

AWARD NUMBER: W81XWH-21-1-0877

TITLE: Stress/Glucocorticoid Mechanisms in Parkinsonian Pathologies

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CONTRACTING ORGANIZATION: University of Cincinnati, Cincinnati, OH

REPORT DATE: October 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

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OMB No. 0704-0188

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1. REPORT DATE October 2022			2. REPORT TYPE Annual		3. DATES COVERED 01Sep2021 - 31Aug2022	
4. TITLE AND SUBTITLE Stress/Glucocorticoid Mechanisms in Parkinsonian Pathologies					5a. CONTRACT NUMBER W81XWH-21-1-0877	
					5b. GRANT NUMBER	
					5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Kim B. Seroogy, PhD E-Mail: kim.seroogy@uc.edu					5d. PROJECT NUMBER	
					5e. TASK NUMBER	
					5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Cincinnati University Hall Goodman Avenue Cincinnati, OH 45220					8. PERFORMING ORGANIZATION REPORT NUMBER	
					10. SPONSOR/MONITOR'S ACRONYM(S)	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012					11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
					12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited	
13. SUPPLEMENTARY NOTES						
14. ABSTRACT A major goal during this research period involved extensive breeding and genotyping of the DAT-Cre line and the GRflox line to generate the DAT-Cre:GRflox (GR knockout in dopaminergic neurons) rat line. We have established a large breeding colony and are generating large cohorts to continue our studies on baseline behavioral assessments, CVS protocols and aSyn injections. Regarding baseline assessments, we performed analysis of locomotor and cognitive behavior in cohorts of male and female DAT-Cre-directed GR deletion (DAT-Cre ^{+/-} :GRflox) and Cre-negative controls (DAT-Cre ^{-/-} :GRflox). Targeted deletion of GR in dopamine neurons reduced locomotor activity in females and impairs initial recall of conditioned fear in males. These results suggest that GR acts in dopamine neurons to regulate locomotor and cognitive responses to stress in a sex-specific manner. Our data implicate GR-dopamine interactions in mediating behaviors relevant to motor and non-motor symptoms in our combined stress/PD model. We also implemented a sophisticated cognitive behavioral test (5-choice serial reaction time task; 5CSRTT). In baseline experiments, we found that rats exposed to single-prolonged stress exhibit impulsive behavior, which is also characteristic of individuals with PD.						
15. SUBJECT TERMS Parkinson's disease, stress, glucocorticoid receptors, dopamine, breeding, motor symptoms, non-motor symptoms, cognitive behavior						
16. SECURITY CLASSIFICATION OF:				17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRDC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U	19b. TELEPHONE NUMBER <i>(include area code)</i>			

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1. INTRODUCTION:

This project will test the overarching hypothesis that chronic stress exacerbates the seeding and propagation of aSyn inclusions, neurodegeneration and associated dysfunction of the dopaminergic nigrostriatal system via deleterious glucocorticoid receptor (GR) mechanisms. The study will determine if GRs expressed specifically in midbrain dopaminergic neurons contribute to dopaminergic degeneration and motor and non-motor behavioral dysfunctions following chronic stress. If our studies do indeed support the concept that stress and GR-mediated mechanisms negatively impact the well-being of remaining nigral dopaminergic neurons in parkinsonian animals, this preclinical information would provide important insights for continued investigations that ultimately may inform medical therapy. Aim 1 is testing the hypothesis that loss of GR in dopaminergic midbrain neurons will block the impact of chronic variable stress (CVS) on seeding and propagation of alpha-synuclein (aSyn) aggregates in the preformed fibril (PFF) model of PD, ameliorating neurodegeneration and behavioral dysfunction (modeling prevention), Aim 2 will test the hypothesis that loss of GR in dopaminergic midbrain neurons prevents the ability of chronic stress to exacerbate progression of PFF-induced neuropathology, behavioral dysfunction and nigrostriatal degeneration (modeling treatment). In both aims, we are using a cell-specific approach employing a conditional GR knockout rat (GRflox) that has been crossed with a DAT-Cre rat to specifically target GR deletion to dopaminergic neurons. The DAT-Cre:GRflox rats will be subjected to a CVS regimen using the state-of-the-art aSyn PFF model of synucleinopathy. We will incorporate a battery of behavioral assays to assess motor and non-motor symptoms relevant to PD. Morphological and stereological analyses will be used to interrogate the integrity of the nigrostriatal pathway at select post-PFF injection time points. Thus, Aims 1 and 2 will elucidate the role of GRs in stress exacerbation of parkinsonism. The overall goal of this project is to gain behavioral, morphological and mechanistic insight into the co-morbidity of PD, stress and depression. Moreover, this research may lead to future therapies that ameliorate affective (i.e. mood, cognition) as well as motor symptoms of PD.

2. KEYWORDS:

Keywords: Parkinson's disease, glucocorticoid receptors, chronic stress, knockout rat, alpha-synuclein preformed fibril model, dopamine, motor symptoms, non-motor symptoms, cognition, neurodegeneration, midbrain

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major goals from SOW:

Specific Aim 1: Test the hypothesis that loss of GR signaling in dopaminergic SNpc neurons will block the impact of chronic stress on seeding and propagation of alpha-synuclein (aSyn) aggregates in the preformed fibril (PFF) model of PD, ameliorating neurodegeneration and behavioral dysfunction (modeling *prevention*).

Major Task 1: Breeding, genotyping, CVS procedure, aSyn PFF injections, behavioral assessment. Timeline: Months 1-18. As of month 12, breeding, genotyping and colony maintenance achieved. CVS, PFF injections and behavioral assessment beginning.

Milestone: Local IACUC approval and HRPO/ACURO approval. Timeline: 1-3 months prior to start date. Completed.

Milestone(s): Completion and analysis of Aim 1 behavioral studies. Manuscript preparation. Continued breeding. Timeline: Month 24. In progress; anticipate completion by month 24.

Major Task 2: Brain sectioning, immunohistochemistry, microscopy, unbiased stereology

Milestone(s): Completion of Aim 1 immunostaining and stereological analyses. Manuscript preparation.
Timeline: Month 24. Not yet performed; anticipate completion by month 24.

Specific Aim 2: Test the hypothesis that loss of GR in dopaminergic SNpc neurons prevents the ability of chronic stress to exacerbate progression of PFF-induced neuropathology, behavioral dysfunction and nigrostriatal degeneration (modeling *treatment*).

Major Task 3: Breeding, genotyping, aSyn PFF injections, CVS procedure, behavioral assessment. Timeline: Months 18-36. Not yet begun.

Milestone(s): Completion and analysis of Aim 2 behavioral studies. Manuscript preparation. Continued breeding. Timeline: Month 36. Not yet begun.

Major Task 4: Brain sectioning, immunohistochemistry, microscopy, unbiased stereology. Timeline: Months 18-36. Not yet begun.

Milestone(s): Completion of Aim 2 immunostaining and stereological analyses. Manuscript preparation. Timeline: Month 36. Not yet begun.

What was accomplished under these goals?

1) Major activities:

The major activities of this reporting period involved extensive breeding and genotyping of the DAT-Cre line and the GRflox line to generate the DAT-Cre:GRflox (GR knockout in dopaminergic neurons) rat line. We have now generated a stable colony and are performing baseline behavioral assessments and starting CVS protocols and aSyn injections. We have taken the opportunity to analyze motor and non-motor behaviors in cohorts of rats with the DAT-Cre-directed GR deletion (see below under Results/Outcomes). In addition, we have performed baseline analysis of rats subjected to stress in the 5CSTT cognitive task.

2) Specific objectives:

Our main objective for this reporting period was to establish a stable, reliable line of DAT-Cre:GRflox rats in sufficient numbers to begin the experimental protocols of Aim 1 (see above). Conducting baseline behavioral assessments was also a goal.

3) Results/Outcomes:

Through our breeding strategy, we have generated the novel DAT-Cre:GRflox line by crossing a conditional GR knockout rat (GRflox) with a DAT-Cre rat to specifically target GR deletion to dopaminergic neurons. This new transgenic model is central to the present studies. We have established a large breeding colony and are currently generating large cohorts to continue our studies.

We performed analysis of locomotor and cognitive behavior in cohorts of male and female DAT-Cre-directed GR deletion (DAT-Cre^{+/-}:GRflox) and Cre negative controls (DAT-Cre^{-/-}:GRflox). We felt it important to perform these data analyses to provide information on DAT-Cre directed GR knockout phenotypes at baseline. Gene deletion in midbrain dopamine neurons was confirmed by immunohistochemistry, demonstrating lack of GR in midbrain neurons expressing tyrosine hydroxylase. Our behavioral data indicate that females with GR deletion show reduced locomotor activity in the open field relative to controls, suggestive of a mild locomotor phenotype. Locomotor activity was not reduced in DAT-Cre GR knockout rats, possibly due to enhanced variability in this group. No effects of DAT-Cre deletion were observed in novel object recognition in either

sex. Examination of cognitive behavior was tested using auditory fear conditioning. We did not observe changes in acquisition of conditioned fear. However, initial retention of fear was impaired in male (but not female) DAT-Cre knockout animals, suggesting a role for GR in initial expression of conditioned fear via dopamine neurons. There was no effect of GR deletion on extinction or reinstatement of fear conditioning. These results provide important data indicating that DAT-Cre directed GR deletion produces functional changes in dopamine-related non-motor behaviors, including locomotion and fear memory. Our data implicate GR-dopamine interaction in mediating behaviors relevant to motor and non-motor symptoms in our stress/PD model. These results were presented in a poster presentation at the annual Society for Neuroscience Meeting held in San Diego, CA on November 12, 2022 (*see Appendix for poster and all data and graphs*).

We also implemented the cognitive behavioral test (5CSRTT) in our group. During this funding period, we purchased the 5CSRTT apparatus and commenced preliminary studies to establish appropriate methods for testing and analysis. In baseline experiments, we found that rats exposed to single-prolonged stress exhibit impulsive behavior, which is also characteristic clinically of individuals with PD. *See Appendix for data and graphs*.

Other achievements:

None to report.

Goals not met:

Due to the delay in obtaining the DAT-Cre rat line from a commercial vendor, our breeding was set back several months. This prevented the timely initiation of CVS and aSyn PFF injections, which are now underway.

What opportunities for training and professional development has the project provided?

Although the project was not specifically designed to provide training and professional development opportunities, this was indeed the case for an undergraduate student as part of the University of Cincinnati (UC) Research Innovation in NeuroScience Education for Underserved Populations (RISE UP) Program. The goal of the RISE UP grant is to equip outstanding undergraduate students from diverse and/or under-privileged backgrounds with research, professional, and social-emotional skills to thrive in cutting-edge neuroscience graduate programs. The RISE UP Program is typically 10 weeks and culminates with a capstone research presentation. Adam Chen, who came to us from Case Western Reserve University, was awarded a summer RISE UP Fellowship under the mentorship of Drs. Herman and Seroogy. As part of his summer research, Mr. Chen was trained in and helped establish the baseline cognitive behavioral test (5CSRTT) in rats and showed that rats exposed to single-prolonged stress exhibit impulsive behavior. This is relevant to PD in that a subset of PD patients experience problems with impulse control. This work was presented by Adam in the program's Capstone Research Presentation (*see Appendix*) and has laid the foundation for our subsequent use of the 5CSRTT to determine the effects of chronic variable stress (CVS) on cognitive performance in the DAT-Cre:GRflox rats.

How were the results disseminated to communities of interest?

The awarding of the current DOD grant and the Parkinson's disease/chronic stress research being performed were disseminated to the public via a podcast interview (Simply Medicine) with the PI and 2 co-investigators on radio station 55KRC.

What do you plan to do during the next reporting period to accomplish the goals?

Now that breeding was successful and a stable colony established, we plan to ramp up CVS procedures, aSyn PFF injections, and behavioral assessments of the DAT-Cre:GRflox rats (and controls) followed by morphological and stereological analyses as outlined in Aim 1 (prevention model), with the goal of having achieved the majority of these tasks completed by the month 24 timeline.

Later in Year 2, we will also begin processing animals for the experiments described in Aim 2 (treatment model), with the goal of having a few cohorts in the pipeline by the month 24 timeline.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

With respect to Parkinson's disease (PD), the generation of the novel animal model by this project, in which an important steroid hormone receptor (glucocorticoid receptor, GR) is deleted in dopaminergic cells, will provide us the means to determine the mechanism by which chronic stress worsens the manifestation and progression of PD.

Our behavioral results in animals lacking the GR in dopaminergic neurons indicate that there are sex differences in glucocorticoid regulation of midbrain dopamine neurons in rat. Targeted deletion of GR in dopaminergic neurons reduces the initial locomotor response to a novel environment in females (but not males) (likely mediated by effects on nigrostriatal DA neurons). However, GR deletion does not affect threat recognition or novel object recognition (working memory) in either sex. Deletion of GR in dopaminergic cells does impair recall of freezing behavior in auditory fear conditioning (a cognitive behavior) in males (but not females). Thus, these data suggest that GR acts in dopaminergic neurons to regulate locomotor and cognitive responses to stress in a sex-specific manner. These findings are likely to have an impact on our subsequent investigations (this project and those of others in the PD field) of the importance of GR in midbrain dopaminergic neurons in governing PD-related motor as well as non-motor behaviors in response to chronic stress. The results also underscore a role for GR in behaviors regulated by dopamine, and provide support for our core hypothesis outlining the necessity of glucocorticoid signaling in impacting stress-induced exacerbation of PD.

Our preliminary cognitive behavioral results in rats subjected to single-prolonged stress show that the stressed rats display impulsive behavior. Impulsive disorders are symptoms of PD in some patients. These findings set the stage for future investigations of chronic stress-induced dysfunctional cognitive behavior in models of PD.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Via radio podcasts and PR news releases (see below), the lay public, including people with Parkinson's disease, is learning how chronic life stress can accelerate the course of neurodegenerative diseases.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

As stated in the approved Updated Budget Justification of the PAIR items prior to the start of the grant, we have changed lines of DAT-Cre rats for increased reliability and stability and needed to purchase them through a commercial vendor (SAGE Labs which was subsequently acquired by Envigo). Although we were told at the start of the grant by Envigo that they had heterozygous (HET) DAT-Cre breeders on hand and could deliver them to us immediately, this was actually not the case. It turns out they had DAT-Cre breeders in the Long Evans rat strain, not in the Sprague Dawley strain as we requested and required, but this was not communicated to us in a timely manner. Bottom line is that the Sprague Dawley rat DAT-Cre line had to be reconstituted by Envigo from cryopreserved DAT-Cre line embryos and then HETs generated and validated to provide breeders. Thus, there was a ~5-6-month delay in providing us the male and female DAT-Cre breeders for us to start our breeding procedure to cross the new DAT-Cre rats with our GR knockout rats (GRflox) to generate the target DAT-Cre:GRflox rats (and various control permutations). Subsequently, we have successfully bred the lines and generated the DAT-Cre:GRflox rats and are maintaining and continually expanding the colony.

This breeding delay led to a subsequent delay in starting the CVS and aSyn PFF procedures. Nevertheless, we were able to collect preliminary baseline motor and non-motor behavioral data that will prove informative for future analyses in the present project. Going forward, we anticipate the project will be on track for the month 24 milestones.

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

No significant changes.

Significant changes in use of biohazards and/or select agents

No significant changes.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Abstract:

Herman, J.P., Martelle, S.E., Chambers, J.B., Lundgren, K.H. and Seroogy, K.B. (2022) Sex difference in glucocorticoid regulation of midbrain dopamine neurons in rat. *Soc. Neurosci. Abst.* #062.03, San Diego, CA. Published.
Acknowledgement of federal (DOD) support: Yes

Other publications, conference papers and presentations.

Nothing to Report

- **Website(s) or other Internet site(s)**

<https://www.uc.edu/news/articles/2022/01/uc-researchers-examine-stress-role-in-parkinsons.html>
This University of Cincinnati website highlights research and other news of its faculty and students.

<https://parkinsonsnewstoday.com/news/research-how-stress-worsens-parkinsons-wins-1m-plus-dod-grant/>
This internet site highlights the latest Parkinson's disease news, research, lifestyle advice, etc.

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Novel animal model generated: DAT-Cre:GRflox rats (i.e. DAT-Cre targeted GR deletion in dopaminergic neurons)

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Kim Seroogy, PhD
Project Role: PI
Researcher Identifier: 0000-0003-2470-7193 (ORCID ID)
Nearest person month worked: 3
Contribution to Project: Dr. Seroogy has overseen and directed the project.
Funding Support: This award.

Name: James Herman, PhD
Project Role: Co-Investigator
Researcher Identifier: 0000-0003-3571-2406 (ORCID ID)
Nearest person month worked: 0.6
Contribution to Project: Dr. Herman has supervised the breeding of the transgenic rat lines and the stress/behavior paradigms for the project.
Funding Support: This award.

Name: Teresa Reyes, PhD
Project Role: Co-Investigator
Researcher Identifier: 0000-0003-3009-3749 (ORCID ID)
Nearest person month worked: 0.6
Contribution to Project: Dr. Reyes has supervised the cognitive behavior component (5-choice serial reaction time task) training for the project.
Funding Support: This award.

Name: Kerstin Lundgren
Project Role: Senior Research Associate
Researcher Identifier: N/A
Nearest person month worked: 9.9 (of a 0.52 FTE)
Contribution to Project: Ms. Lundgren has performed work in the area of breeding and behavior.
Funding Support: This award.

Name: Ben Packard
Project Role: Research Associate
Researcher Identifier: N/A
Nearest person month worked: 5.6
Contribution to Project: Mr. Packard has performed work in the area of breeding and genotyping of the transgenic rat lines and the stress/behavior paradigms for the project.
Funding Support: This award.

Name: Claire Cantelon
Project Role: Research Assistant
Researcher Identifier: N/A
Nearest person month worked: 3.26
Contribution to Project: Ms. Cantelon has performed work in the area of establishing the 5-choice serial reaction time task in rats for the project.
Funding Support: This award.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Active Other Support

PI: Kim Seroogy, PhD

No change.

Co-I: James Herman, PhD

Two new awards (listed below). Two previously active grants from NIH and the VA have closed. The new awards are unrelated to the current DOD grant (i.e. no scientific or budgetary overlap) and do not impact Dr. Herman's effort on the project.

*Title: Cortical Mechanisms of Traumatic Stress

*Major Goals: This proposal tests neurocircuit and molecular mechanisms of underlying severe stress-induced behavioral deficits, using an animal model of PTSD pathology.

*Status of Support: Active *Project Number: 1R01MH127835-01A1

*Name of PD/PI: Herman *Source of Support: NIH

*Primary Place of Performance: University of Cincinnati

*Project/Proposal Start and End Date: 09/2022 – 08/2027

*Total Award Amount (including Indirect Costs):

*Person Months (Calendar/Academic/Summer) per budget period: 4.2 cal months

*Title: Glucocorticoid Receptor Mechanisms of Traumatic Stress Pathology

*Major Goals: This proposal examines the role prefrontal cortex stress hormone (glucocorticoid) receptors in driving development of PTSD behavioral pathologies, ranging from anxiety symptoms, exaggerated fear responses and cognitive dysfunction.

*Status of Support: Active *Project Number: BX005923

*Name of PD/PI: Herman *Source of Support: VA Merit Award

*Primary Place of Performance: Veterans Administration, Cincinnati

*Project/Proposal Start and End Date: 04/2022 – 03/2026

*Total Award Amount (including Indirect Costs):

*Person Months (Calendar/Academic/Summer) per budget period: 4.69 cal months

Co-I: Teresa Reyes, PhD

One new award (listed below). Other active support unchanged. The new award is unrelated to the current DOD grant (i.e. no scientific or budgetary overlap) and does not impact Dr. Reyes' effort on the project.

*Title: Biomedical Postbaccalaureate Research Education Program at the University of Cincinnati College of Medicine (PREP@UC)

*Major Goals: The PREP@UC program is a one-year paid internship designed to help diverse college graduates enter a doctoral program and achieve a career as a PhD-Level biomedical scientist.

*Status of Support: Active

*Project Number: R25GM134976 (Contact PI: Reyes)

*Name of PD/PI: Teresa M. Reyes *Source of Support: NIH

*Person Months (Calendar/Academic/Summer) per budget period: 1.2 cal months

*Primary Place of Performance: University of Cincinnati

*Project/Proposal Start and End Date: 9/21/2021 - 08/31/2025

*Total Award Amount (including Indirect Costs):

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES:

See below.

Glucocorticoid Regulation of Dopamine of Midbrain Dopamine Neurons in Rat: Sex Differences

Glucocorticoid receptors (GR) are expressed in midbrain dopamine (DA) neurons and are thought to be involved in transducing negative effects of glucocorticoids on cell survival and cognitive behavior in Parkinson's disease (PD) models. To test involvement of GR in mediating motor and non-motor behaviors relevant to PD, we generated a rat GR knockout in dopamine neurons by crossing GRflox rats with a DAT-Cre driver line. We tested initial responses to a novel environment as a measure of locomotion and anxiety-linked behavior, novel object recognition as a test of working memory, and auditory fear conditioning. (*Note: this work was presented as a poster at the 2022 Society for Neuroscience meeting in San Diego, CA; Abstract #062.03. See full poster format at end of Appendix*)

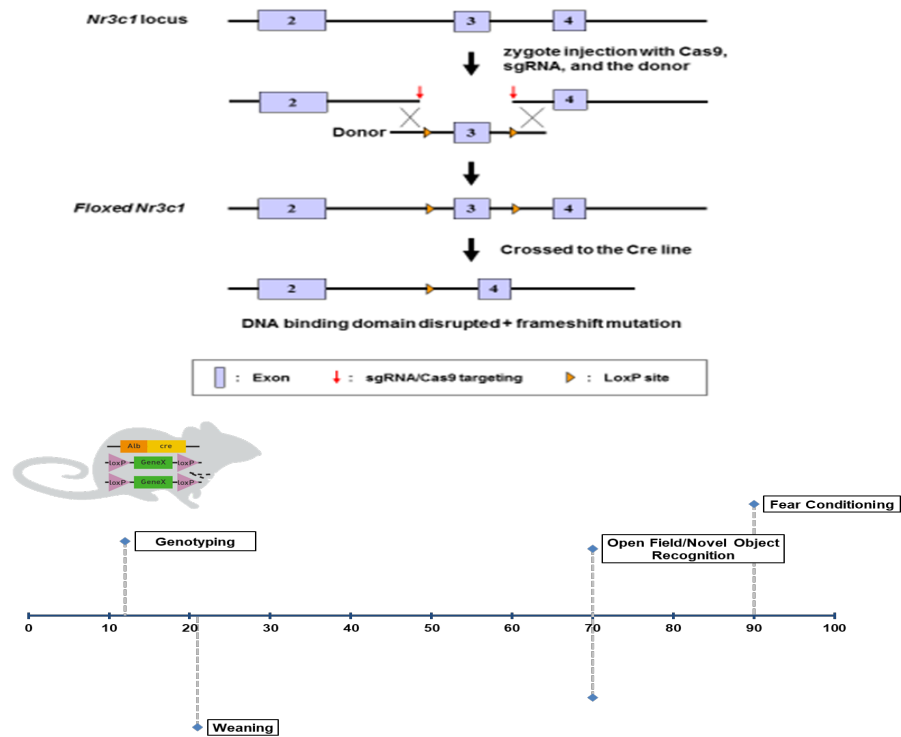


Fig. 1. Strategy for generation of conditional DAT-Cre nr3c1 (GR) knockout rats and experimental timeline. All animals received an open field/novel object recognition (OF/NOR) test, followed by exposure to an auditory fear conditioning (FC) test. For the OF/NOR test, the initial response to the open field was assessed (over 5 minutes), followed by exposure to a novel object. Recognition was tested the next day. Testing was performed on 10 Cre+ (GR knockout) females, 9 Cre- females (controls), 15 Cre+ males and 7 Cre- males. Sexes were analyzed separately.

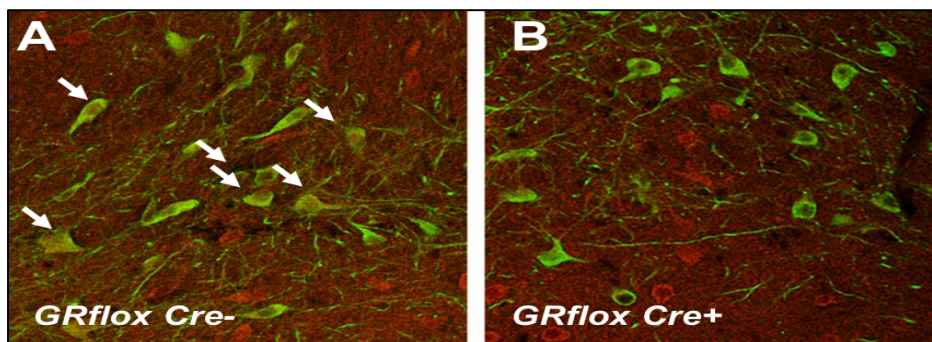


Fig. 2. Cell-specific deletion of GR in substantia nigra pars compacta (SNpc) dopaminergic neurons. A. TH (green) and GR (red) immunostaining in DAT-Cre-negative GRflox rats. Colocalization of GR is evident in the vast majority of TH-positive neurons in this field. B. Absence of TH/GR colocalization in nigral TH neurons of DAT-Cre-positive GRflox animals. Note GR-positive (red) nuclei in A, but clear nuclei in B.

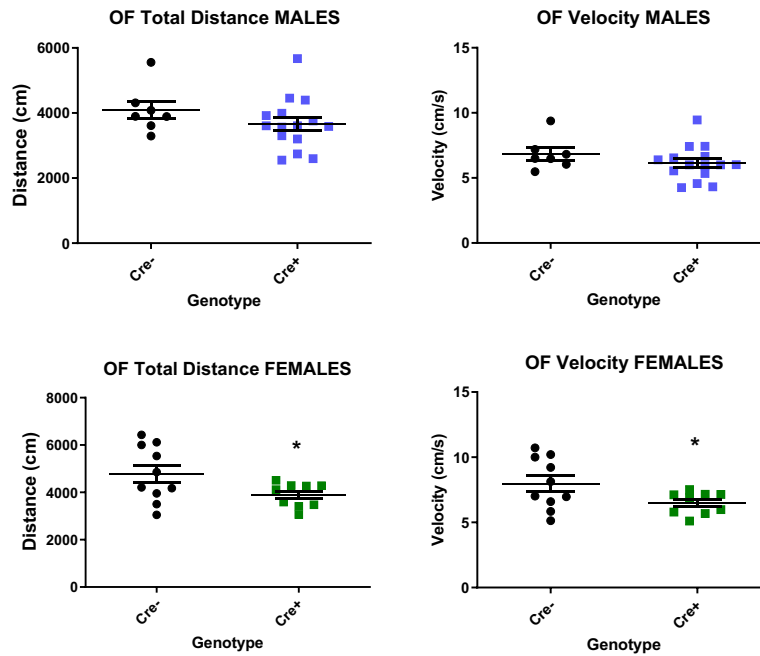


Fig. 3. DAT-Cre-targeted GR deletion in DA neurons reduces movement speed and total distance traveled upon initial exposure to a novel open field in females. There were no effects on center time or entry, and no effects on novel object recognition on day 2. * $p < 0.05$

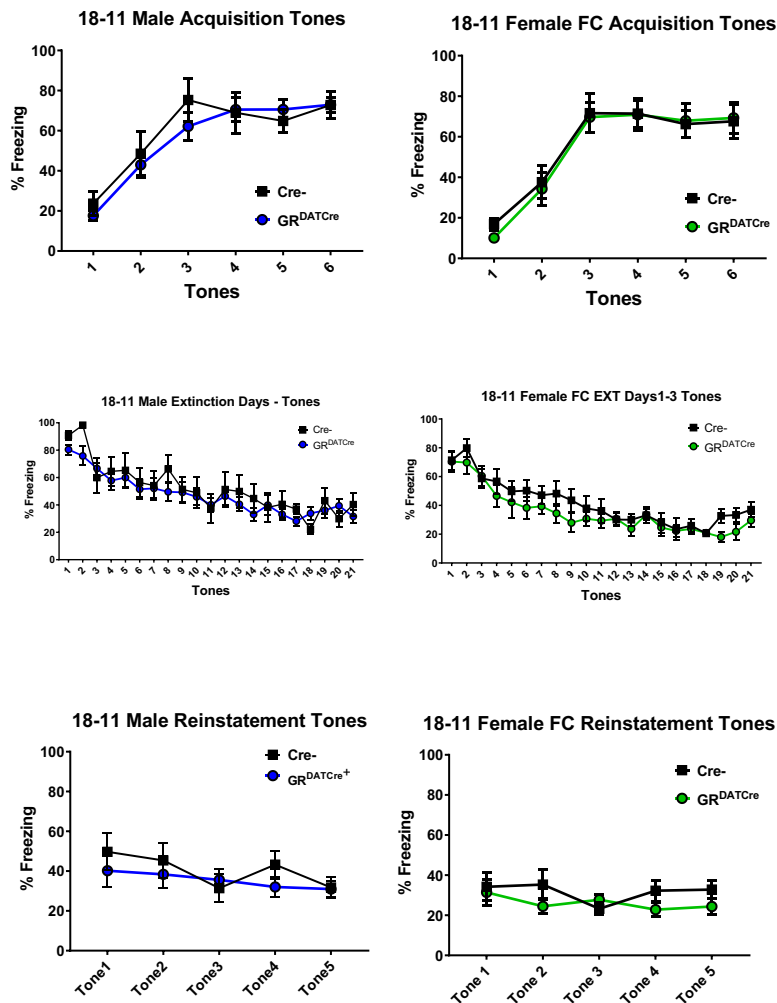


Fig. 4. DAT-Cre-targeted GR deletion in DA neurons did not affect acquisition, extinction or reinstatement of conditioned fear in either male or female rats.

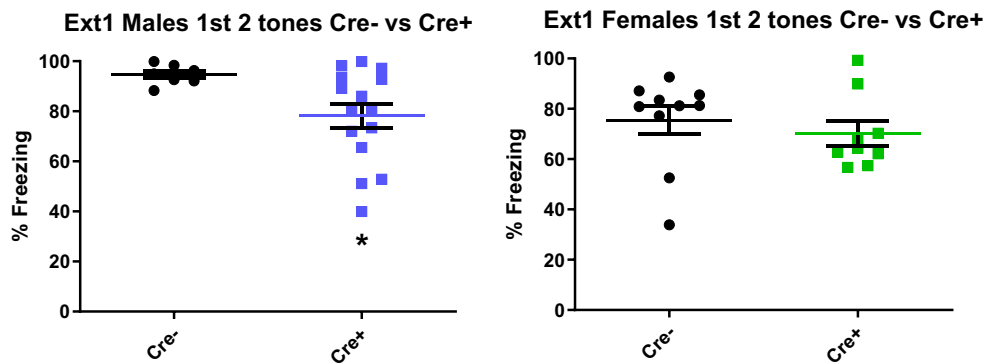


Fig. 5. DAT-Cre-targeted GR deletion in DA neurons differentially affected the initial freezing response to tone the day following acquisition training in males. The data suggest that GR deletion transiently impairs recall of shock-tone pairing in males. * $p < 0.05$

Summary and Conclusions from this work:

- Targeted deletion of GR in DA neurons reduces the initial locomotor response to a novel environment in females
- GR deletion does not affect threat assessment or novel object recognition in either sex
- Deletion of GR in DA neurons impairs initial recall of freezing behavior in auditory fear conditioning in males
- The data suggest that GR acts in DA neurons to regulate locomotor and cognitive responses to stress in a sex-specific manner

Funding:

This work was supported by the DOD Office of the Assistant Secretary of Defense for Health Affairs through the Neurotoxin Exposure Treatment Parkinson's (NETP) Program, Investigator-Initiated Research Award W81XWH-21-1-0877. Other funding sources included the Kerman Family Fund for Parkinson's Research, the Selma Schottenstein Harris Lab for Research in Parkinson's Disease, the Gardner Family Center for Parkinson's Disease and Movement Disorders, and the Parkinson's Disease Support Network - Ohio, Kentucky & Indiana.

Stress Impairs Performance in the 5-Choice Serial Reaction Time Task

Single-prolonged stress (SPS) in rats elicits aversive behaviors exhibited by humans (anxiety, depression, fear responses, social avoidance). However, its role in detrimental behaviors characteristic of subpopulations of PD patients, such as impulsivity and inattention, has not been studied. Here, we subjected rats to SPS (restraint stress for 2 hours, forced swim for 20 minutes, ether anesthesia for 5 minutes). Ten days following the SPS, we compared the cognitive behavioral performance of control vs SPS rats ($n = 10$ male rats /group) in the 5-choice serial reaction time task (5CSRTT). As shown below, results demonstrate that rats exposed to SPS exhibit impulsive behavior, but not inattentive behavior. These findings lay the foundation for our subsequent investigations in this project of chronic stress-induced dysfunctional cognitive behavior in our combined model of stress/PD.

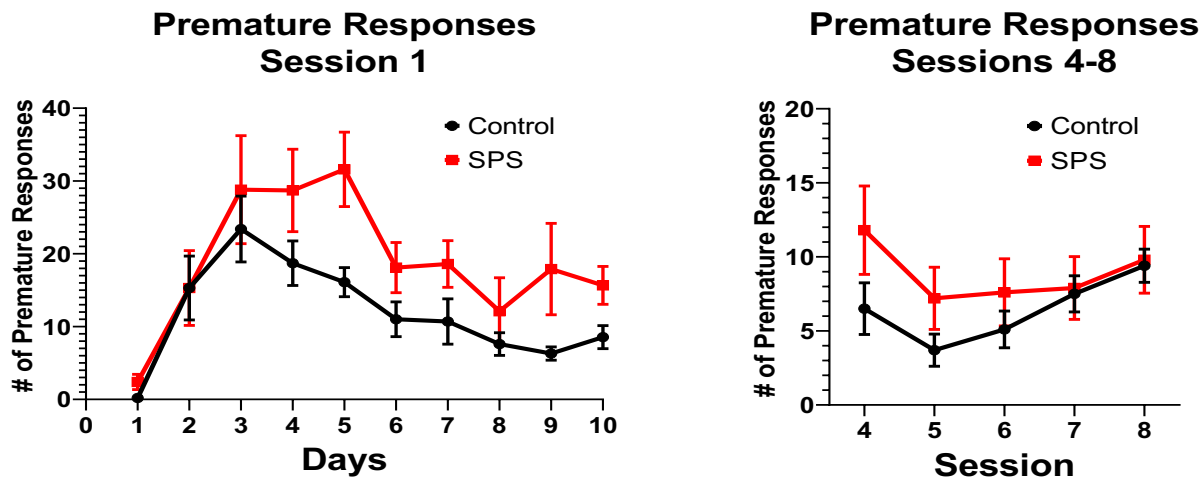


Fig. 1. Rats subjected to SPS exhibit impulsive behavior in the 5CSRTT

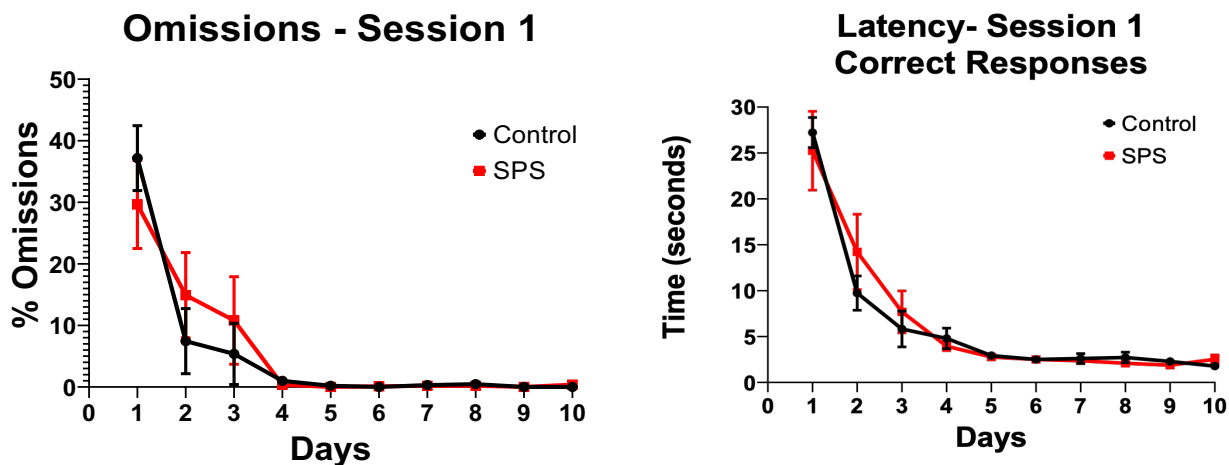


Fig. 2. Rats subjected SPS do not exhibit inattentive behavior in the 5CSRTT

transducing negative effects of glucocorticoids on cell survival and cognitive behavior in Parkinson's disease (PD) models. To test involvement of GR in mediating motor and non-motor behaviors relevant to PD, we generated a rat GR knockout in dopamine neurons by crossing GR flox rats with a DAT1-Cre driver line. We tested initial responses to a novel environment as a measure of locomotion and anxiety-linked behavior, novel object recognition as a test of working memory, and auditory fear conditioning.

Methods

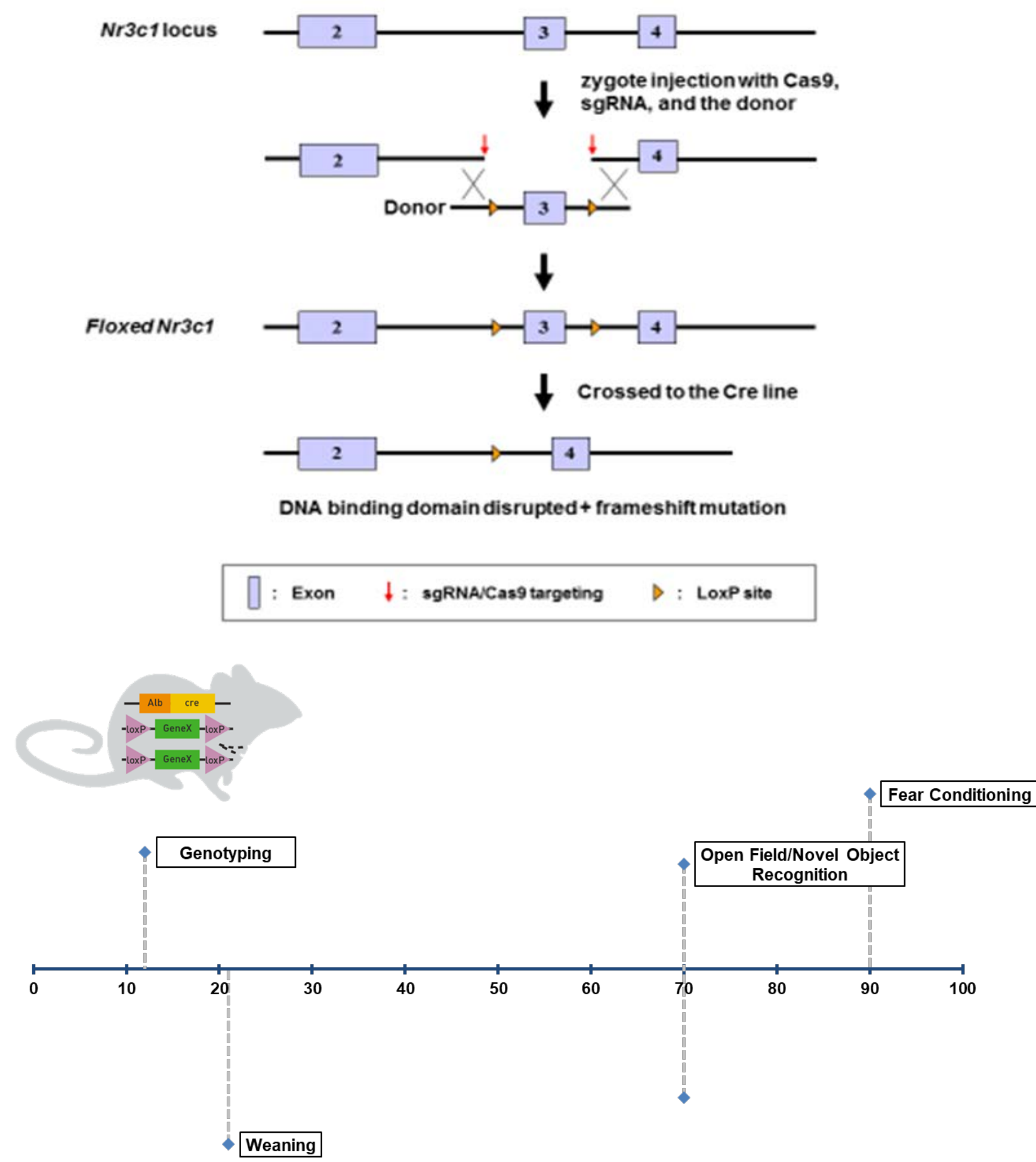


Fig. 1. Strategy for generation of conditional DAT1-Cre nr3c1 (GR) knockout rats and experimental timeline. All animals received an open field/novel object recognition (OF/NOR) test, followed by exposure to an auditory fear conditioning (FC) test. For the OF/NOR test, the initial response to the open field was assessed (over 5 minutes), followed by exposure to a novel object. Recognition was tested the next day. Testing was performed on 10 Cre+ (GR knockout) females, 9 Cre- females (controls), 15 Cre+ males and 7 Cre- males. Sexes were analyzed separately.

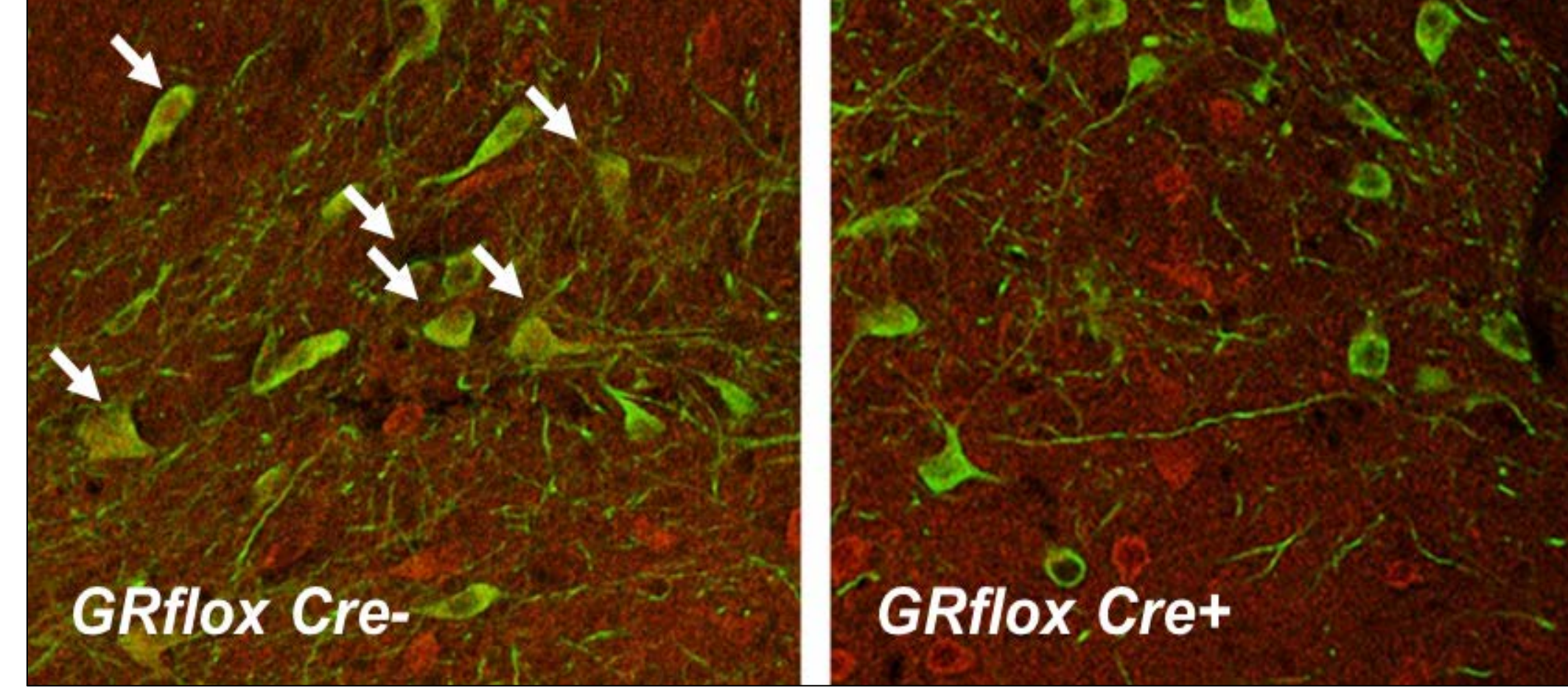


Fig. 2. Cell-specific deletion of GR in substantia nigra pars compacta (SNpc) dopaminergic neurons. A. TH (green) and GR (red) immunostaining in DAT-Cre-negative GRflox rats. Colocalization of GR is evident in the vast majority of TH-positive neurons in this field. B. Absence of TH/GR colocalization in nigral TH neurons of DAT-Cre-positive GRflox animals. Note GR-positive (red) nuclei in A, but clear nuclei in B.

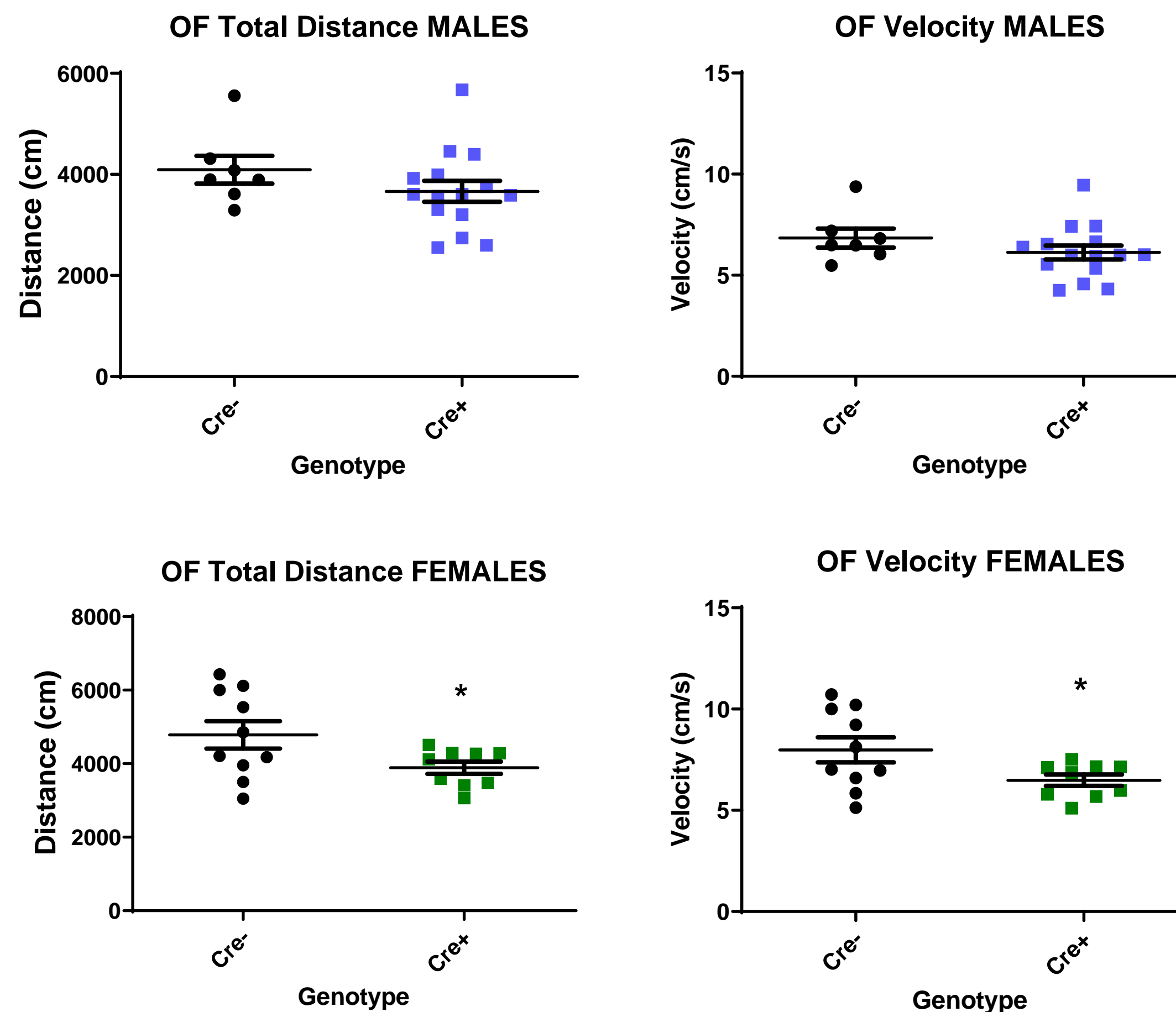


Fig. 3. DAT-Cre-targeted GR deletion in DA neurons reduces movement speed and total distance traveled upon initial exposure to a novel open field in females. There were no effects on center time or entry, and no effects on novel object recognition on day 2. * $p < 0.05$

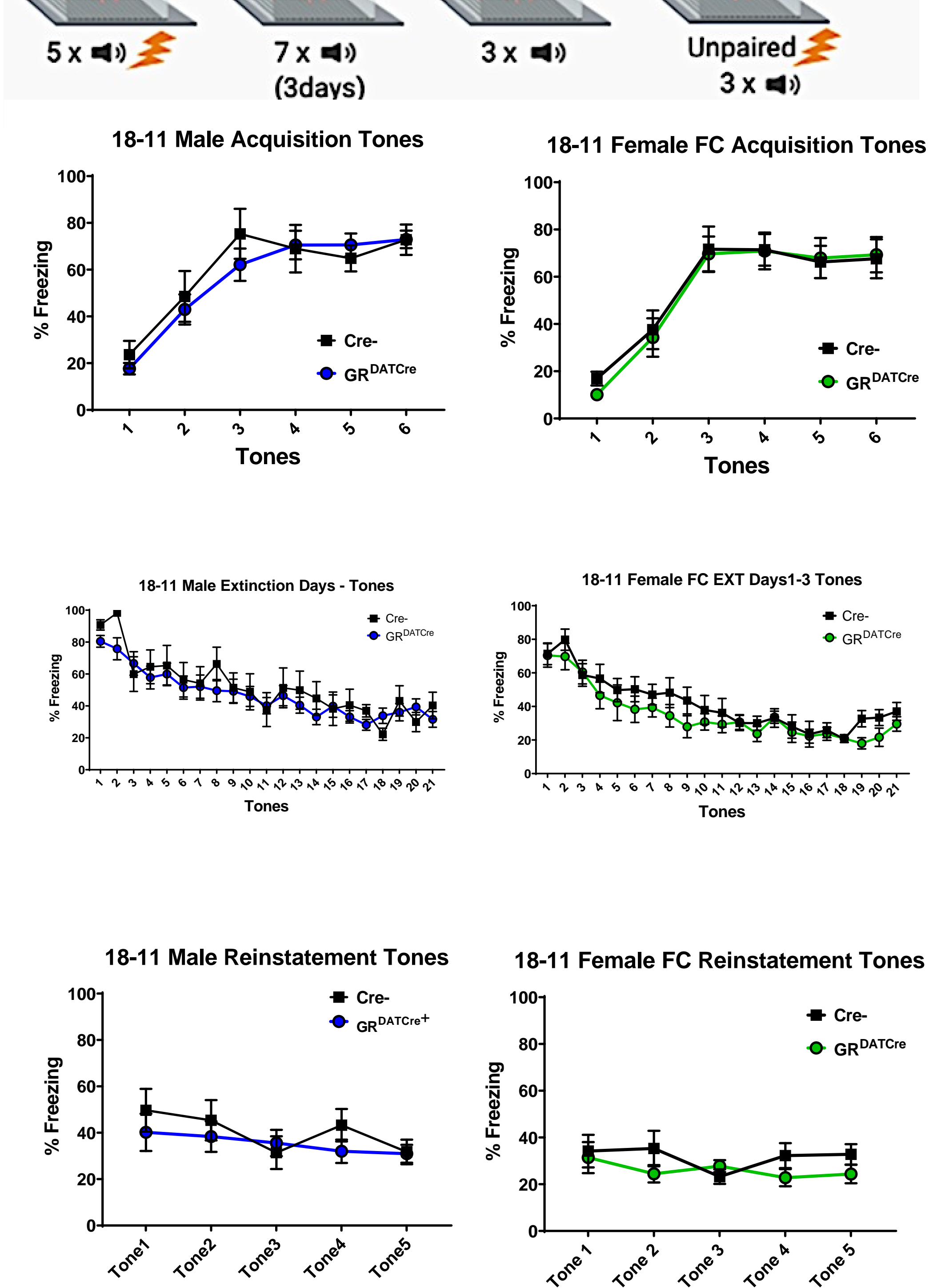


Fig. 4. DAT-Cre-targeted GR deletion in DA neurons did not affect acquisition, extinction or reinstatement of conditioned fear in either male or female rats.

This work was supported by the DOD Office of the Neurotoxin Exposure Treatment Parkinson's (NETP) 0877. Other funding sources included the Kerman Lab for Research in Parkinson's Disease, the G and the Parkinson's Disease Support Network -