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Chronic corticosterone aggravates behavioral and neuronal symptomatology in a mouse model of alpha-synuclein pathology

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ABSTRACT

Debilitating, yet underinvestigated nonmotor symptoms related to mood/emotion, such as depression, are common in Parkinson's disease. Here, we explore the role of depression and of the amygdala, a brain region robustly linked to mood/emotion, in synucleinopathy. We hypothesized that mood/emotional deficits might accelerate Parkinson's disease–linked symptomatology, including the formation of α-synuclein pathology. We combined elevated corticosterone treatment, modeling chronic stress and depression, with a model of seeded α-synuclein pathology in mouse striatum and assessed behavioral parameters with a focus on mood/emotion, and neuropathology. We report behavioral resilience against α-synuclein proteinopathy in the absence of additional insults, potentially based on hormesis/conditioning mechanisms. Elevated corticosterone, however, reversed α-synuclein pathology–induced behavioral adaptations and was associated with increased dopaminergic cell loss as well as aggravated α-synuclein pathology in specific brain regions, such as the entorhinal cortex. These findings point to elevated glucocorticoids as a risk factor for Parkinson's disease progression and highlight the potential of glucocorticoid level reducing strategies to slow down disease progression in synucleinopathy.

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1. Introduction

Synucleinopathies, such as Parkinson's disease, are neurodegenerative diseases characterized by the accumulation and aggregation of the presynaptic protein α-synuclein and neuronal loss in the affected brain regions (neocortical, limbic, and nigrostriatal circuities) (Lashuel et al., 2013; Spillantini et al., 1997). Under physiological conditions, α-synuclein is believed to play roles in synaptic transmission (Abeliovich et al., 2000; Burre et al., 2010), exocytosis (Logan et al., 2017), and mitochondrial function (Ludtmann et al., 2016). In Parkinson's disease brains, α-synuclein undergoes conformational changes that render the protein prone to aggregation. Mutations (Kruger et al., 1998; Polymeropoulos et al., 1997; Zarranz et al., 2004), duplication (Singleton et al., 2003), or triplication (Ibanez et al., 2004) of the gene coding for α-synuclein, *SNCA*, is sufficient to cause α-synuclein misfolding and aggregation and early-onset forms of Parkinson's disease. Disease-associated mutations enhance α-synuclein aggregation in vitro and promote the formation of Lewy body and Lewy neuritis–like pathology in neuronal and animal models of Parkinson's disease (Polymenidou and Cleveland, 2012). Although overexpression of wild-type or disease-associated mutants of α-synuclein in rodents or nonhuman primates recapitulates many pathological and motor features of Parkinson's disease, none of these models reproduce the full spectrum of pathological and clinical features of the disease (Dawson et al., 2018).

Recent findings showed the propagation of α-synuclein pathology from host tissues to mesencephalic transplants grafted into Parkinson's disease patient's brains (Kordower et al., 2008; Li et al., 2008) and subsequent studies provided robust evidence for interneuronal transmission of α-synuclein-pathology (Desplats et al., 2009; Volpicelli-Daley et al., 2011). Furthermore, α-synuclein pathology can be induced by inoculation of α-synuclein aggregates (seeds) and be propagated through the central nervous system via a prion-like mechanism (Luk et al., 2012; Masuda-Suzukake et al., 2013; Mougenot et al., 2012). This hypothesis has been tested in rodents treated with different forms of recombinant α-synuclein aggregates (Rey et al., 2016a) or with α-synuclein aggregates derived from postmortem Parkinson's disease (Recasens et al., 2014) or

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Multiple Systems Atrophy (Peng et al., 2018; Prusiner et al., 2015) patient brains. Injection of α-synuclein preformed fibrils (PFFs) in different brain regions, such as the striatum (Luk et al., 2012), the olfactory bulb (Rey et al., 2016b), or the substantia nigra (Masuda-Suzukake et al., 2013) induces pronounced α-synuclein pathology propagation. Interestingly, in these models, the amygdala is among the brain regions most severely affected by α-synuclein pathology. In our hands, the amygdala consistently exhibited the highest density of α-synuclein pathology between 1 and 6 months after α-synuclein PFF injection into the striatum. These observations are consistent with previous studies on postmortem brains from patients with Parkinson's disease and other neurodegenerative diseases, suggesting that the amygdala is particularly prone to the formation of α-synuclein pathology (Nelson et al., 2018; Popescu et al., 2004), which occurs there as early as α-synuclein pathology is observed in the substantia nigra. Whether such pathology in the amygdala elicits clinical symptoms or if it contributes to disease progression remains unclear. Several common comorbidities of Parkinson's disease (sometimes preceding motor symptoms) are, however, linked to the amygdala. Among them are anxiety and depression disorders (Kalia and Lang, 2015; Sagna et al., 2014; Shiba et al., 2000), which have been linked to altered physiology and anatomy of both, the amygdala and dopaminergic systems (Thobois et al., 2017). Similarly, chronic stress and glucocorticoid dysbalance change amygdala physiology and indeed are involved in the development of anxiety and depression (de Kloet et al., 2016; Janak and Tye, 2015; Kavushansky and Richter-Levin, 2006; Sandi and Richter-Levin, 2009).

Despite the prominent involvement of the amygdala in common prodromal emotional/mood pathologies in Parkinson's disease, the coincidental vulnerability of the amygdala to α-synuclein pathology and reports on chronic stress as a risk factor for neurodegeneration in mouse models of Parkinson's disease (Hemmerle et al., 2014; Wu et al., 2016), the role of elevated glucocorticoids and of the amygdala in the pathogenesis of Parkinson's disease, and other synucleinopathies are poorly understood.

Toward addressing this knowledge gap, we sought to investigate whether mood/emotional deficits (elicited by chronic corticosterone administration) might facilitate Parkinson's disease–linked symptomatology, such as the formation of α-synuclein pathology. In addition, we assessed the effects of α-synuclein pathology formation in the amygdala on mood and emotion-linked behaviors.

For this purpose, we used a model of α-synuclein pathology based on intrastriatal injection of α-synuclein PFFs. PFF injection induces prominent pathology propagation throughout the brain, including the amygdala. We combined this model with chronic corticosterone administration, which robustly result in a depressive phenotype (Bacq et al., 2012; David et al., 2009; Gourley et al., 2008). We investigated the possibility that emotion/mood pathology (induced by chronic heightening of corticosterone) and α-synuclein pathology might facilitate each other and that they might synergistically increase behavioral deficits related to motor and nonmotor symptoms of Parkinson's disease. To study behaviors associated with amygdala dysfunction, we applied a wide array of behavioral tests, which were also complemented with tests for motor performance and histological analyses of dopaminergic cell loss, as well as α-synuclein pathology formation and propagation to different brain regions.

2. Materials and methods

2.1. Animals and surgical procedure

C57BL/6JRj male mice were ordered at an age of 8 weeks (Elevage Janvier, 22.0–26.2 g body weight) and allowed to acclimate to the animal house for at least 2 weeks. They were kept at 23 °C (40% humidity) in a 12 h/12h light/dark cycle with free access to standard laboratory rodent chow and water, 3 animals per cage in individually ventilated cages, equipped with a paper role and plastic house for enrichment.

All animal experimentation procedures were approved by the Cantonal Veterinary Authorities (Vaud, Switzerland) and performed in compliance with the European Communities Council Directive of 24 November 1986 (86/609EEC). Every effort was taken to minimize the number of animals used. The ARRIVE guidelines were followed.

Surgical procedures were performed at an age of 3 months (23.4–29.7 g for vehicle-treated and 21.7–33 g for corticosterone-treated animals). Wild-type α-synuclein PFFs (5 μg in 2 μL phosphate buffered saline (PBS)) or PBS (2 μL) were stereotaxically injected unilaterally into the right dorsal striatum (coordinates: AP +0.4, ML +2, DV −2,6).

After behavioral experiments (N = 9 per group, N = 17–18 per group for elevated plus maze), animals were killed by an overdose of thiopental (150 mg/kg) perfused with heparinized saline (0.9%) and fixed with 4% paraformaldehyde for immunohistochemistry and histological studies.

Body weight and fat and lean mass (Echo MRI) of animals were continuously assessed throughout the experiments.

For pilot time course studies of α-synuclein propagation (Fig. 1A), 18 animals were injected with PFFs as described previously, and 6 animals per group were sacrificed at 30, 60, or 90 days after injection.

2.2. Corticosterone treatment

Corticosterone (Sigma) was dissolved in 0.45% hydroxypropyl-b-cyclodextrin (Sigma). Either corticosterone (35 mg/L) in hydroxypropyl-b-cyclodextrin or hydroxypropyl-b-cyclodextrin alone (vehicle) was administered to animals in drinking water starting 4 weeks before surgery and then continuously until sacrifice of the animals as described elsewhere (Bacq et al., 2012).

2.3. Behavioral tests

All behavioral tests were performed in the morning (8 am-1 pm), unless stated otherwise. Camcorders (Sony) were used to record behavior, where applicable. Animals of the different experimental and control groups were assigned randomized numbers for each behavioral test. Randomized individuals were then stratified in a way that the same number of animals per group was tested per 25% of testing time to avoid circadian rhythm bias. Scoring was performed blinded.

2.3.1. Elevated plus maze

Mice were habituated to the experimental room for at least 45 min. They were then placed in the central area of an elevated plus maze (65 cm above the floor, with 2 open and 2 enclosed arms) and allowed to explore the maze for 5 minutes. Maze was cleaned with 5% ethanol between runs. Exploratory behavior, the time spent in each arm or the center, and zone transitions were recorded using the center of the mouse as criterium for zone sojourn. Lux in distal parts of open arms was 12.1–12.2 and 8.7 at the mid junction. EthoVision software (Noldus) was used to score behavior.

2.3.2. Open field and novel object test

Light intensity was adjusted to 7 lux in the center of squared boxes. Mice were habituated to the experimental room for more than 30 minutes before the test and were then placed in the open-field arena. After 10 minutes, a novel object (transparent drinking bottle) was placed in the middle of the arena and mice were again allowed to explore freely for 5 minutes. Distance traveled and the time spent in the different areas defined in the arena (wall, intermediate and center) were recorded. EthoVision software (Noldus) was used to score behavior.

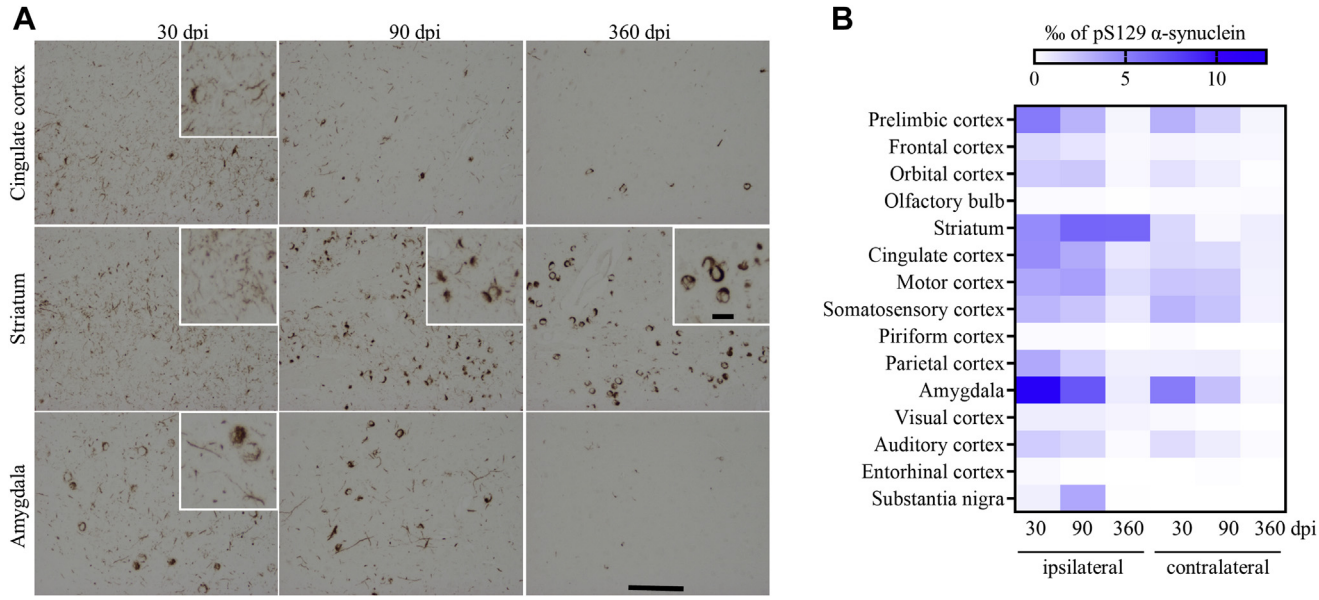

Fig. 1. Brain region–dependent differences in α-synuclein pathology. (A) α-synuclein pS129 immunoreactivity in the cingulate cortex, striatum, and amygdala (each in the hemisphere of injection) 30, 90, and 360 days postinjection (dpi) derived from a pilot study after unilateral, intrastriatal injection of α-synuclein preformed fibrils (PFFs) in naïve, wild-type mice. (B) Quantification of α-synuclein pS129 immunodensity over time in different affected brain regions. Scale bars in (A) are 100 μm (A) and 20 μm (insets).

For the methods and protocols of the following behavioral tests, see supplementary materials and methods section: marble burying test, fear conditioning tests, forced swim test, resident intruder test, rotarod test, activity cage, and saccharine preference test.

2.4. Immunohistochemistry and imaging

For full protocols and antibodies, please refer to supplementary materials. Briefly, 4-μm-thick, paraffin-embedded sections of paraformaldehyde-fixed brains were used for immunohistochemistry after epitope retrieval in trisodium citrate buffer. Immunofluorescence or 3,3′-diaminobenzidine–revelation was applied.

2.5. Statistical analyses

Data are presented as means ± SD, except for behavioral tests, in which means ± SEM are presented. Heat maps are based on mean values (of α-synuclein phosphorylated at serine 129 [pS129] immunodensities for brain regions). Statistical tests applied for the different experiments are given in figure legends. Two-way repeated measurement ANOVAs were calculated for comparisons between groups across different time points/intervals. Regular 2-way ANOVAs were applied to compare corticosterone/vehicle and PFF/PBS conditions. Tukey's post hoc tests were used to correct for multiple comparisons. One-sample t-tests against 50% chance were calculated to assess saccharin preference. Mann-Whitney tests were used to compare pS129 immunodensities between the corticosterone and vehicle conditions. Pearson coefficients were calculated for correlation studies. Microsoft Office Excel and GraphPad Prism 8 were used to present statistical results. p values <0.05 were considered as significant. Please refer to supplementary material for detailed presentation of statistical results.

3. Results

3.1. Experimental design and rational

To investigate the relationship between α-synuclein pathology and potential brain region–related behavioral deficits at time points of high α-synuclein aggregation load, behavioral tests were conducted 1–2 months after PFF injection. In our experiments, we consistently observe a peak of α-synuclein pS129-positive aggregate levels, in particular in the amygdala, but also in several cortical regions between 1 and 3 months after intrastriatal α-synuclein PFF injection (Fig. 1A). At this time, α-synuclein pS129-positive neurons are also detected in the substantia nigra, a brain region containing neuronal populations that are highly vulnerable in Parkinson's disease. With the exceptions of the injection site (striatum) and the substantia nigra, immunodensities of pS129 tend to decrease over time after this initial peak (Fig. 1B).

Mice chronically treated with corticosterone were injected unilaterally with either α-synuclein PFFs (hereafter referred to as PFF(C) mice) or PBS [PBS(C) mice] into the dorsal striatum by stereotactic surgery. The preparation and characterization of the PFFs are described in supplementary material and methods and in supplementary Fig. 1. Vehicle-treated mice are referred to as PBS mice or PFF mice, respectively. After surgery, corticosterone/vehicle treatment was continued until shortly before sacrifice (Fig. 2A).

Treatment with corticosterone induced depressive-like phenotypes in the forced swim test (Fig. 2B and suppl. Fig. 2) and saccharine preference test (Fig. 2C). It also elicited pronounced effects on body fat content, body shape, and weight gain after surgery (Fig. 2D and E), as described previously (David et al., 2009; Rebuffe-Scrive et al., 1992), as well as on drinking and feeding behavior (Fig. 2F and G; note that corticosterone/vehicle treatment was stopped for the home cage activity tests). Although corticosterone treatment significantly changed depressive-like phenotypes, fat content, weight gain, and drinking and feeding behavior, PFF injection did not influence these parameters.

3.2. Corticosterone treatment reverses behavioral changes in the elevated plus maze after α-synuclein PFF injection

Elevated corticosterone has previously been shown to affect mood and emotional behavior (Bacq et al., 2012; David et al., 2009; Gourley et al., 2008) and induced depressive-like behaviors in the present study (Fig. 2B and C). Given our observation of pronounced α-synuclein pathology in brain regions implicated in mood and

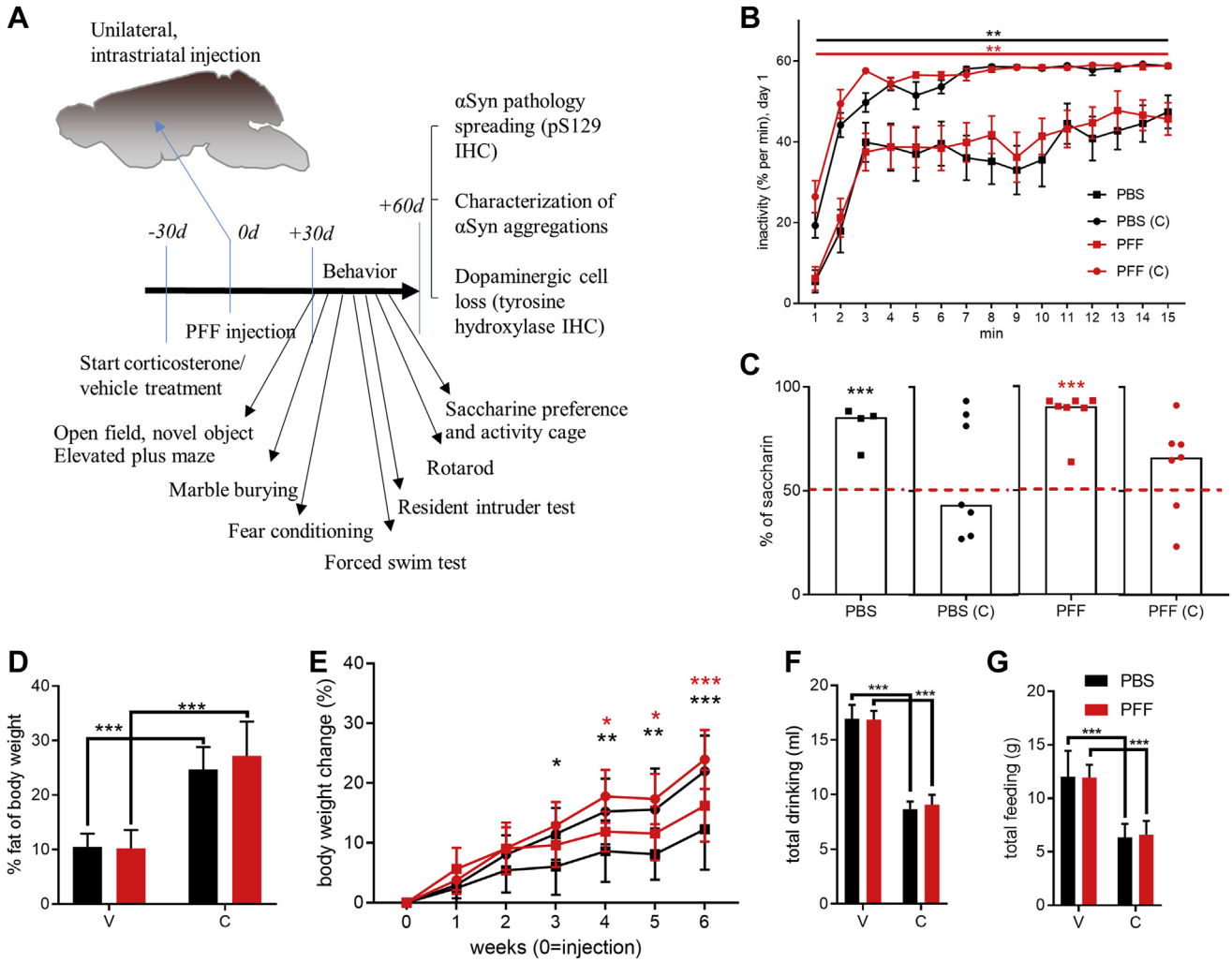

Fig. 2. Experimental setup and corticosterone effects. (A) Experimental setup: Preformed fibril (PFF) or vehicle (PBS) was injected in mice continuously treated with either corticosterone (35 mg/L) or vehicle (V; 0.45% hydroxypropyl-β-cyclodextrin) in the drinking water throughout the experiment, starting 1 month before PFF/PBS injection. Animals were subjected to a series of behavioral tests lasting for about another month, after which brains were processed for histology (IHC = immunohistochemistry). (B) Corticosterone (C)-treated animals spent significantly more time inactive (floating) in the forced swim test, indicating depressive-like behavior. A 2-day protocol of the forced swim test was applied as detailed in supplementary Fig. 2A. (C) Strong preference for saccharine was observed for vehicle-treated animals, such preference was absent in corticosterone-treated animals, suggesting anhedonia (median is indicated, 1-sample t-tests against 50% chance were calculated): PFF(C): $p = 0.20$, PFF (V): $p < 0.001$, PBS(C): $p = 0.54$, PBS (V): $p = 0.007$. (D) Fat content normalized to body weight was significantly higher in corticosterone groups (mean ± SD), accompanied by (E) increased body weight gain. (F) Drinking and (G) feeding were decreased after corticosterone treatment (corticosterone treatment was discontinued during activity measurements in the home cage). Two-way repeated measurement ANOVAs were calculated for B and E, and regular 2-way ANOVAs were applied in D, F, and G with Tukey's post hoc tests. Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. N = 9 per group for B, D, and E. N = 4–8 for C, F, and G. Details of statistical tests in supplementary table 2.

emotional behavior, such as the amygdala, we investigated anxiety-like behaviors in conditions of elevated corticosterone levels and α-synuclein pathology. We assessed basal spontaneous anxiety and exploratory reactivity to novelty in the elevated plus maze and open field test. A moderately hypoanxious phenotype for PFF mice was observed in the elevated plus maze (significantly less time spent in the closed arms, with the time spent in the open arms being not significantly changed), which was reversed by corticosterone treatment (Fig. 3A–F). PFF(C) animals also moved less in the elevated plus maze (Fig. 3D). No significant differences were observed in the open field test, (Fig. 3G–I). Novel object test (Fig. 2J–L) and marble burying test (data not shown), used to assess neophobia and anxiety, were altered neither by PFF injection nor by corticosterone treatment.

The involvement of the amygdala in fear-related and aggressive behavior (Coccaro et al., 2007; Rogan et al., 1997) and the high levels of α-synuclein pathology in the amygdala (Fig. 1) prompted us to assess potential impairments of these behaviors in the presence or absence of α-synuclein pathology and at normal or elevated corticosterone conditions. We observed no differences in a fear-conditioning protocol and in a resident intruder aggression test across all conditions (Supplementary Fig. 3 and 4).

Taken together, PFF injection resulted in a moderately hypoanxious phenotype one month later, which was reversed by corticosterone treatment but did not affect neophobia, fear, and aggressive behaviors.

3.3. Corticosterone treatment aggravates α-synuclein pathology and dopaminergic cell loss in α-synuclein PFF-injected mice

We next investigated the influence of heightened corticosterone in combination with injection of PFFs on the pathological hallmarks of Parkinson's disease: α-synuclein pathology and dopaminergic cell loss. No α-synuclein pS129 immunoreactivity was detected in PBS-injected controls. In PFF-injected animals, highest α-synuclein pS129 densities were observed in the

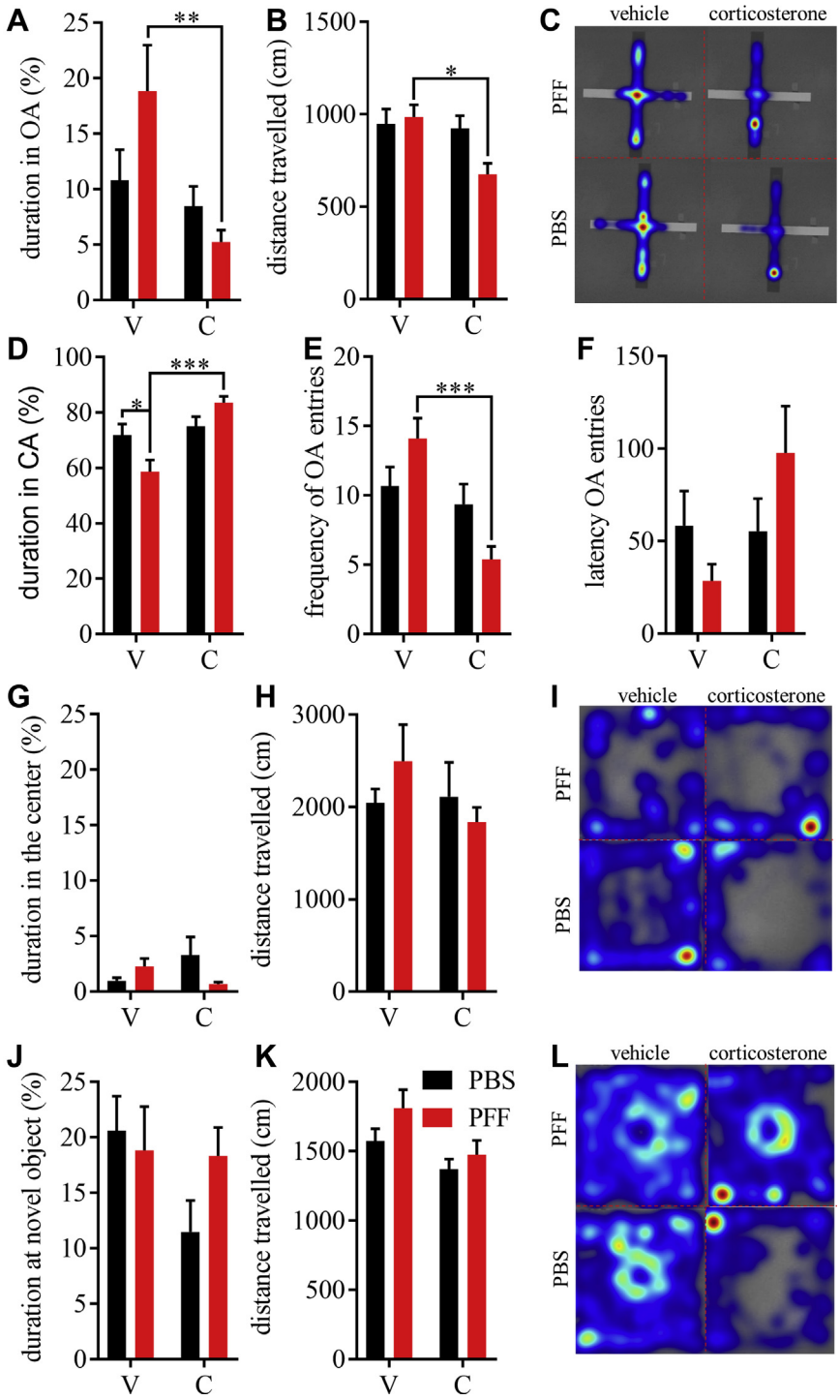

Fig. 3. Corticosterone (C) treatment reverses PFF-induced behavioral changes in the elevated plus maze. A nonsignificant trend toward hypoanxiety-like behavior in the elevated plus maze (EPM) of PFF-injected mice was reversed by C-treatment, in the open arms (OAs) (A). PFF-injected, C-treated mice moved less in the 7 minutes lasting EPM test as compared with vehicle (V)-treated and PFF-injected animals (B). Representative heat maps visualizing visited areas in the EPM are depicted in (C). Between V-treated groups, PFF-injected animals spent significantly less time in the closed arms (CA) of the EPM, an effect reversed by C-treatment (D). Mice injected with PFF and pretreated with V visited the open arms more often (E) and tended to move to the open arms sooner than C-treated and PFF-injected mice (F). In the open field test, no significant differences in time spent in the center (G), distance traveled (H), or visited area patterns (I) were observed. Despite a trend of lower interest into a novel object of the C-treated PBS group, no significant differences between groups were observed for time spent at the novel object (J) or distance traveled in the field (K). Representative heat maps visualizing movement in the novel object test are depicted in (L). N = 17–18 per group for A–F, N = 9 per group for G–I. Regular 2-way ANOVAs with Tukey's post hoc tests are presented. Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Details of statistical tests in supplementary table 3.

amygdala, prelimbic cortex, and substantia nigra for both corticosterone- and vehicle-treated groups (Fig. 4A). Interestingly, α-synuclein pS129 signal in the entorhinal cortex was significantly higher in the PFF(C) group as compared with the PFF group, where it was almost absent (Fig. 4B). In addition, in the auditory cortex, α-synuclein pS129 density was significantly higher in PFF(C) mice, whereas differences in other brain regions were small (Fig. 4A).

60 days after PFF injection, the total distance traveled during 3 days in the activity cage was similar across groups (Supplementary Fig. 5A). Reduction of motor coordination in the rotarod test of PFF(C) mice was mainly due to corticosterone treatment (Supplementary Fig. 5B). Although no significant tyrosine-hydroxylase (TH) immunoreactive cell loss in the substantia nigra was observed in PFF mice, corticosterone treatment resulted in decreased ipsilateral versus contralateral TH-positive cell numbers (Fig. 4C and D). In line with these results, the density of overall nigral TH immunoreactivity was reduced ipsilaterally (hemisphere of injection) in PFF(C) mice (Supplementary Fig. 5C,D). α-Synuclein pS129 immunodensity (Fig. 4B) was similar between PFF and PFF(C) animals.

The α-synuclein aggregates were resistant to proteinase K and were detected by antibodies for α-synuclein pS129 and for the N-terminal part of α-synuclein (1-20) (Fig. 4E). These aggregates colocalized with the macroautophagy marker p62 and ubiquitin in the substantia nigra of PFF and PFF(C) mice (Supplementary Fig. 5E).

Altogether, these results suggest that chronic corticosterone treatment aggravates neurodegeneration after PFF injection without significantly affecting α-synuclein pathology, as assessed quantitatively (Fig. 4A and B) or qualitatively by pS129 immunoreactivity (Fig. 4E and Supplementary Fig. 5E), in the substantia nigra or general motor behavior (Supplementary Fig. 5A and B).

4. Discussion

Despite their profound impact on the quality of patient life (Castrioto et al., 2016), nonmotor symptoms, including affective disorders, remain understudied compared with motor symptoms in Parkinson's disease and their impact on pathology formation and disease progression remains unclear. Previous studies in rodent models of Parkinson's disease demonstrated a potential link with affective disorders (Campos et al., 2013) (Caudal et al., 2015), for example, chronic mild stress–induced depression worsened neurochemical and behavioral outcomes in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin) model of Parkinson's disease (Janakiraman et al., 2016). Chronic stress and stress-associated heightened glucocorticoid levels are known risk factors for affective disorders (de Kloet et al., 2016; Sandi and Richter-Levin, 2009), and potential risk factors for neurodegeneration, for example, in mouse Parkinson's disease models (Hemmerle et al., 2014; Wu et al., 2016). Recently, also an association between peripheral α-synuclein mRNA levels and major depression has been reported in patients (Rotter et al., 2019). Despite the associations between glucocorticoid levels, affective disorders, and Parkinson's disease, the relationship of models of (depression inducing) heightened glucocorticoid and α-synuclein pathology formation and propagation have not been

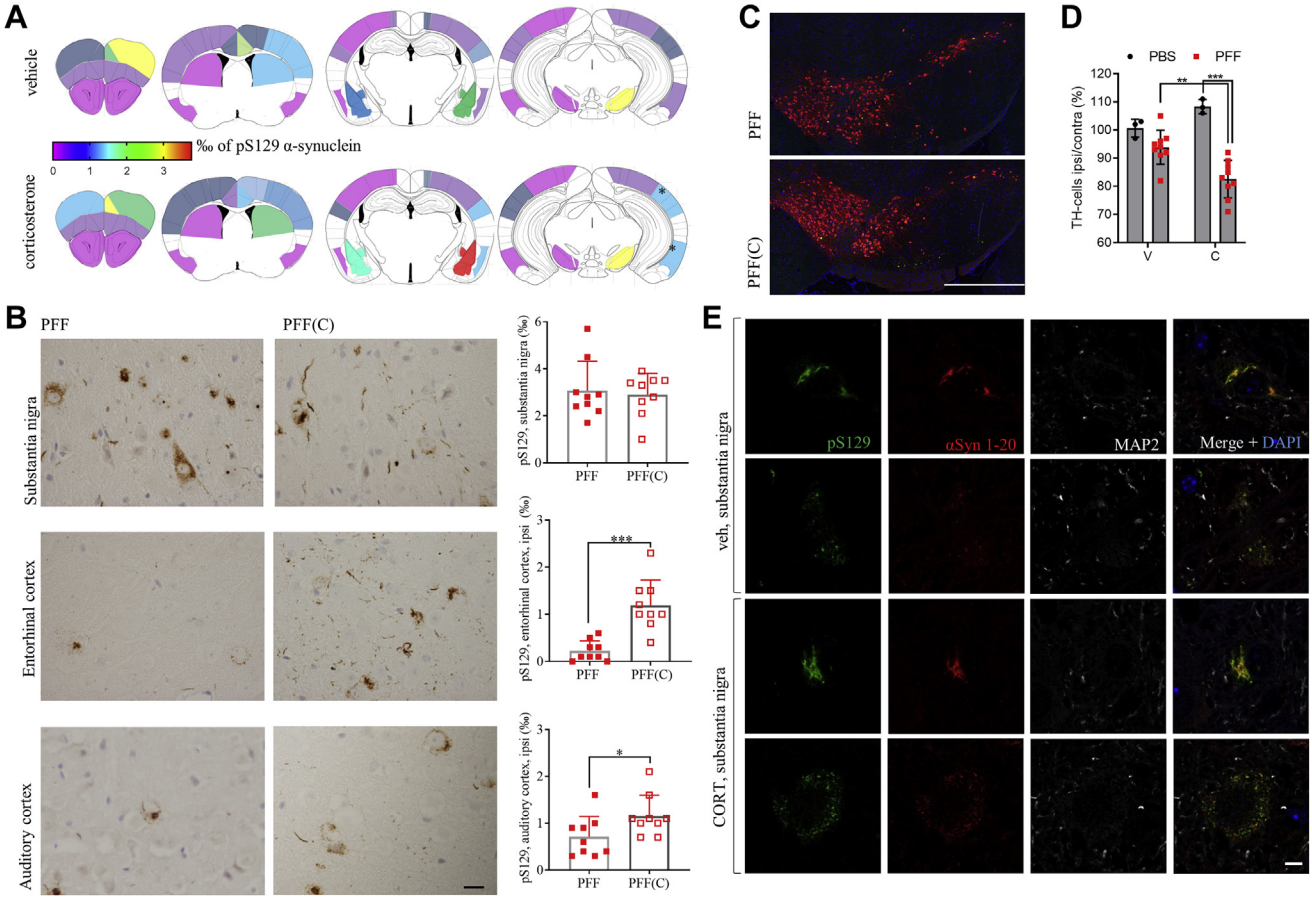

Fig. 4. α-Synuclein preformed fibril (PFF) injections aggravated neuropathology after chronic corticosterone (C). (A) Heat maps reflecting mean α-synuclein pathology across the brain (density of α-synuclein pS129 immunoreactivity in ‰ of area). Right hemisphere is the hemisphere of injection. Images represent coronal brain sections across the rostrocaudal axis. Significant differences in α-synuclein pathology (pS129 density) were observed in the ipsilateral entorhinal (B; Mann-Whitney test, $p < 0.001$) and auditory cortex (B; Mann-Whitney test, $p < 0.018$) between corticosterone- [PFF(C)] versus vehicle- [PFF] pretreated animals—marked with asterisks in (A) but not in the substantia nigra (B). (C) Micrographs of ipsilateral (i.e., hemisphere of injection) midbrains including the substantia nigra (TH in red, pS129 in green, and DAPI in blue) and (D) cell counts of ipsilateral substantia nigra TH-positive neurons normalized to contralateral cell count (2-way ANOVA with Tukey's post hoc tests, $F_{interaction}(1, 20) = 11{,}90$, $p = 0.003$, $F_{PFF\ vs\ PBS}(1, 20) = 34{,}94$, $p < 0.001$). Values reflect mean cell counts of 6 sections per brain each. (E) Characterization of aggregates in the substantia nigra using immunostaining for α-synuclein pS129, α-synuclein 1–20, and microtubule-associated protein 2 (MAP2) after proteinase K treatment. Note the two clearly distinguishable aggregation forms: fibrillar (arrowheads) and granular (asterisks). Scale bars represent 20 μm (B), 500 μm (C), and 5 μm (E). N = 9 per PFF group (N = 3 for PBS groups). Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

assessed previously. On the basis of these observations, we decided to investigate the interplay of elevated corticosterone and α-synuclein pathology in the amygdala as a potential focal point of these 2 conditions.

To this end, we applied a well-established mouse model of α-synuclein pathology induced by intrastriatal injection of α-synuclein PFFs and combined it with a model of chronic corticosterone treatment, robustly causing depressive-like phenotypes.

We report surprisingly modest effects of α-synuclein pathology formation and spreading on behaviors associated with amygdala function at time points of strong α-synuclein pathology in the amygdala. α-Synuclein pathology induced hypoanxiety in the elevated plus maze was, however, reversed by chronic corticosterone treatment. Chronic corticosterone treatment elicited clear depressive-like and physiological effects, as depicted in Fig. 2. These results were confirmed by partial least squares (PLS) discriminant analysis, which demonstrated in an unbiased way very clear effects of the corticosterone treatment (Suppl. Fig. 6). Corticosterone treatment was further associated with aggravated α-synuclein pathology in specific brain regions and dopaminergic cell loss in the substantia nigra after PFF injection.

As an important side note, different models of genetically or chronic stress-induced depression (Barkus, 2013) exhibit different physiological and molecular outcomes, such as reduced body weight gain (contrary to the model applied in this study) in chronic unpredictable stress models (Monteiro et al., 2015) or pronounced immunological effects in chronic social defeat stress (Menard et al., 2017). We cannot exclude that different forms of depression will therefore differentially affect α-synuclein pathology. In addition, cortisol/corticosterone plays a multifaceted role in depression (Herbert, 2013), for example, related to the circadian rhythm. Although most studies indicate higher levels of diurnal cortisol patterns in depressed individuals (Bhagwagar et al., 2005; Carroll et al., 2007; Dienes et al., 2013; Pruessner et al., 2003; Vreeburg et al., 2009), there is also evidence for lower or similar morning cortisol in depressed patients than in controls (Huber et al., 2006; Stetler and Miller, 2005).

4.1. Corticosterone treatment reverses effects of α-synuclein PFF injection on behavior in the elevated plus maze

The extensive behavioral and neuropathological characterization allowed ample correlative studies (Suppl. Fig. 7 and 8), which were followed up by PLS regression analysis (Suppl. Fig. 9).

Chronic corticosterone treatment surprisingly reversed moderate hypoanxious-like behaviors, significant for time spent in closed arms in the elevated plus maze, induced by α-synuclein PFF injection. PLS regression analysis revealed that higher α-synuclein pathology in particular in the cingulate cortex and the striatum was correlated with more time spent in the closed arms in this test (Suppl. Fig. 9B). Respective PLS components indicate this effect to be significantly more pronounced in the corticosterone condition (Suppl. Fig. 9C), confirming the results of blunted hypoanxiety. Hypoanxiety has been described previously in models of early stages of Parkinson's disease (George et al., 2008; Graham and Sidhu, 2010) and might reflect changes in dopamine signaling (Ardouin et al., 2009). We hypothesize that chronic corticosterone treatment impaired dynamic adaptations to α-synuclein pathology, thereby preventing potential transient bursts in dopamine signaling that might occur because of α-synuclein's suspected function as negative regulator of dopamine release (Abeliovich et al., 2000; Nemani et al., 2010). In line with this assumption is a recent report on enhanced presynaptic activity of neurons in the presence of α-synuclein inclusions (Froula et al., 2018). We did not observe hypoanxious-like behaviors in the other anxiety tests applied. This could be due to the additional exposure to altitude in the elevated plus maze, suggesting that the described hypoanxiety-like effects are more likely to be detectable in stressful conditions, potentially indicating altered coping with stress.

4.2. α-Synuclein pathology does not alter amygdala-related behaviors such as fear behavior or aggression

A high level of α-synuclein pathology in the amygdala has been reported in patients suffering from Parkinson's disease and other neurodegenerative diseases (Nelson et al., 2018), as well as in several α-synuclein pathology models (Luk et al., 2012; Masuda-Suzukake et al., 2013; Recasens et al., 2014; Rey et al., 2016b) and in this study. The amygdala is importantly involved in emotional behavior and depression in general (Janak and Tye, 2015) and in Parkinson's disease in particular (Castrioto et al., 2016). Therefore, we assessed amygdala-related emotional and mood behaviors in mice treated chronically with corticosterone and thus exhibiting depressive-like phenotypes, after induction of α-synuclein pathology.

We did not observe altered α-synuclein pathology in the amygdala after PFF injection and corticosterone treatment. Despite the severe α-synuclein pathology observed in all PFF-treated animals, most behaviors associated with the amygdala (e.g., fear, aggression) were unaffected. These results suggest that α-synuclein aggregates by themselves do not immediately impair amygdala physiology. This is in line with recent observations that hippocampus-dependent behavior is not altered by the induction of severe hippocampal α-synuclein pathology either (Nouraei et al., 2018). Alternatively, it is possible that, at the time points after treatments at which the present study took place, the relevant neuronal circuits are resilient or plastic enough to prevent general physiological deterioration or behavioral alterations. Over time, only some particularly vulnerable neuronal populations—such as dopaminergic neurons in the substantia nigra—succumb to degeneration. As previously suggested (Fares et al., 2016) for primary neurons seeded with PFFs, toxicity might be conferred to the aggregations in the presence of additional insult factors or during late stages of Lewy body formation and maturation (i.e., the transition from fibrils to Lewy bodies).

4.3. Corticosterone treatment aggravates α-synuclein pathology and dopaminergic cell loss in α-synuclein PFF-injected mice

Striatal α-synuclein PFF injection caused pronounced brain α-synuclein pathology, which was aggravated in distinct brain regions after corticosterone treatment, most notably in the entorhinal cortex. Interestingly, the entorhinal cortex is severely affected by Lewy pathology in many Parkinson's disease (Jellinger, 2003; Mattila et al., 2000) and dementia with Lewy Bodies (Gómez-Tortosa et al., 2000) patients. Here, α-synuclein pS129 immunoreactivity in the entorhinal cortex for PFF-injected mice was negatively correlated with feeding, drinking, hedonic behavior, and movement in the elevated plus maze, whereas positive correlations were observed for body fat content and depressive-like behavior in the forced swim test, which is interesting in the light of antidepressive effects demonstrated by activation of the entorhinal cortex (Yun et al., 2018). In accordance with the pronounced differences of α-synuclein pathology in the entorhinal cortex, the PLS regression component of this brain region (Suppl. Fig. 9C) also differed most profoundly between corticosterone and vehicle conditions, with the factors body fat content, food and drink intake, saccharine preference, and velocity in the elevated plus maze most strongly loading on the differentiating component (Suppl. Fig. 9B). Correlation patterns for forced swim test and activity cage parameters were intriguingly inverted in the visual, as compared to the entorhinal cortex. Our results point to the entorhinal cortex as being a particularly vulnerable brain region for α-synuclein pathology in conditions of glucocorticoid imbalance. Due

to the prominent role of the entorhinal cortex' in cognition and the potential role of entorhinal Lewy pathology in cognitive deficits in Parkinson's disease (Kovari et al., 2003; Mattila et al., 2000), future studies on patients assessing the effect of chronic stress and depression on cognitive performance and α-synuclein pathology in the entorhinal cortex will be of interest.

No significant neuronal loss in the substantia nigra was observed in PFF mice, which is in line with previous reports, in which neurodegeneration was detected only ~180 days after injection (Luk et al., 2012). PFF(C) mice presented with a relative decrease in TH-positive neurons in the substantia nigra of the hemisphere of injection as compared with the contralateral substantia nigra. Thus, heightened corticosterone levels resulted in aggravated α-synuclein pathology and nigral neurodegeneration after PFF injection. Motor behavior was not significantly impaired during the investigated time interval, suggesting that the reduction of cells (~20% fewer dopaminergic cells in the ipsilateral as compared with the contralateral substantia nigra) was not sufficient to trigger motor dysfunction. This is in line with the requirement of massive functional impairment of the dopaminergic nigrostriatal system before occurrence of motor symptoms in patients (Bernheimer et al., 1973).

4.4. Physiology and behavior are altered in conditions of heightened corticosterone in mice exhibiting α-synuclein pathology

PLS discriminant analysis differentiated the PFF(C) condition much better from the PFF condition than PFF from PBS. This supports the notion that α-synuclein pathology by itself does not strongly impact on the measured behavioral parameters. Strikingly, α-synuclein pathology at its peak even induced hypoanxious-like behavior. In addition, although substantia nigra α-synuclein pathology correlated negatively with distance traveled in the activity cage (similar effects of somatosensory and cingulate cortices), it correlated positively with performance on the rotarod (note that differences across groups on the rotarod were due to corticosterone treatment but not due to PFF treatment, Suppl. Fig. 5B). Clearly, this correlative result has to be interpreted with caution. Should these effects be reproducible, however, this could be due to an initially protective role of α-synuclein pathology in the substantia nigra. Should the pS129 pathology bearing neurons be the vulnerable ones, this could indicate that mice with higher a priori motor performance are able to handle pS129 pathology in a better way. Several reports demonstrating protective effects of exercise interventions on pathology formation and dopaminergic neurodegeneration in Parkinson's disease models are in favor of this hypothesis (Gerecke et al., 2010; Jang et al., 2017; Shin et al., 2017; Zhou et al., 2017).

PLS regression analysis confirmed additionally differential effects of PFF and PFF(C) groups in all analyzed brain regions; in the substantia nigra, we identified increased associated fear behaviors (freezing in the cue and context test), sniffing behavior in the resident intruder test, and anxiety-like behavior in the open field test (latency to enter the center) as more positively correlated with α-synuclein pathology in the corticosterone group. Interestingly, α-synuclein pathology in the prelimbic cortex was differentially associated with aggressive and fear behaviors (more negatively associated in PFF(C) mice), as well as with body fat content and weight gain (more positively associated in PFF(C) mice). Taken together, all these results demonstrate divergent physiological and behavioral alterations in heightened corticosterone conditions in PFF-injected mice, potentially mediated by corticosterone-suppressed beneficial adaptations, leading to higher vulnerability of PFF(C) mice to α-synuclein pathology and neurodegeneration (Fig. 5). These (hormetic) adaptations might be mediated or impaired, for example, by metabolic or synaptic changes (Nouraei et al., 2018; Subramaniam et al., 2014) or gut dysfunction (Dodiya et al., 2018) as recently suggested. Hormetic adaptations by exposure of the system to exogenous sublethal challenges have been proposed as potential mechanisms for protection against α-synuclein pathology, or to delay it (Fouillet et al., 2012; Govindan et al., 2018; Leak, 2018; Matus et al., 2012; Mollereau et al., 2016). Here, we propose that α-synuclein pathology itself might have the potential to serve as a preconditioning agent inducing hormesis. This hypothesis is supported by previous studies implicating α-synuclein in inflammatory preconditioning (Koller et al., 2017; Roodveldt et al., 2013), which could also explain corticosterone's effect of countering these adaptations through its well-known anti-inflammatory actions (Floman and Zor, 1976; Hong and Levine, 1976).

5. Conclusions

We report aggravated α-synuclein pathology and neurodegeneration in mice injected with α-synuclein PFFs in a condition of heightened corticosterone, suggesting heightened glucocorticoid levels as a risk factor for the development of the neuropathological hallmarks of Parkinson's disease and potential target for treatment. Taken together, our findings suggest that chronic corticosterone treatment reduces the ability of the mouse brain to adapt to the proteostatic stress of intrastriatal injection of α-synuclein PFFs, resulting in lower thresholds for α-synuclein pathology handling and nigral neurodegeneration. Further studies aimed at elucidating the vulnerability factors of specific brain regions to α-synuclein pathology, and why at some point resilience fails and neurodegeneration (such as in the substantia nigra) occurs, are needed and will greatly enhance our understanding of the role of α-synuclein pathology in the pathogenesis of Parkinson's disease and synucleinopathies.

Based on our results, we propose that α-synuclein pathology in the absence of additional clinical (e.g., depression, chronic stress)

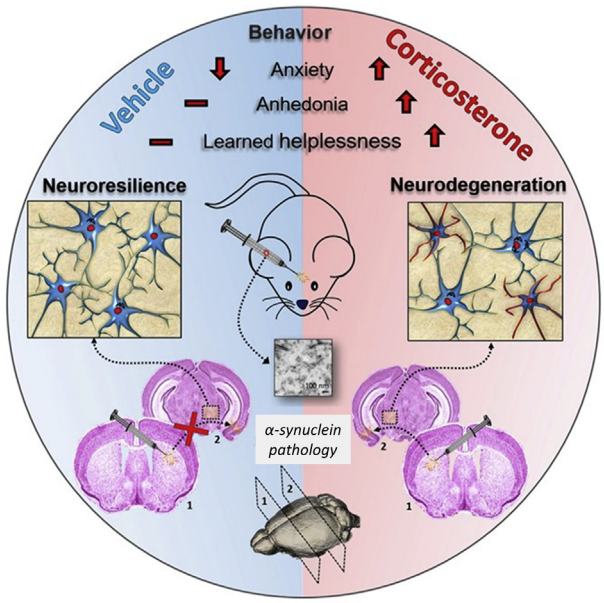

Fig. 5. Working model of corticosterone effects in disease progression after PFF injection. Injection of α-synuclein PFFs triggers α-synuclein pathology formation. Chronic administration of corticosterone induced depressive-like phenotypes, for example, in the forced swim test (learned helplessness) and saccharine preference test (anhedonia) (see Fig. 1). If no corticosterone was administered, we hypothesize beneficial (hormetic) adaptations to take place, which are abolished by corticosterone treatment. We believe this and other adaptive processes to be involved in the induction of mildly hypoanxious behaviors (less time spent in the closed arms of the elevated plus maze), protection from neurodegeneration, and limitation of α-synuclein pathology in distinct brain regions, such as the entorhinal cortex. Abbreviation: PFF, preformed fibrils.

and molecular (mitochondrial function, oxidative stress, and so forth) risk factors is not immediately noxious, maybe even triggering transient protective hormetic adaptations and aiding in the clearance of aggregates.

Disclosure

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All authors have read the manuscript, the paper has not been previously published (however, it is available on the preprint-server bioRxiv), and it is not under simultaneous consideration by another journal. Nobody but the named authors have written the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2019.08.007.

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