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Report

Direct Regulation of Adult Brain Function by the Male-Specific Factor SRY

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Summary

The central dogma of mammalian brain sexual differentiation has contended that sex steroids of gonadal origin organize the neural circuits of the developing brain [1]. Recent evidence has begun to challenge this idea and has suggested that, independent of the masculinizing effects of gonadal secretions, XY and XX brain cells have different patterns of gene expression that influence their differentiation and function [2]. We have previously shown that specific differences in gene expression exist between male and female developing brains and that these differences precede the influences of gonadal hormones [3]. Here we demonstrate that the Y chromosome-linked, male-determining gene *Sry* is specifically expressed in the substantia nigra of the adult male rodent in tyrosine hydroxylase-expressing neurons. Furthermore, using antisense oligodeoxynucleotides, we show that *Sry* downregulation in the substantia nigra causes a statistically significant decrease in tyrosine hydroxylase expression with no overall effect on neuronal numbers and that this decrease leads to motor deficits in male rats. Our studies suggest that *Sry* directly affects the biochemical properties of the dopaminergic neurons of the nigrostriatal system and the specific motor behaviors they control. These results demonstrate a direct male-specific effect on the brain by a gene encoded only in the male genome, without any mediation by gonadal hormones.

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Results and Discussion

Localization of SRY in the Brain

To examine the expression of *Sry* mRNA in the adult mouse brain, we analyzed consecutive coronal sections by radioactive in situ hybridization. Specific labeling of *Sry* was observed in the substantia nigra (SN) of the midbrain and the medial mammillary bodies (MMB) of the hypothalamus of male, but not female, mice (Figures 1A–1C). Lower levels of *Sry* expression were observed sparsely throughout the cortex. Primers that distinguish between circular and linear *Sry* mRNA forms were used [4] in RT-PCR, confirming that *Sry* mRNA transcripts in the midbrain and cortex of the adult male brain were of the linear form (data not shown). Examination of brains of male and female *Sry*-enhanced green fluorescent protein (EGFP) transgenic adult mice, in which 7.7 kb of the *Sry* promoter drives the expression of the reporter EGFP [5], also revealed positive *Sry* expression in the SN (Figures 1D and 1E). This observation confirms our in situ hybridization results and suggests that regulatory sequences for brain expression of *Sry* are contained within 7.7 kb of the start transcription. These data are consistent with previous reports of *Sry* expression in the adult mouse hypothalamus, midbrain, and cortex [6, 7] but provide a detailed survey of its expression.

Sry protein was observed in all regions of the SN, with most SRY immunoreactive cells in the pars compacta region of adult male mouse (Figure 1F [SNc]) and rat brains (Figure S1). Sry staining was observed in both the cytoplasm and the nucleus of cells; the nucleus is the presumed site of action of SRY as a DNA binding transcription factor. To determine whether SRY was colocalized with tyrosine hydroxylase (TH) in the SN, we performed double-label immunohistochemistry for SRY and TH (Figure 1F). Ten percent of all cells (DAPI stained) were SRY positive. All SRY-positive cells were also TH-positive neurons. Thus, SRY protein is produced in the adult male mouse SN and is localized exclusively in TH-positive neurons. Sry staining was absent in the female mouse SN. (Figure 1G).

Before evaluating the role of Sry function in the SN, we first tested whether the number of TH neurons in the rat SN was sexually dimorphic. In females, the number of TH-ir neurons was 20% less than the number in age-matched male controls (Figure 2C, $p = 0.032$). This indicates that the expression of TH, the rate-limiting enzyme of the dopaminergic pathway, is sexually dimorphic in rats, which correlates with previous findings in mice [8].

Effects of *Sry* on TH Neurons in the SN and Striatum

To investigate the effect of *Sry* on TH neurons in vivo and consequent motor behavior in rats, we specifically downregulated *Sry* in the SN by an antisense targeting method [9]. *Sry* antisense oligodeoxynucleotides (ODN; see Table 1) were microinfused unilaterally into the SNs of male rats. As a control, the contralateral SN was infused with *Sry* sense ODN. Infusion of *Sry*

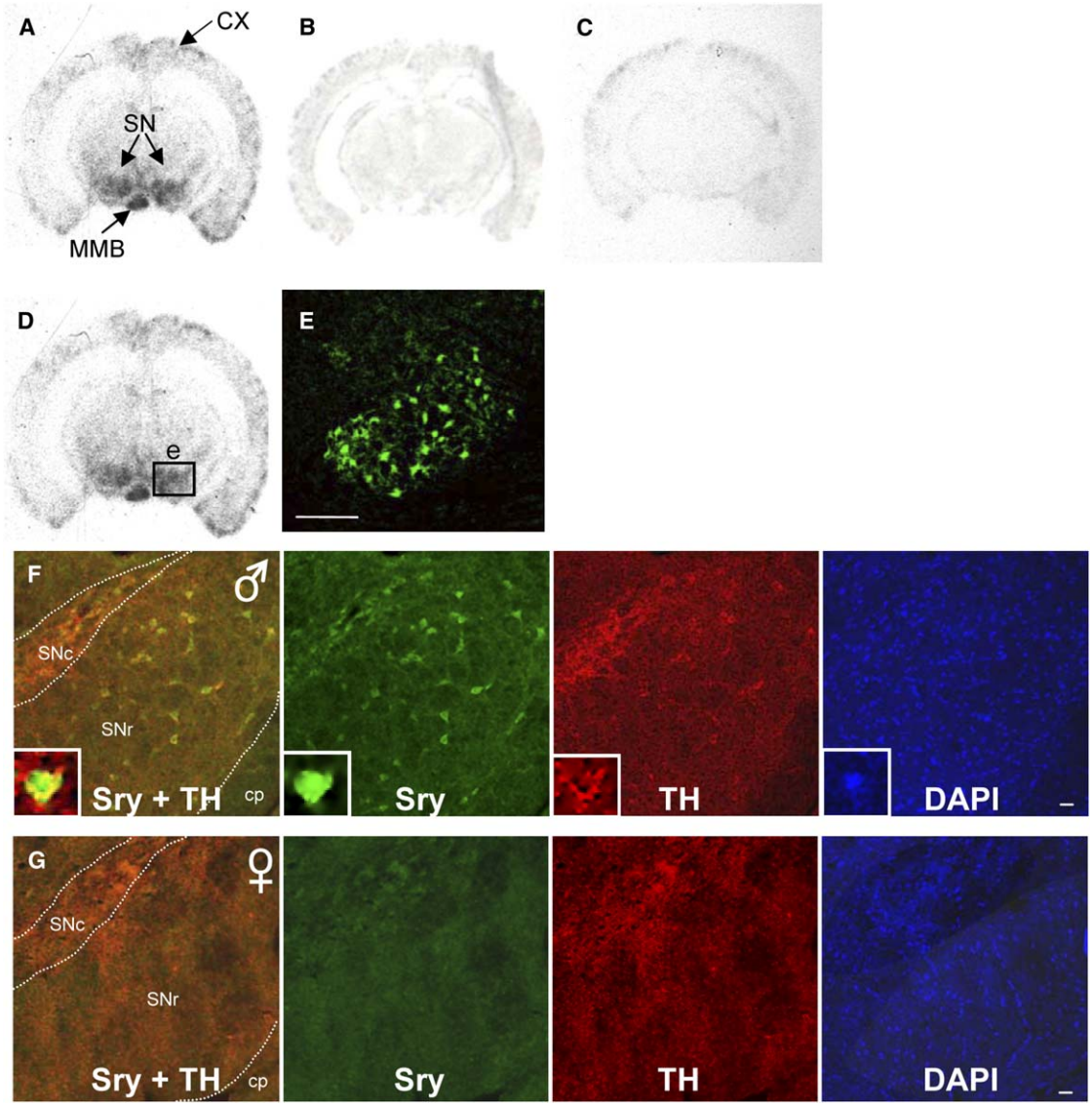

Figure 1. Localization of Sry in Specific Regions of the Adult Mouse Brain

(A) In situ hybridization using either labeled antisense or sense *Sry* cRNA probes localized *Sry* expression specifically to the medial mammillary bodies (MMB), substantia nigra (SN), and to a lesser extent in the cortex (CX).

(B) Control *Sry* sense probes revealed no signal in any regions on comparable sections in the adult male mouse brain.

(C) Antisense *Sry* probes revealed no specific hybridization in adult female mouse brains.

(D and E) *Sry* expression in the SN (E) was confirmed with *Sry*-EGFP transgenic mice. The box in panel (D) is indicative of the region represented in (E).

(F) In the adult male mouse SN, Sry protein (green) localized to both the cytoplasm and nuclei of TH-ir neurons (red). The inset shows a representative TH- and Sry-ir neuron (magnified). All cells in a representative field for Sry/TH co-labeling (n = 4923) were counted and scored. The scale bar represents 25

(G) Sry staining was absent in adult female mouse SN.

The scale bar represents 100 μm (E) and 25 μm (F and G).

antisense ODN into the SN reduced the number of TH-ir neurons by 38% in comparison to the side infused with sense ODN, as quantified with an optical fractionator sampling design [10] (Figures 2A, 2B, and 2D, p = 0.0022). A higher magnification indicates that neural degeneration in TH-positive cells had not occurred (Figure 2B, inset). RT-PCR confirmed the decrease in *Sry* mRNA expression in vivo in rats infused with antisense *Sry* ODN (Figure S2). Adjacent coronal brain sections stained with thionin (Nissl staining) revealed no difference in the number of cells resembling neurons in treated versus untreated SNs (Figure S3). Furthermore, females treated unilaterally with *SRY* antisense ODN and contralaterally with sense ODN displayed no difference in the number of SN TH-ir neurons (Figure 2E), suggesting that the *Sry* antisense cocktail used in this study was specific for the *Sry* gene. These results suggest that the reduction of TH-positive neurons in males was due

Table 1. Base Sequences and Positions of Antisense and Sense Oligodeoxynucleotides

ODN	Sequence	Target Region of mRNA
Antisense ODN 1	*GCG*CTTGACATGGCCCTC*CAT*	+1 to +21
Antisense ODN 2	*CAT*GGGGCGCTTGACATGGC*CC*	+5 to +27
Antisense ODN 3	GGCCCTCCATGCTATCTAGA	−10 to +10
Control (sense) ODN 1	*ATG*GAGGGCCATGTCAAG*CG*C	+1 to +21
Control (sense) ODN 2	*A*GGGCCATGTCAAGCGCCCC*AT*	+5 to +27
Control (sense) ODN 3	TCTAGATAGCATGGAGGGCC	−10 to +10

Numbering starts at the initiation codon (the complementary triplet is underlined). Italic nucleotides are phosphorothioated.

to a reduction in TH expression rather than a loss of TH-ir neurons.

TH neurons in the SN project to the striatum and provide dopamine innervation. Thus, we tested whether *Sry* antisense ODN infusions in the SN altered TH immunostaining in the striatum. Quantification of striatal TH-ir by optical density demonstrated that treatment with antisense ODN reduced TH immunostaining 26% in comparison to SRY sense control sides (Figure 2F, p = 0.042).

To demonstrate that the *Sry* actions on TH neurons observed in our studies were specific, we investigated whether the expression of Nur-related factor 1 (Nurr1) was affected by our ODN infusions. Nurr1 is expressed in the midbrain of developing mice at embryonic day 10.5, a time before TH is detected, and continues to be expressed into adulthood. In Nurr1 knockout mice, developing neurons do not acquire dopaminergic markers such as TH, and they ultimately die as development progresses [11, 12]. Using adjacent brain slides that

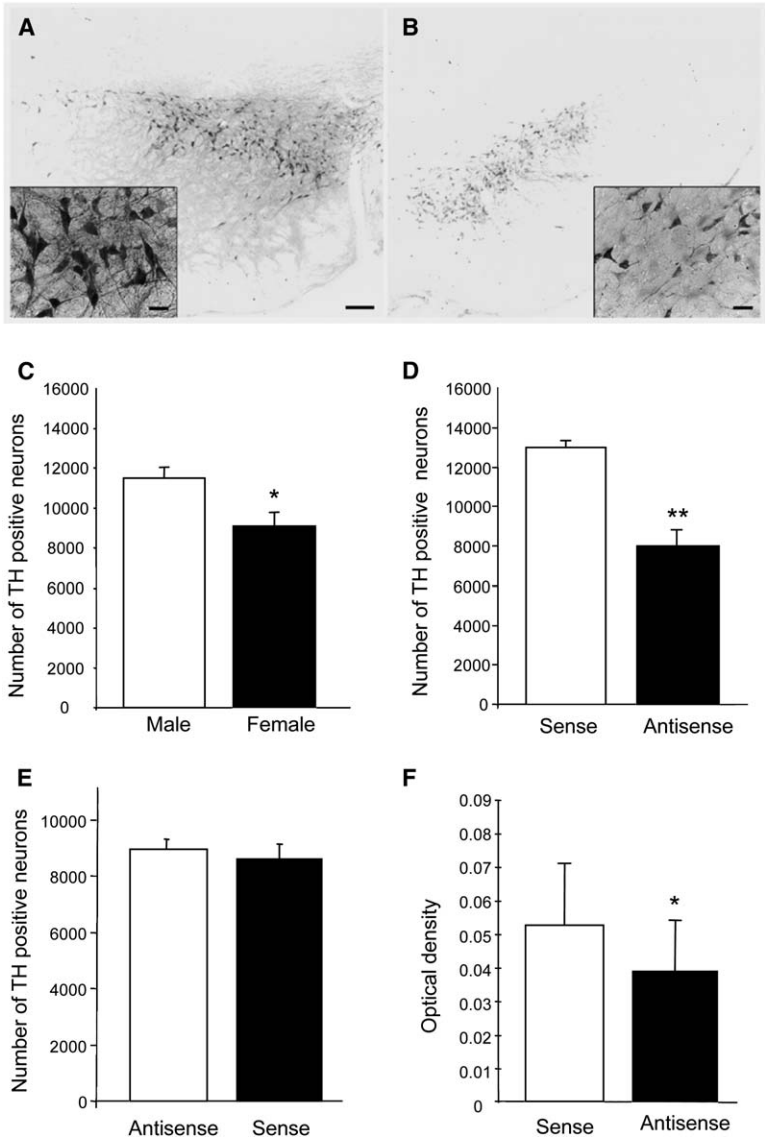

Figure 2. *Sry* Antisense ODN Decreases the Number of TH-ir Neurons in the SN

(A) Unilateral infusions of *Sry* sense ODN had no effect on the number of TH-ir neurons and served as an internal control.

(B Unilateral infusions of *Sry* antisense ODN significantly reduced the number of TH-ir neurons in the SN. Panels (A) and (B) show TH-ir labeling from the same animal. Inset: 63× magnification.

(C) In a comparison of age-matched control male and female rats, the number of TH-positive neurons in the SN of females was 20% smaller than in males (mean ± SEM; n = 4 for each group).

(D) Infusions of *Sry* antisense ODN in the SN reduced the number of TH-ir neurons by 38% in comparison to sense-ODN-infused SN (mean ± SEM; n = 6).

(E) No difference between *Sry* antisense or sense ODN was observed in the number of TH-ir neurons in female rats (mean ± SEM; n = 4).

(F) Optical density measured a 26% decrease in striatal TH-ir insides infused with *Sry* antisense ODN versus those infused with sense ODN (n = 5).

The scale bar represents 200 μm and 10 μm (insets).

Table 2. Data from Sensorimotor-Behavioral Tests

	Pre-ODN Infusion			Post-ODN Infusion			Recovery		
	Contra	Ipsi	I − C	Contra	Ipsi	I − C	Contra	Ipsi	I − C
Akinesia test (mean number of steps/10 s ± SEM)	13.8 ± 0.5	14.0 ± 1.3	0.2 ± 1.8	7.3 ± 1.5	17.7 ± 2.3	10.4 ± 3.8	13.0 ± 0.2	12.5 ± 0.2	−0.5 ± 0.4
Limb-use asymmetry test (mean percent of limb use ± SEM)	64.8 ± 6.6	35.2 ± 6.6	−29.6 ± 13.2	42.4 ± 9.6	57.6 ± 9.6	15.2 ± 19.2	N/A	N/A	N/A

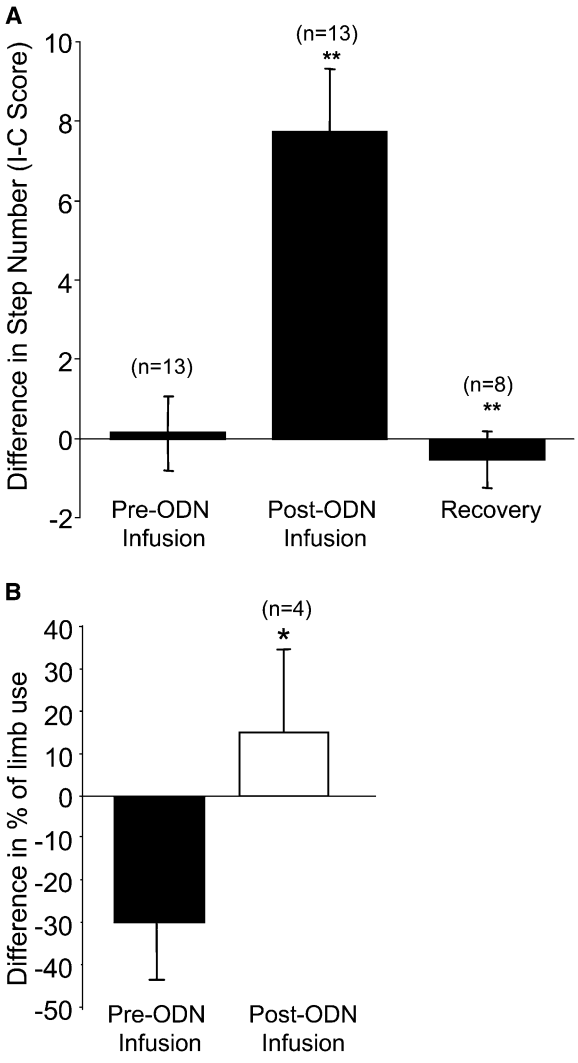

Figure 3. *Sry* Downregulation in the SN Induces Deficits in Sensorimotor Behaviors

(A) Akinesia tests revealed a significant decrease in the number of steps taken by the contralateral forelimb in animals infused with *Sry* antisense ODN. Unilateral downregulation of TH-positive neurons caused animals to use their contralateral forelimbs by 58.8% more than pre-*Sry* antisense ODN infusions. The overall index of the akinesia score (I − C; mean ± SEM) of 0.2 pre-ODN infusion reflects equal usage of both limbs. A significant, positive I − C score of 10.4 observed in post-ODN infusion experiments revealed a significant bias for ipsilateral limb use. After 7 days of no ODN infusion, an I − C score of −0.5 was observed, indicating full recovery of ipsilateral limb usage.

(B) Limb-use asymmetry test also revealed a 35% (mean ± SEM) decrease in limb use after infusions of *Sry* antisense ODN. Animals demonstrated an ipsilateral-forelimb bias for usage in cylinder exploration after infusions of *Sry* antisense ODN, as determined by a significant I − C score. The reluctance toward contralateral forelimb usage correlates with the downregulation of TH-positive neurons in the SN. I − C is a single overall score that is obtained by subtracting the use value of the contralateral limb from that of the ipsilateral limb.

have shown differences in TH-ir in the SN, we did not observe any differences between antisense-*Sry*-ODN-infused brains and sense-ODN-infused brains (data not shown) when we performed immunostaining with Nurr1 antibody, suggesting that *Sry* does not directly affect the expression of *Nurr1*.

SRY Effects on Sensorimotor Function

A decrease in SN dopamine causes quantifiable deficits in sensorimotor function [13]. The effect of *Sry* antisense ODN in the SN on specific motor functions was assessed by the akinesia and limb-use asymmetry tests [14]. In the akinesia test, animals with unilateral dopamine depletions take fewer steps with their forelimb contralateral to the depletion than they do with their ipsilateral forelimb. Unilateral *Sry* antisense infusions reduced the number of steps taken by the contralateral forelimbs by 58.8% (Table 2). A pre-ODN infusion I − C (ipsilateral minus contralateral) score of 0.2 almost reflected equal usage of both forelimbs in stepping when individual limbs were examined. However, after ODN infusion, animals stepped more readily when ipsilateral limbs were isolated, as represented by a significant positive I − C of 10.4, suggesting a statistically significant deficit in contralateral limb usage (Figure 3A, $p < 0.001$). In addition, animals with unilateral *Sry* antisense ODN infusions exhibited a similar decrease in TH expression in the SN, and this decrease was associated with asymmetrical limb usage. In this test, we observed a 26.4% decrease in contralateral limb usage after infusion of *Sry* antisense ODN (Table 2). The negative I − C score during pre-ODN infusion trials (−29.6 ± 13.2) indicated an inherent bias by the animals toward favoring their contralateral limb during cylinder exploration. However, the tendency of limb usage switched to significantly favoring the ipsilateral limb that was associated with sense ODN treatments during post-ODN trials (Figure 3B, $p = 0.013$). Both sets of behavior tests exhibited similar trends where animals uniformly decreased their contralateral limb usage and began

favoring the use of their ipsilateral limbs. These data suggest that *Sry* downregulation in the SN reduces motor behaviors and are entirely consistent with the effect of Sry on TH expression in the nigrostriatal system.

To verify that the decrease in TH-ir neurons in the SN, and the consequent motor-behavior deficit observed in our rats, were not attributed to generalized neurotoxicity brought about by the administration of antisense ODN, we assessed animals for motor behavior 7 days after termination of the antisense ODN treatment. Because the effect of the ODN should be transient, some, if not all, recovery of both the activity of the *Sry* gene product and motor behavior should occur. Using the akinesia test, we observed no difference in contralateral or ipsilateral limb usage when the I − C value returned to near zero (−0.5 ± 0.7) (Figure 3A, p = 0.001) after 7 days of no ODN infusions, indicating a complete recovery in contralateral limb usage.

Conclusions

The experiments described here provide evidence that the testis-determining gene *Sry* is expressed and translated in specific regions of the male mammalian adult brain and that its role in the SN is to maintain biochemical and motor functions of dopaminergic neurons. These regions interestingly correspond to areas of the brain that are subject to sexual differentiation [15]. Modulation of TH expression is complex and involves transcriptional control, alternative RNA processing, and regulation of RNA stability [16]. Our data indicate that *Sry* may control TH expression in the SN via both direct and indirect mechanisms. Direct transcriptional control of TH by *Sry* is possible in a subset of cells co-expressing the proteins and is supported by the reported Sry-mediated activation of the *TH* gene promoter and by in vitro transfection experiments [17]. Sry might act to increase the number of TH-positive neurons in the SN to a male level. When *Sry* is absent, as is the case in females, the number of TH-positive neurons remains at a lower level. However, this does not explain why downregulation of *Sry* causes nigrostriatal deficits because females do not exhibit overt motor dysfunction. An alternative view is that *Sry* could compensate for a factor that is only present in females and maintains TH expression in SN neurons. Estrogens have been shown to influence TH in dopaminergic neurons: Short-term administration of estradiol benzoate increases the levels of TH in dopaminergic neurons [18], and short-term ovariectomy results in a reversible decrease in the number of TH-positive neurons [19]. The role of *Sry* in the brain, to generate sex differences in behavior, as well as to compensate for other (e.g., hormonally induced) sex differences [20], is consistent with the dual-function hypothesis for neural sex differences [21]. The precise molecular mechanisms by which Sry affects dopaminergic neurons remain to be elucidated. Demonstration of a specific function for Sry in the SN may provide a new model for a direct genetic effect on a brain sexual dimorphism that is not mediated by the effects of gonadal hormones.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures and three figures and are available with this article online at http://www.current-biology.com/cgi/content/full/16/4/415/DC1/.

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References

1. Morris, J.A., Jordan, C.L., and Breedlove, S.M. (2004). Sexual differentiation of the vertebrate nervous system. Nat. Neurosci. *7*, 1034–1039.
2. Arnold, A.P., and Burgoyne, P.S. (2004). Are XX and XY brain cells intrinsically different? Trends Endocrinol. Metab. *15*, 6–11.
3. Dewing, P., Shi, T., Horvath, S., and Vilain, E. (2003). Sexually dimorphic gene expression in mouse brain precedes gonadal differentiation. Brain Res. Mol. Brain Res. *118*, 82–90.
4. Mayer, A., Mosler, G., Just, W., Pilgrim, C., and Reisert, I. (2000). Developmental profile of Sry transcripts in mouse brain. Neurogenetics *3*, 25–30.
5. Albrecht, K.H., and Eicher, E.M. (2001). Evidence that Sry is expressed in pre-Sertoli cells and Sertoli and granulosa cells have a common precursor. Dev. Biol. *240*, 92–107.
6. Lahr, G., Maxson, S.C., Mayer, A., Just, W., Pilgrim, C., and Reisert, I. (1995). Transcription of the Y chromosomal gene, Sry, in adult mouse brain. Brain Res. Mol. Brain Res. *33*, 179–182.
7. Mayer, A., Lahr, G., Swaab, D.F., Pilgrim, C., and Reisert, I. (1998). The Y-chromosomal genes SRY and ZFY are transcribed in adult human brain. Neurogenetics *1*, 281–288.
8. Vadasz, C., Baker, H., Fink, S.J., and Reis, D.J. (1985). Genetic effects and sexual dimorphism in tyrosine hydroxylase activity in two mouse strains and their reciprocal F1 hybrids. J. Neurogenet. *2*, 219–230.
9. Landgraf, R. (1996). Antisense targeting in behavioural neuroendocrinology. J. Endocrinol. *151*, 333–340.
10. Gundersen, H.J., Bagger, P., Bendtsen, T.F., Evans, S.M., Korbo, L., Marcussen, N., Moller, A., Nielsen, K., Nyengaard, J.R., Pakkenberg, B., et al. (1988). The new stereological tools: Disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. APMIS *96*, 857–881.
11. Zetterstrom, R.H., Solomin, L., Jansson, L., Hoffer, B.J., Olson, L., and Perlmann, T. (1997). Dopamine neuron agenesis in Nurr1-deficient mice. Science *276*, 248–250.
12. Saucedo-Cardenas, O., Quintana-Hau, J.D., Le, W.D., Smidt, M.P., Cox, J.J., De Mayo, F., Burbach, J.P., and Conneely, O.M. (1998). Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. Proc. Natl. Acad. Sci. USA *95*, 4013–4018.
13. Whishaw, I.Q., Woodward, N.C., Miklyaeva, E., and Pellis, S.M. (1997). Analysis of limb use by control rats and unilateral DA-depleted rats in the Montoya staircase test: movements, impairments and compensatory strategies. Behav. Brain Res. *89*, 167–177.
14. Schallert, T., Fleming, S.M., Leasure, J.L., Tillerson, J.L., and Bland, S.T. (2000). CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. Neuropharmacology *39*, 777–787.
15. Vadasz, C., Kobor, G., Kabai, P., Sziraki, I., Vadasz, I., and Lajtha, A. (1988). Perinatal anti-androgen treatment and genotype affect the mesotelencephalic dopamine system and behavior in mice. Horm. Behav. *22*, 528–539.
16. Kumer, S.C., and Vrana, K.E. (1996). Intricate regulation of tyrosine hydroxylase activity and gene expression. J. Neurochem. *67*, 443–462.

17. Milsted, A., Serova, L., Sabban, E.L., Dunphy, G., Turner, M.E., and Ely, D.L. (2004). Regulation of tyrosine hydroxylase gene transcription by Sry. Neurosci. Lett. *369*, 203–207.
18. Serova, L.I., Maharjan, S., Huang, A., Sun, D., Kaley, G., and Sabban, E.L. (2004). Response to tyrosine hydroxylase and GTP cyclohydrolase I gene expression to estrogen in brain catecholaminergic regions varies with mode of administration. Brain Res. *1015*, 1–8.
19. Leranth, C., Roth, R.H., Elsworth, J.D., Naftolin, F., Horvath, T.L., and Redmond, D.E., Jr. (2000). Estrogen is essential for maintaining nigrostriatal dopamine neurons in primates: implications for Parkinson's disease and memory. J. Neurosci. *20*, 8604–8609.
20. De Vries, G.J. (2004). Minireview: Sex differences in adult and developing brains: compensation, compensation, compensation. Endocrinology *145*, 1063–1068.
21. De Vries, G.J., and Boyle, P.A. (1998). Double duty for sex differences in the brain. Behav. Brain Res. *92*, 205–213.