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RESEARCH ARTICLE

Topography and collateralization of dopaminergic projections to primary motor cortex in rats

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Abstract Dopaminergic signaling within the primary motor cortex (M1) is necessary for successful motor skill learning. Dopaminergic neurons projecting to M1 are located in the ventral tegmental area (VTA, nucleus A10) of the midbrain. It is unknown which behavioral correlates are encoded by these neurons. The objective here is to investigate whether VTA-M1 fibers are collaterals of projections to prefrontal cortex (PFC) or nucleus accumbens (NAc) or if they form a distinct pathway. In rats, multiple-site retrograde fluorescent tracers were injected into M1, PFC and the core region of the NAc and VTA sections investigated for concomitant labeling of different tracers. Dopaminergic neurons projecting to M1, PFC and NAc were found in nucleus A10 and to a lesser degree in the medial nucleus A9. Neurons show high target specificity, minimal collateral branching to other than their target area and hardly cross the midline. Whereas PFC- and NAc-projecting neurons are indistinguishably intermingled within the ventral portion of dopaminergic nuclei in middle and caudal midbrain, M1-projecting neurons are only located within the dorsal part of the rostral midbrain. Within M1, the forelimb representation receives sevenfold more dopaminergic projections than the hindlimb representation. This strong rostro-caudal gradient as well as the topographical preference to dorsal structures suggest that projections to M1 emerged late in the development of the dopaminergic systems in and form a functionally distinct system.

Keywords Motor cortex · Dopamine · VTA · Rat

Introduction

Apart from the well-characterized connection between substantia nigra and dorsal striatum, dopaminergic neurons within the midbrain also innervate limbic and neocortical structures (Fallon 1981; Swanson 1982). By providing information about the individual value and significance of environmental stimuli, these dopaminergic projections profoundly influence behavior and modulate higher brain functions (Arias-Carrion et al. 2010; Bromberg-Martin et al. 2010). Between species, pronounced differences in magnitude and complexity of cortical dopaminergic innervation have to be taken into account. Presumably as a consequence of neocortical development, dopaminergic innervation of cortex is more widespread in humans and non-human primates when compared to rodents (Berger et al. 1991), emphasizing the importance of dopaminergic signaling for higher cognitive processes.

Beside the well-defined dopaminergic projections to prefrontal cortex (PFC) and limbic structures (e.g., nucleus accumbens or amygdala), the primary motor cortex (M1) also harbors dopaminergic terminals (for review see (Luft and Schwarz 2009). Recently, the dopaminergic connection between midbrain and M1 has been linked to a function, as

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the integrity of this pathway is a necessity for successful motor learning in rats (Hosp et al. 2011).

The objective here is to characterize the recently described dopaminergic midbrain to M1 connection with respect to topography of originating neurons, collateralization and homogeneity of innervation within M1. Thus, retrograde fluorescent tracers were injected into multiple sites including M1, PFC and NAc—two well-defined target regions of the meso-cortico-limbic system—in combination with immunohistochemistry against tyrosine hydroxylase (TH), a marker enzyme for dopaminergic neurons. We show that apart from similarities regarding high target specificity and distribution among midbrain nuclei, M1-projecting neurons can be distinguished from those innervating PFC and NAc due to their unique topographical features.

Materials and methods

Animals and experiments

Adult male Long-Evan rats ($n = 11$, 8–10 weeks, 250–350 g, Centre d'Elevage R. Janvier, Le Genest-St. Isle, France) were used for all experiments. Animals were housed individually in a 12/12-h light/dark cycle (light on: 8 p.m., off: 8 a.m.). After surgery, animals were returned to their home cages and killed after a period of 7 days. All experiments were conducted in accordance with Swiss regulations and were approved by the Committee for Animal Experimentation of the Canton of Zürich (license number 207/2008, section 2g).

Surgical procedures and tracer injection

Surgical procedures and the tracer injection were described previously (Hosp et al. 2011). In brief, a craniotomy over the right hemisphere was performed (coordinates for the motor cortex with respect to bregma; 3 mm posterior, 6 mm anterior, 5 mm lateral, 0.5 mm medial) under ketamine (75 mg/kg, i.p.) and xylazine anesthesia (10 mg/kg, i.p.) with the rats fixated in a computer-controlled stereotaxic instrument (Dual Benchmark Angle One; Harvard Apparatus). Additional ketamine doses (30 mg/kg, i.p.) were administered if necessary. Body temperature was controlled using a heating pad. Buprenorphin (0.01 mg/kg, i.p.) was given after surgery for pain relief. After craniotomy, the dura was gently removed and tracer injections were performed using a digitally controlled microliter injection pump (Ultramicropump 3, World Precision Instruments, Berlin, Germany). Stereotactic coordinates were defined according to Paxinos and Watson (1997). All rats ($n = 11$) received 200-nl injections of the retrograde fluorescent tracer Fast Blue (FB; EMS-Polyloyp, Gross-Umstadt, Germany; 1 % suspension in 0.1 M PB and 2 % DMSO; injection-speed: 100 nl/s; cannula: 26 ga) at five adjacent injection positions around the center of M1 forelimb representation (depth: 900 μm; coordinates with respect to bregma: 2.5 mm lateral, 2.5 mm). After each injection, the needle was left in place for 5 min before being slowly retracted. Subsequently, 200 nl of the second retrograde fluorescent tracer Micro Ruby (MR; 3,000 kD dextran; Sigma-Aldrich Chemie GmbH, Buchs, Switzerland; 10 % suspension in aqua dest; injection speed: 5 nl/s; cannula: 34 ga) were injected into the center of either (1) *M1 hindlimb representation* (M1-FL/HL group): $n = 4$ rats, 5 depots, depth: 900 μm, coordinates with respect to bregma: 1.5 mm lateral, 1.5 mm posterior; (2) *Prefrontal cortex* (M1-FL/PFC group): $n = 3$ rats, 5 depots, depth: 1.8 mm, coordinates with respect to bregma: 0.7 mm lateral, 3–4 mm anterior. It has to be admitted that the existence of a prefrontal cortex in rodents that is homologous to the dorsolateral prefrontal cortex of higher mammals is a matter of debate: Rose and Woolsey proposed the idea that PFC is unique to primate species due to the presence of projections from the medio-dorsal thalamic nucleus (Rose and Woolsey 1948). However, there is anatomical and functional evidence that PFC also exists in rats, even though it is less differentiated when compared to primates (Uylings et al. 2003). The coordinates used for this study target the prelimbic cortex (PL), a structure that is considered to be an integral part of the PFC in rodents (Leonard 1969; Preuss 1995). (3) *core of Nucleus accumbens* (M1-FL/NAc group): $n = 4$ rats, 3 depots, depth: 7.1 mm, coordinates with respect to bregma: 1.8 mm lateral, 2.0 mm anterior. The location of different injection sites is indicted schematically in Supplementary Fig. 1. After surgery, bone flaps were replaced and fixated using bone cement (FlowLine, Heraus Kulzer, Dormagen, Germany).

During the preparation of brain slices with the cryotome, the placement of tracer deposits could be visually identified. For FB-injections (26 ga cannula), a placement defect could be seen macroscopically on the cortical surface. For injections of MR (34 ga cannula), the tracks of the needle were too fine to allow a visual detection. Thus, brain slices (50 μm) containing tracer depots were sampled and subsequently embedded. The precision of tracer placement was then confirmed microscopically using a low magnification objective (5×/0.5 EC Plan-Neofluar objective, Zeiss AG, Jena, Germany; Supplementary figure 2).

Immunohistochemical procedures and data analysis

Seven days after tracer injection, animals were deeply sedated (pentobarbital, 50 mg/kg i.p.) and perfused transcardially with 4 % paraformaldehyde (PFA). Brains were quickly removed and kept in 4 % PFA for 24 h, transferred to 30 % sucrose solution for 3–4 days before rapid freezing in 2-methyl butan. Coronal sections (50 μm)

5–7 mm posterior to bregma containing midbrain dopaminergic regions were prepared using a cryostat (Leica Microsystems GmbH, Wetzlar, Germany). Free floating sections were rinsed three times in 0.05 M tris-buffered saline (TBS), treated with 3 % H_2O_2 for 30 min, washed three times in 0.05 M TBS, then rinsed in 0.1 % Triton for 10 min, and blocked for 30 min in 10 % fetal cow serum. The monoclonal mouse anti-tyrosine hydroxylase was obtained commercially (Chemicon International, Temecula, USA, cat. MAB 318). According to the manufacturer's information, the antibody recognizes an epitope on the outside of the regulatory N-terminus of the protein. Furthermore, there is no cross-reactivity with other members of aromatic amino hydroxylases such as dopamine-beta-hydroxylase, phenylalanine hydroxylase or tryptophan hydroxylase. This primary antibody was previously used for detection of TH-positive neurons within the rats' midbrain (Hosp et al. 2011). There, topographical distribution and number of these neurons were very similar to earlier reports (German and Manaye 1993). Sections were incubated with primary anti-TH antibody (TH; 1:200 diluted in 0.05 M TBS and 5 % fetal cow serum) for 24 h at 4 °C under agitation, then washed three times in 0.05 M TBS and subsequently incubated with a goat anti-mouse FITC-coupled secondary antibody (1:200 diluted in 0.05 M TBS and 2.5 % fetal cow serum; Zymed Laboratories, San Francisco, CA, USA) at 4 °C for 90 min. Sections were mounted with Vectashield (Vector Laboratories Inc., Burlingame, USA) and analyzed using a fluorescent microscope (Axioplan II, Zeiss AG, Jena, Germany; equipped with a motorized x–y stage; 20×/0.5 EC Plan-Neofluar objective).

As TH is the rate-limiting enzyme not only for the synthesis of DA but also for epinephrine and norepinephrine, TH-positivity labels dopaminergic, adrenergic and noradrenergic neurons. However, the latter two types may be negligible as global TH positivity is not affected by specifically destroying epinephrinergic and norepinephrinergic neurons (Descarries et al. 1987), i.e., the vast majority of TH-containing neurons are dopaminergic. Because TH was confirmed to be the most reliable marker for dopaminergic neurons in the rodents' midbrain (Margolis et al. 2010), dopaminergic midbrain nuclei were identified based on TH positivity. The nomenclature of dopaminergic structures was adopted from Dahlstrom and Fuxe (1964) and German and Manaye (1993): nucleus A8 contains the retrorubral field (RRF), nucleus A9 contains the substantia nigra pars compacta (SNC) and pars reticulata (SNR). Nucleus A10 finally contains the ventral tegmental area (VTA: including nucleus paranigralis, parabrachial pigmented nucleus and rostral linear nucleus raphe), the central linear nucleus (CLi) and the interfascicular nucleus (IF).

For analysis, every fifth section was taken into account. Thus, sections were equally spaced (fixed distance of 200 μm between subsequent slices) with a randomly chosen starting point. Thus, a total of eight midbrain slices were prepared per animal. Initially, the contour of each slice and the borders of dopaminergic nuclei were traced using the Neurolucida software (Version 8.21.6, MicroBrightField Inc., Williston, VT, USA). Subsequently, the entire slice was scanned step-by-step for FB or MR-positive neurons. FB-, MR- and FB + MR-labeled neurons were counter-checked for TH positivity (for illustration see Fig. 1a–c) and marked within the reconstructed midbrain slice. The number of differentially labeled neurons per anatomical region was displayed using NeuroExplorer version 4.7 (MicroBrightField Inc., Williston, VT, USA) and extrapolated for the entire structure. Graphs were created using Prism version 5.0 (GraphPad Inc., San Diego, CA, USA). Numerical results are expressed as mean and SEM.

Results

Dopaminergic projections from midbrain to M1 forelimb representation

Seventeen percent of the neurons projecting from the midbrain to the M1 forelimb representation were also positive for TH (total number of FB-positive neurons: 3,122 ± 579; range: from 1,220 to 7,925. Number of neurons double-labeled for FB and TH: 537 ± 74; range: from 427 to 883; $n = 11$ animals). The vast majority of these double-labeled neurons was located ipsilateral to the injected side (ipsilateral: 522 ± 73 neurons = 97 %; contralateral: 15 ± 3 neurons = 3 %; $n = 11$ rats; Fig. 2). Seventy-nine percent of TH/FB double-labeled neurons were located in the ipsilateral nucleus A9 (VTA: 378 ± 49 neurons = 70 %; CLi: 45 ± 8 neurons = 8 %; IF: 4 ± 1 neurons = 1 %), 18 % of TH/FB double-labeled neurons are located within the ipsilateral nucleus A10 (SNC: 80 ± 19 neurons = 15 %; SNR: 16 ± 9 neurons = 3 %), only a negligible number of double-labeled neurons are present within the ipsilateral nucleus A8 (RRF: 1 ± 0.5 neurons). The largest amount of double-labeled neurons was observed in the rostral midbrain (61 % from −5 to −6 mm with respect to bregma). Their density decreased in rostro-caudal direction (Fig. 3a). Double-labeled neurons were also more frequently found laterally in rostral sections (lateral VTA and medial SNC) and medially in caudal sections (medial VTA and CLi; Fig. 2).

Dopaminergic projections from midbrain to M1 hindlimb representation

In contrast to the M1 forelimb representation, only 2 % of the neurons projecting to M1 hindlimb showed immunoreactivity against TH. These neurons were also positive for

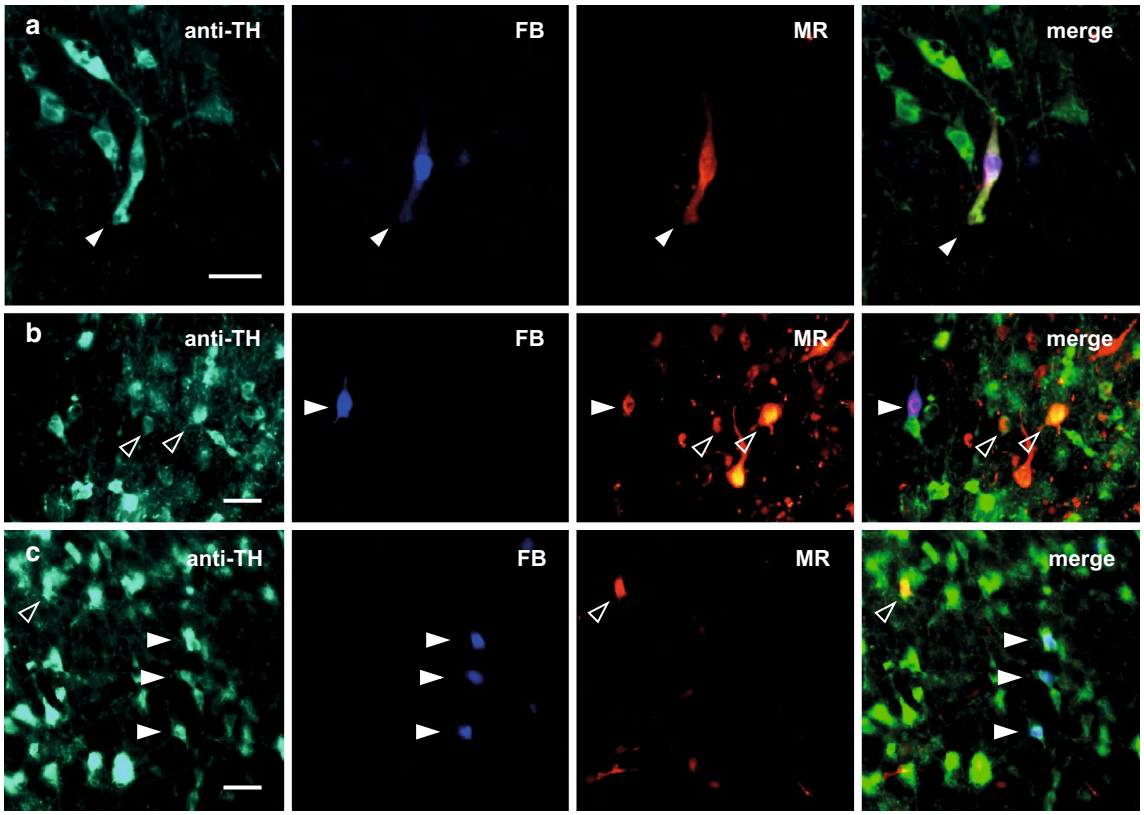

Fig. 1 Identification of retrograde traced dopaminergic neurons in VTA. **a** *White arrowheads* indicate a triple-labeled neuron, positive for anti-tyrosine hydroxylase (anti-TH), Fast Blue (FB) and Micro Ruby (MR). Image derived from ID 3329, M1-FL/NAc group. For **a**–**c**: images were taken with a light microscope (×20 magnification), *scale bars* 30 μm. **b** *White arrowheads* indicate double-labeled neurons positive for anti-TH and MR. *Open arrowheads* indicate a double-labeled neuron positive for FB and MR. Image derived from ID 3332, M1-Fl/NAc group. **c** *White arrowheads* indicates a double-labeled neuron positive for anti-TH and MR. *Open arrowheads* indicate double-labeled neurons positive for anti-TH and FB. Image derived from ID 3278, M1-FL/PFC group

FB—the tracer injected into the forelimb representation—indicating a projection to both fore- and hindlimb representation of M1 from the same VTA neuron (total number of MR-positive neurons: 565 ± 60; range: from 437 to 693. Number of neurons triple-labeled for MR, TH and FB: 9 ± 3; range: from 6 to 14; $n = 4$ animals). Forty-two percent of the neurons projecting to the M1 hindlimb representation were FB-positive. These findings suggest substantial collateralization from TH-negative and TH-positive midbrain neurons between representations in M1 (total number of MR-positive neurons: 565 ± 60; range: from 437 to 693. Number of neurons double-labeled for MR and FB: 234 ± 33; range: from 187 to 299; $n = 4$ animals; Fig. 4).

Dopaminergic projections from midbrain to PFC

Thirty percent of the neurons projecting from midbrain toward PFC were TH positive (total number of MR-positive neurons: 2,249 ± 695; range: from 1,638 to 3,358. Number of neurons double-labeled for MR and TH: 665 ± 39; range: from 605 to 709; $n = 3$ animals). Ninety-six percent of the TH-positive neurons projecting from midbrain to PFC were ipsilateral (ipsilateral: 636 ± 33 neurons; contralateral: 29 ± 6 neurons = 4 %; $n = 3$ rats; Fig. 5). Seventy-seven percent of these were located in nucleus A9 (VTA: 421 ± 6 neurons = 63 %; CLi: 80 ± 14 neurons = 12 %; IF: 16 ± 1 neurons = 2 %), 14 % in A10 (SNC: 84 ± 23 neurons = 13 %; SNR: 5 ± 2 neurons = 1 %) and 5 % in A8 (RRF: 30 ± 10 neurons). Only a negligible number of triple-labeled neurons could be detected, indicating very limited co-innervation of a single dopaminergic neuron to both PFC and M1 (total number of neurons positive for MR, TH and FB: 5 ± 2; $n = 3$ animals, i.e., 0.2 % of all MR-positive neurons). Most TH-positive neurons projecting to PFC were in a different topographical location than those projecting to the M1 forelimb area (Fig. 5): M1-projecting neurons were located dorsally and PFC-projecting neurons ventrally. The highest density of M1-projecting neurons was found in the rostral midbrain (61 % of neurons were located from −5 to −6 mm with respect to bregma), the highest density of PFC-projecting neurons was found in more caudal parts

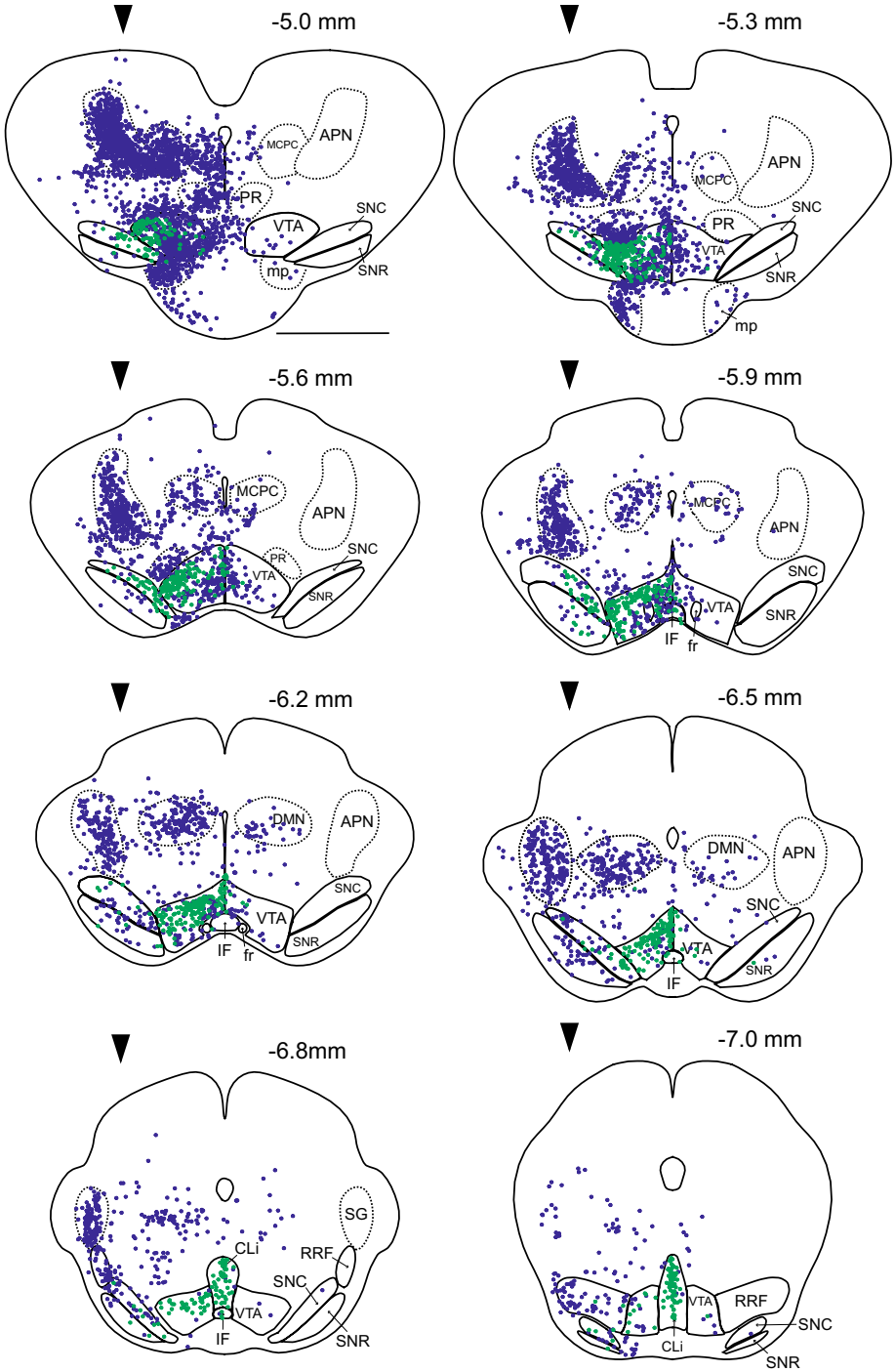

Fig. 2 Retrograde tracing from M1 forelimb areas identifies dopaminergic neurons in the midbrain. Superposition of double-labeled neurons (*green*) and Fast Blue-labeled neurons (*blue*) in eight representative sections (positions relative to bregma) derived from eleven animals. The *arrowhead* indicates the tracer-injected side. *APN* anterior pretectal nucleus, *CLi* central linear nucleus, *DMN* deep mesencephalic nucleus, *fr* fasciculus retroflexus, *IF* interfascicular nucleus, *MCPC* magnocellular nucleus of posterior commissure, *mp* mammillary peduncle, *PR* prerubral field, *RRF* retrorubral field, *SG* suprageniculate thalamic nucleus, *SNC* substantia nigra pars compacta, *SNR* substantia nigra pars reticulata, and *VTA* ventral tegmental area. *Scale bar* 1 mm

of the midbrain (70 % from −6 to −7 mm with respect to bregma; Fig. 3).

Dopaminergic projections from midbrain to NAc

Sixty-four percent of the neurons projecting from midbrain to NAc were TH-positive (total number of MR-positive neurons: 1,204 ± 208; range: from 1,533 to 885. Number of neurons double-labeled for MR and TH: 773 ± 124; from 579 to 999; $n = 4$ animals). Ninety-seven percent of the TH-positive neurons projecting from midbrain to NAc (TH/MR-double labeled) were located ipsilaterally (ipsilateral: 748 ± 115 neurons; contralateral: 25 ± 9 neurons = 3 %; $n = 4$ rats; Fig. 6), 91 % in A9 (VTA: 589 ± 95 neurons = 76 %; CLi: 81 ± 5 neurons = 11 %; IF: 29 ± 9 neurons = 4 %), 6 % in A10 (SNC: 40 ± 12 neurons = 5 %; SNR: 6 ± 3 neurons = 1 %), and very few in A8 (RRF: 5 ± 2 neurons). Only a negligible amount of triple labeled

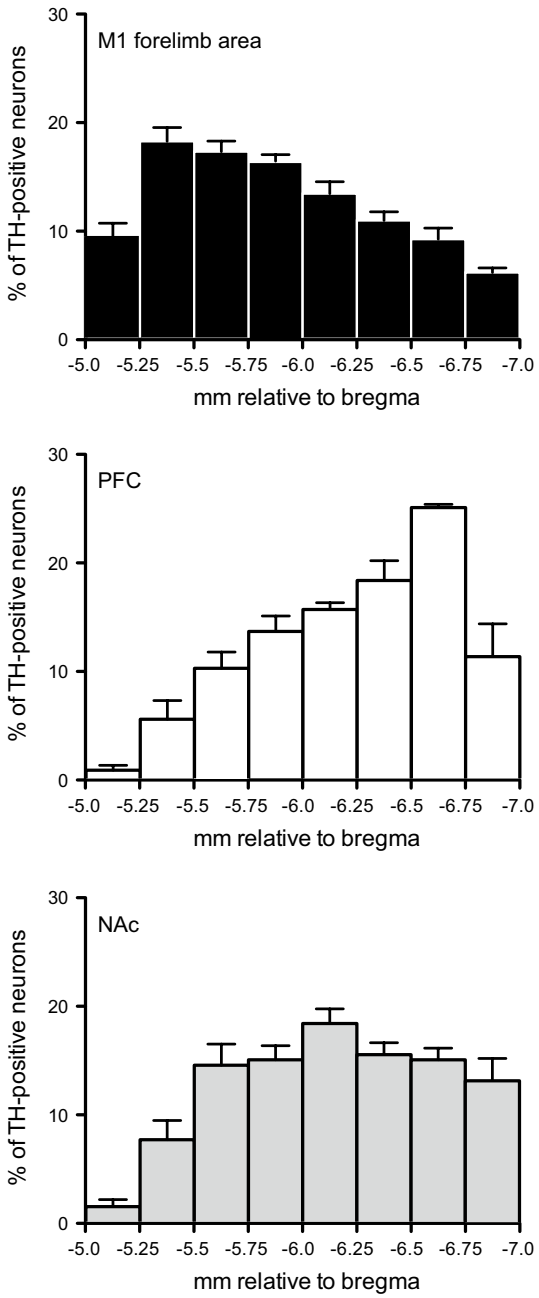

Fig. 3 Distribution of double-labeled neurons within the midbrain along the rostro-caudal axis. Positions are indicated relative to bregma. *Error bars* indicate SEM. For M1-forelimb area $n = 11$; for PFC $n = 3$; for NAc $n = 4$

neurons could be detected within this group, indicating limited co-innervation of a single VTA neuron to both NAc and M1 (total number of neurons positive for MR, TH and FB: 6 ± 1; $n = 4$ rats, i.e., 0.5 % of all MR-positive neurons). TH-positive neurons projecting to NAc were topographically distinguishable from those projecting to the M1 forelimb area (Fig. 6): M1-projecting neurons were located dorsally, and NAc-projecting neurons were found ventrally. While M1-projecting neurons were most densely distributed in the rostral midbrain (61 % from −5 to −6 mm with respect to bregma), NAc-projecting neurons were mainly found in the middle portion of the midbrain (63 % from −5.5 to −6.5 mm with respect to bregma; Fig. 3).

Discussion

Topography and collateralization of dopaminergic projection to M1

Dopaminergic neurons projecting to M1 are located in the nucleus A10 (particularly VTA) and to a lesser extent, in the lateral portions of nucleus A9 (particularly SNC). Along the rostro-caudal axis, the density of neurons decreases and shifts from the lateral part of the VTA toward medial dopaminergic nuclei (CLi and IF). The projection specifically targets the ipsilateral motor cortex and shows only sparse collaterals to other cortical (PFC) or limbic structures (NAc). Although the dopaminergic projections to M1 shares typical anatomical features of meso-cortico-limbic systems with respect to origin and low collateralization (Swanson 1982; Takada and Hattori 1987), M1-projecting neurons form a population that can be clearly distinguished from dopaminergic cells that target PFC/NAc due to their exclusive topographical location within the dopaminergic midbrain nuclei (coronal plane: dorsal for M1 vs. ventral for PFC/NAc; sagittal plane: rostral for M1 vs. caudal for PFC/NAc). In rats, selective lesion of these neurons interferes with motor learning, but did not affect motivation and locomotion (Hosp et al. 2011). Thus, these lesions predominantly depleted the dopaminergic input in M1, whereas DA supply of PFC and NAc that depends on more ventro-caudal portions of VTA remained largely intact.

Within M1, the density of dopaminergic projections markedly decreases from frontal (forelimb area) toward caudal parts (hindlimb area). These observations are in good agreement with the rostro-caudal gradient known for the cortical distribution of dopamine concentrations (Kehr et al. 1976), dopaminergic terminals (Descarries et al. 1987) and DA-receptors (Martres et al. 1985; Dawson et al. 1986a, b).

As a general rule, phylogenetic younger dopaminergic neurons are appended dorsally to phylogenetic older dopaminergic midbrain nuclei (Bjorklund and Dunnett 2007), and the density of dopaminergic terminals in more caudal cortical areas increases during phylogenetic development (Berger et al. 1991). M1 is located caudally along the rostro-caudal axis when compared to the PFC and limbic structures. Furthermore, M1-projecting dopaminergic neurons are located in dorsal portions of the DA midbrain nuclei, whereas PFC- or NAc-projecting neurons can be found at more ventral sites. This topographical distribution

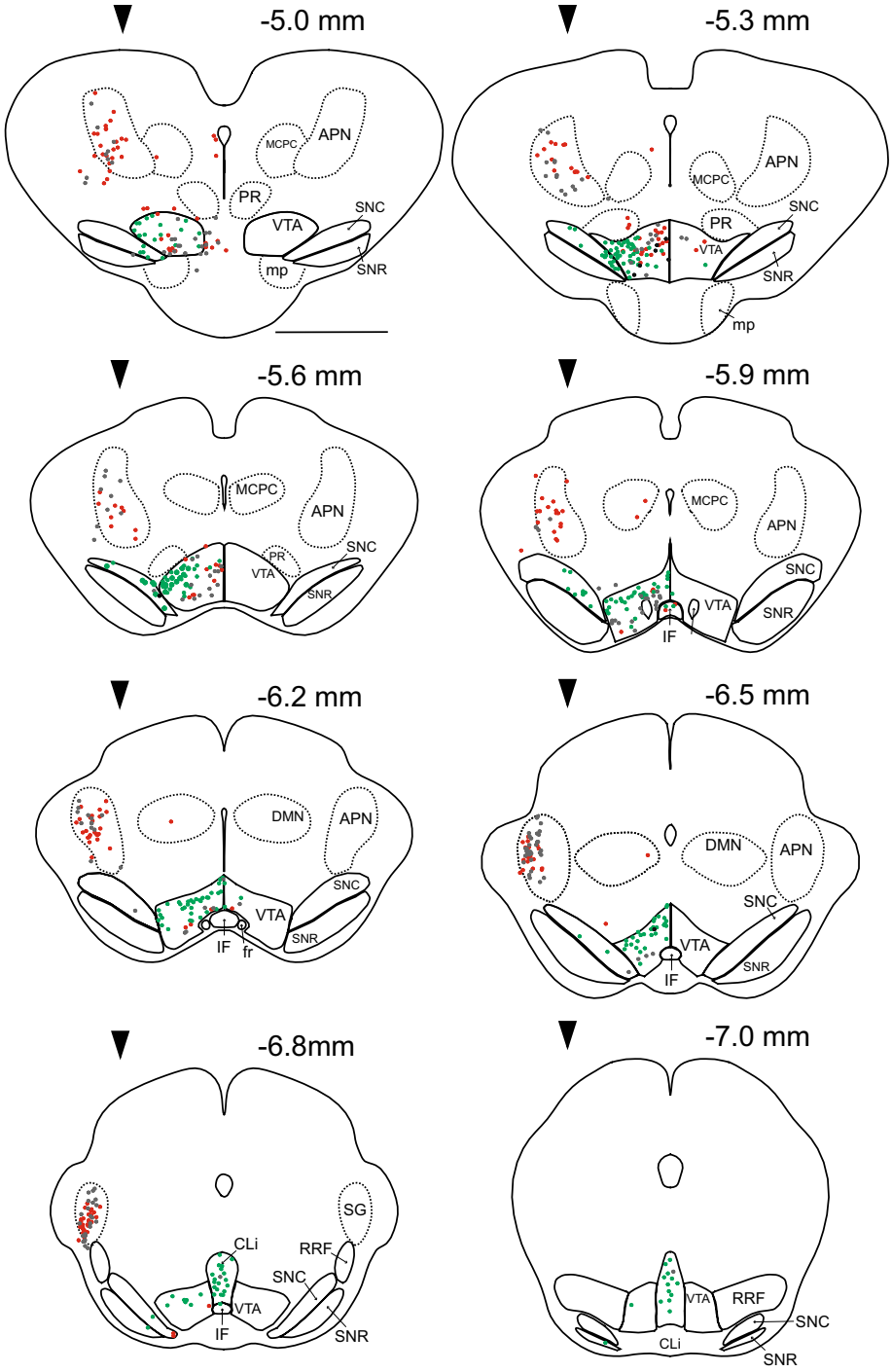

Fig. 4 Retrograde tracing from M1 forelimb and hindlimb position (M1-FL/HL group). Superposition of reconstructed sections derived from four animals. *Green* TH-positive neurons projecting to the M1 forelimb area (FB/TH-positive). *Black* TH-positive neurons projecting to both, M1 fore- and hindlimb representation (FB/MR/TH-positive). *Gray* TH-negative neurons projecting to both, M1 fore- and hindlimb representation (FB/MR-positive). *Red* TH-negative neurons projecting to M1 hindlimb area (MR-positive). TH-negative neurons projecting to M1 forelimb representation (FB-positive) were not shown for the sake of clarity. Positions are provided relative to bregma, the *arrowhead* indicates the tracer-injected side. *APN* anterior pretectal nucleus, *CLi* central linear nucleus, *DMN* deep mesencephalic nucleus, *fr* fasciculus retroflexus, *IF* interfascicular nucleus, *MCPC* magnocellular nucleus of posterior commissure, *mp* mammillary peduncle, *PR* prerubral field, *RRF* retrorubral field, SG suprageniculate thalamic nucleus, *SNC* substantia nigra pars compacta, *SNR* substantia nigra pars reticulata, and *VTA* ventral tegmental area. *Scale bar* 1 mm

of origin and target region suggests that the dopaminergic innervation of M1 developed later in phylogeny than those of limbic structures or PFC.

Functional aspects of the dopaminergic midbrain-M1 projections

The dopaminergic system is thought to provide information about the evaluation of environmental stimuli with respect to individual value and significance. Whereas particular classes of dopaminergic neurons are coupled to a specific content (e.g., motivational value or motivational saliency (Bromberg-Martin et al. 2010), the time course of dopamine release is thought to encode whether a stimulus is pleasant ("rewarding") or aversive ("punishing"; (Schultz 2007a, b). Recently, dopaminergic projections from midbrain to M1 have been shown to be a prerequisite for motor skill learning (Molina-Luna et al. 2009; Hosp et al. 2011).

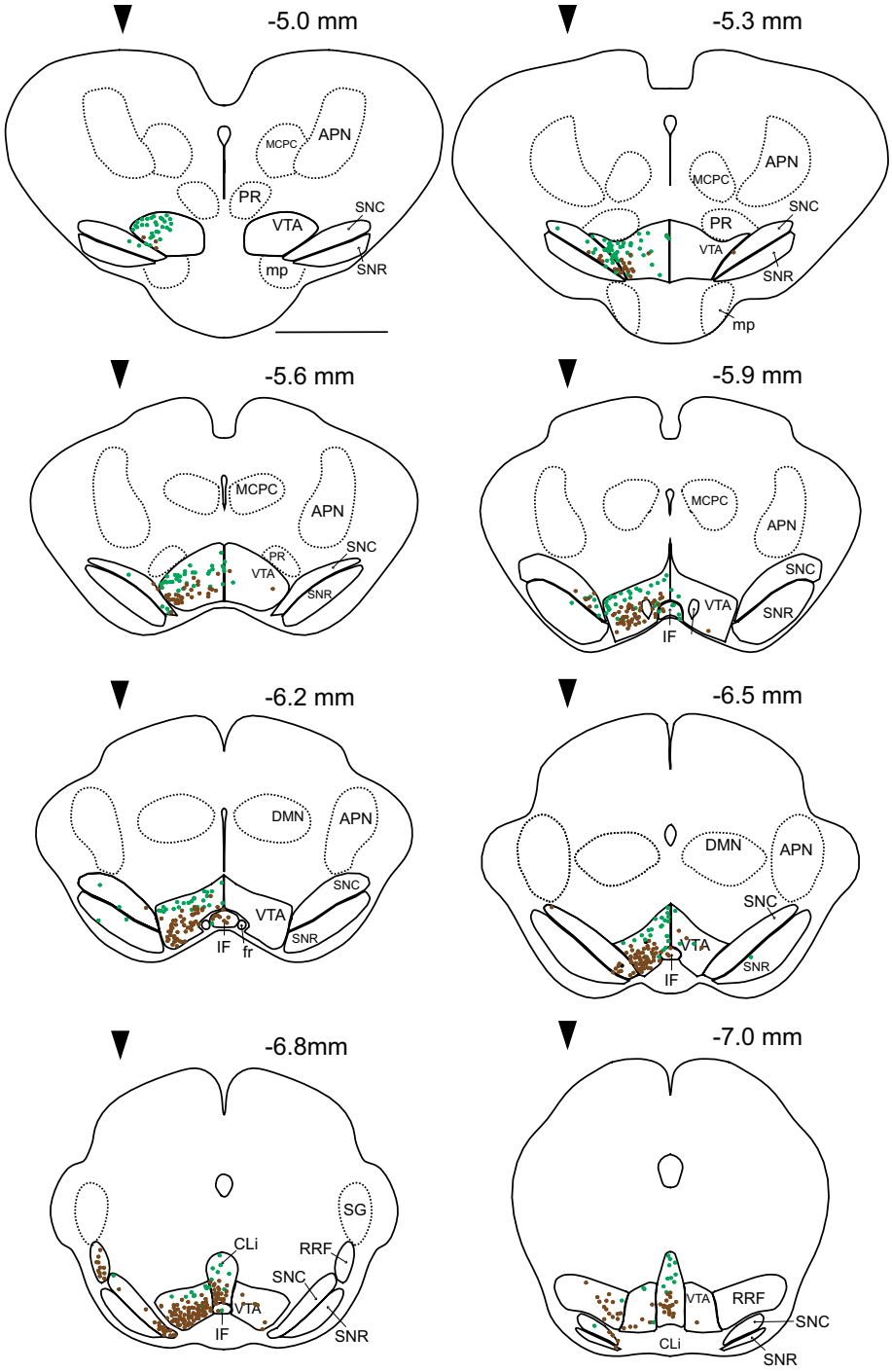

Fig. 5 Retrograde tracing from M1 forelimb representation and PFC (M1-FL/PFC group). Superposition of reconstructed sections derived from three animals. *Green* TH-positive neurons projecting to the M1 forelimb area (FB/TH-positive). *Brown* TH-positive neurons projecting to PFC (MR/TH-positive). *Black* TH-positive neurons projecting to both M1 forelimb representation and PFC (FB/MR/TH-positive). TH-negative neurons projecting to M1 forelimb representation (FB-positive) or PFC (MR-positive) are not shown for the sake of clarity. Positions are given relative to bregma, the *arrowhead* indicates the tracer-injected side. *APN*, anterior pretectal nucleus, *CLi* central linear nucleus, *DMN* deep mesencephalic nucleus,*fr*, fasciculus retroflexus, *IF* interfascicular nucleus, *MCPC* magnocellular nucleus of posterior commissure, *mp*, mammillary peduncle, *PR* prerubral field, *RRF* retrorubral field, *SG* supragenic-ulate thalamic nucleus, *SNC* substantia nigra pars compacta, *SNR* substantia nigra pars reticulata, and *VTA* ventral tegmental area. *Scale bar* 1 mm

M1 is thought to be a key structure for the storage of the motor memory trace (Luft et al. 2004; Monfils et al. 2005) and dopaminergic signaling promotes plasticity in M1 circuitry at various levels (for review see also Hosp and Luft 2013): (1) at the network level, dopamine increases M1 excitability and integrity of motor maps (Hosp et al. 2009), a prerequisite for successful motor learning (Conner et al. 2003). (2) At the cellular level, dopamine induces the expression of c-Fos (Hosp et al. 2011), a transcription factor related to motor learning (Kleim et al. 1996). (3) At the level of synaptic transmission, dopamine is essential for the formation of long-term potentiation (LTP), a key mechanism for motor learning in M1 (Rioult-Pedotti et al. 2000). Thus, the dopaminergic projections from midbrain to M1 may couple reward-related external stimuli to successful acquisition of novel movement sequences. However, the environmental cues triggering dopamine release in M1 remain to be identified.

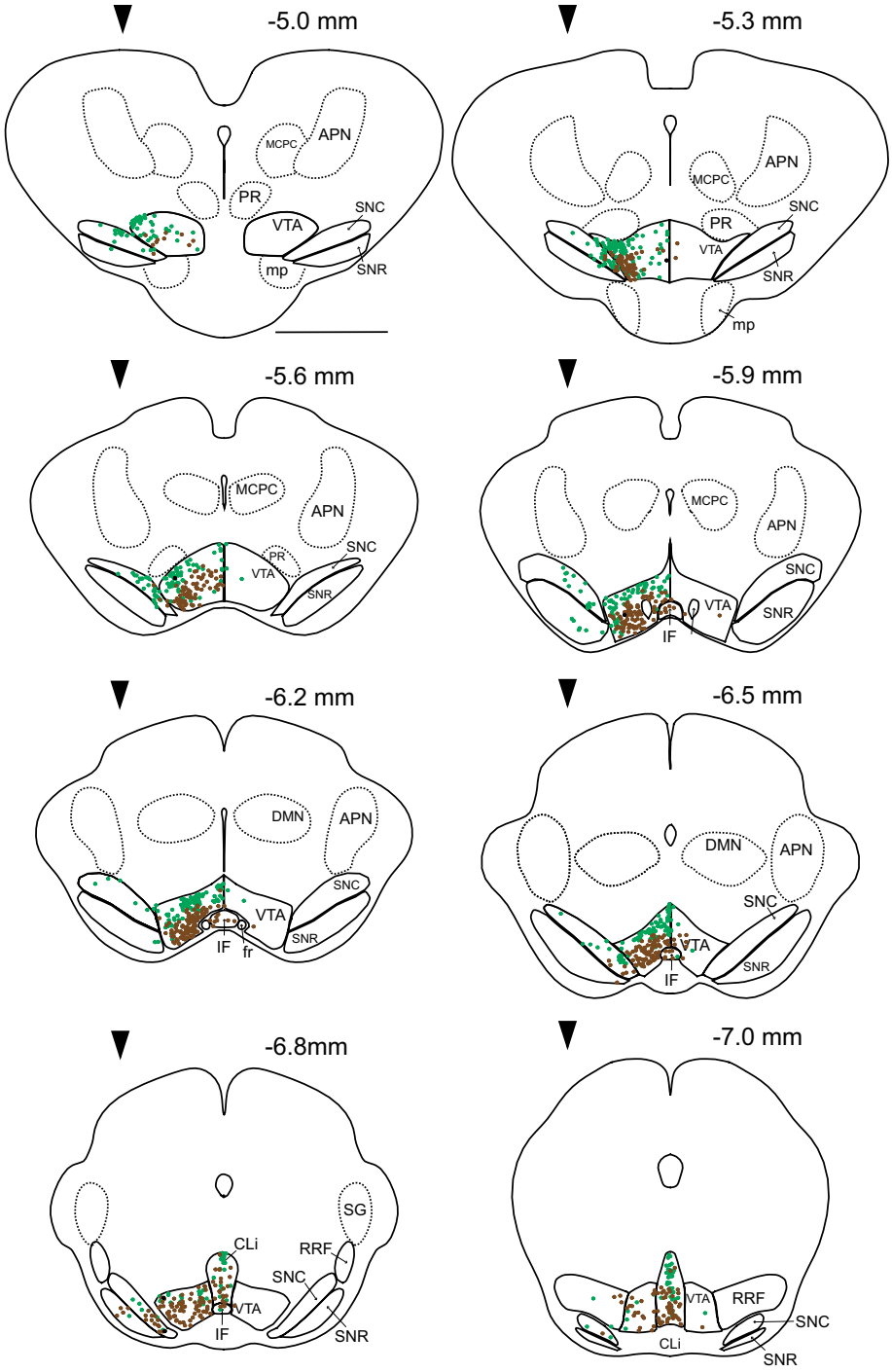

Fig. 6 Retrograde tracing from M1 forelimb representation and NAc (M1-FL/NAc group). Superposition of reconstructed sections derived from four animals. *Green* TH-positive neurons projecting to the M1 forelimb area (FB/TH-positive). *Brown* TH-positive neurons projecting to NAc (MR/TH-positive). *Black* TH-positive neurons projecting to both, M1 forelimb representation and NAc (FB/MR/TH-positive). TH-negative neurons projecting to M1 forelimb representation (FB-positive) or NAc (MR-positive) are not shown for the sake of clarity. Positions are provided relative to bregma, the *arrowhead* indicates the tracer-injected side. *APN*, anterior pretectal nucleus, *CLi* central linear nucleus, *DMN* deep mesencephalic nucleus,*fr*, fasciculus retroflexus, *IF* interfascicular nucleus, *MCPC* magnocellular nucleus of posterior commissure, *mp*, mammillary peduncle, *PR* prerubral field, *RRF* retrorubral field, *SG* suprageniculate thalamic nucleus, *SNC* substantia nigra pars compacta, *SNR* substantia nigra pars reticulata, and *VTA* ventral tegmental area. *Scale bar* 1 mm

In rats, dopaminergic projections are especially confined to deep cortical layers of the agranular cortex (Descarries et al. 1987). There, DA affects the activity of layer V motor neurons and the cortico-spinal tract (Awenowicz and Porter 2002; Vitrac et al. 2014). Thus, besides supporting plasticity, dopaminergic projections to M1 are ideally suited to control the cortico-fugal output of the primary motor cortex. The fact that only rostral parts of M1 including the forelimb representation receive strong dopaminergic input rats, whereas caudal portions containing the hindlimb area are innervated by only few dopaminergic neurons, may indicate that dopamine is needed for precise forelimb movements as opposed to automatized hindlimb motion.

Methodological considerations

The fluorescent tracers Fast Blue (FB) and Micro-Ruby (MR) were used in this study. FB is one of the most

frequently used fluorescent agents for retrograde tracing experiments. It is taken up by axon terminals rather than fibers of passage (Kobbert et al. 2000) and exerts a cytoplasmic labeling of neurons 1–2 weeks after its injection (Novikova et al. 1997). Among all fluorescent tracers, FB is characterized by its high staining effectiveness and good tissue penetration (Choi et al. 2002). MR is a dextran (MW 3,000 kD) conjugated to the fluorophore rhodamine. Dextran conjugates penetrate bypassing fibers and axon terminals at the injection site (Jiang et al. 1993; Reiner et al. 2000). In rats, maximal labeling can be expected 7 days after (Novikova et al. 1997). Whereas higher molecular dextrans (>10,000 kD) are predominantly used for anterograde tracing, low molecular weight dextrans such as MR are the preferred substances for retrograde tracing and retrograde labeling of neuronal cell bodies (Reiner et al. 2000). Furthermore, axonal uptake is more effective in low molecular dextrans leading to a higher staining efficacy (Luby-Phelps et al. 1986). Whereas the superficial intracortical injections could be performed with a high precision, a certain degree of spillage along the needle track has to be taken into account for injections into PFC and NAc (Supplementary Figure 2b and c). For injections into the PFC, tracer was confined to the prelimbic and the cingulate cortex that are both substructures of the PFC homolog in rodents (Uylings et al. 2003). For injections into the NAc, some spillage of tracer could be detected within the striatum (Supplementary Figure 2c). Dopaminergic neurons projecting to the striatum are mainly located within the ipsilateral substantia nigra/nucleus A9 (Fallon 1981). However, only few retrogradely labeled neurons are present in the substantia nigra after injections into the NAc (Fig. 6), arguing against a major tracer displacement. Thus, even though some degree of spillage of tracer could be observed along the needle track, this should not have significantly confounded the results of this study.

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