

culture to be used for sequential expansion. The combined microglial yield from four separate passages yields an average of  $8 \times 10^6$  cells, a 10,000-fold expansion from the initial microglial population.

Fig. 6. Comparative analysis of microglial expansion from different regions of the neonatal brain. Neonatal brains were harvested from postnatal day 3 mice, and their brains dissected as described in panel A (superior view), with dashed lines indicating incisions. The cerebellum was removed and cultured, while the forebrain region was further dissected so as to separate the cortex from the SVZ (coronal view). Panel B) Microglial yields from passage 1, 2, and 3 cultures (4 agitation isolations per passage) were combined and are presented as fold expansion as compared to the number of microglia present at passage 1 prior to induction of microglial proliferation.

Fig 7. Adult SVZ cultures are capable of producing isolatable microglia in the presence of MPM. 4 hours after attachment to treated coverslips and subsequent immunostaining all attached cell types were found to be positive for CD11b (A) and CD45 (B), with both ramified and amoeboid cell types present. Blue in both panels is DAPI. Scale bars in both panels =  $50 \mu\text{m}$ .

Supplementary Figure 1: Analysis of microglia in established SVZ cultures. Microglia exist in confluent SVZ cultures grown in NGM prior to the addition of MPM. DIC imaging of one such monolayer (A) reveals cells with an amoeboid morphology present on the surface of the established monolayer. Immunostaining of the same culture for the microglia marker CD45 reveals that the amoeboid cells are microglia (Panel B: DIC/fluorescent overlay. CD45/green; DAPI/blue). Scale bars in both panels =  $50 \mu\text{m}$ .

Supplementary Figure 2: Isolated microglia from MPM-induced SVZ cultures are CD11b positive. Following 24 hours of attachment and subsequent immunostaining attached cells are positive for CD11b, with both ramified and amoeboid cell types present (CD11b/red, DAPI/blue). Scale bar =  $50 \mu\text{m}$ .