Supplemental Data

Recently, expression of GFP with N-terminal palmitoylation site of GAP-43 was reported to induce filopodia formation and branching of the dendrites in cultured neurons (Gauthier-Campbell, 2002). However, we found no obvious increase in the number of dendritic branches palGFP-expressing SNc neurons when we compared them with GFP-expressing neurons or SNc neurons reported in rat brain (Juraska et al., 1977; Preston et al., 1981; Tepper et al., 1987), although observation of the proximal dendrites were interfered with the extracellularly leaked palGFP. Furthermore, almost no thin filopodia-like processes were observed on palGFP-labeled dendritic processes.

Next, to examine the effect of palGFP expression on axonal morphology, we carefully compared nigrostriatal axon fibers labeled with palGFP and those with GFP. No obvious differences were found between axon fibers labeled with palGFP and those with GFP in the striatum (Fig. 1E, F) or other brain regions except that the fine detail of palGFP-labeled fibers was much clearer than those with GFP-labeled ones. Neither growth cone-like structures nor filopodia-like processes were observed on palGFP-labeled axons in the striatum. Furthermore, palGFP- and GFP-labeled axon fibers of SNc dopamine neurons were compared in width and inter-branch interval of intrastratal axon fibers. These data were measured with Neurolucida apparatus (MBF Bioscience, Williston, VT) attached to microscope Vanox (Olympus, Tokyo, Japan). The width of a randomly selected axon fiber was determined with 100x objective lens (SP PlanApo, oil immersion, NA = 1.35; Olympus) as the mean value of widths at 3 to 5 points on the fiber outside of the varicosities. Randomly selected axon fibers were traced three-dimensionally under the Neurolucida-attached microscope with 20x objective lens, and their inter-branch interval was measured between neighboring branch points. The fiber width and inter-branch interval of 80 palGFP-labeled intrastratal axon fibers, which were the summation of 10 fibers each selected randomly from eight nigrostriatal dopamine neurons listed in Table 1, were $0.83 \pm 0.13 \mu m$ (mean $\pm$ S.D.) and $30.9 \pm 21.6 \mu m$, respectively, and those of 80 randomly selected GFP-labeled fibers were $0.82 \pm 0.11 \mu m$ and $31.2 \pm 19.4 \mu m$, respectively. The differences were not statistically significant ($p = 0.72$ for fiber width and 0.92 for inter-branch interval; two-sided $t$-test). We, therefore, concluded that palGFP expression did not have an obvious effect on axonal
morphology at least in nigrostriatal dopamine neurons of adult brain.

References

