**Distribution Agreement**

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_

Samuel J. Rose Date

**A new mouse model of l-DOPA-responsive dystonia**

By

Samuel J. Rose

Doctor of Philosophy

Graduate Division of Biological and Biomedical Science

Neuroscience

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Ellen J. Hess, PhD

Advisor

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

H.A. Jinnah, MD/PhD

Committee Member

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Gary W. Miller, PhD

Committee Member

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Yoland Smith, PhD

Committee Member

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

David Weinshenker, PhD

Committee Member

Accepted:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Lisa A. Tedesco, Ph.D. Dean of the James T. Laney School of Graduate Studies

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date

**A new mouse model of l-DOPA-responsive dystonia**

By

Samuel J. Rose

B.S., University of Georgia, 2008

Advisor: Ellen J. Hess, PhD

An abstract of a dissertation submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

In partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Neuroscience

2015

**Abstract**

A new mouse model of l-DOPA-responsive dystonia

By

Samuel J. Rose

Dystonia is a neurological movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements or postures. Abnormal dopamine neurotransmission is associated with many different dystonic disorders. For instance, mutations in genes critical for the synthesis of dopamine, including GTP cyclohydrolase 1and tyrosine hydroxylasecause l-DOPA-responsive dystonia. Despite evidence that implicates abnormal dopamine neurotransmission in dystonia, the precise nature of the dopaminergic defects that result in dystonia is not known. To better understand these defects, we generated a knockin mouse model of l-DOPA-responsive dystonia that recapitulates the human p.381Q>K TH mutation (c.1141C>A). Mice homozygous for this mutation (DRD mice) had reduced TH activity throughout the brain and striatal dopamine concentration that were ~1% of normal. Although the gross anatomy of the nigrostriatal dopaminergic neurons was normal in DRD mice, the microstructural target of corticostriatal synapses was affected; corticostriatal input in DRD mice showed a shift away from synapses on dendritic spines towards dendrites themselves. DRD mice displayed the core behavioral features of the human disorder, including dystonia that worsened throughout the course of the active phase, and improvement in the dystonia in response to both l-DOPA and trihexyphenidyl. Administration of D1- or D2-type dopamine receptor agonists reduced the dystonic movements while administration of D1- or D2-type dopamine receptor antagonists worsened the dystonia, suggesting that both receptors mediate the dystonic movements. Further, D1-dopamine receptors were supersensitive; adenylate cyclase activity, locomotor activity and stereotypy were exaggerated in DRD mice in response to the D1-dopamine receptor agonist SKF 81297. D2-dopamine receptors responses were blunted or altered in DRD mice with an increase in adenylate cyclase activity and blunted behavioral responses after challenge with the D2-dopamine receptor agonist quinpirole. Together, the findings here implicate developmental dopamine loss within a specific range in the development of dystonia. Further, they implicate maladaptive changes to dopamine receptor responses as important factors for this disorder.

**A new mouse model of l-DOPA-responsive dystonia**

By

Samuel J. Rose

B.S., University of Georgia, 2008

Advisor: Ellen J. Hess, PhD

A dissertation submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

In partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Neuroscience

2015

**Table of Contents**

***Chapter 1: Introduction***

Dystonia\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ 1

Neuroanatomical substrates of dystonia\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ 4

Basal ganglia function and dysfunction in dystonia\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ 5

Cerebellar dysfunction in dystonia\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ 10

Dysfunctional DA neurotransmission as a common molecular pathway in dystonia\_\_11

Tyrosine hydroxylase\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_15

DA receptors\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ 16

DA and the development of the basal ganglia\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_19

Evidence for DA dysfunction in dystonia\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_20

DRD as a disorder prototypical of dystonia arising from DA dysfunction\_\_\_\_\_\_\_\_\_\_\_23

DRD: Clinical features\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_24

Mutations in tetrahydrobiopterin synthesizing enzymes\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_25

Mutations in TH\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_25

Animal models closely resembling DRD\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_27

Neonatal 6-OHDA-treated rats\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_27

MPTP-treated primates\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_29

Mice modeling impaired BH4 synthesis\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_29

Mutant TH lines\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_31

Summary and guiding questions of thesis work\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_31

***Chapter 2: In vivo molecular, neurochemical, and anatomical effects of the p.382Q>K mutation in TH***

Abstract\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_33

Introduction\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_34

Methods\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_35

Results\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_42

PCR conformation of DRD knockin allele\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_42

Generating homozygous DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_46

DRD mice exhibit reduced TH activity and brain catecholamines\_\_\_\_\_\_\_\_\_\_\_50

Normal gross anatomy of midbrain and striatum in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_54

Microstructural changes to corticostriatal and thalamostriatal connectivity\_\_\_\_56

Discussion\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_60

***Chapter 3: Behavioral phenotype of the DRD mice: special emphasis on diurnal fluctuations and drug responses.***

Abstract\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_64

Introduction\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_65

Methods\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_66

Results\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_70

Dystonic movements in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_70

Neurochemistry correlates with diurnal fluctuations\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_75

Behavioral responses to indirect catecholamine agonists\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_79

Discussion\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_87

***Chapter 4: DA receptor-mediated responses in DRD mice***

Abstract\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_93

Introduction\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_94

Methods\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_96

Results\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_100

DA receptors mediate dystonic movements\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_100

Abnormal DA receptor responses in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_102

*Ex vivo* DA receptor expression and second messenger responses\_\_\_\_\_\_\_\_107

Discussion\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_110

***Chapter 5: General Discussion***

DRD mice: contribution to the field\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_115

Dystonia vs. parkinsonism\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_117

DA receptor mechanisms specific to dystonia\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_120

Future directions\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_121

References\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_123

**List of Figures**

1. Structures of the basal ganglia and related circuitry in mouse\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_6

2. Schematic of presynaptic DA terminal\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_14

3. Conformation of knockin and resolution of Neo\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_44

4. RNA expression of DRD allele\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_45

5. Pre and postnatal death of DRD mice on a C57BL6/J background\_\_\_\_\_\_\_\_\_\_\_\_\_\_47

6. Viability of DRD mice on F2D2B6 background\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_49

7. *In vivo* TH activity in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_52

Table 1. Regional monoamine concentration\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_53

8. Gross anatomy of nigrostriatal pathway in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_55

9. Glutamatergic cortico- and thalamo-striatal immunostraining\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_58

10. Glutamatergic cortico- and thalamo-striatal terminals in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_59

11. Dystonic movements in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_72

12. Time of day-dependent differences in motor behavior\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_73

13. Diurnal fluctuations in DA metabolism\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_77

14. Amphetamine response in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_80

15. l-DOPA response in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_82

Table 2. Effect of l-DOPA and l-DOPS on monoamines\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_83

16. Regional specificity of l-DOPA effects\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_85

17. l-DOPS response in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_85

18. THP response in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_86

19. Abnormal movement response to DA receptor-specific agonists and antagonists\_101

20. Behavioral sensitivity to D1DAR agonism in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_104

21. Behavioral responses to D2DAR agonism in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_105

22. Cataleptic response to DA receptor antagonists in DRD mice\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_106

Table 3. qRT-PCR for DA receptors\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_108

Table 4. DA receptor binding\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_108

23. *Ex vivo* striatal adenylate cyclase activity\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_109

**List of Abbreviations**

l-DOPA = 3,4- l-dihydroxyphenylalanine

DRD = l-DOPA-responsive dystonia

DA = dopamine

NE = norepinephrine

DOPAC = 3,4-dihydroxypheynlacetic acid

5-HT = serotonin

5-HIAA = 5-hydroxyindoleacetic acid

l-DOPS = 3,4-l-dihydroxyphenylserine

BH4 = tetrahydrobiopterin

cAMP = cyclic adenosine monophosphate

GCH1 = guanosine triphosphate cyclohydrolase 1

TH = tyrosine hydroxylase

DAT = dopamine transporter

VMAT2 = vesicular monoamine transporter 2

AADC = aromatic acid decarboxylase

D1DAR = D1-type DA receptor

D2DAR = D2-type DA receptor

AC = adenylate cyclase

vGluT1 = vesicular glutamate transporter 1

vGluT2 = vesicular glutamate transporter 2

SNc = substantia nigra pars compacta

SNr = substantia nigra pars reticulata

VTA = ventral tegmental area

GPi = internal globus pallidus

GPe = external globus pallidus

STN = subthalamic nucleus

MSN = medium spiny neuron

Ctx = cerebral cortex

Hipp = hippocampus

Cbm = cerebellum

MAO = monamine oxidase

COMT = catechol-o-methyltransferase

PKA = cAMP-dependent protein kinase A

ERK = extracellular signal-regulated kinase

CaMKII = CaM-phospokinase 2

DARPP-32 = 32-kD DA and cAMP-regulated phosphoprotein

DBS = deep brain stimulation

LTD = long term depression

6-OHDA = 6-hydroxydopamine

MPTP = 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine

PD = Parkinson’s disease

LID = l-DOPA-induced dyskinesia

HPLC = high performance liquid chromatography

FDG-PET = [18F]-fluorodeoxyglucose positron-emission tomography

fMRI = functional magnetic resonance imaging

Neo = Neomycin resistance cassette

PCR = polymerase chain reaction

qRT-PCR = quantitative reverse transcriptase PCR

TBS = Tris-buffered saline

NGS = normal goat serum

s.c. = subcutaneous