Neuroscience Letters 651 (2017) 79–87

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Stress and corticosterone alter synaptic plasticity in a rat model of Parkinson's disease

YongXin Hao, Aref Shabanpoor, Gerlinde A. Metz*

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

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.

ARTICLE INFO

Article history:
Received 1 February 2017
Received in revised form 26 April 2017
Accepted 28 April 2017
Available online 1 May 2017

Keywords:
6-hydroxydopamine
Stress response
Skilled movement
Tyrosine hydroxylase
Synaptophysin
Calcyon
Glucocorticoid receptor

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 non-skilled 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 of PD. The findings suggest a role for stress in motor and non-motor symptoms of PD and stress response.

1. Introduction

Parkinson's disease (PD) is a progressive, incurable, and the second most common neurodegenerative disorder characterized by degeneration of dopaminergic neurons in the substantia nigra (SN) pars compacta [11]. The resulting reduction in striatal dopamine leads to the typical Parkinsonian syndrome that includes tremor, bradykinesia, rigidity, and cognitive deficits [68,9]. The pathogenesis of PD in most cases is unclear [19,68,17]. Studies indicated, however, that many cases of PD may result from an interaction of genetic and environmental causes [36]. Notably, stress represents one of the earliest proposed causes of PD which can potentially trigger or hasten the underlying neurodegeneration [6]. Chronic stress, as a function of elevated glucocorticoid (GC) levels, promotes a pro-inflammatory state, activates microglia and ultimately promotes death of dopaminergic neurons in the SN [13,77]. Furthermore, stress may exacerbate neurodegenerative events and motor deficits in a rat model of PD [67]. These findings are supported by the clinical observation that stress hormones, such as GCs, are positively associated with gait deficits in PD patients [7,68] by activating glucocorticoid receptors (GR) in motor areas of the brain [1].

* Corresponding author at: Canadian Centre for Behavioural Neuroscience, University of Lethbridge, 4401 University Drive, Lethbridge, AB, T1K 3M4, Canada.
E-mail address: gerlinde.metz@uleth.ca (G.A. Metz).

http://dx.doi.org/10.1016/j.neulet.2017.04.063
0304-3940/

Aside from the prominent impact of stress and GCs on motor function [50,67], stress and GCs also are potent modulators of gene expression, which in turn affects neuronal plasticity and neurodegeneration [31,10,77] in frontal cortex [39], prefrontal cortex [8], hippocampus [87], amygdala [33], and hypothalamus [24,39,8]. Stress may also regulate dopaminergic function [77] and through GC-GR interaction contribute to dopaminergic neurodegeneration via modulating the inflammatory response of microglia [61]. Accordingly, GR density has been attributed a role in neurodegeneration and progression of clinical symptoms in PD [61]. In addition, both GR and also the mineralocorticoid receptor (MR) participate in fine motor control and therefore may directly affect motor symptoms of PD [28]. 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 [86,64]. 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.

2. Materials and methods

2.1. Subjects

This experiment involved 18 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 × 25.5 × 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-h 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.

2.2. 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.

2.3. Physiological manipulations and stress procedures

2.3.1. 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 h prior to behavioural training/testing [50].

2.3.2. Restraint stress

Animals were individually placed in Plexiglas tubes (5 cm inner diameter) for 20 min [50,34]. Restraint stress took place in the morning hours between 8 and 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.

2.3.3. Nigrostriatal 6-OHDA lesion

Thirty minutes prior to surgery, rats received 25 mg/kg i.p. desmethylimipramine (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 [49,51] 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 [56,47].

2.4. Behavioural testing

2.4.1. Open field task

2.4.1.1. 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 (Fig. 1A).

2.4.1.2. 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.

2.4.1.3. 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.

2.4.2. Reaching movement performance

2.4.2.1. Apparatus. Animals were trained and tested in a transparent Plexiglas box according to earlier descriptions [48]. 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 (Fig. 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