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H 知的財産権の出願・登録状況

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2. 実用新案登録

なし

3. その他

なし

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Distinct Roles of Synaptic Transmission in Direct and Indirect Striatal Pathways to Reward and Aversive Behavior

Takatoshi Hikida,[1,2] Kensuke Kimura,[1,3] Norio Wada,[1] Kazuo Funabiki,[1] and Shigetada Nakanishi[1,*]
[1]Department of Systems Biology, Osaka Bioscience Institute, 6-2-4 Furuedai, Suita, Osaka 565-0874, Japan
[2]PRESTO, Japan Science and Technology Agency (JST), 4-1-8 Honcho Kawaguchi, Saitama 332-0012, Japan
[3]Department of Biological Sciences, Kyoto University Faculty of Medicine, Sakyo-ku, Kyoto 606-8501, Japan
*Correspondence: snakanis@obi.or.jp
DOI 10.1016/j.neuron.2010.05.011

SUMMARY

In the basal ganglia, convergent input and dopaminergic modulation of the direct striatonigral and the indirect striatopallidal pathways are critical in rewarding and aversive learning and drug addiction. To explore how the basal ganglia information is processed and integrated through these two pathways, we developed a reversible neurotransmission blocking technique, in which transmission of each pathway was selectively blocked by specific expression of transmission-blocking tetanus toxin in a doxycycline-dependent manner. The results indicated that the coordinated modulation of these two pathways was necessary for dopamine-mediated acute psychostimulant actions. This modulation, however, shifted to the predominant roles of the direct pathway in reward learning and cocaine sensitization and the indirect pathway in aversive behavior. These two pathways thus have distinct roles: the direct pathway critical for distinguishing associative rewarding stimuli from nonassociative ones and the indirect pathway for rapid memory formation to avoid aversive stimuli.

INTRODUCTION

The basal ganglia are the key neural substrates that control motor balance and reward-based learning (Graybiel, 2000; Wickens et al., 2003). Dysfunction of the basal ganglia leads to devastating neurological disorders such as Parkinson's disease, Huntington's disease, and drug addiction (Albin et al., 1989; Chesselet and Delfs, 1996; Hyman et al., 2006). The striatal projection neurons are GABA-containing medium-sized spiny neurons (MSNs), which are divided into two subpopulations, i.e., striatonigral neurons in the direct pathway and striatopallidal neurons in the indirect pathway (Albin et al., 1989; Alexander and Crutcher, 1990; Graybiel, 2000). The inputs of these two pathways converge at the substantia nigra pars reticulata (SNr) and control the dynamic balance of the basal ganglia-thalamocortical circuitry (Deniau et al., 2007; Graybiel, 2000). Accumulated evidence has indicated vast differences in the expression profiles of functional proteins and intracellular signaling molecules between these two subpopulations of MSNs (Gerfen et al., 1990; Heiman et al., 2008; Surmeier et al., 2007; Valjent et al., 2009). The striatonigral neurons selectively express substance P (SP) and D1 dopamine receptor, in marked contrast to the confined expression of enkephalin (Enk) and D2 dopamine receptor in the striatopallidal neurons (Gerfen et al., 1990; Graybiel, 2000; Surmeier et al., 2007). Gene targeting and pharmacological studies have shown that the D1 receptor-expressing direct pathway acts more predominantly than the D2 receptor-expressing indirect pathway in adaptive responses to cocaine and other dopamine agonists (Baker et al., 1996, 1998; Caine et al., 2007; Smith et al., 2002; Welter et al., 2007; Xu et al., 2000, but see also Miner et al., 1995). However, these techniques would have global effects on many other brain regions as well. Furthermore, two types of MSNs are morphologically indistinguishable and exist in a similar number (Surmeier et al., 2007). This similarity has made it extremely difficult to determine how the neural information is processed and integrated through these two pathways.

To explore the regulatory mechanisms of the basal ganglia function, we adopted a gene-manipulating technique termed reversible neurotransmission blocking (RNB) (Wada et al., 2007; Yamamoto et al., 2003). For this technique, we used previously developed transgenic mice (TN mice), in which the expression of tetanus toxin light chain (TN) was controlled by the tetracycline-responsive element (TRE) in a tetracycline-derivative doxycycline (DOX)-dependent manner (Wada et al., 2007; Yamamoto et al., 2003). TN is a bacterial toxin that cleaves the synaptic-vesicle-associated VAMP2 protein (Schiavo et al., 1992) and abolishes neurotransmitter release from synaptic vesicles (Wada et al., 2007). SP and Enk are specifically expressed in striatonigral and striatopallidal neurons, respectively, but are also distributed in other brain regions (Cuello and Kanazawa, 1978; Miller and Pickel, 1980). To restrict the expression of the tetracycline-repressive transcription factor (tTA) to striatonigral and striatopallidal neurons, we combined with the adeno-associated virus- (AAV-) mediated gene expression system, which has been reported to effectively express incorporated transgenes in MSNs and modify properties of these cells in a transgene-dependent manner (Lerchner et al., 2007; Pulipparacharuvil et al., 2008). We constructed two types of AAVs, termed V-S-tTA and V-E-tTA, in which expression of tTA was

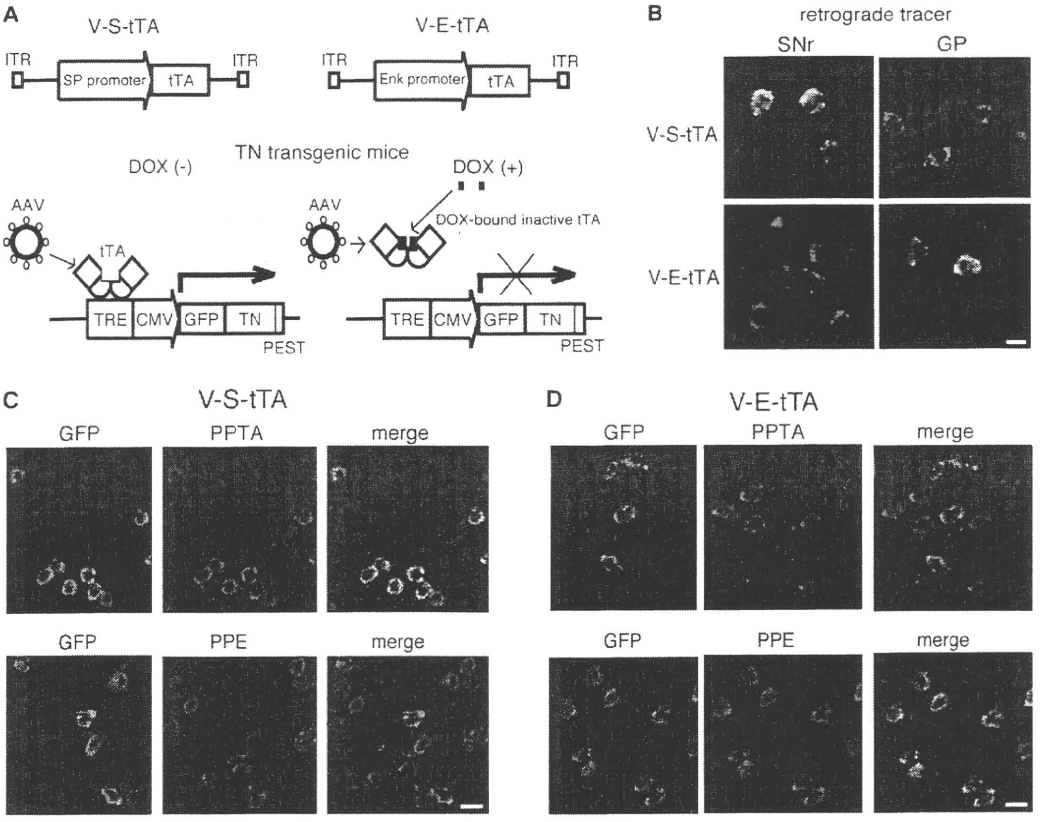

Figure 1. RNB Technique and Selective Expression of TN in Striatonigral or Striatopallidal Neurons

(A) Schema of cell-type-specific reversible expression of TN. The V-S-tTA and V-E-tTA viruses incorporated the *flag*-tagged *tTA* gene following the SP and Enk promoters, respectively. The TN transgenic mice contained the *GFP-TN* fusion gene. When the striatum was transfected with the recombinant virus, the expression of the GFP-TN was driven by the interaction of tTA with the TRE under the DOX-free condition, but this expression was abolished by DOX treatment. The expression of TN was confined to the striatonigral and the striatopallidal neurons by transfection with V-S-tTA and V-E-tTA, respectively. ITR, inverted terminal repeat; CMV, cytomegalovirus promoter; PEST, the degradation-facilitating PEST sequence.

(B) Cell-type-specific expression of flag-tTA. The CTB-Alexa 594 retrograde tracer was injected into the SNr or GP of the V-S-tTA-transfected and V-E-tTA-transfected wild-type mice. Two weeks after the injection, coronal sections of the striatum were immunostained with anti-flag antibody, and the retrograde tracer (red) and the flag-tagged tTA (green) were visualized. Scale bar, 10 μm.

(C and D) Cell-type-specific expression of TN. Coronal sections of the V-S-tTA-transfected (C) and the V-E-tTA-transfected TN mice (D) were double-immunostained with the GFP and the indicated antibodies. Scale bar, 10 μm.

directed by the SP or the Enk promoter, respectively. When these recombinant viruses were injected into the striatum of TN mice, TN was specifically expressed and in turn separately blocked neurotransmission in striatonigral and striatopallidal neurons. This investigation has indicated that transmission blockade of either of the two pathways abolished the psychostimulant-induced acute responses but that the blockade of the direct and indirect pathways caused selective impairments of the reward-based and aversive behavior, respectively. Synaptic transmission of the two pathways is thus coordinated, but each plays a distinct role in controlling adaptive mechanisms involved in the basal ganglia function.

RESULTS

Cell-Type-Specific Expression of TN

The selective and reversible blockade of synaptic transmission of the two types of MSNs in vivo was achieved by combining the TN transgenic mice (Wada et al., 2007; Yamamoto et al., 2003) with the recombinant AAV transfection technique (Figures 1A and S1, available online). In the TN mice, the expression of the fusion protein of TN and green fluorescent protein (GFP) was driven by the TRE and was induced by interaction with the tTA (Figure 1A). The AAV-mediated gene expression system (V-S-tTA or V-E-tTA) was used to restrict the SP- or Enk-derived expression of tTA within the striatum (Figure 1A). With this strategy, the SP or Enk-promoter-derived tTA would activate expression of the GFP-TN transgene in specific striatal neurons. The administration of DOX would then terminate the expression of TN and allow recovery of synaptic transmission through newly synthesized VAMP2 (Figure 1A).

The success of our strategy relied on accurate and mutually exclusive expression of TN in one of the MSN types. We first examined whether the SP and Enk promoters would faithfully direct the expression of tTA in striatonigral and striatopallidal neurons, respectively, in wild-type mice (Figure 1B). The V-S-tTA or

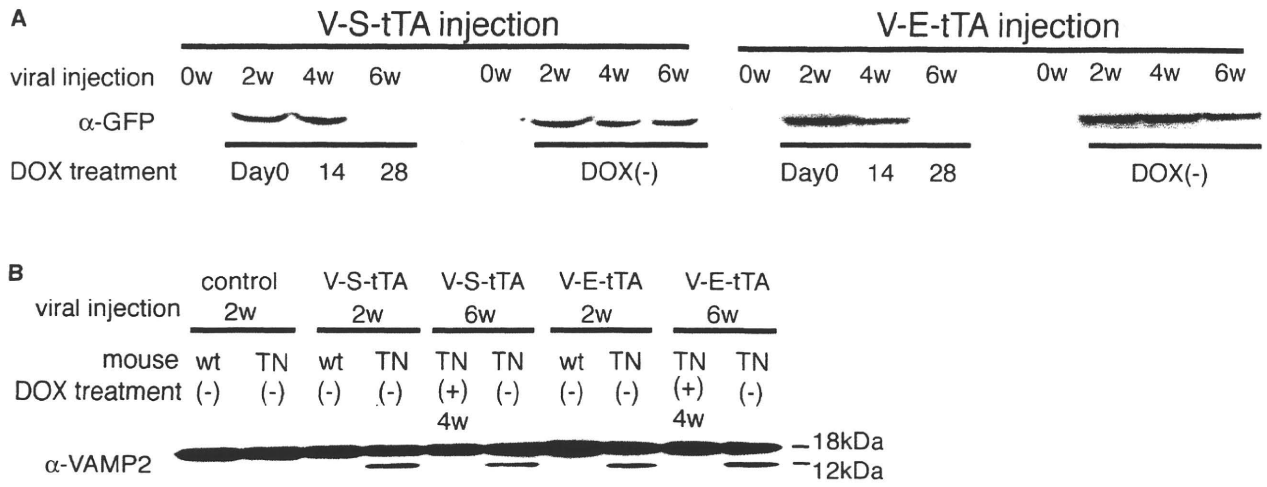

Figure 2. Reversible Expression of TN

(A) Two weeks after the viral injection, TN mice were treated or not with DOX. At the indicated days after the viral injection or DOX treatment, the striatum was isolated, and cell lysates were prepared and then immunoblotted with the GFP antibody.

(B) Animals were treated as in (A), and striatal cell lysates were prepared and then immunoblotted with the N-terminal VAMP2 antibody.

V-E-tTA virus was injected into the striatum. The cholera toxin subunit B (CTB)-Alexa 594 retrograde tracer was then injected into either the globus pallidus (GP) or the SNr of these mice. Two weeks after the injection, the retrograde tracer and the flag-tagged tTA were visualized in serial sections of the striatum by fluorescence and immunostaining with anti-flag antibody, respectively (Figure 1B). Immunoreactivity of the flag-tagged tTA was exclusively seen in striatonigral neurons of the V-S-tTA-transfected mice, which were retrogradely labeled from the SNr; the tTA-positive cells were 68.6% ± 1.0% of retrogradely labeled cells (n = 3). Conversely, the immunoreactivity was restricted to the striatopallidal neurons of the V-E-tTA-transfected mice, which were retrogradely labeled from the GP; the tTA-positive cells were 66.2% ± 3.1% of retrogradely labeled cells (n = 3).

We then examined the tTA-dependent, cell-type-specific expression of TN by injecting the recombinant virus into the striatum of the TN and wild-type mice. Upon double immunostaining of the TN mice 2 weeks after V-S-tTA injection, immunoreactivity of the TN-GFP fusion protein was exclusively seen in SP-immunoreactive but not in Enk-immunoreactive neurons (Figure 1C); the GFP-immunoreactive cells were 74.2% ± 1.9% of SP-immunoreactive striatonigral neurons (n = 4). This immunoreactive colocalization was reversed in the V-E-tTA-transfected TN mice (Figure 1D); the GFP-immunoreactive cells were 71.1% ± 0.9% of Enk-immunoreactive striatopallidal neurons (n = 4). In controls, no GFP immunoreactivity was observed in any other striatal interneurons (Figure S2), nor in the virus-transfected wild-type striatum. Although viral tropism may also contribute to specific expression of transgenes in MSNs, the results indicated that TN was exclusively expressed in striatonigral or striatopallidal neurons, depending on the transfection with the V-S-tTA or V-E-tTA virus, respectively.

Reversibility of TN Expression and VAMP2 Cleavage

We next examined the DOX-mediated reversibility of TN expression and cleavage of VAMP2 in TN mice. The V-S-tTA or V-E-tTA virus or the tTA-free control virus was injected into one side of the striatum of wild-type and TN mice. Two weeks after the viral injection, DOX was continuously administered or the animals were left untreated. At 2, 4, or 6 weeks after the viral injection, the striatum was dissected from DOX-treated and DOX-untreated animals. No anatomical alterations with Nissl staining nor changes in patterns and intensities of immunoreactivity of dopamine transporter and hybridization signals of *SP* and *Enk* mRNAs were observed at the striatum in virus-transfected TN mice regardless of treatment or not with DOX (Figure S3). Immunoblot analysis showed intense GFP immunoreactivity in both the V-S-tTA-injected and the V-E-tTA-injected TN mice at least up to 6 weeks after the viral injection (Figure 2A). This immunoreactivity completely disappeared 4 weeks after DOX treatment in both cases (Figure 2A). No GFP immunoreactivity was seen in striatal sections transfected with the control AAV or in those of wild-type mice transfected with either the V-S-tTA or V-E-tTA (data not shown). The expression of TN was thus reversibly controlled in a DOX-dependent manner.

The reversibility of the TN-mediated cleavage of VAMP2 was examined by immunoblot analysis of striatal lysates with antibody against N-terminal VAMP2 (Figure 2B). The intact 18 kDa VAMP2 was significantly cleaved to the 12 kDa N-terminal fragment in TN mice with either V-S-tTA or V-E-tTA transfection. This cleavage disappeared 4 weeks after DOX treatment (Figure 2B). Upon densitometric measurement, cleavage of the 18 kDa VAMP2 was calculated to be about 30% in both virus-transfected TN mice (n = 2). If striatonigral and striatopallidal neurons are assumed to exist in a 1:1 ratio within the striatum, the densitometric calculation indicated that about two-thirds of the VAMP2 was cleaved in targeted cells in both viral transfections. This value was consistent with more than 70% of GFP-positive target cells in the virus-transfected striatum (Figures 1C and 1D). No VAMP2 cleavage was detected in the virus-transfected wild-type mice nor in the control virus-transfected TN mice (Figure 2B).

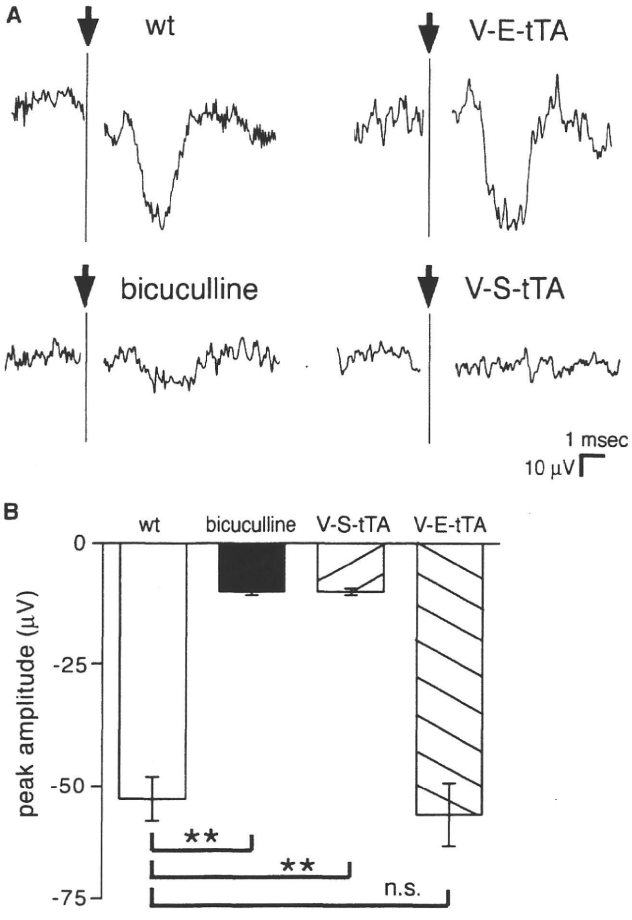

Figure 3. Selective Blockade of Striatonigral Transmission in the V-S-tTA-Transfected TN Mice

(A) Field potentials recorded in the SNr of wild-type and virus-transfected TN mice after striatal stimulation. For bicuculline treatment, 5 mM bicuculline methochloride was perfused into the SNr 15 min before striatal stimulation. The arrow denotes time of striatal stimulation.

(B) Peak amplitudes of bicuculline-sensitive, short-latency responses (<10 ms). Columns and error bars represent the mean ± SEM (n = 4–5). **p < 0.01; n.s., not significant.

Electrophysiological Characterization of the Virus-Transfected TN Mice

We tested transmission blockade of the virus-transfected TN mice by extracellular recordings of the GP or the SNr in anesthetized animals after electrical stimulation of the striatum. The striatal stimulation evoked a rapid and monophasic response in the SNr of wild-type mice (Figure 3A). This response was inhibited by perfusion with the GABA receptor antagonist bicuculline methochloride into the SNr (Figure 3A), confirming GABA-mediated transmission from the striatum to the SNr in wild-type mice. Importantly, the striatal stimulation failed to evoke an electrophysiological response in the V-S-tTA-transfected TN mice but induced a normal level of response in the V-E-tTA-transfected TN mice (Figures 3A and 3B). These results indicate that neurotransmission of the direct pathway was selectively blocked in the V-S-tTA-transfected TN mice. Extracellular recordings of the GP after striatal stimulation of anesthetized wild-type mice were also conducted, but this attempt failed to identify GABA-mediated transmission from the striatum to the GP. This failure was due to close localization of the GP from the striatum, so that electrical excitation within the striatum hampered identification of the striatum-stimulated response in the GP. However, the blockade of striatopallidal transmission was confirmed by a different approach that examined the striatopallidal transmission-mediated upregulation of c-*fos* mRNA in the ventral pallidum (VP) (see Figure 5E). These results demonstrate that neurotransmission of the direct and indirect pathways was selectively blocked in the V-S-tTA-transfected and the V-E-tTA-transfected TN mice, respectively.

Abnormal Turning Behavior by Blocking Either the Striatonigral or Striatopallidal Transmission

The two types of MSNs exert opposing effects on the SNr neurons (Deniau et al., 2007; Graybiel, 2000). Consequently, deficit of the striatonigral transmission on one side of the striatum induces abnormal turning ipsilateral to the impaired side, whereas deprivation of one side of the striatopallidal transmission elicits contralateral turning (Kaneko et al., 2000; Pycock, 1980) (Figure S4). To examine the DOX-dependent, reversible motor imbalance in the virus-transfected TN mice, we injected the recombinant virus unilaterally into the left side of the striatum. Two weeks after the injection, DOX was continuously administered or the animals were left untreated. The abnormal turning behavior was then analyzed by forcing the animals to rotate on a hemispherical container. In the absence of DOX treatment, unilaterally V-S-tTA-injected TN mice all showed rotation ipsilateral to the side of the viral injection (Figure 4A). Conversely, contralateral rotation was induced in unilaterally V-E-tTA-injected TN mice (Figure 4B). Consistent with the TN expression ontogeny (Figure 2A), the abnormal turning behavior remained up to 2 weeks and then disappeared 4 weeks after DOX treatment in both the V-S-tTA and V-E-tTA-injected TN mice (Figures 4A and 4B). In the virus-injected wild-type mice, the abnormal turning never occurred, irrespective of DOX treatment (Figures 4A and 4B). The abnormal turning was thus not only induced in a TN-expression-dependent manner but also was consistent with the coordinated role of the striatonigral and striatopallidal transmission in motor balance (Figure S4). The results described above all support the conclusion that synaptic transmission of the direct and indirect pathways was selectively and reversibly blocked in our model animals. We hereafter refer to the direct-pathway-blocked and the indirect-pathway-blocked TN mice as the D-RNB and I-RNB mice, respectively.

Critical Role of Both Striatonigral and Striatopallidal Transmission on Psychostimulant-Induced Acute Responses

Psychostimulants methamphetamine and cocaine both stimulate the dopamine system in the striatum and enhance acute responses of locomotor activity (Hyman et al., 2006). So we examined whether the methamphetamine-induced acute hyperlocomotion would be influenced in the D-RNB and I-RNB mice by bilateral injection of the V-S-tTA or V-E-tTA virus, respectively, into the striatum including the nucleus accumbens (NAc)

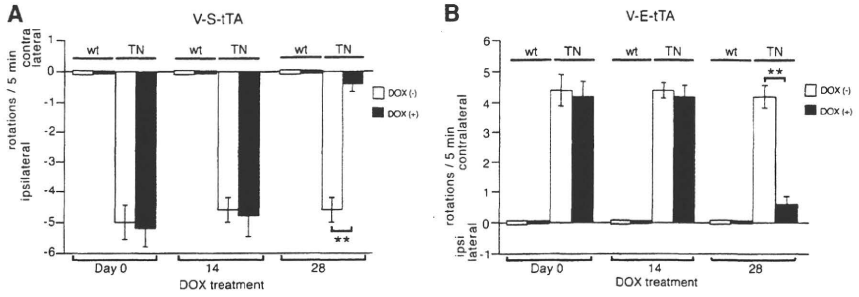

Figure 4. Abnormal Rotation of Unilaterally Virus-Transfected TN Mice

The V-S-tTA virus (A) or the V-E-tTA virus (B) was injected into the left striatum of the TN and wild-type mice. Two weeks after the viral injection, the animals were treated or not with DOX. Numbers of rotations were counted for a 5 min period at days 0, 14, and 28 after the start of the DOX treatment. Columns and error bars represent the mean ± SEM (n = 5 each). **p < 0.01.

(Figures 5A and 5B). The D-RNB and I-RNB mice showed no abnormal locomotor activity under the ordinary condition. The wild-type mice showed a significant methamphetamine-induced acute hyperlocomotion regardless of the viral transfection and DOX treatment. Similarly, hyperlocomotion was induced in the TN-transgenic mice before the viral injection. Remarkably, blockade of either the direct or the indirect pathway abrogated the methamphetamine-induced acute hyperlocomotion. Importantly, when DOX was administered for 4 weeks, the methamphetamine-induced hyperlocomotion recovered in both the D-RNB and I-RNB mice to levels comparable to those of the wild-type mice. In controls, the injection of saline alone had no effect on locomotor activity in any experimental stages of the D-RNB or I-RNB mice.

The cocaine-induced acute hyperlocomotion was also examined by bilateral viral injection into the NAc, the ventral part of the striatum that is the central neural substrate for cocaine actions (Di Chiara and Imperato, 1988) (Figures 5C and 5D). In remarkable contrast to the wild-type mice, neither the D-RNB mice nor the I-RNB mice showed the cocaine-induced acute hyperlocomotion immediately after a single cocaine administration. Synaptic transmission of both pathways is thus required for psychostimulant-enhanced acute responses.

The dopamine stimulation induced by cocaine administration excites the pallidal neurons of the indirect pathway and suppresses the SNr neurons, which receive convergent inputs from the direct and indirect pathways (Albin et al., 1989; Graybiel, 2000). This modulatory effect on the VP and SNr neurons was examined by quantitative in situ hybridization analysis of the activity-dependent up- and downregulation of c-*fos* mRNA at the corresponding brain regions 30 min after cocaine administration (Marshall et al., 1998). This analysis showed that c-*fos* mRNA was upregulated in the VP of both the wild-type and the D-RNB mice but not in the VP of the I-RNB mice (Figure 5E). Furthermore, the cocaine treatment downregulated c-*fos* mRNA in the SNr of the wild-type mice but failed to show such downregulation in both the D-RNB and I-RNB mice (Figure 5F). This finding further confirmed the conclusion that the input transmission from the striatum/NAc to the SNr was impaired by selective blockade of the direct and indirect pathways in the D-RNB and I-RNB mice, respectively.

Differential Control of Cocaine-Induced Adaptation by Striatonigral and Striatopallidal Transmission

Repeated cocaine administration induces neural adaptation of the dopamine system and causes a progressive increase in locomotor activity, called locomotor sensitization (Hikida et al., 2001, 2003; Kalivas and Stewart, 1991). When the recombinant virus was bilaterally injected into the NAc, the I-RNB mice showed delayed cocaine-induced sensitization but reached hyperlocomotor levels comparable to those of the wild-type mice on days 3–5 (Figure 6B). In contrast, the D-RNB mice showed not only reduced locomotor activity in the early stage but also markedly attenuated locomotor sensitization by repeated cocaine administration (Figure 6A). The partial reduction in locomotor sensitization could have resulted from insufficient transfection of the NAc with the V-S-tTA virus; but this interpretation seems to be unlikely, because a large number of the SP-immunoreactive striatonigral cells were GFP positive (Figure 1C), and acute cocaine-induced hyperlocomotion was abolished in the D-RNB mice (Figure 5C).

The adaptive response to chronic cocaine administration persists even after omission of cocaine administration (Grimm et al., 2001; Hikida et al., 2003). To examine this long-lasting adaptive response, we treated animals with cocaine for 5 days and then treated them with DOX from day 6 to day 33 in the absence of cocaine administration (Figures 6A and 6B). They were then challenged with cocaine administration on day 33. In both the wild-type and I-RNB mice, the locomotion was significantly higher on day 33 than the initial cocaine-induced locomotion on day 1 (Figure 6B). Importantly, the cocaine-induced locomotion of the D-RNB mice was strikingly higher on day 33 than the locomotion on day 5 and became comparable to that of the wild-type mice (Figure 6A). These results indicate that the adaptive mechanism remained effective even when synaptic transmission was blocked in the striatonigral and striatopallidal neurons.

The distinct influences of the two pathways on the adaptive responses were further analyzed by conditioned place preference (CPP), which is NAc-mediated associative learning (Tzschentke, 2007). Animals were conditioned by repeated cocaine administration in one of two chambers that differed visually and textually (Figures 6C and 6D). Before conditioning, all animals showed no preference in visiting the two chambers. After conditioning with cocaine for 3 days, blockade of the direct pathway significantly reduced the cocaine-induced CPP (Figure 6C). In contrast, the same manipulation of the indirect pathway showed the ability to induce CPP, which was comparable to that of the wild-type mice (Figure 6D). Both hyperlocomotion and CPP analyses indicate that the direct pathway plays a predominant role in adaptive behaviors of cocaine sensitization.

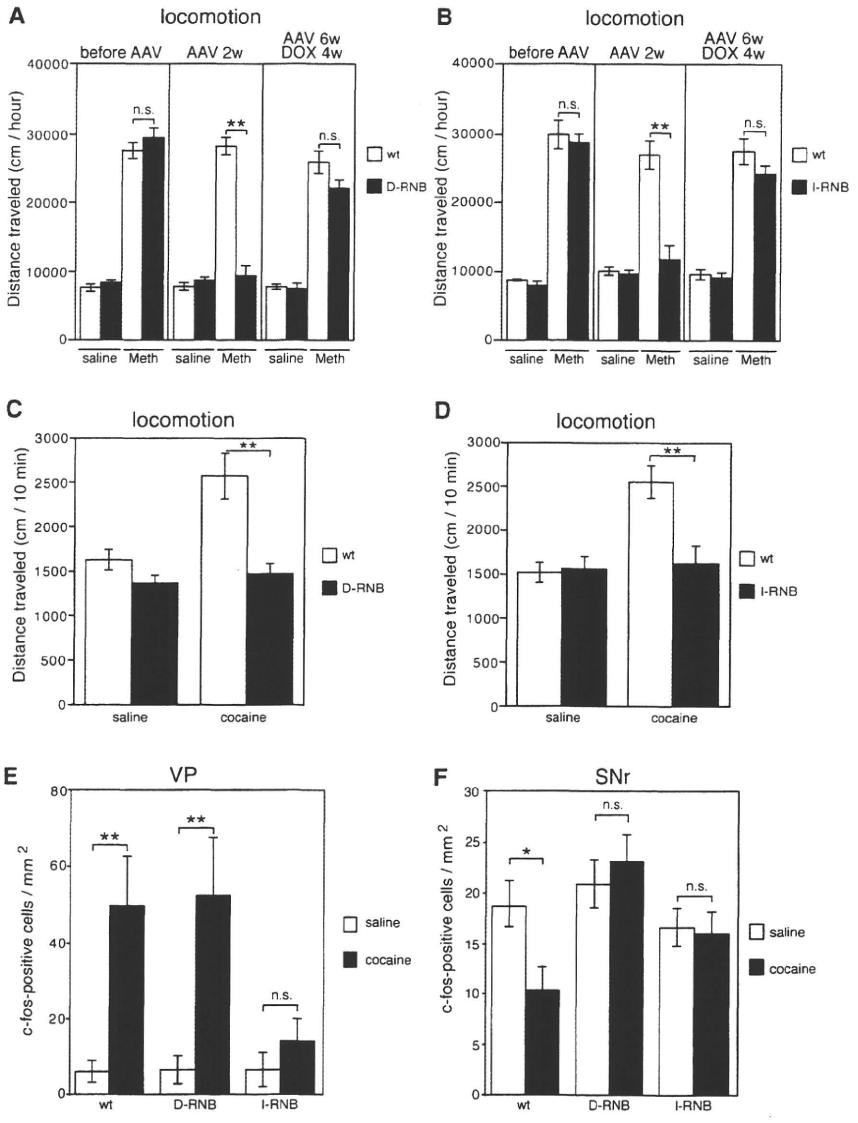

Figure 5. Effects of Blockade of Transmission on Methamphetamine-Induced and Cocaine-Induced Acute Responses

(A and B) Two weeks after bilateral injection of the V-S-tTA virus (A) or the V-E-tTA virus (B) into the striatum, the animals were treated or not with DOX for 4 weeks (n = 5 or 6). Locomotion was measured for 60 min immediately after i.p. injection of methamphetamine (Meth, 2 mg/kg) or saline before and 2 and 6 weeks after the viral transfection.

(C and D) Two weeks after the viral injection into the NAc, locomotion was measured for 10 min immediately after i.p. injection of cocaine (10 mg/kg) or saline (n = 8 each).

(E and F) Mice were treated with cocaine as in (C) and (D), and coronal sections of the brain were prepared 30 min after cocaine or saline treatment and subjected to in situ hybridization with [^{35}S]-labeled c-*fos* cRNA. c-*fos* mRNA-positive cells were counted in the VP (E) and the SNr (F) from four to six brain sections of two to four animals and averaged. Columns and error bars represent the mean ± SEM. *$p < 0.05$, **$p < 0.01$; n.s., not significant.

Distinct Roles of Striatonigral and Striatopallidal Transmission in Reward-Based and Aversive Behavior

Dopamine neurons recorded in vivo exhibit two different patterns of firings, i.e., a burst of phasic firing and a slow single-spike or tonic firing (Grace et al., 2007; Schultz, 2007). The phasic firing is responsible for stimulating the low-affinity D1 receptor and is believed to be the functionally relevant signal involved in reward-related behavior (Grace et al., 2007; Mirenowicz and Schultz, 1994). The tonic firing is crucial for modulating the high-affinity D2 receptor and has been reported to be suppressed by aversive stimuli (Grace et al., 2007; Mirenowicz and Schultz, 1996; Ungless et al., 2004). The D-RNB and I-RNB mice showed a normal preference for chocolate over a standard food, when they were freely given access to the two foods (Figure S5). So we extended the CPP test to the naturally occurring appetitive reward learning (Figures 7A and 7B). Animals were trained by pairing a standard food with one chamber and a palatable chocolate food with the other chamber. After conditioning for 3 days, the wild-type and the I-RNB mice learned to visit the chocolate-paired chamber with no statistical difference between the two groups (Figure 7B). In contrast, this learning ability was markedly impaired in the D-RNB mice (Figure 7A). The direct pathway is thus critical in the adaptive mechanism of the naturally occurring reward learning.

The effect of transmission blockade on aversive learning was then examined by the one-trial inhibitory avoidance task. In this task, mice received electric shocks, when they entered from a light chamber to a preferred dark chamber. Aversive behavior was then tested 24 hr later by measuring latencies, in which the mouse avoided entering an electrically shocked dark chamber. The wild-type mice avoided entering the dark chamber after experiencing electric shocks. This aversive behavior was not impaired in the D-RNB mice (Figure 7C). In contrast, the I-RNB mice failed to show aversive behavior and entered the dark chamber with no significant difference before and after electric shocks (Figure 7D). The indirect pathway is thus critical for evoking aversive behavior.

To exclude the possibility that the deficit in aversive behavior was due to an impaired fear system, we examined the freezing response after applying electric footshocks (Figure 7E). During the three 1 min intervals after electric footshocks, all three animals showed immediate postshock freezing that increased by repeated presentation of footshocks. There was no statistical difference in extent or pattern of freezing among wild-type, I-RNB, and D-RNB mice.

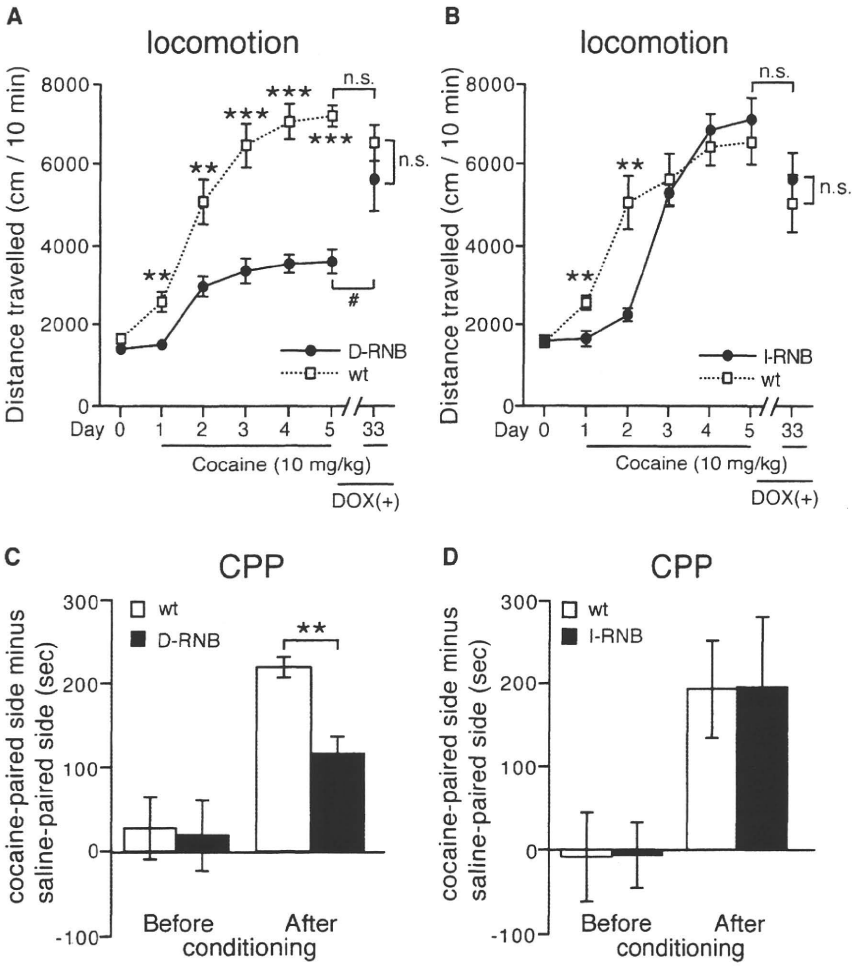

Figure 6. Impairment of Cocaine Sensitization in the D-RNB Mice

The V-S-tTA virus (A and C) or the V-E-tTA virus (B and D) was bilaterally injected into the NAc of wild-type and TN mice.

(A and B) Two weeks after the viral injection, the animals received i.p. saline once a day and were habituated to a novel chamber for 3 days. Cocaine (10 mg/kg) was then i.p. injected once a day from day 1 to day 5, and immediately after the cocaine injection, the locomotor activity was counted for a 10 min period. These animals were treated with DOX from day 6 to day 33, and on day 33, locomotor activity was counted for a 10 min period immediately after 10 mg/kg cocaine injection. Marks and error bars represent the mean ± SEM (n = 8 each). $^{**}p < 0.01$, $^{***}p < 0.001$ (WT versus RNB); #p < 0.05, (D-RNB on day 5 versus day 33); n.s., not significant. Repeated-measured ANOVA showed a significant difference between the virus-injected wild-type and RNB mice (in A, for genotype, $p < 0.001$; for day, $p < 0.001$; interaction genotype × day, $p < 0.001$; in B, for genotype, $p = 0.723$, for day, $p < 0.001$, interaction genotype × day, $p < 0.001$).

(C and D) CPP was developed by repeated cocaine (10 mg/kg) administration for 3 days and measured on day 4. Columns and error bars represent the mean ± SEM (C, n = 8 each; D, n = 6 or 7). $^{**}p < 0.01$.

It has been reported that repeated cocaine administration induces LTD in local field potentials of the NAc via D2 receptor stimulation (Goto and Grace, 2005a). We tested whether the cocaine sensitization could disrupt the synaptic plasticity involved in aversive behavior. In this test, cocaine was administered to wild-type mice for 5 days, and after a 3 day cocaine-free interval, aversive behavior was tested by the one-trial inhibitory avoidance task (Figure 7F). This test clearly indicated that cocaine sensitization severely impaired aversive behavior as compared with saline-injected control animals. Thus, similar to blockade of the indirect pathway, cocaine sensitization that alters the adaptive mechanisms of striatopallidal transmission causes impairment of aversive behavior. The striatonigral and striatopallidal transmission thus plays differential roles in reward-based and aversive behavior, respectively.

DISCUSSION

This study has established genetic manipulation that allowed determination of distinct roles of striatonigral and striatopallidal transmission in the basal ganglia circuitry. The expression of TN under the control of the SP and Enk promoters was mutually exclusive in the two types of MSNs; and the DOX-dependent TN expression was reflected in reversible cleavage of VAMP2, which is indispensable for transmitter release from the synaptic vesicles (Wada et al., 2007). The electrophysiological and c-*fos* mRNA analyses indicated that the selective expression of TN separately blunted transmission in the direct and indirect pathways. Furthermore, abnormal but reverse turning was induced by unilateral injection of the recombinant viruses, in agreement with the classical regulatory model of the two pathways in motor balance (Gerfen et al., 1990; Graybiel, 2000; Pycock, 1980). Thus, our gene-manipulating technique allowed selective and reversible blockade of striatonigral and striatopallidal transmission in a DOX-dependent manner.

This investigation has disclosed not only the coordinated function of these two pathways in acute psychostimulant responses but also unexpected, distinct roles of each pathway in reward-based and aversive behavior. The predominant role of the direct pathway in cocaine-induced adaptive responses is generally consistent with previous reports based on gene targeting and pharmacological analyses (Baker et al., 1996, 1998; Caine et al., 2007; Smith et al., 2002; Welter et al., 2007; Xu et al., 2000; but see also Miner et al., 1995). However, if the coordinated regulation by these two pathways, as is generally accepted, is a key mechanism of rewarding and aversive learning behavior, blunting one of the pathways would impair the learning ability of both rewarding and aversive behavior.

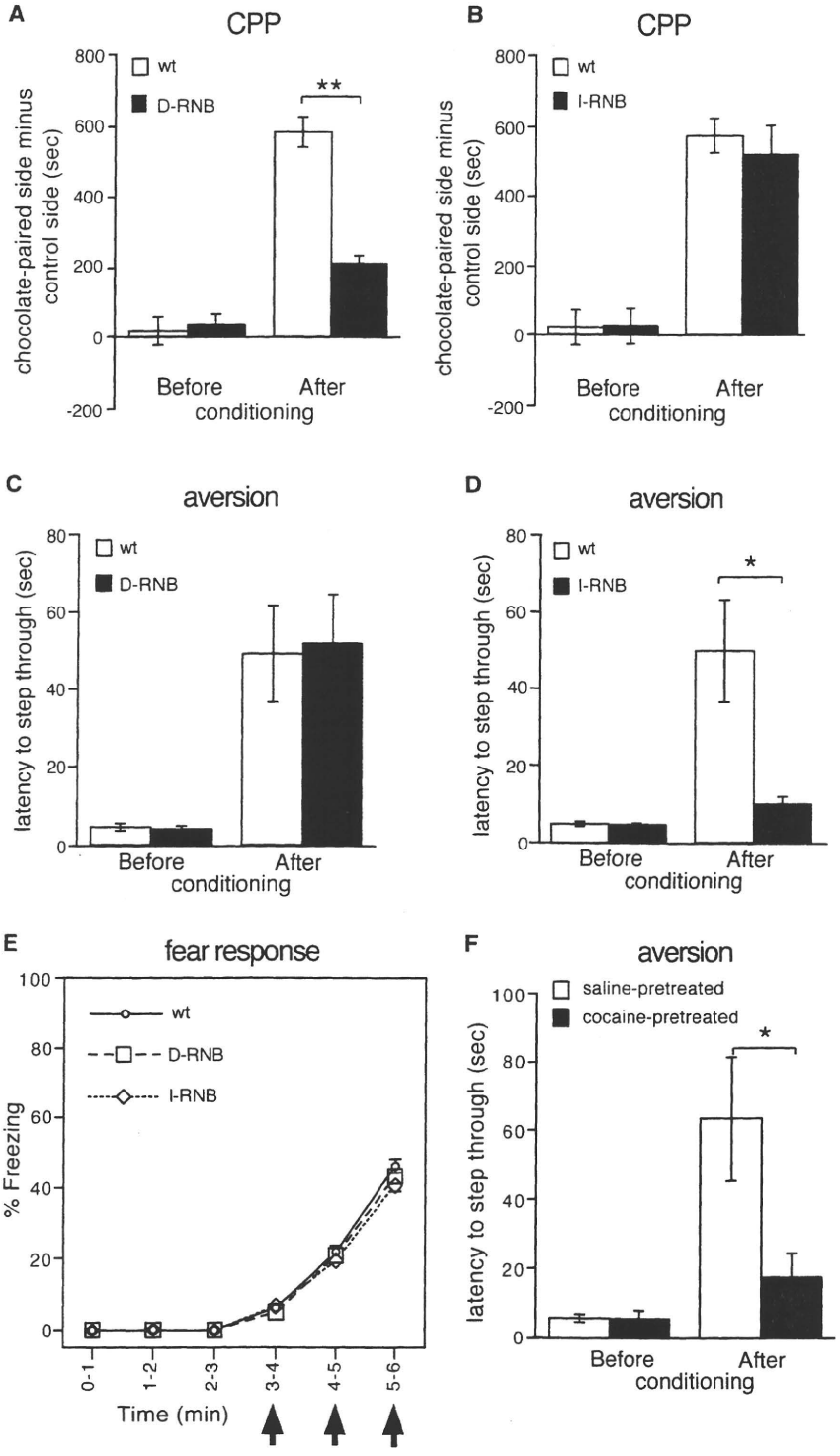

Figure 7. Analysis of Striatonigral and Striatopallidal Transmission in Reward-Based and Aversive Learning Behavior

(A and B) Two weeks after the viral injection into the NAc, animals were trained by pairing a standard food with one chamber and a chocolate food with the other chamber for 3 days. CPP for appetitive reward was then measured on day 4 (n = 6 each).

(C and D) Two weeks after the viral injection into the NAc, retention of aversive memory was tested by the one-trial inhibitory avoidance task. When mice moved from a light chamber to a preferred dark chamber, electric shocks were delivered. Memory retention was tested 24 hr later by measuring latencies for the animals to enter the dark chamber (n = 6 or 7).

(E) Percentage freezing was determined for the 1 min period by giving electric shocks at 3, 4, and 5 min (arrows) (n = 4–9). ANOVA with repeated-measures revealed a significant increase in post-stimulus freezing but no statistical difference among the three groups of mice (for genotype, $p = 0.297$; for time, $p < 0.001$; interaction genotype × time, $p = 0.53$).

(F) Wild-type mice received cocaine (10 mg/kg) for 5 days. After a cocaine-free interval for 3 days, memory retention for aversive stimuli was analyzed as described in (C) and (D) (n = 6 or 7). Columns and error bars represent the mean ± SEM. $^*p < 0.05$, $^{**}p < 0.01$.

In contrast, the present investigation has disclosed that each pathway has a differential and selective role in rewarding and aversive behavior. Because the aversive and rewarding behaviors were tested 1 day after footshocks and 3 days after training, respectively, impairments of these behaviors could have resulted from different temporal effects of transmission blockade on these behaviors. However, this possibility is unlikely, because blockade of each pathway abolished the initial stage of cocaine-induced hyperlocomotion of naive mice. In reward experiments, animals were allowed to choose freely between two chambers. In contrast, in aversive experiments, animals need to inhibit a prepotent response to enter into a preferred dark room. Impairments of aversive behavior could thus reflect an impulsivity when animals are faced with decision conflict. The indirect pathway may thus be more widely involved in behaviors of self-control in the so-called "NoGo" pathway (Frank, 2005; Frank et al., 2007).

The cocaine-induced adaptive response is exerted by multiple processes, at least triggering, execution, and storage of addictive behaviors (Hyman et al., 2006). The present study has indicated that the adaptive mechanism is endowed and saved during blockade of the direct pathway and induces normal levels of cocaine-mediated hyperlocomotion upon recovery of this transmission. Interestingly, it has been reported that the pharmacological inactivation of the striatum impairs the conditioned