Supporting Information

Order of Excitation:

In two-photon excitation, the fluorescence counts have a quadratic dependence on the excitation power. For a 2 mM aqueous dopamine solution, the logarithm of the fluorescence vs. the logarithm of the excitation intensity can be fit well by a straight line with a slope of 1.95 ± 0.03 (first eight points, Fig. S1). This shows that our optical scheme causes two-photon excitation of dopamine. The deviation from linearity at higher excitation powers denotes saturation. The saturation power is estimated to be around 25 mW at the back aperture of the objective.

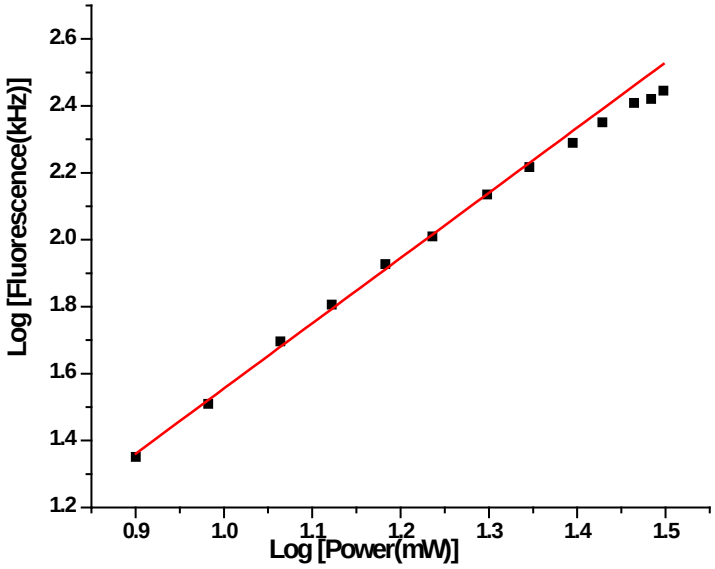

Figure S1 : **Fluorescence signal as a function of excitation laser power.**

The logarithm of fluorescence counts (kHz) obtained from 2 mM dopamine solution plotted as a function of the logarithm of excitation laser power (mW) (squares). The first eight points are fit to a straight line (red).

Sensitivity of detection:

To find the detection sensitivity of our method we performed two-photon detection of dopamine solution with gradual dilution. Fig. S2 shows the fluorescence signal for 0.05 mM to 0.8 mM dopamine (detected with 15 mW laser power at the back aperture of the objective), which yields a slope of 6.7 ± 0.2. Experiment at higher concentrations (0.4 mM- 25 mM, Fig. S2 inset) yields similar slope of 6.8± 0.3. We find that dopamine can be reliably detected above background at concentrations ≥100 μM. However, with cellular background, the effective detection limit will be higher.

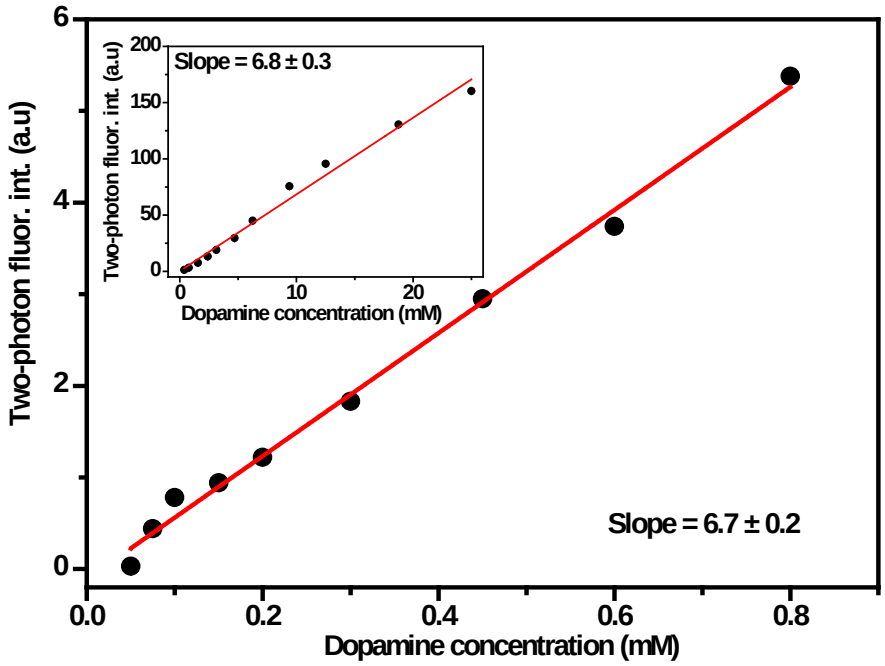

Figure S2: **Sensitivity of dopamine detection.**

*Two-photon fluorescence intensity of dopamine solution detected as a function of concentration. The slope for 0.05-0.8 mM dopamine solution (*6.7 ± 0.2*) is similar to that at higher concentrations (0.4-25 mM, slope: 6.8*± 0.3*), presented in the inset.*

Absorption and emission spectra of dopamine and related UV-fluorophores:

Tyrosine, phenylalanine and tryptophan, abundant in proteins, have considerable absorption at 260-280 nm, hence will also get two-photon excited at 540 nm. Also these molecules are mid-UV (tyrosine, phenylalanine) or near-UV (tryptophan) fluorescent, hence may get detected by our method. Absorption and emission spectra of these UV-fluorophores along with dopamine (100 μM solutions in water) are presented in Fig. S3. Fluorescence emission was recorded with 270 nm excitation in a fluorimeter (Fluoromax 3, Horiba Scientific, Japan). However, these molecules are not packed into small spot-like objects at high concentrations. Dopamine is packed at 100's of mM concentrations in vesicles, and can therefore be imaged over the background of these fluorophores.

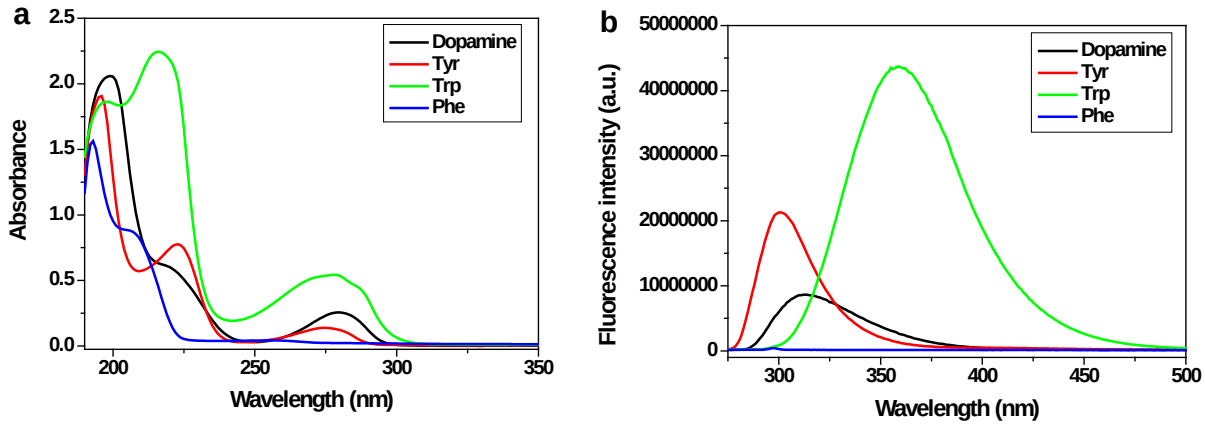

Figure S3: **Absorption and emission spectra of UV-fluorophores.**

(a) Absorption and (b) fluorescence emission spectra of 100 μM solutions of dopamine (black), tyrosine (red), tryptophan (green), phenylalanine (blue) in water.

Estimation of cellular dopamine content by mass spectrometry:

We analyze the dopamine content of MN9D (or Glia) cells using a HPLC coupled mass spectrometer as described in the manuscript. Fig. S4a shows a representative elution profile corresponding to dopamine (protonated mass of 154, upper panel, black) and isotopically labeled dopamine (protonated mass of 157, lower panel, red) from MN9D cell extract. Both dopamine and isotopically labeled dopamine co-elute from the cell extracts (22.5-24.5 min of elution, shaded gray in Fig. S4a). Figure S4b shows example mass spectra for the cell extracts of MN9D (upper panel, red), untreated glia (middle panel, blue) and glia incubated with 10mM dopamine for 4 hr (bottom panel, black). The mass spectrum shows a clear peak at a mass of 154 (expected mass of protonated dopamine, Fig. S4b, red, upper panel), while this mass peak is negligible in the glia (Fig. S4b, blue, middle panel). The peak increases upon incubation with dopamine (Fig. S4b, black, bottom panel). MN9D cells are estimated to contain a substantial amount of dopamine (386 ± 18 ng/mg of the dry mass of the cells), but this amount is significantly lower in the glia (26 ± 13 ng/mg) ($p < 0.001$). The dopamine content in glia incubated with dopamine (170 ± 71 ng/mg) is significantly higher than untreated glia ($p<0.01$).

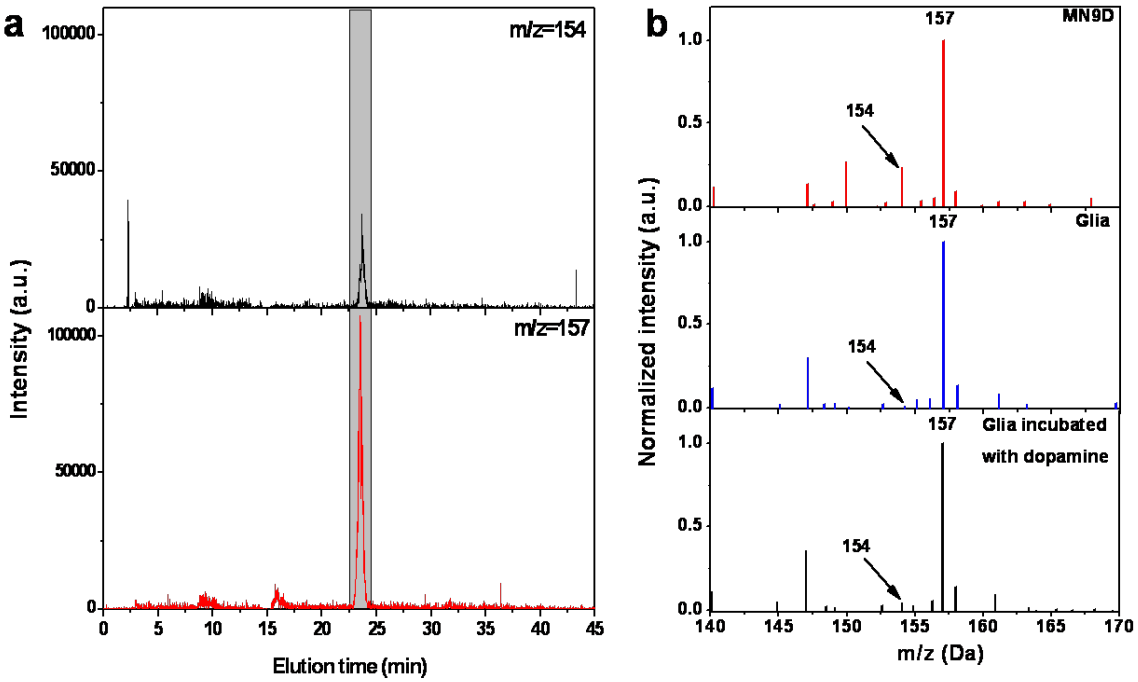

Figure S4: **Mass spectroscopic analysis of intracellular dopamine.**

(a) *Representative elution profile of dopamine (protonated, m/z= 154, upper panel, black) and isotopically labeled dopamine (protonated, m/z= 157, lower panel, red) in MN9D cell extract. The gray shaded region corresponds to 22.5-24.5 min of elution.* ***(b)*** *Example mass spectra*

obtained from the cell extracts of MN9D (upper panel, red), glia (middle panel, blue) and glia incubated with dopamine for 4 hr (bottom panel, black). The protonated masses of dopamine (154) and isotopically labeled dopamine (157) are marked. All the spectra are normalized to 1 for m/z= 157.

Dopaminergic neurons are primarily localized in SN:

We compare the images taken from the SN with an arbitrarily chosen region ~2 mm away from it on the dorsal side. Neurons with bright vesicular structures are clearly visible in the SN (Fig. S5a). In comparison, images recorded away from the SN in the same brain section (Fig. S5b) contain very few bright structures, indicating that cells containing bright vesicles are mostly localized in the SN region, as would be expected if the signal arises from dopamine.

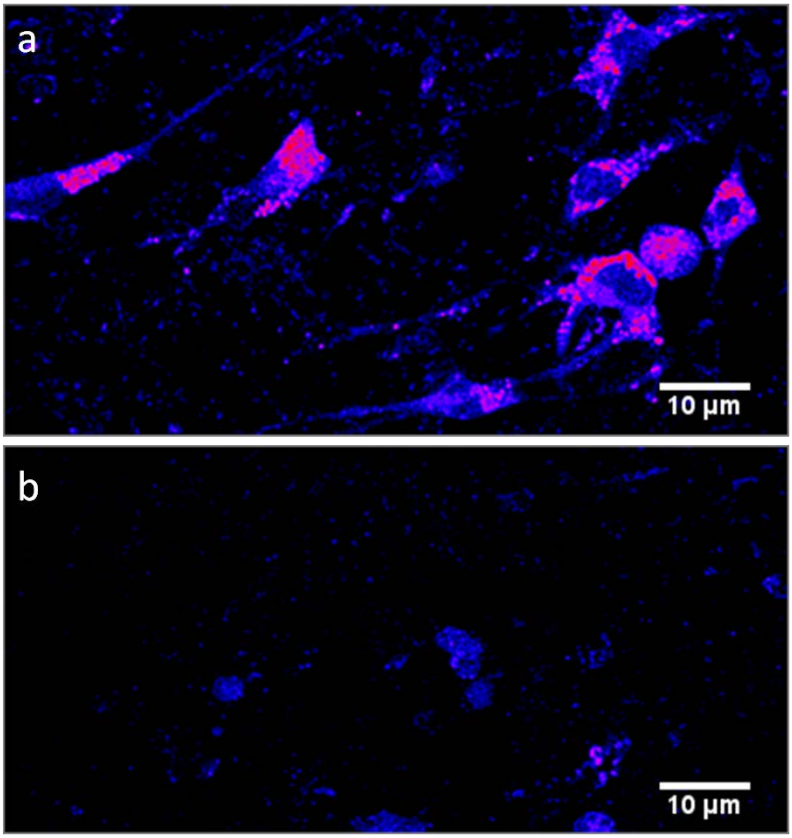

Figure S5: Dopaminergic neurons are primarily localized in SN

(a) *High-resolution two-photon image of a region of the SN with many bright neurons containing dopamine vesicles.* ***(b)*** *Control region ~2mm away from the SN, where the neurons appear mostly dark.*

Three-dimensional dopamine imaging through thick tissue:

High resolution two-photon dopamine imaging can be performed in brain tissue at depth of at least 25 µm. Fig. S6 shows the montage of Z-sections of the SN of a live mid-brain section (25 µm) recorded every 2 µm. Numerous neurons with distinct punctate vesicles/vesicle clusters appear at various depths, thus revealing the 3D arrangement of the dopaminergic neurons. This demonstrates the ability of our technique to non-invasively image the mid-ultraviolet fluorescence of dopaminergic neurons with high three-dimensional resolution several cell layers inside live brain tissue.

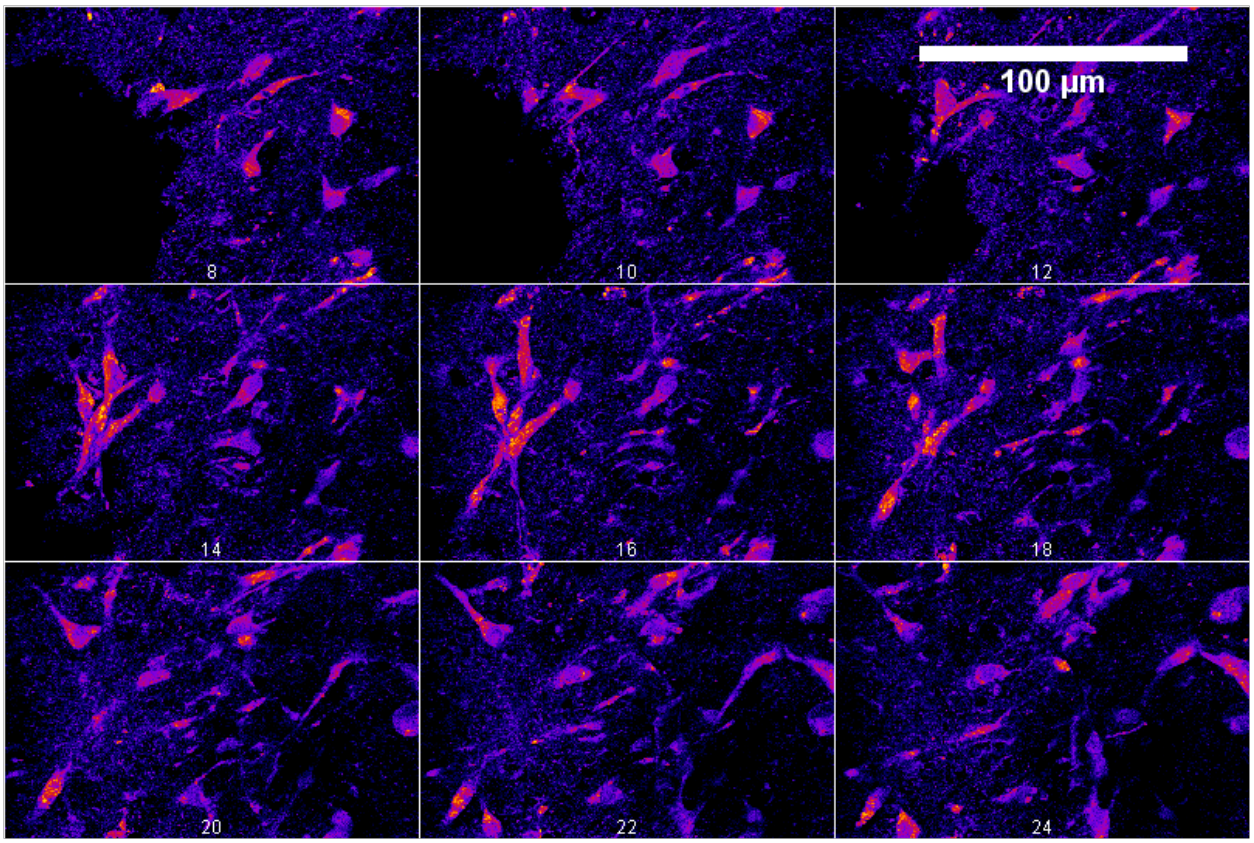

Figure S6: Dopamine imaging through thick tissue

Montage of high-resolution two-photon images of SN in a live brain tissue at 2 µm depth interval. The depth of the image is indicated in each panel. Dopamine could be successfully imaged at depths of at least ~ 25 µm.