The hTH-GFP rat: a novel model for the study of Parkinson's disease

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INTRODUCTION

Currently, there are a number of transgenic mouse lines that have been used to study Parkinson disease (PD), including those in which rat or human tyrosine hydroxylase (TH) and dopamine transporter (DAT) have been engineered to drive expression of EGFP reporter protein expression in midbrain dopamine (DA) neurons of the substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA) and in their respective terminals in the striatum and cortex. These models have enabled the study of PD, particularly when systemic MPTP is used to generate damage to DA neurons.

However, until now, there has been no transgenic reporter rat lines to facilitate these studies in vivo and in vitro. Thus, the Michael J. Fox Foundation (MJFF) partnered with Thomas Jefferson University and Taconic to generate new transgenic rat lines carrying 12kb of the human TH gene promoter driving EGFP. With the high levels of endogenous GFP, these novel hTH-GFP rat reporter lines allow for anatomical visualization and microdissection of the rat midbrain into SNpc and/or VTA and detailed analysis of midbrain DA neurons and axonal projections after toxin treatment in vivo and for purification of these neurons by FACS for in vitro studies.

MATERIALS AND METHODS

Five founder rat lines were generated using a 12kb hTH-GFP construct. Plasmid phTH-12kb-EGFP (pMAK 288-12) places the hTH promoter upstream of the EGFP reporter gene. DNA fragments including 10.794 kb of the distal hTH promoter, 1.168 kb of proximal hTH promoter were ligated to produce plasmid phTH-12kb-EGFP which was placed upstream of the EGFP reporter gene (Kessler et al., 2003).

RESULTS

hTH-GFP expression in the midbrain area of founder transgenic rat lines

Rat Line	12108		12121		12141		12142		12155-F	
	TH staining	GFP	TH staining	GFP	TH staining	GFP	TH staining	GFP	TH staining	GFP
Traditional CA sites										
Hypothalamus (A11–14)										
Arcuate n.	+++	-	+++	+	++	-	+++	-	++	-
Periventricular n.	++	+	+	+	++	+	++	-	++	-
Paraventricular n.	++	+	++	+	++	-	++	-	++	+
Zona incerta	+++	+++	+++	+++	+++	+++	+++	-	+++	++
Olfactory bulb	+++	+++	+++	+++	+++	+++	+++	-	+++	+
Substania nigra (A9)	+++	+++	+++	+++	+++	+++	+++	+	+++	+++
VTA(A10)	+++	+++	+++	+++	+++	+++	+++	+	+++	+++
Dorsal raphe n.	+	-	++	+	++	++	++	-	+	-
Locus ceruleus (A6)	++	-	++	+	++	-	++	-	++	-
A5	++	-	++	++	++	+	++	-	++	-
A2	++	+	++	++	++	+	++	-	++	-
A1	++	++	++	++	++	+	++	-	++	-
Area postrema	++	+	++	++	++	-	++	-	++	-
Adrenal gland	ND	ND	ND	ND	++	-	++	-	++	-
Striatum	++	++	++	++	++	++	++	-	++	++
Nontraditional CA sites										
Anteriao olfactory n.	-	+	-	+++	-	+	-	-	-	-
N. accumbens	-	+	-	+	-	-	-	-	-	-
Hippocampus	-	+	-	+	-	-	-	-	-	-
Cortex	-	+	-	+	-	-	-	-	-	-
Supraoptic n.	-	+	-	++	-	+	-	-	-	-
Habenular n.	-	++	-	++	-	-	-	-	-	-
Medial mammillary n.	-	+	-	+	-	-	-	-	-	-

Table 1. hTH-GFP expression was closely correlated with TH expression in the adult midbrains of Lines 12108, 12121, 12141, females of Line 12155. However, GFP was poorly expressed in the adult midbrains of Line 12142 and the males of Line 12155. Several lines exhibited hTH-GFP expression in other DA structures (striatum, olfactory bulb, hypothalamus). Only Line 12121 showed low levels of GFP in the noradrenergic LC nucleus and no lines expressed GFP in the adrenal medulla. Ectopic expression was low in all lines except Line 12121.

Line 12141 expresses high levels of hTH-GFP expression in TH+ DA neurons of the adult midbrain.

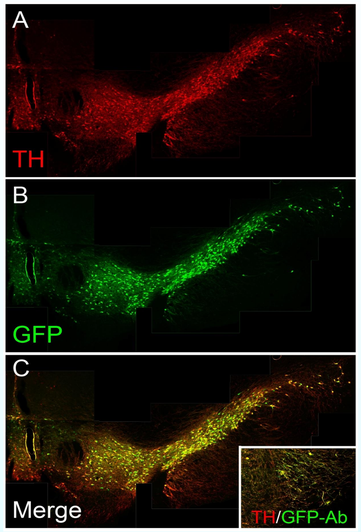


Figure 1. In particular Line 12141 faithfully showed TH staining (A) and high levels of endogenous GFP (B) fluorescence in midbrain cell bodies of the substantia nigra (SN) and ventral tegmental area (C). The inset of Panel (C) shows that the GFP antibody could detect signal in the fine dendritic processes emanating from the cell bodies into the SN pars reticulata (SNpr), which were not as easily visualized by endogenous GFP fluorescence.

Line 12141 shows hTH-GFP expression in other DA regions with little ectopic GFP in non-DA regions.

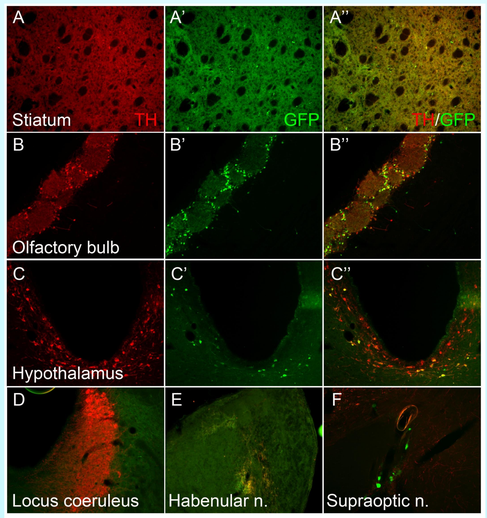


Figure 2. hTH-GFP expression matched TH expression in adult striatum (A-A"), olfactory bulb (B-B") of Line 12141. However, GFP expression was scarce in the hypothalamus (C-C") and absent in the locus coeruleus (D) while TH staining was robust. Ectopic GFP expression is seldom observed in Non-TH expressing areas such as habenular nucleus (E) and supraoptic nucleus (F) of Line 12141.

Line 12141 expresses GFP along with other mDA markers during development.

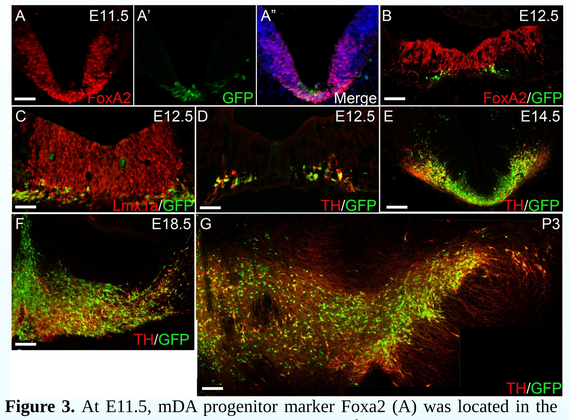


Figure 3. At E11.5, mDA progenitor marker Foxa2 (A) was located in the ventral midbrain ventricular zone while GFP (A') was visible in only a few cells in this region. At E12.5, Foxa2 (B), Lmx1a (C) and TH (D) were clearly located in the central ventral midbrain. TH was co-labeled with GFP in the ventral midbrain. At all stages examined, E14.5 (E), E18.5 (F) and P3 (G), GFP expression was well-correlated with TH in the midbrain.
Scale bar = 50 µm in A, C, D. Scale bar = 100 µm in B, E, F, G.

E14. 5 mDA neurons from Line 12141 can be highly enriched by FACS.

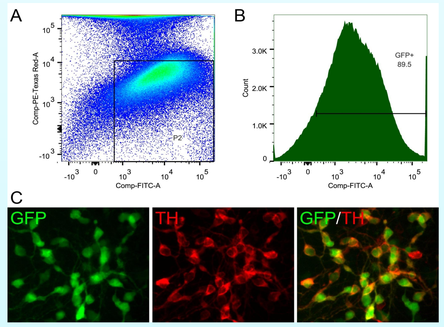
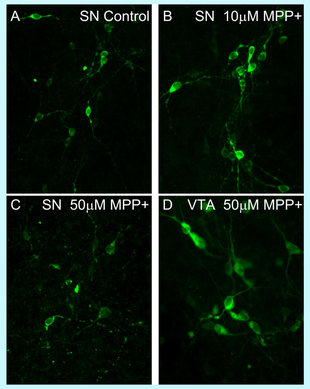


Figure 4. Gating for live GFP⁺ cells was established by utilizing positive controls for GFP and propidium iodide (PI) stained cells (not shown). The gate, P2 (A), excluded weakly GFP⁺ cells and any PI⁺ (dead) cells. This allowed for the collection of approximately 90% of GFP⁺ cells (B). Collected cells were plated at a density of 300,000cells/well, grown 3 days, fixed and TH-immunostained. TH and GFP co-labeled the same cells (C), as seen *in vivo*.

Treatment of Line 12141 with the neurotoxin MPP+ causes selective hTH-GFP+ neuronal death in the SN in vitro

Figure 5. E14.5 hTH-GFP+ SN and VTA neurons were grown for 7 days in defined media. (A) Control SN neurons were treated with media+PBS. (B-C) SN neurons were treated with MPP⁺ at 10 & 50µM for 48 hours. Neurons treated with 10µM MPP+ (B) exhibited healthy, undamaged morphology, while 50µM (C) treated SN neurons were fragmented and dead. Control (not shown) or 50µM MPP⁺ treated (D) VTA neurons exhibited normal, healthy morphology.



hTH-GFP rats in Line 12141 accurately models aspects of the disease in vivo.

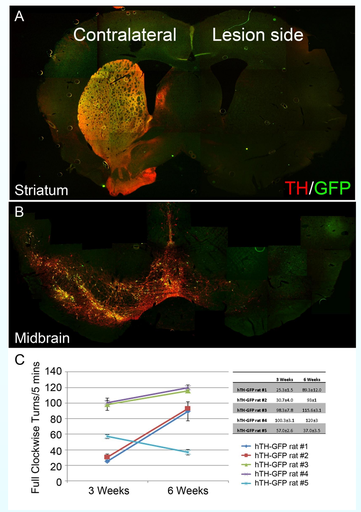


Figure 6. Six weeks after 6-OHDA lesion, GFP or TH expression was undetectable on the lesion side while both GFP and TH expression was unaffected in the contralateral striatum (A) and midbrain (B). Rotation tests were performed at 3 weeks and 6 weeks after lesion. The scores are listed and plotted in Panel (C). Most rats exhibited a robust lesion with an average of more than 50 full clockwise turns per 5 minutes.

Summary

Together these results have demonstrated that the hTH-GFP rat, particularly line 12141, exhibits high level specific EGFP fluorescence in DA brain structures (ie. SN, VTA, striatum, olfactory bulb, hypothalamus) with minimal ectopic expression elsewhere in the brain. This hTH-GFP reporter rat should serve as an important new model, having several major benefits over their wild type counterparts:

First, these rats allow for the microdissection of the embryonic mesencephalon in a fluorescence microscope, making it possible to segregate PD-susceptible DA neurons of the SN from PD-resistant DA neurons of the VTA for studies of disease pathogenesis in culture.

Second, the hTH-GFP reporter rat can be used to further purify GFP+ DA neurons by FACS sorting, dramatically enriching their yield in culture.

Thirdly, because GFP expression was more easily detected than TH immunostaining at early developmental stages, the reporter rat may be particularly useful for early embryonic studies as TH+ dopamine neurons arise from a population of Foxa2+ floor plate cells co-expressing the DA-fate gene Lmx1a.

Finally, the hTH-GFP reporter rat will greatly facilitate studies on the most widely used in vivo model of PD, the 6-OHDA rat.

ACKNOWLEDGEMENTS

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