

Fig.S1

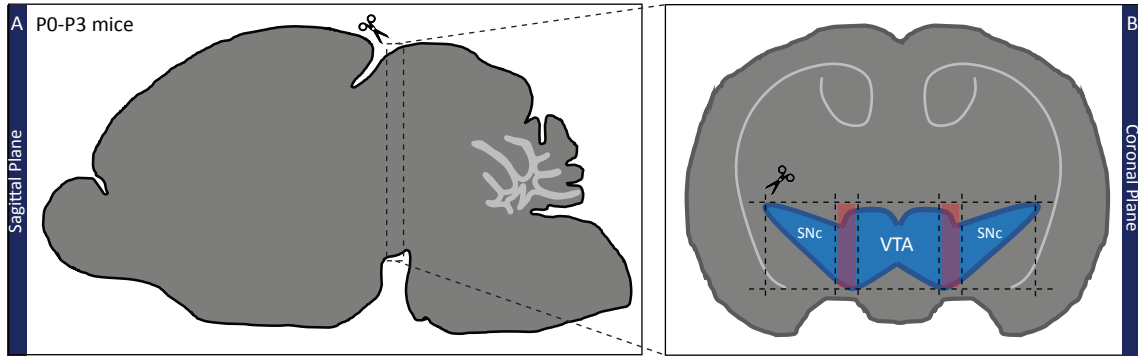


Fig. S2

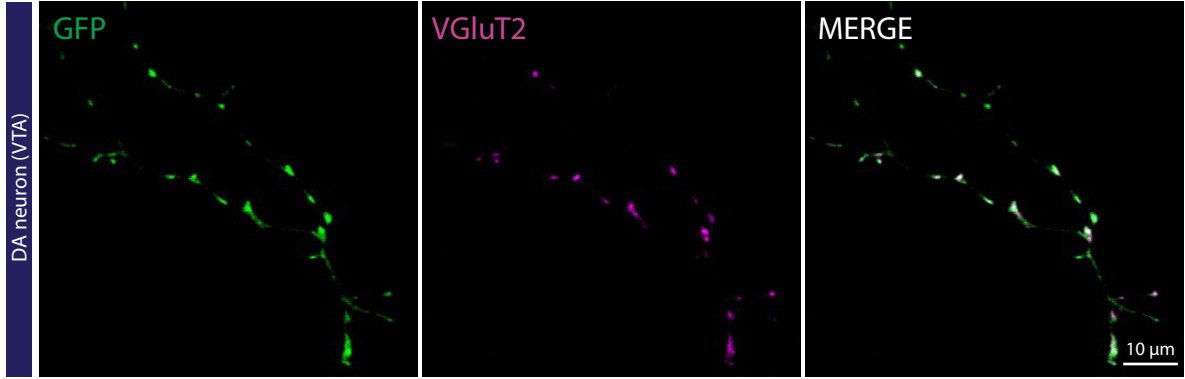


Fig. S3

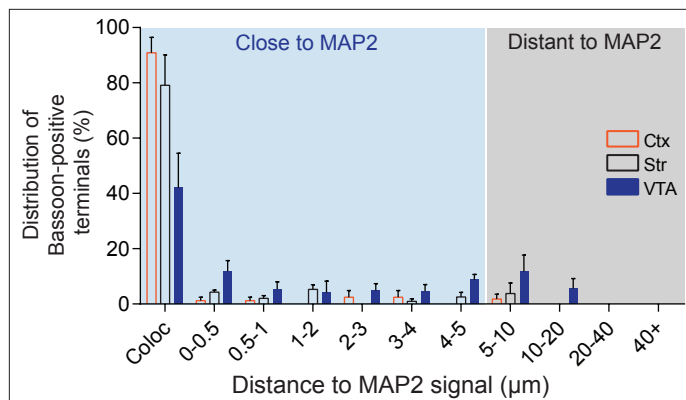


Fig. S4

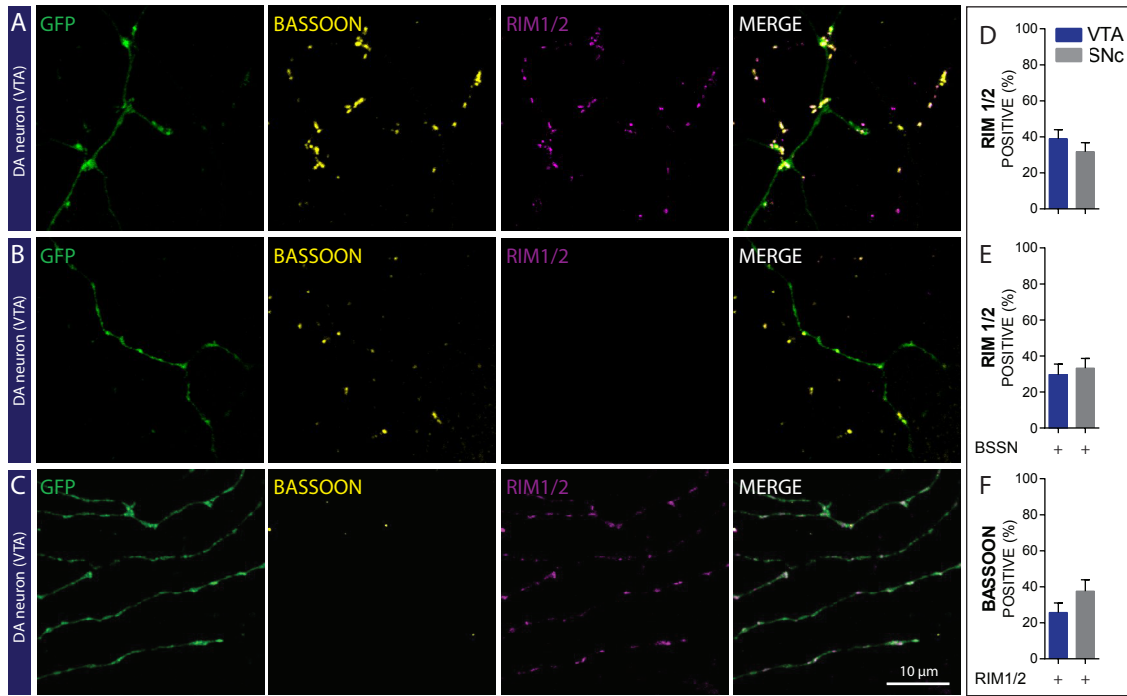


Fig. S5

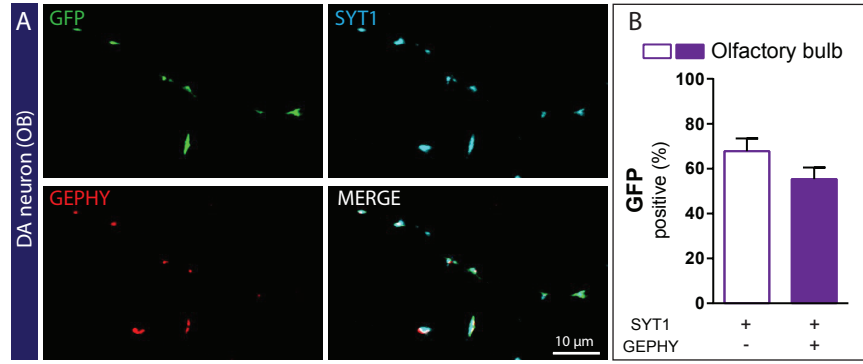
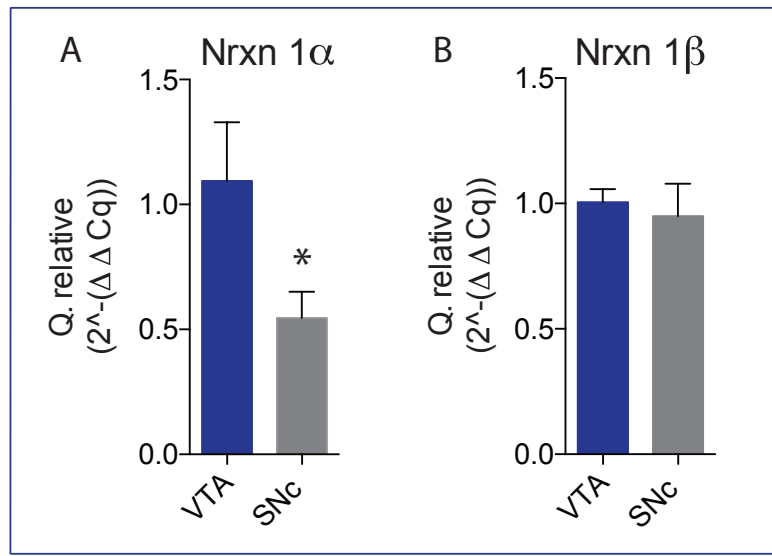


Fig.S6



1 **SUPPLEMENTAL FIGURE LEGENDS**

2

3 **Supplemental Fig. S1 - Schematic representation of the forebrain and midbrain dissection. A-**

4 The forebrain was first isolated by dissection along the dashed line. **B-** Schematic representation
5 of a coronal view of the dissected forebrain. The dashed lines represent the parts used to prepare
6 the primary cultures of VTA and SNc DA neurons. The red squares represent parts that were
7 excluded.

8

9 **Supplemental Fig. S2 – DA neurons express VGlut2 at some of the axon terminals.**

10 Photomicrographs illustrating GFP positives axonal varicosities along the axonal arborization of a
11 DA neuron expressing VGlut2.

12

13 **Supplemental Fig. S3 – Bassoon distribution along the axonal arbor of DA neurons compared to**

14 **glutamatergic and GABAergic neurons.** Histograms representing the mean +/- SEM from 3
15 different fields from 3 different neurons for each structure (VTA, cortex (Ctx) and striatum (Str)).
16 Each histogram includes 137 terminals from cortical neurons, 134 terminals from striatal neurons
17 and 612 terminals from VTA DA neurons.

18

19 **Supplemental Fig. S4 – The active zone proteins RIM 1/2 and bassoon are partially segregated**

20 **in distinct sets of DA terminals. A-** Photomicrographs illustrating GFP positives axonal varicosities
21 established by VTA DA neurons expressing bassoon and co-expressing RIM1/2. **B-**
22 Photomicrographs illustrating GFP positives axonal varicosities established by VTA DA neurons

23 expressing bassoon but not RIM1/2. **C-** Photomicrographs illustrating GFP positives axonal
24 varicosities established by VTA DA neuron expressing RIM1/2 but not bassoon. **D-** Bar graph
25 representing the proportion (%) of VTA and SNc GFP positive axonal varicosities expressing
26 RIM1/2. **E-** Bar graph representing the proportion (%) of DAergic GFP positive axonal varicosities
27 positive for bassoon and colocalizing with RIM1/2. **F-** Bar graph representing the proportion (%)
28 of DAergic GFP positive axonal varicosities positive for RIM1/2 and colocalizing with bassoon. The
29 bar graphs represent the mean + SEM. VTA n=28 SNc n=30; from 2 different cultures. The number
30 of observations represents the number of fields from individual neurons examined

31

32 **Supplemental Fig. S5 – Olfactory Bulb DA neurons establish terminals that are mainly synaptic.**

33 **A** – Photomicrographs illustrating GFP positives varicosities along the axonal arborization of an
34 olfactory bulb DA neuron expressing Syt1 and colocalizing with gephyrin. **B** – Bar graph
35 representing the proportion (mean +/- SEM) of GFP/Syt1 positive axonal varicosities colocalizing
36 with gephyrin (purple) or not colocalizing with gephyrin (white), established by olfactory bulb DA
37 neurons. VTA n=27 SNc n=27; from 3 different cultures. The number of observations represents
38 the number of fields from individual neurons examined.

39

40 **Supplemental Fig. S6 - Nr1x1a and Nr1x1b genes are expressed in DA neurons.** Relative

41 quantification by qPCR of mRNA encoding for Nr1x1a (**A**) and Nr1x1b (**B**), for both VTA and SNc

42 DA neurons.