Published in final edited form as:

Neurosci Lett. 2017 June 09; 651: 79–87. doi:10.1016/j.neulet.2017.04.063.

Stress and Corticosterone Alter Synaptic Plasticity in a Rat Model of Parkinson's Disease

YongXin Hao, Aref Shabanpoor, and Gerlinde A. Metz

Canadian Centre for Behavioural Neuroscience, University of Lethbridge, Lethbridge, AB T1K3M4, Canada

Abstract

As a major influence on neuronal function and plasticity, chronic stress can affect the progression and symptoms of neurodegenerative conditions, such as Parkinson's disease (PD). Here we investigated the influence of unilateral dopamine depletion and stress on dopamine-related hallmarks of stress response and neuronal plasticity in a rat model of PD. Animals received either restraint stress or a combination of adrenalectomy and corticosterone (CORT) supplementation to clamp circulating glucocorticoid levels for three weeks prior to unilateral nigrostriatal dopamine depletion. Rats were tested in skilled and gross motor function up to three weeks post-lesion. Midbrain mRNA expression assessments included markers of dopamine function and neuroplasticity, such as tyrosine hydroxylase (TH), synaptophysin (SYN), calcyon, and glucocorticoid receptor (GR). Along with impaired motor performance, stress and clamped CORT partially preserved TH expression in both substantia nigra (SN) and ventral tegmental area (VTA), but differentially modulated the expression of SYN, calcyon and GR mRNA in midbrain and cortical areas. Stress reduced synaptophysin mRNA expression in SN/VTA, and elevated calcyon mRNA optical density in both non-lesion and lesion hemispheres. Stress and CORT increased GR mRNA in the non-lesion SN/VTA, while in the lesion hemisphere GR mRNA was only elevated by CORT. In the motor cortex and striatum, however, GR was higher in both hemispheres under both experimental conditions. These findings suggest that stress and stress hormones differentially affect dopaminergic function and neuroplasticity in a rat model PD. The findings suggest a role for stress in motor and non-motor symptoms of PD and stress response.

Keywords

6-hydroxydopamine; stress response; skilled movement; tyrosine hydroxylase; synaptophysin; calcyon; glucocorticoid receptor

Corresponding Address: Gerlinde A. Metz, Ph.D., Canadian Centre for Behavioural Neuroscience, University of Lethbridge, 4401 University Drive, Lethbridge, AB, Canada T1K 3M4, gerlinde.metz@uleth.ca.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Parkinson's disease (PD) is a progressive, incurable, and the second most common neurodegenerative disorder characterized by progressive degeneration of dopaminergic neurons in the substantia nigra (SN) pars compacta (Dauer and Przedborski, 2003; Eyhani-Rad, 2012). The resulting reduction in striatal dopamine leads to the typical Parkinsonian syndrome that includes tremor, bradykinesia, rigidity, and cognitive deficits (Smith et al., 2002, Connolly and Lang, 2014). The pathogenesis of PD in most cases is unclear (Gibberd and Simmonds, 1980; Smith et al., 2002, Djamshidian and Lees, 2014). Studies indicated, however, that many cases of PD may result from an interaction of genetic and environmental causes (Klein, 2012; Goldman, 2014). Notably, stress represents one of the earliest proposed causes of PD which can potentially trigger or hasten the underlying neurodegeneration (Charcot, 1878). Chronic stress, as a function of elevated glucocorticoid (GC) levels, promotes a pro-inflammatory state, activates microglia and ultimately leads to death of dopaminergic neurons in the SN (de Pablos et al., 2014; Vyas et al., 2016). Furthermore, stress may exacerbate neurodegenerative events and motor deficits in a rat model of PD (Smith et al., 2008). These findings are supported by the clinical observation that stress hormones, such as GCs, are positively associated with gait deficits in PD patients (Charlett et al., 1998; Smith et al., 2002) by activating glucocorticoid receptors (GR) in motor areas of the brain (Ahima and Harlan, 1990).

Aside from the prominent impact of stress and GCs on motor function (Metz et al., 2005a; Smith et al., 2008), stress and GCs also are potent modulators of gene expression, which in turn affects neuronal plasticity and neurodegeneration (Joels et al., 2004, Datson et al., 2012, Vyas et al., 2016) in frontal cortex (Lee et al., 2006), prefrontal cortex (Chen et al., 2008), hippocampus (Zuena et al., 2008), amygdala (Kim and Han, 2006), and hypothalamus (Harris et al., 2006, Lee et al., 2006, Chen et al., 2008). Stress may also regulate dopaminergic function (Vyas, 2016) and through GC-GR interaction contribute to dopaminergic neurodegeneration via modulating the inflammatory response of microglia (Morale, 2004; Ros-Bernal, 2011; Herrera, 2015). Accordingly, GR density has been attributed a role in neurodegeneration and progression of clinical symptoms in PD (Ros-Bernal et al., 2011). In addition, both GR and also the mineralocorticoid receptor (MR) participate in fine motor control and therefore may directly affect motor symptoms of PD (Jadavji et al., 2011). Stress therefore may represent one of the most critical clinical variables determining the risk, onset and progression of PD.

The present study investigated the impact of chronic mild stress on hallmarks of clinical symptoms, dopaminergic function and neuronal plasticity in a rat model of PD. The activity of the HPA axis in rats was manipulated by either restraint stress, or the combination of adrenalectomy (ADX) and corticosterone (CORT) supplementation to clamp circulating GC levels for three weeks prior to unilateral nigrostriatal dopamine depletion using the neurotoxin 6-hydroxydopamine 6-OHDA (Zigmond et al., 1989; Schober, 2004). Rats were tested in skilled and gross motor function up to three weeks post-lesion along with *in situ* hybridization examination of mRNA expression of molecular hallmarks reflective of dopaminergic function and plasticity. Assessments included GR mRNA density as a marker of hypothalamic-pituitary-adrenal (HPA) axis regulation, the rate limiting enzyme in

dopamine synthesis, tyrosine hydroxylase (TH), the synaptic vesicle glycoprotein synaptophysin (SYN) and the neuron-specific vesicular protein calcyon in the midbrain as markers of synaptic plasticity and functionality.

Materials and Methods

Subjects

This experiment involved 24 male young adult Long-Evans hooded rats raised at the University of Lethbridge vivarium. The animals were housed pairwise in standard polycarbonate shoebox cages ($45.5 \times 25.5 \times 20$ cm) on corn cob bedding (Bed-o'Cobs 1/8"). The housing room was maintained at 20° C and relative humidity of 30% on a 12-hour light/dark cycle with light starting at 7:30 AM.

Prior to the experiments, the rats were placed on a restricted diet to maintain body weights at 90–95% of their baseline weight to encourage participation in the reaching task. Supplementary food was given daily in their home cages five hours after behavioural testing to maintain body weight. Animals were weighed daily. All procedures were performed according to standards set by the Canadian Council of Animal Care and approved by the University of Lethbridge Animal Welfare Committee.

Experimental Design

Following pre-training in the skilled reaching task for three weeks, individual rats were matched for reaching success and randomly assigned to one of the following groups: restraint stress (STRESS; $n=6$), a combination of adrenalectomy and corticosterone treatment to clamp the physiological stress response (CORT; $n=6$), and the remaining animals were considered non-treated lesion controls (CONTROL; $n=6$).

The STRESS and CORT treatments were performed daily for a period of three weeks prior to the lesion. All animals received a unilateral nigrostriatal 6-OHDA lesion. STRESS and CORT treatments continued daily up to three weeks post-lesion. Within-subject comparisons were used to assess the treatment effects without and with lesion. During the entire period, animals were tested daily in the skilled reaching task in the morning hours. At the end of the post-lesion test period, animals were video recorded in the skilled reaching task for qualitative analysis of movement performance. At this time, animals were also video recorded in an open field task for analysis of motor activity and exploration. After completion of behavioural tests rats were euthanized and brain tissues were collected on day 21 post-lesion.

Physiological Manipulations and Stress Procedures

Adrenalectomy and CORT Administration—Adrenal glands were removed bilaterally to suppress endogenous production of CORT. To clamp CORT levels, 5 mg of CORT (Sigma-Aldrich, St. Louis, MO, USA) was mixed with cookie crumbs, reaching food pellets, water and peanut oil. CORT was administered once daily in the morning 1 hour prior to behavioural training/testing (Metz et al., 2005a).

Restraint Stress—Animals were individually placed in Plexiglas tubes (5 cm inner diameter) for 20 min (Metz et al., 2005a; Kirkland et al., 2008). Restraint stress took place in the morning hours between 8–10 AM one hour prior to behavioural testing. Restraint stress was applied daily for a period of six weeks, starting three weeks prior to pre-lesion up to 3 weeks post-lesion.

Nigrostriatal 6-OHDA Lesion—Thirty minutes prior to surgery, rats received 25 mg/kg i.p. desmethylinipramine (Sigma Aldrich, St. Louis, MO). The rats were then anesthetized with isoflurane (4% for induction, 1.5% for maintenance). The neurotoxic lesions of the nigrostriatal bundle were performed with injections of 6-hydroxydopamine hydrobromide (2 µl of 4 mg/ml in 0.9% saline with 0.02% ascorbic acid (Metz et al., 2001; Metz et al., 2005b) at the following coordinates: 4.0 mm posterior to bregma, 1.5 mm lateral to the midline, and 8.5 mm ventral to the skull surface, with the skull flat between lambda and bregma. The injection rate was set at 1 µl/min with 5 min allowed for diffusion (Paxinos and Watson, 1998, Mercanti et al., 2012).

Behavioural Testing

Open Field Task

Apparatus: The open field arena (100 × 100 × 18 cm) was made of opaque black Plexiglas. The bottom of the arena was divided into 16 zones (22 × 22 cm) by white masking tape (Figure 1A).

Testing: Each rat was individually placed in the middle of the open field arena and video recorded for 5 min. The number of fields rats travelled in the arena was recorded on day 21 post-lesion.

Analysis: Video recordings were scored for activity (total number of fields entered) by an experimenter blind to the experimental condition. Entered fields were scored when more than 50% of the animal's body crossed a subdivision of the open field.

Reaching Movement Performance

Apparatus: Animals were trained and tested in a transparent Plexiglas box according to earlier descriptions (Metz and Whishaw, 2000). Rats were trained to reach for food pellets (45 mg precision pellets, Bioserv, Frenchtown, NJ) placed on a shelf attached to the outside of the front wall (Figure 2A). Two small indentations on the upper side of the shelf, each aligned with one side of the slit, served as indentations to hold the food pellets.

Once rats began reaching, pellets were placed in the indentation contralateral to the limb with which the rat reached. After each reach, animals were required to walk to the back of the box and come to the front again in order to reposition themselves at the food aperture prior to the next reach.

Testing: Pre-training was performed daily for three weeks until success rates reached asymptotic levels according to earlier descriptions (Metz and Whishaw, 2000). Subsequent baseline testing was performed for five successive days, for which values were averaged

("baseline"). During pre- and post-lesion testing rats continued to be tested daily. In each training and test session rats reached for 20 food pellets. On day 21 post-lesion, reaching performance was video recorded with a Canon digital camera set at a shutter speed of 1/1000 for qualitative analysis of the movement patterns. For the filming, the apparatus was illuminated by a two-arm cold light source (Zeiss KL 1500, Carl Zeiss Inc., Jena, Germany) and a white light source (Caselite, Lowel Inc., Brooklyn, NY). Tapes were analyzed frame-by-frame on a Sony digital video recorder.

Analysis: Reaching performance was scored by an experimenter blind to the experimental conditions using frame-by-frame inspection of the videotapes. The quantitative analysis included the number of hits and misses in each session. An attempt was defined as a forelimb movement toward the pellet. The number of attempts were counted and analysed (Metz and Whishaw, 2000). A success was recorded if an animal grasped a food pellet on the first attempt and withdrew the paw with the pellet through the slit to consume the food (Metz and Whishaw, 2002a). Data are shown as percentage of baseline values.

For qualitative analysis, the first three successful reaches were scored according to reaching movement patterns using a 35-point scoring system developed by Metz and Whishaw (2000). The score was used to rate eleven major components of a reaching movement (Orient, Limb lift, Digits close, Aim, Advance, Digits open, Pronation, Grasp, Supination I, Supination II, and Release) and their 35 subcomponents. Each of the 35 subcomponents was rated on a 3-point scale: a score of 0 was given if this movement component was completely absent, a score of 0.5 was given if the movement was present but abnormal, and a score of 1 was given if the movement was normal (Metz and Whishaw, 2000).

In situ Hybridization

Tissue Preparation: The animals were rapidly decapitated, and their brains immediately removed, frozen on dry ice, and stored at -80°C . Serial $16\text{ }\mu\text{m}$ coronal cryostat sections were thaw-mounted onto Superfrost plus microscope slides (VWR, Edmonton, AB, Canada) from the level of striatum (Bregma 1.20 mm – Bregma -0.26 mm) and SN (Bregma -4.80 mm – Bregma -5.80 mm) (Paxinos and Watson, 1998).

Probes and Labelling: ^{35}S -labelled cRNA antisense probes were prepared via *in vitro* transcription with ^{35}S -uridine-5'-thiotriphosphate (UTP). Templates for transcription were part of rat GR, TH, SYN and calcyon cDNAs as described previously (GR: Diaz et al., 1997, Diaz et al., 1998); TH: (Berod et al., 1987); calcyon: (Thome et al., 2001, Zelenin et al., 2002)).

In situ hybridization: All sections labeled for a specific probe were handled simultaneously throughout the process. Briefly, frozen tissue sections were fixed in 4% paraformaldehyde for 4 min at 4°C , then prehybridized for 30 min at room temperature with buffer followed by hybridization using specific probes. Hybridization was performed in $1 \times 10^6\text{ cpm/section}$ with a ^{35}S -Uridine 5'-triphosphate (UTP)-labeled RNA probe. Hybridization condition was 12–14 hr at 50°C for GR, MR (Diaz et al., 1998); 18 hr at 42°C for both TH and SYN (Berod et al., 1987, Marqueze-Pouey et al., 1991); and 14–16 hr at 55°C for calcyon (Heijtz

et al., 2007). After hybridization, sections were washed at 60° C for GR, and 55° C for TH, SYN and calcyon, respectively. Sections were then treated with RNase (1 µg/ml; Roche, Laval, QC, Canada) for 1 hour at 37° C, dehydrated and dried. Sections were exposed to BioMax MR-1 film (Kodak) and stored at room temperature for 1–2 weeks. Films were developed in a 1:4 dilution of Ilford 2150XL developer (Ilford Photo, UK) for 3 minutes, fixed (1:4 dilution for 2 min, Ilford 2150XL fixer), and rinsed. Films were then air dried before being scanned for quantification.

Quantification of in Situ Hybridisation

GR, TH, and SYN mRNA expression was quantified in the SN and the ventral tegmental area, along with GR and calcyon mRNA expression in striatum and the motor cortex, by measuring optical density on autoradiograms. Images were captured with a flatbed scanner (Perfection 1260, Epson, Long Beach, CA), saved in TIF format, and processed with Adobe Photoshop 6.0 (Adobe Systems Inc., Mountain View, CA). Processing of images included color inversion. Optical density was measured using the public domain NIH Image program (Version 1.63, National Institutes of Health, Bethesda, MA).

Statistical Analysis

Statistical analysis was performed using SPSS 22 for Windows 8.1 (IBM Corporation, Armonk, NY, USA). Behavioural measurements were analyzed using analysis of variance (ANOVA). The optical density of brain areas was subjected to a two-way multivariate ANOVA (MANOVA) with CORT, and STRESS treatment as factors. Independent-samples t-tests were used for post-hoc comparisons and paired t-tests for within-group comparisons between baseline and post-lesion performance. Additionally, chi-square tests were conducted to determine possible relationships between the dependent variables. The results are presented as means ± standard error of the mean (SEM). A p-value of less than 0.05 was chosen as the significance level.

Results

Stress Increases Open Field Activity

The number of fields entered in an open field was counted on day 21 postoperative. There was a significant effect of treatment in the number of fields entered ($F(2,11)=3.82$, $p<0.05$). In particular, STRESS animals were significantly more active than CORT and CONTROL animals (both p 's <0.05 ; Figure 1B). There was no significant difference between CORT and CONTROL rats.

Stress Impairs Skilled Reaching Accuracy

There was a significant group effect in the number of attempts performed ($F(2,11)=4.85$, $p<0.05$). The lesion increased the number of reaching attempts in CONTROL animals when compared to pre-lesion tests ($p>0.05$; Figure 2B). Both pre- and post-lesion, the STRESS and CORT animals performed about 2–3 times more reaching attempts than CONTROL rats to grasp a single food pellet. ($p<0.05$ for STRESS, $p<0.01$ for CORT rats).

The qualitative reaching movement performance was scored on the first three successful reaches in a session on day 21 post-lesion. Movement patterns in STRESS animals were significantly lower compared to CONTROL animals and compared to baseline values ($p<0.05$; Figure 2C). In particular, STRESS animals showed abnormalities in rotatory limb movements, such as supinating the paw to bring the food to the mouth, and difficulty opening the digits to release the food pellet.

Stress and clamped CORT Partially Reduce the Impact of a 6-OHDA Lesion

The unilateral 6-OHDA infusion led to a reduction in TH mRNA expression in both SN ($F(4,20)=1.8$, $p<0.001$) and ventral tegmental area ($F(4,20)=5.4$, $p<0.001$) in the lesion hemisphere as indicated by optical density units (Figures 3A, 3B). Follow-up within-group comparisons showed that TH mRNA expression in all groups was significantly lower in the lesion hemisphere than in the non-lesion hemisphere in SN (CONTROL: $t=3.51$, $p<0.001$; STRESS: $t=1.11$, $p<0.001$; CORT: $t=1.15$, $p<0.001$) and ventral tegmental area (CONTROL: $t=1.24$, $p<0.001$; STRESS: $t=1.60$, $p<0.001$; CORT: $t=8.38$, $p<0.001$).

Post-hoc comparisons showed that optical density for TH mRNA in the SN in the lesion hemisphere of STRESS rats was significantly higher than in CONTROL animals ($t=-2.99$, $p<0.001$). In addition, TH mRNA optical density was significantly higher in both the non-lesion and the lesion sides in CORT animals versus the CONTROL group (non-lesion: $t=-2.38$, $p<0.001$; lesion: $t=-4.29$, $p<0.001$). Stress animals showed higher optical density only in the lesion hemisphere ($t=1.30$, $p<0.001$).

Similarly, TH mRNA optical density in the ventral tegmental area was higher in STRESS than in CONTROL animals in the lesion hemisphere ($t=-5.89$, $p<0.001$) while it was higher in both non-lesion and lesion hemispheres in the CORT group (non-lesion: $t=-1.60$, $p<0.001$; lesion: $t=-2.93$, $p<0.001$).

Stress Exacerbates Loss of Midbrain Synaptophysin mRNA after 6-OHDA Lesion

The expression of SYN mRNA showed an overall significant difference among groups in both SN ($F(4,16)=12.93$, $p<0.001$) and ventral tegmental area (VTA) ($F(4,16)=23.42$, $p<0.001$; Figures 4A, 4B). SYN mRNA expression was lower on the lesion side than on the non-lesion side in SN (CONTROL: $t=4.29$, $p<0.05$; STRESS: $t=5.6$, $p<0.05$; CORT: $t=3.94$, $p<0.05$) and VTA (STRESS: $t=4.35$, $p<0.05$; CORT: $t=10.17$, $p<0.05$).

SYN mRNA levels in STRESS rats compared to CONTROL animals were significantly reduced in the SN (non-lesion: $t=5.56$, $p<0.05$; lesion: $t=4.05$, $p<0.05$) and VTA (non-lesion: $t=3.82$, $p<0.05$; lesion: $t=4.30$, $p<0.05$). CORT animals compared to CONTROL rats had higher optical density units of SYN mRNA in both SN (non-lesion: $t=-6.56$, $p<0.05$) and VTA (non-lesion: $t=6.05$, $p<0.05$; lesion: $t=-7.64$, $p<0.001$). In comparison to CORT rats, STRESS-treated animals also showed significantly lower SYN mRNA density in both SN (non-lesion: $t=11.63$, $p<0.001$; lesion: $t=3.66$, $p<0.05$) and VTA (non-lesion: $t=18.79$, $p<0.001$; lesion: $t=13.70$, $p<0.001$).

Stress and CORT Increase Calcyon mRNA Expression in Motor Cortex and Striatum

The optical density of calcyon mRNA expression among groups was significantly different in motor cortex ($F(4,16)=4.56$, $p<0.05$) and dorsolateral striatum ($F(4,16)=9.54$, $p<0.001$; Figures 5A, 5B). Optical density was significantly higher on the lesion than on the non-lesion side among groups in motor cortex (CONTROL: $t=-5.91$, $p<0.05$; STRESS: $t=-4.26$, $p<0.05$; CORT: $t=-3.81$, $p<0.05$) and in striatum (CONTROL: $t=-7.17$, $p<0.05$; STRESS: $t=-5.30$, $p<0.05$).

In the motor cortex, calcyon mRNA expression was higher in CORT animals compared to CONTROL rats (non-lesion: $t=-2.66$, $p<0.05$; lesion: $t=-4.10$, $p<0.05$). In STRESS animals higher levels were limited to the non-lesion hemisphere ($t=-2.62$, $p<0.05$). Overall, calcyon levels in the lesion hemisphere in the CORT group were higher than in the STRESS group ($t=2.86$, $p<0.05$).

In the striatum, calcyon mRNA expression was significantly higher in both non-lesion and lesion sides in STRESS (non-lesion: $t=-9.32$, $p<0.001$; lesion: $t=-8.00$, $p<0.001$) and CORT animals (non-lesion: $t=-7.23$, $p<0.001$; lesion: $t=-5.18$, $p<0.001$) as compared to CONTROL animals.

Stress and CORT Increase GR Density in Motor Areas after 6-OHDA Lesion

GR mRNA expression in the midbrain of 6-OHDA lesion animals was affected by STRESS and CORT treatments. The expression of GR mRNA showed overall significant differences among groups in both the SN ($F(4,18)=1.93$, $p<0.001$) and VTA ($F(4,16)=2.22$, $p<0.001$; Figures 6A, 6B). In the SN, GR mRNA expression in the lesion hemisphere was significantly lower than in the non-lesion hemisphere (CONTROL: $t=2.60$, $p<0.001$; STRESS: $t=6.81$, $p<0.05$; CORT: $t=5.00$, $p<0.05$) and VTA (STRESS: $t=9.28$, $p<0.05$; CORT: $t=7.37$, $p<0.05$) except for the CONTROL animals ($t=-1.75$, $p=0.18$).

Compared to the CONTROL group, CORT animals showed higher expression of GR mRNA in both SN (non-lesion: $t=-9.48$, $p<0.001$; lesion: $t=-10.11$, $p<0.001$) and VTA (non-lesion: $t=-11.11$, $p<0.001$; lesion: $t=-9.44$, $p<0.001$; Figures 6A, 6B). Compared to the STRESS group, GR mRNA expression in the CORT group was also higher (SN: non-lesion: $t=4.88$, $p<0.05$; lesion: $t=11.59$, $p<0.01$; VTA: non-lesion: $t=4.6$, $p<0.05$; lesion: $t=8.89$, $p<0.001$). In addition, GR mRNA expression in the non-lesion hemisphere among STRESS rats was higher than in CONTROL animals (SN: $t=-6.02$, $p<0.001$; VTA: $t=-2.99$, $p<0.001$), while there was no difference in the lesion hemisphere.

Furthermore, GR mRNA expression showed a significant overall group difference in the motor cortex ($F(4,14)=6.81$, $p<0.001$) and striatum ($F(4,16)=0.72$, $p<0.05$; Figures 7A, 7B). Optical density of GR mRNA expression on the lesion side was significantly higher than on the non-lesion side in motor cortex (CONTROL: $t=-2.61$, $p<0.01$; STRESS: $t=-7.45$, $p<0.01$; CORT: $t=-1.92$, $p<0.001$) and in the striatum (CONTROL: $t=-6.7$, $p<0.05$; STRESS: $t=-8.91$, $p<0.01$; CORT: $t=-6.22$, $p<0.01$). CORT animals showed elevated GR mRNA levels in both hemispheres compared to CONTROL animals in both motor cortex (non-lesion: $t=-1.56$, $p<0.001$; lesion: $t=1.48$, $p<0.001$) and striatum (non-lesion: $t=-1.34$, $p<0.001$; lesion: $t=-1.33$, $p<0.001$). Compared to STRESS animals, CORT rats also

displayed reduced GR mRNA expression in non-lesion motor cortex ($t=-6.00$, $p<0.01$), but increased expression in lesion motor cortex ($t=4.75$, $p<0.01$) and in both striatal hemispheres (non-lesion: $t=8.18$, $p<0.001$; lesion: $t=4.02$, $p<0.01$). Furthermore, STRESS rats also showed significant increases in both hemispheres as compared to CONTROL rats in both motor cortex (non-lesion: $t=-6.02$, $p<0.001$; lesion: $t=-5.82$, $p<0.01$) and striatum (non-lesion: $t=-9.15$, $p<0.001$; lesion: $t=-6.27$, $p<0.01$).

Discussion

The present data support the notion that stress is a necessary variable to be considered in the pathogenesis and treatment of Parkinson's disease. Here we show that following unilateral dopamine depletion in a rat model, chronic physical stress and targeted CORT manipulation modulate motor function in association with altered expression of genes related to neuronal plasticity. Both stress and clamped CORT supplementation increased open-field activity and exaggerated skilled movement impairments induced by dopamine depletion, though stress at times appeared to have a greater effect. Although stress and clamped CORT partially protected TH expression in both SN and VTA against the neurotoxic insult, they differentially modulated the expression of SYN, calcyon and GR mRNA in midbrain and cortical areas. GR in SN and VTA was affected by both stress and CORT clamping in the non-lesion hemisphere, but in the lesioned hemisphere only CORT clamping raised GR levels in the motor cortex and striatum, however, GR was higher in both hemispheres under both experimental conditions.

As previously shown (Jadavji et al., 2006; Smith et al., 2008), stress exacerbates the behavioural impairments after unilateral dopamine depletion in rats. The current study replicated these findings, showing a significant lesion-induced reduction in both qualitative and quantitative scoring of fine motor performance. Additionally, chronic exposure to stress altered locomotor activity to a larger degree than clamped elevated CORT levels. This observation indicates that while CORT exposure mimics a specific component of the stress response in behaviour, the endocrine complexity of the response to a physical stressor gives rise to additional behavioural and neurochemical responses.

It is important to note that adrenalectomy does not only remove endogenous CORT production but also adrenaline and noradrenaline production, which represent central components of the sympathetic adrenal medullary (SAM) axis. Thus, the latter two stress-responsive hormones are likely responsible for the behavioural differences seen between STRESS and CORT rats; especially increased activity in STRESS rats in the open field task. The findings suggest that treating rats with ADX combined with CORT clamp reduces the physiological response to stress on the HPA axis, excluding the SAM axis.

At the molecular level, the response of catecholamines such as dopamine and their related machinery is closely influenced by stress (Sabban and Kvetnanský, 2001). This rate-limiting enzyme of dopamine synthesis is considered the major determinant of midbrain dopamine levels (Daubner, 2011). Stress may profoundly increase dopaminergic activity (Pani, 2000), which then stimulates biosynthesis via TH in the VTA and to a lesser extent in SN (Serova et al., 1999). Here, the SN and VTA in 6-OHDA lesion animals reveal reduced TH levels,

which is in accordance with earlier studies of short- and long-term upregulation of TH mRNA transcription by most types of stress (Alterio et al., 2001; Wong and Tank, 2007; Tank et al., 2008). Evidence suggests a complex regulation of TH expression at all levels, transcriptional, post-transcriptional, translational and post-translational related to stress (Kumer and Vrana 1996; Xu et al. 2007). Here, exposure of animals to stress or CORT partially preserved TH mRNA levels, however, which concurs with previous findings (Serova et al., 1999).

The present observations agree with the idea that stress and glucocorticoids affect degenerative and neuroplastic adaptive changes in the nigrostriatal motor system (Brunelin, 2008; de Pablos et al., 2014; Vyas et al., 2016). Chronic stress can accelerate neuronal loss in the midbrain dopamine system and exaggerate motor dysfunction in 6-OHDA lesion rats (Smith et al., 2008). It is possible that stress-induced reduction in neuronal plasticity may hamper motor learning and compensatory processes in the dopamine-depleted brain, thus further exaggerating motor loss. While GCs may directly affect neurodegenerative events and plasticity (Marchetti et al., 2005), these can also occur through microglia activation and upregulated inflammatory responses (de Pablos et al., 2014), through oxidative stress (Lotharius et al., 2005; Jiang et al., 2016) or through changes in neurotrophic factor levels (Scaccianoce et al., 2004).

Stress and elevated GC levels may also exert beneficial effects in the nigrostriatal system. Clamping of CORT in the present study ameliorated TH loss, it is possible that the particular CORT concentration in the present study promoted neuroprotective properties or adaptive neuroplasticity as described for other GC treatments (e.g., Wojtal et al., 2006). Accordingly, low levels of CORT supplementation in ADX-treated animals has been shown to enhance neuroprotection (Qiu et al., 2012) which may occur through moderating the inflammatory response associated with 6-OHDA-induced neurodegeneration which can also be seen in human PD (Theodore and Maragos, 2015). The immune response after 6-OHDA lesion involves activated microglia, but also infiltration of other immune cells such as macrophages (Tentillier et al., 2016). Consequently, GC administration may promote neuroprotection through their well-documented anti-inflammatory effects (Silva et al., 2009; Tentillier et al., 2016). In addition, neurotrophic factor expression in striatum, including brain-derived neurotrophic factor, may also occur independently of GR-mediated mechanisms (Schulte-Herbruggen et al., 2006).

The present findings suggest further molecular mechanisms for stress-induced neuroplasticity. In general, unilateral dopamine depletions induce cortical changes in gene expression in a bilateral pattern (Rodriguez-Puertas et al., 1999; Steiner and Kitai, 2001; Orioux et al., 2002). In turn, stress diminished SYN mRNA expression in both hemispheres, indicating compromised integrity and synaptic plasticity of nigral cells. SYN is an integral protein of the synaptic vesicle membrane and is required for vesicle fusion and neurotransmitter release (Navone et al., 1986; Greengard et al., 1993; Alder et al., 1995). Alterations in SYN expression have been linked to stress-associated metaplastic changes (Kim and Yoon, 1998), altered synaptic terminal structure (Magarinos et al., 1997), and long-term potentiation and depression (Huang et al., 2005). Our findings confirm that SYN

expression is stress-responsive (Thome et al., 2001; Xu et al., 2004), which might mediate some of the pathophysiological and behavioural effects of stress in the basal ganglia.

Although SYN levels were found to be unaffected by PD in humans (Girault et al., 1989), stress might synergistically limit neuronal plastic responses following dopamine depletion by reducing SYN expression. It has been shown that SYN levels reflect the amount of neurotransmitter release (Alder et al., 1995), which would indicate reduced dopamine availability causing movement deficits. These findings indicate reduced function of nigral afferents and, thus, reduced efficacy of dopamine producing neurons. Furthermore, limited SYN expression might indicate reduced plastic properties of nigral neurons thus abolishing post-lesion behavioural plasticity (i.e., compensation) particularly in stress-treated animals. Thus, the expression of SYN may be a central molecular component of stress-induced changes in synaptic plasticity in the motor system and associated behavioral alterations. Interestingly, the regulation of synaptic vesicle proteins may be relevant to disorders such as psychoses and depression that are sensitive to stress and involve changes in neural and synaptic plasticity (Eastwood et al 1995; Honer et al 1999). Hence, the regulation of SYN and also calcyon could represent a potential mechanism of the development of non-motor symptoms in PD patients (Mollenhauer et al., 2013; Balestrino and Martinez-Martin, 2017).

Calcyon is a membrane protein associated with the D1 and D5 receptors (Lezcano and Bergson, 2002; Zelenin et al., 2002). Its function was suggested to modulate intracellular calcium signaling and signal transduction (Lezcano and Bergson, 2002; Tang and Bezprozvanny, 2004). It is expressed in prefrontal cortex, hippocampus and in the motor system such as striatum, SN and cerebellum in primate and rat (Zelenin et al., 2002; Oakman and Meador-Woodruff, 2004). Out of the latter, calcyon density in SN was noted to be particularly high (Oakman and Meador-Woodruff, 2004). Furthermore, calcyon forms a ternary complex with D1 dopamine receptor (D(1)DR1) and thus regulates D(1)DR trafficking (Ha, 2012). Calcyon may also have a potential role as a partner of clathrin light chains implicated in endocytosis and synaptic plasticity in PD (Xiao, 2006; Heijtz, 2007). Stress- and CORT-treated groups both showed significantly elevated calcyon mRNA levels in their motor cortices and striatum, suggesting that stress-regulated calcyon expression may be related to the gross and fine movement deficits after 6-OHDA lesion. Accordingly, calcyon expression can be directly linked to behavioural change (Trantham-Davidson et al., 2008).

A possible upstream link to generate stress-related gene expression patterns is GR expression. The present findings show global upregulation of GR mRNA in the motor system in response to chronic CORT manipulation. Given the role of GR in the adaptive response to stress (Groeneweg et al., 2011), it can be postulated that elevated levels of GR expression represent an adaptive response to persistent CORT presence due to clamping. Given the global response of GR to CORT supplementation, stress effects on GR density were most prominent in the lesion hemisphere. This suggests that the non-steroidal components of the HPA response may be involved in mitigating GR overexpression. Furthermore, it has been previously shown that GR and MR signaling in the motor system interact to regulate negative feedback and determine motor outcome (Jadavji et al., 2011). It

is possible that MR function or its expression levels may have adjusted to chronic CORT clamping in a way to influence motor outcome or the rate of neuronal degeneration.

A possible explanation for different GR expression patterns in SN/VTa and motor cortex/striatum might be that GR influence the dopaminergic neurotransmission by an indirect mechanism, which possibly involves intermittent neurotransmitter release (Czyrak, 2001). Other possible mechanisms might involve greater vulnerability of dopaminergic midbrain subpopulations to stress (Parlato, 2014) or GR-mediated plasticity that contributes to stress-induced sensitization (Vendruscolo, 2012). Moreover, GR-dependent interaction between dopaminergic and dopaminergic populations was reported in the mesocorticolimbic circuit following stress in response to socially defeated mice (Barik, 2013). It is also possible that GR/MR signaling in the motor system interact to regulate negative feedback and influence motor outcome (Jadavji et al., 2011) and thus affect the rate of neuronal degeneration.

While previous studies have investigated the effects of 6-OHDA noradrenergic system lesions with GR mRNA expression (Maccari et al., 1990; Yau and Seckl, 1992) the present study is the first to evaluate changes of nigrostriatal 6-OHDA lesion on this central component of the HPA axis. The GR upregulation in response to stress and CORT elevation may represent a protective or adaptive mechanism in response to elevated CORT conditions. For example, a recent study showed that GR is critical for transcriptional regulation of the mitochondrial genome (Hunter et al., 2016) which may be particularly vital for mitochondrial function and survival in PD (Kupsch et al., 2014; Tuon et al. 2015). Accordingly, GR density has been attributed a role in neurodegenerative regulation in PD (Ros-Bernal et al., 2011), which may exacerbate symptoms and neuronal loss in Parkinson's disease.

Conclusion

The present findings show that a 6-OHDA lesion results in characteristic changes in GR density as a central component of HPA axis regulation, thus potentially altering the stress response in a dopamine-depleted brain. Furthermore, we demonstrate that genes related to neuronal plasticity and synaptic function are altered by the loss of nigrostriatal dopamine and by synergistic effects of stress and stress hormones. Synaptophysin and calcyon are stress-responsive factors, which may be central in producing some of the pathophysiological effects of stress in the motor system. The observed changes may be relevant to the motor and non-motor symptoms of PD that are modulated by stress and neuroplastic adaptations to stress. The stress-evoked alteration of dopamine-related hallmarks of synaptic and neuronal plasticity offer important considerations for the identification of disease pathways and novel therapeutic approaches for PD.

Acknowledgments

The authors are grateful to Keri Colwell for technical assistance. The authors thank Dr. Rochellys Diaz-Heijtz, Karolinska Institutet, Stockholm, Sweden, for generously providing the GR and calcyon probes; Dr. Jaques Mallet, CNRS, Paris, France, for providing the TH probe; and Dr. Johannes Thome, Central Institute of Mental Health, Mannheim, Germany, for providing the synaptophysin probe. This research was supported by the National Institutes of Neurological Disorders and Stroke, NIH Grant # NS043588, by the Natural Sciences and Engineering Research

Council of Canada grant #05519, and by the Canadian Institutes of Health Research grants #102652 and #363195 (G.M.).

References

- Ahima RS, Harlan RE. Charting of type II glucocorticoid receptor-like immunoreactivity in the rat central nervous system. *Neuroscience*. 1990; 39:579–604. [PubMed: 1711170]
- Alder J, Kanki H, Valtorta F, Greengard P, Poo MM. Overexpression of synaptophysin enhances neurotransmitter secretion at *Xenopus* neuromuscular synapses. *J Neurosci*. 1995; 15:511–519. [PubMed: 7823159]
- Alterio J, Mallet J, Biguet NF. Multiple complexes involved in tyrosine hydroxylase mRNA stability in rat adrenal medulla, after reserpine stimulation. *Mol Cell Neurosci*. 2001; 17:179–89. [PubMed: 11161478]
- Balestrino R, Martinez-Martin P. Neuropsychiatric symptoms, behavioural disorders, and quality of life in Parkinson's disease. *J Neurol Sci*. 2017; 373:173–78. [PubMed: 28131182]
- Brunelin J, d'Amato T, van Os J, Cochet A, Suaud-Chagny MF, Saoud M. Effects of acute metabolic stress on the dopaminergic and pituitary-adrenal axis activity in patients with schizophrenia, their unaffected siblings and controls. *Schizophr Res*. 2008; 100:206–11. [PubMed: 18155448]
- Charcot JM. Lectures on the Diseases of the Nervous System. 1878
- Charlett A, Dobbs RJ, Purkiss AG, Wright DJ, Peterson DW, Weller C, Dobbs SM. Cortisol is higher in parkinsonism and associated with gait deficit. *Acta Neurol Scand*. 1998; 97:77–85. [PubMed: 9517856]
- Chen JX, Tang YT, Yang JX. Changes of glucocorticoid receptor and levels of CRF mRNA, POMC mRNA in brain of chronic immobilization stress rats. *Cell Mol Neurobiol*. 2008; 28:237–244. [PubMed: 17636405]
- Connolly BS, Lang AE. Pharmacological treatment of Parkinson disease: a review. *JAMA*. 2014; 311:1670–1683. [PubMed: 24756517]
- Datson NA, Speksnijder N, Mayer JL, Steenbergen PJ, Korobko O, Goeman J, de Kloet ER, Joels M, Lucassen PJ. The transcriptional response to chronic stress and glucocorticoid receptor blockade in the hippocampal dentate gyrus. *Hippocampus*. 2012; 22:359–371. [PubMed: 21184481]
- Dauer W, Przedborski S. Parkinson's Disease: Mechanisms and Models. *Neuron*. 2003; 39:889–909. [PubMed: 12971891]
- Davie CA. A review of Parkinson's disease. *Br Med Bull*. 2008; 86:109–127. [PubMed: 18398010]
- de Pablos RM, Herrera AJ, Espinosa-Oliva AM, Sarmiento M, Muñoz MF, Machado A, Venero JL. Chronic stress enhances microglia activation and exacerbates death of nigral dopaminergic neurons under conditions of inflammation. *J Neuroinflammation*. 2014; 11:34. [PubMed: 24565378]
- DeVries AC, Joh HD, Bernard O, Hattori K, Hurn PD, Traystman RJ, Alkayed NJ. Social stress exacerbates stroke outcome by suppressing Bcl-2 expression. *Proc Natl Acad Sci U S A*. 2001; 98:11824–11828. [PubMed: 11553785]
- Diaz R, Brown RW, Seckl JR. Distinct ontogeny of glucocorticoid and mineralocorticoid receptor and 11 β -hydroxysteroid dehydrogenase types I and II mRNAs in the fetal rat brain suggest a complex control of glucocorticoid actions. *J Neurosci*. 1998; 18:2570–2580. [PubMed: 9502816]
- Diaz R, Sokoloff P, Fuxe K. Codistribution of the dopamine D3 receptor and glucocorticoid receptor mRNAs during striatal prenatal development in the rat. *Neurosci Lett*. 1997; 227:119–122. [PubMed: 9180218]
- Djamshidian A, Lees AJ. Can stress trigger Parkinson's disease? *Journal of neurology, neurosurgery, and psychiatry*. 2014; 85:878–881.
- Eastwood SL, Burnet PW, Harrison PJ. Altered synaptophysin expression as a marker of synaptic pathology in schizophrenia. *Neuroscience*. 1995; 66:309–319. [PubMed: 7477874]
- Gibberd FB, Simmonds JP. Neurological disease in ex-Far-East prisoners of war. *Lancet*. 1980; 2:135–137. [PubMed: 6105303]
- Girault JA, Raisman-Vozari R, Agid Y, Greengard P. Striatal phosphoproteins in Parkinson disease and progressive supranuclear palsy. *Proc Natl Acad Sci U S A*. 1989; 86:2493–2497. [PubMed: 2928345]

- Greengard P, Valtorta F, Czernik AJ, Benfenati F. Synaptic vesicle phosphoproteins and regulation of synaptic function. *Science*. 1993; 259:780–785. [PubMed: 8430330]
- Groeneweg FL, Karst H, de Kloet ER, Joels M. Rapid non-genomic effects of corticosteroids and their role in the central stress response. *J Endocrinol*. 2011; 209:153–167. [PubMed: 21357682]
- Ha CM, Park D, Han JK, Jang JI, Park JY, Hwang EM, Seok H, Chang S. Calcyon forms a novel ternary complex with dopamine D1 receptor through PSD-95 protein and plays a role in dopamine receptor internalization. *J Biol Chem*. 2012; 287:31813–31822. [PubMed: 22843680]
- Harris RB, Palmondon J, Leshin S, Flatt WP, Richard D. Chronic disruption of body weight but not of stress peptides or receptors in rats exposed to repeated restraint stress. *Horm Behav*. 2006; 49:615–625. [PubMed: 16423352]
- Heijtz RD, Alexeyenko A, Castellanos FX. Calcyon mRNA expression in the frontal-striatal circuitry and its relationship to vesicular processes and ADHD. *Behav Brain Funct*. 2007; 3:33. [PubMed: 17623072]
- Honer WG, Falkai P, Chen C, Arango V, Mann JJ, Dwork AJ. Synaptic and plasticity-associated proteins in anterior frontal cortex in severe mental illness. *Neuroscience*. 1999; 91:1247–1255. [PubMed: 10391432]
- Hunter RG, Seligsohn M, Rubin TG, Griffiths BB, Ozdemir Y, Pfaff DW, Datson NA, McEwen BS. Stress and corticosteroids regulate rat hippocampal mitochondrial DNA gene expression via the glucocorticoid receptor. *Proc Natl Acad Sci U S A*. 2016; 113:9099–104. [PubMed: 27457949]
- Jadavji NM, Supina RD, Metz GA. Blockade of mineralocorticoid and glucocorticoid receptors reverses stress-induced motor impairments. *Neuroendocrinology*. 2011; 94:278–290. [PubMed: 22024815]
- Jadavji NM, Kolb B, Metz GA. Enriched environment improves motor function in intact and unilateral dopamine-depleted rats. *Neuroscience*. 2006; 140:1127–1138. [PubMed: 16678972]
- Jiang T, Sun Q, Chen S. Oxidative stress: A major pathogenesis and potential therapeutic target of antioxidative agents in Parkinson's disease and Alzheimer's disease. *Prog Neurobiol*. 2016; 147:1–19. [PubMed: 27769868]
- Joels M, Karst H, Alfarez D, Heine VM, Qin Y, van Riel E, Verkuy M, Lucassen PJ, Krugers HJ. Effects of chronic stress on structure and cell function in rat hippocampus and hypothalamus. *Stress*. 2004; 7:221–231. [PubMed: 16019587]
- Kim JJ, Yoon KS. Stress: metaplastic effects in the hippocampus. *Trends Neurosci*. 1998; 21:505–509. [PubMed: 9881846]
- Kim KS, Han PL. Optimization of chronic stress paradigms using anxiety- and depression-like behavioral parameters. *J Neurosci Res*. 2006; 83:497–507. [PubMed: 16416425]
- Kirkland SW, Coma AK, Colwell KL, Metz GA. Delayed recovery and exaggerated infarct size by post-lesion stress in a rat model of focal cerebral stroke. *Brain Research*. 2008; 1201:151–160. [PubMed: 18308294]
- Kitraki E, Karandrea D, Kittas C. Long-lasting effects of stress on glucocorticoid receptor gene expression in the rat brain. *Neuroendocrinology*. 1999; 69:331–338. [PubMed: 10343174]
- Klein C, Westerberger A. Genetics of Parkinson's Disease. *Cold Spring Harbor Perspectives in Medicine*. 2012; 2:a008888. [PubMed: 22315721]
- Kumer SC1, Vrana KE. Intricate regulation of tyrosine hydroxylase activity and gene expression. *J Neurochem*. 1996; 67:443–62. [PubMed: 8764568]
- Kupsch A, Schmidt W, Gizatullina Z, Debska-Vielhaber G, Voges J, Striggow F, Panther P, Schwegler H, Heinze HJ, Vielhaber S, Gellerich FN. 6-Hydroxydopamine impairs mitochondrial function in the rat model of Parkinson's disease: respirometric, histological, and behavioral analyses. *J Neural Transm (Vienna)*. 2014; 121:1245–57. [PubMed: 24627045]
- Lee SY, Kang JS, Song GY, Myung CS. Stress induces the expression of heterotrimeric G protein beta subunits and the phosphorylation of PKB/Akt and ERK1/2 in rat brain. *Neurosci Res*. 2006; 56:180–192. [PubMed: 16949170]
- Leussis MP, Andersen SL. Is adolescence a sensitive period for depression? Behavioral and neuroanatomical findings from a social stress model. *Synapse*. 2008; 62:22–30. [PubMed: 17957735]

- Lezcano N, Bergson C. D1/D5 dopamine receptors stimulate intracellular calcium release in primary cultures of neocortical and hippocampal neurons. *J Neurophysiol.* 2002; 87:2167–2175. [PubMed: 11929934]
- Lotharius J, Falsig J, van Beek J, Payne S, Dringen R, Brundin P, Leist M. Progressive degeneration of human mesencephalic neuron-derived cells triggered by dopamine-dependent oxidative stress is dependent on the mixed-lineage kinase pathway. *J Neurosci.* 2005; 25:6329–42. [PubMed: 16000623]
- Maccari S, Le Moal M, Angelucci L, Mormede P. Influence of 6-OHDA lesion of central noradrenergic systems on corticosteroid receptors and neuroendocrine responses to stress. *Brain Res.* 1990; 533:60–65. [PubMed: 2128197]
- Magarinos AM, Verdugo JM, McEwen BS. Chronic stress alters synaptic terminal structure in hippocampus. *Proc Natl Acad Sci U S A.* 1997; 94:14002–14008. [PubMed: 9391142]
- Marchetti B, Serra PA, L'Episcopo F, Tirolo C, Caniglia S, Testa N, Cioni S, Gennuso F, Rocchitta G, Desole MS, Mazzarino MC, Miele E, Morale MC. Hormones are key actors in gene x environment interactions programming the vulnerability to Parkinson's disease: glia as a common final pathway. *Ann N Y Acad Sci.* 2005; 1057:296–318. [PubMed: 16399902]
- Marqueze-Pouey B, Wisden W, Malosio ML, Betz H. Differential expression of synaptophysin and synaptoporin mRNAs in the postnatal rat central nervous system. *J Neurosci.* 1991; 11:3388–3397. [PubMed: 1941089]
- Mercanti G, Bazzu G, Giusti P. A 6-hydroxydopamine in vivo model of Parkinson's disease. *Methods Mol Biol.* 2012; 846:355–364. [PubMed: 22367825]
- Metz GA, Whishaw IQ. Skilled reaching an action pattern: stability in rat (*Rattus norvegicus*) grasping movements as a function of changing food pellet size. *Behavioural brain research.* 2000; 116:111–122. [PubMed: 11080542]
- Metz GA, Farr T, Ballermann M, Whishaw IQ. Chronic levodopa therapy does not improve skilled reach accuracy or reach range on a pasta matrix reaching task in 6-OHDA dopamine-depleted (hemi-Parkinson analogue) rats. *Eur J Neurosci.* 2001; 14:27–37. [PubMed: 11488946]
- Metz GA, Jadavji NM, Smith LK. Modulation of motor function by stress: a novel concept of the effects of stress and corticosterone on behavior. *Eur J Neurosci.* 2005a; 22:1190–1200. [PubMed: 16176362]
- Metz GA, Tse A, Ballermann M, Smith LK, Fouad K. The unilateral 6-OHDA rat model of Parkinson's disease revisited: an electromyographic and behavioural analysis. *Eur J Neurosci.* 2005b; 22:735–44. [PubMed: 16101755]
- Mollenhauer B, Sixel-Döring F, Storch A, Schneider C, Hilker R, Kalbe E. Early recognition of Parkinson's disease. Objectifiable non-motor symptoms and biomarkers. *Nervenarzt.* 2013; 84:918–26. [PubMed: 23831930]
- Navone F, Jahn R, Di Gioia G, Stukenbrok H, Greengard P, De Camilli P. Protein p38: an integral membrane protein specific for small vesicles of neurons and neuroendocrine cells. *J Cell Biol.* 1986; 103:2511–2527. [PubMed: 3097029]
- Oakman SA, Meador-Woodruff JH. Calcyon transcript expression in macaque brain. *J Comp Neurol.* 2004; 468:264–276. [PubMed: 14648684]
- Pani L, Porcella A, Gessa GL. The role of stress in the pathophysiology of the dopaminergic system. *Mol Psychiatry.* 2000; 5:14–21. [PubMed: 10673764]
- Paxinos, G., Watson, C. *The Rat Brain in Stereotaxic Coordinates.* Academic Press; 1998.
- Perovic M, Mladenovic A, Rakic L, Ruzdijic S, Kanazir S. Increase of GAP-43 in the rat cerebellum following unilateral striatal 6-OHDA lesion. *Synapse.* 2005; 56:170–174. [PubMed: 15765516]
- Qiu G, Spangler EL, Wan R, Miller M, Mattson MP, So KF, de Cabo R, Zou S, Ingram DK. Neuroprotection provided by dietary restriction in rats is further enhanced by reducing glucocorticoids. *Neurobiol Aging.* 2012; 33:2398–410. [PubMed: 22226488]
- Rasmusson AM, Shi L, Duman R. Downregulation of BDNF mRNA in the hippocampal dentate gyrus after re-exposure to cues previously associated with footshock. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology.* 2002; 27:133–142. [PubMed: 12093587]

- Rodriguez-Puertas R, Herrera-Marschitz M, Koistinaho J, Hokfelt T. Dopamine D1 receptor modulation of glutamate receptor messenger RNA levels in the neocortex and neostriatum of unilaterally 6-hydroxydopamine-lesioned rats. *Neuroscience*. 1999; 89:781–797. [PubMed: 10199613]
- Ros-Bernal F, Hunot S, Herrero MT, Parnadeau S, Corvol JC, Lu L, Alvarez-Fischer D, Carrillo-de Sauvage MA, Saurini F, Coussieu C, Kinugawa K, Prigent A, Hoglinger G, Hamon M, Tronche F, Hirsch EC, Vyas S. Microglial glucocorticoid receptors play a pivotal role in regulating dopaminergic neurodegeneration in parkinsonism. *Proc Natl Acad Sci U S A*. 2011; 108:6632–6637. [PubMed: 21467220]
- Sabban EL, Kvetnansky R. Stress-triggered activation of gene expression in catecholaminergic systems: dynamics of transcriptional events. *Trends Neurosci*. 2001; 24:91–98. [PubMed: 11164939]
- Scaccianoce S, Mattei V, Del Bianco P, Gizzi C, Sorice M, Hiraiwa M, Misasi R. Hippocampal prosaposin changes during stress: a glucocorticoid-independent event. *Hippocampus*. 2004; 14:275–280. [PubMed: 15132426]
- Schober A. Classic toxin-induced animal models of Parkinson's disease: 6-OHDA and MPTP. *Cell Tissue Res*. 2004; 318:215–24. [PubMed: 15503155]
- Serova L, Danailov E, Chamas F, Sabban EL. Nicotine infusion modulates immobilization stress-triggered induction of gene expression of rat catecholamine biosynthetic enzymes. *J Pharmacol Exp Ther*. 1999; 291:884–892. [PubMed: 10525113]
- Silva C, Fuxe K, Chadi G. Involvement of astroglial fibroblast growth factor-2 and microglia in the nigral 6-OHDA parkinsonism and a possible role of glucocorticoid hormone on the glial mediated local trophism and wound repair. *J Neural Transm Suppl*. 2009; 2009:185–202.
- Smith, Jadavji NM, Colwell KL, Katrina Perehudoff S, Metz GA. Stress accelerates neural degeneration and exaggerates motor symptoms in a rat model of Parkinson's disease. *Eur J Neurosci*. 2008; 27:2133–2146. [PubMed: 18412632]
- Smith AD, Castro SL, Zigmond MJ. Stress-induced Parkinson's disease: a working hypothesis. *Physiol Behav*. 2002; 77:527–531. [PubMed: 12526994]
- Steiner H, Kitai ST. Unilateral striatal dopamine depletion: time-dependent effects on cortical function and behavioural correlates. *Eur J Neurosci*. 2001; 14:1390–1404. [PubMed: 11703467]
- Tang TS, Bezprozvanny I. Dopamine receptor-mediated Ca(2+) signaling in striatal medium spiny neurons. *J Biol Chem*. 2004; 279:42082–42094. [PubMed: 15292232]
- Tank AW, Xu L, Chen X, Radcliffe P, Sterling CR. Post-transcriptional regulation of tyrosine hydroxylase expression in adrenal medulla and brain. *Ann N Y Acad Sci*. 2008; 1148:238–48. [PubMed: 19120116]
- Tentillier N, Etzerodt A, Olesen MN, Rizalar FS, Jacobsen J, Bender D, Moestrup SK, Romero-Ramos M. Anti-Inflammatory Modulation of Microglia via CD163-Targeted Glucocorticoids Protects Dopaminergic Neurons in the 6-OHDA Parkinson's Disease Model. *J Neurosci*. 2016; 36:9375–9390. [PubMed: 27605613]
- Theodore S, Maragos W. 6-Hydroxydopamine as a tool to understand adaptive immune system-induced dopamine neurodegeneration in Parkinson's disease. *Immunopharmacol Immunotoxicol*. 2015; 37:39339–9.
- Thome J, Pesold B, Baader M, Hu M, Gewirtz JC, Duman RS, Henn FA. Stress differentially regulates synaptophysin and synaptotagmin expression in hippocampus. *Biol Psychiatry*. 2001; 50:809–812. [PubMed: 11720700]
- Trantham-Davidson H, Vazdarjanova A, Dai R, Terry A, Bergson C. Up-regulation of calcyon results in locomotor hyperactivity and reduced anxiety in mice. *Behavioural brain research*. 2008; 189:244–249. [PubMed: 18295356]
- Tuon T, Souza PS, Santos MF, Pereira FT, Pedroso GS, Luciano TF, De Souza CT, Dutra RC, Silveira PC, Pinho RA. Physical training regulates mitochondrial parameters and neuroinflammatory mechanisms in an experimental model of Parkinson's disease. *Oxid Med Cell Longev*. 2015; 2015:261809. [PubMed: 26448816]

- Vyas S, Rodrigues AJ, Silva JM, Tronche F, Almeida OF, Sousa N, Sotiropoulos I. Chronic Stress and Glucocorticoids: From Neuronal Plasticity to Neurodegeneration. *Neural Plast.* 2016; 2016:6391686. [PubMed: 27034847]
- Wang HL, Morales M. Corticotropin-releasing factor binding protein within the ventral tegmental area is expressed in a subset of dopaminergic neurons. *J Comp Neurol.* 2008; 509:302–318. [PubMed: 18478589]
- Whishaw IQ, Pellis SM. The structure of skilled forelimb reaching in the rat: a proximally driven movement with a single distal rotatory component. *Behavioural brain research.* 1990; 41:49–59. [PubMed: 2073355]
- Wojtal K, Trojnar MK, Czuczwar SJ. Endogenous neuroprotective factors: neurosteroids. *Pharmacol Rep.* 2006; 58:335–340. [PubMed: 16845207]
- Wong DL, Tank AW. Stress-induced catecholaminergic function: transcriptional and post-transcriptional control. *Stress.* 2007; 10:121–30. [PubMed: 17514580]
- Xu H, He J, Richardson JS, Li XM. The response of synaptophysin and microtubule-associated protein 1 to restraint stress in rat hippocampus and its modulation by venlafaxine. *J Neurochem.* 2004; 91:1380–1388. [PubMed: 15584914]
- Xu L, Chen X, Sun B, Sterling C, Tank AW. Evidence for regulation of tyrosine hydroxylase mRNA translation by stress in rat adrenal medulla. *Brain Res.* 2007; 1158:1–10. [PubMed: 17543899]
- Yau JL, Seckl JR. Central 6-hydroxydopamine lesions decrease mineralocorticoid, but not glucocorticoid receptor gene expression in the rat hippocampus. *Neurosci Lett.* 1992; 142:159–162. [PubMed: 1454210]
- Zelenin S, Aperia A, Diaz Heijtz R. Calcyon in the rat brain: cloning of cDNA and expression of mRNA. *J Comp Neurol.* 2002; 446:37–45. [PubMed: 11920718]
- Zigmond MJ, Berger TW, Grace AA, Stricker EM. Compensatory responses to nigrostriatal bundle injury. Studies with 6-hydroxydopamine in an animal model of parkinsonism. *Mol Chem Neuropathol.* 1989; 10:185–200. [PubMed: 2504173]
- Zuena AR, Mairesse J, Casolini P, Cinque C, Alema GS, Morley-Fletcher S, Chiodi V, Spagnoli LG, Gradini R, Catalani A, Nicoletti F, Maccari S. Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats. *PLoS One.* 2008; 3:e2170. [PubMed: 18478112]

Research Highlights

This manuscript presents the following research highlights:

- Chronic stress exacerbates motor symptoms of dopamine depletion in a rat model;
- Stress and corticosterone modify mRNA expression of dopamine and neuroplasticity factors;
- Stress may affect motor and non-motor symptoms of PD and stress response.

Figure 1. Chronic stress increases open field activity

(A) Photograph illustrating the open field task consisting of an arena to encourage exploratory activity. (B) The number of fields rats entered on day 21 post-lesion showed hyperactivity among STRESS animals when compared to CONTROL and clamped CORT animals. Asterisk indicates significance: * $p < 0.05$, unpaired t-test compared to CONTROL and CORT groups.

Figure 2. Stress exacerbates skilled movement impairments

(A) Photographs illustrating the skilled reaching task in which rats were required to grasp and retrieve individual food pellets with the forelimb contralateral to the 6-OHDA lesion.

(B) STRESS and clamped CORT increased the number of reaching attempts required to grasp a single food pellet on days 7 pre-lesion and day 21 post-lesion. Note that there is no habituation to stress between the two testing time points. (C) Movement scores of STRESS animals on day 21 post-lesion were diminished compared to clamped CORT and control rats. Note that STRESS treatment exerted chronic adverse effects on both quantitative (reaching attempts) and qualitative (movement score) performance. The dotted line indicates levels of baseline performance. Asterisks indicate significances: * $p < 0.05$, ** $p < 0.01$, unpaired t-test compared to CONTROL or baseline, respectively.

Figure 3. Tyrosine hydroxylase (TH) mRNA optical density in substantia nigra (A) and ventral tegmental area (B)

The 6-OHDA-induced dopamine depletion caused a significant reduction in TH mRNA in the lesion hemisphere. STRESS and clamped CORT treatments were associated with higher TH levels in substantia nigra and ventral tegmental area particularly in the lesion hemisphere. Asterisks indicate significances: *** $p < 0.001$, unpaired t-test compared to the respective CONTROL group.

Figure 4. Synaptophysin mRNA optical density in substantia nigra (A) and ventral tegmental area (B)

The 6-OHDA-induced dopamine depletion led to an overall reduction in synaptophysin mRNA in the lesion hemisphere. STRESS was associated with lower synaptophysin TH levels in substantia nigra and ventral tegmental area in the non-lesion and lesion hemisphere while CORT had rather the opposite effect. Asterisks indicate significances: * $p < 0.05$, *** $p < 0.001$, unpaired t-test.

Figure 5. Calcyon mRNA optical density in motor cortex (A) and dorsolateral striatum (B) CORT group rats showed higher calcyon mRNA in the non-lesion motor cortex and non-lesion and lesion striatum as compared to non-stress CONTROL animals. STRESS animals were similarly affected except that the lesion hemisphere was not different. Asterisks indicate significances: * $p<0.05$, *** $p<0.001$, unpaired t-test.

Figure 6. Glucocorticoid receptor (GR) mRNA optical density in substantia nigra (A) and ventral tegmental area (B)
 STRESS led to higher GR mRNA expression only in the non-lesion hemisphere. CORT animals showed higher expression of GR mRNA in both hemispheres in substantia nigra and ventral tegmental area as compared with controls. Asterisks indicate significances: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, unpaired t-test.

Figure 7. Glucocorticoid receptor (GR) mRNA optical density in motor cortex (A) and dorsolateral striatum (B)

Both STRESS and CORT upregulated GR mRNA expression in both hemispheres of motor cortex and striatum as compared to controls. Elevation by CORT in general was higher than that induced by STRESS. Asterisks indicate significances: ** $p < 0.01$, *** $p < 0.001$, unpaired t-test.