagittal 7- $\mu$ m-thick paraffin sections from two additional normal human brains and, as before, we can confirm the presence of a residual ventricular extension with an ependymal-like lining of cells in the VONS (see Fig. 1, H and I). This structure is a consistent finding; however, it has varying patency in the adult postmortem brain. Although the anatomical structure is in place with an attenuated central cavity, determining its physiological patency in the human brain will be difficult.

Finally, Sanai *et al.* (3) claim our findings on magnetic resonance imaging (MRI) to be misleading because two previous MRI (12, 13) studies failed to show the same result in the olfactory bulb. Results from the analyses of both normal and hydrocephalic patients were included in our study with identical results concerning the imaging of the olfactory tract and bulb. Neither of the studies referenced by Sanai *et al.* (3) used even remotely similar spin-echo sequences needed to detect small volumes of fluid, and therefore it would have been quite remarkable and unfair to expect those studies to have revealed a fluid-filled olfactory ventricle using inappropriate MRI parameters. The figures shown in (12) and (13) do not even show fluid accentuation in the vitreous humor. Therefore it remains unclear to us how these investigators could be expected to

detect fluid (12–14). As a positive control, we have also been able to detect fluid in the central canal of the spinal cord using this technique. We have published our MRI parameters and methodology, and we welcome others to further validate these findings.

Although caution must always be exercised when performing human brain studies, it is also important to use techniques appropriate to the question being asked. Because the RMS exists in rodents and nonhuman primates, one must go to exhaustive lengths before concluding that a comparable pathway does not exist in the human brain. Furthermore, the presence of a human RMS exemplifies the presence of common biological processes across mammals and, in particular, demonstrates that rodent neurogenesis studies have relevance to humans.

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