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Research paper

Parkinsonian GM2 synthase knockout mice lacking mature gangliosides develop urinary dysfunction and neurogenic bladder

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ABSTRACT

Parkinson's disease is a neurodegenerative disorder that reduces a patients' quality of life by the relentless progression of motor and non-motor symptoms. Among the non-motor symptoms is a condition called neurogenic bladder that is associated with detrusor muscle underactivity or overactivity occurring from neurologic damage. In Parkinson's disease, Lewy-body-like protein aggregation inside neurons typically contributes to pathology. This is associated with dopaminergic neuron loss in substantia nigra pars compacta (SNc) and in ventral tegmental area (VTA), both of which play a role in micturition. GM1 gangliosides are mature glycosphingolipids that enhance normal myelination and are reduced in Parkinson's brain. To explore the role of mature gangliosides *in vivo*, we obtained GM2 Synthase knockout (KO) mice, which develop parkinsonian pathology including a loss of SNc dopaminergic neurons, which we reconfirmed. However, bladder function and innervation have never been assessed in this model. We compared GM2 Synthase KO and wild type (WT) littermates' urination patterns from 9 to 19 months of age by counting small and large void spots produced during 1 h tests. Because male and female mice had different patterns, we evaluated data by sex and genotype. Small void spots were significantly increased in 12–16 month GM2 Synthase KO females, consistent with overactive bladder. Similarly, at 9–12 month GM2 KO males tended to have more small void spots than WT males. As GM2 Synthase KO mice aged, both females and males had fewer small and large void spots, consistent with detrusor muscle underactivity. Ultrasounds confirmed bladder enlargement in GM2 Synthase KO mice compared to WT mice. Tyrosine hydroxylase (TH) immunohistochemistry revealed significant dopaminergic loss in GM2 Synthase KO VTA and SNc, and a trend toward TH loss in the GM2 KO periaqueductal gray (PAG) micturition centers. Levels of the nerve growth factor precursor, proNGF, were significantly increased in GM2 Synthase KO bladders and transmission electron micrographs showed atypical myelination of pelvic ganglion innervation in GM2 Synthase KO bladders. Cumulatively, our findings provide the first evidence that mature ganglioside loss affects micturition center TH neurons as well as proNGF dysregulation and abnormal innervation of the bladder. Thus, identifying therapies that will counteract these effects should be beneficial for those suffering from Parkinson's disease and related disorders.

1. Introduction

Parkinson's disease (PD) is an aging disorder that affects millions of people worldwide. The hallmark pathology of PD includes the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and pathological alpha-synuclein (aSyn) aggregation in Lewy bodies/Lewy neurites (Shulman et al., 2011). The substantia nigral pars compacta (SNc) dopaminergic neuron loss ultimately leads to movement problems, however, non-motor symptoms like urinary dysfunction are also common in PD (Winge et al., 2006). While not yet widely appreciated,

Abbreviations: aSyn, alpha-synuclein; A53T, alanine to threonine mutant human aSyn; BDNF, brain derived neurotrophic factor; GDNF, glial cell line derived neurotrophic factor; HMW, high molecular weight; HS, high salt buffer; HST, high salt triton-X 100 buffer; KO, knockout; MAG, myelin associated glycoprotein; NGF, nerve growth factor; PAG, periaqueductal gray; PD, Parkinson's disease; proNGF, pro nerve growth factor; RIPA, radio immunoprecipitation assay buffer; SNc, substantia nigra pars compacta; Tg, transgenic; TH, tyrosine hydroxylase; VTA, ventral tegmental area; WT, wild type

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there is growing evidence that the dopaminergic neurons of the ventral tegmental area (VTA) are also damaged in PD (Alberico et al., 2015; Harrison et al., 2016; Rinne et al., 1990). Up to 83% of those with idiopathic PD also exhibit urinary dysfunction with symptoms of nocturia, incontinence, urgency, and/or poor bladder emptying (Campos-Sousa et al., 2003). This type of dysfunction, also known as neurogenic bladder, results from damage to central, peripheral, and/or autonomic nervous system inputs to the bladder (Dubow, 2007; Ginsberg, 2013; Hall et al., 2012; Lemack et al., 2000). Neurogenic bladder has been associated with abnormal nerve growth factor (NGF) levels (Hamill et al., 2012), with NGF proposed as a neurogenic bladder biomarker (Seth et al., 2013). Interestingly, the precursor form of NGF, proNGF, is the main NGF found in adult human brain (Al-Shawi et al., 2007), and proNGF overexpression is associated with sympathetic and cholinergic system degeneration in animal models (Cuello, 2012). ProNGF has also been shown to be implicated in bladder dysfunction (Ryu et al., 2018). Moreover, loss of modulatory inputs arising from SNc and VTA dopaminergic neurons can affect brain micturition centers in a manner to negatively impact bladder function (Kitta et al., 2008; Soler et al., 2011; Yoshimura et al., 2003). Together the data suggest that evaluating brain regions associated with micturition, bladder function, bladder volumes, as well as aSyn and NGF levels in bladder of GM2 Synthase deficient parkinsonian mice will be informative.

Among other major molecules that regulate neurodevelopment and neuronal function are the gangliosides, which are sialic acid-bearing glycosphingolipids that are highly expressed in brain (Schnaar, 2010). There are four major mature gangliosides that contribute to myelination and neurotransmission, GM1 (mono, with one sialic acid), GD1a and GD1b (di, with two sialic acids), and GT1b (tri, with three sialic acids) (Palmano et al., 2015; Posse de Chaves and Sipione, 2010; Sturgill et al., 2012). In fact, axon growth and stability utilize a lectin-glycan system composed of gangliosides that interact with myelin associated glycoprotein (MAG) (Schnaar, 2010). MAG, is one of the major sialic acid-binding lectin molecules in brain that binds directly to GD1a and GT1b in a manner to optimize cell-cell recognition and structure, as well as neuronal signaling (Schnaar and Lopez, 2009). Moreover, deficiencies in GD1a and GT1b have been shown to contribute to neurodegeneration (Ohmi et al., 2014) and GM1 levels in substantia nigra dopaminergic neurons are lower than normal in PD brain (Hadaczek et al., 2015; Wu et al., 2012a). Recent data further confirm that levels of immature GM3 gangliosides are also elevated in PD serum (Chan et al., 2017). Treating PD patients with GM1 or the GM1-analogue, LIGA-20 can significantly reduce PD motor symptoms (Schneider et al., 1998; Schneider et al., 2010). These data imply that loss of GM1 gangliosides contributes significantly to PD pathogenesis, though the impact of GM1/GM2 ganglioside loss on bladder is unexplored.

Transgenic *B4galnt1* null mice entirely lack expression of GM2 synthase (EC 2.4.1.92), the enzyme that catalyzes the second step in ganglioside biosynthesis to convert immature GM3, GD3, and GT3 gangliosides to mature GM1, GD1, and GT1 gangliosides (Sheikh et al., 1999). The GM2 synthase KO mice have low MAG expression, demyelination, and develop age onset motor impairment (Chiavegatto et al., 2000; Schnaar and Lopez, 2009; Sheikh et al., 1999). More recently, motor impairment in GM2 Synthase KO (−/−) mice was shown to be associated with nigrostriatal dopaminergic aSyn-pathology (Wu et al., 2011) and model parkinsonism. Similar parkinsonian changes also occur in the heterozygous (+/−) GM2 Synthase mice (Wu et al., 2012a), making both models appropriate for PD research. Based on these findings we hypothesized that parkinsonian GM2 Synthase KO (−/−) mice would develop PD-like urinary dysfunction due to aSyn pathology and trophic factor dysregulation in the bladder.

2. Materials and methods

2.1. Animals

Heterozygous *B4galnt1* (+/−) breeders were generously provided by Drs. Ledeen and Wu of Rutgers School of Medicine (Newark, NJ, USA), to generate our mouse colony. Genotyping was performed by PCR as previously described (Wu et al., 2001). For this study, we evaluated a cohort of male and female WT (+/+) mice that express two copies of GM2 Synthase, and GM2 Synthase KO mice that totally lack GM2 Synthase (−/−) expression, from 9 to 23 months of age. Animals were maintained on 12-h light/dark cycles with access to food and water *ad libitum* except during 1 h urinary pattern tests as described below. All experiments were conducted using protocols approved by the Texas Tech University Health Sciences Center Institutional Animal Care and Use Committee (IACUC) in accordance with AALAC and National Institutes of Health guidelines.

2.2. Behavioral tests and other measures

2.2.1. Open field

(N = 20, 9 WT and 11 KO). Locomotor activity was assessed using a TruScan™ open field activity monitor (Coulbourn Instruments, Whitehall, PA, USA). Mice were acclimated to the test room for 15 min prior to being placed individually in the center of the arena with total movement monitored for 15 min. Total movement was measured as successive coordinate movements made across the floor plane while mice were continuously active. Mice were tested in random order on two independent occasions essentially as previously described (Farrell et al., 2014)

2.2.2. Urination patterns

(N = 26, 15 WT and 11 KO). Food and water were removed during tests. Five independent tests were conducted per mouse per group at each time point. Each mouse was individually placed in a clean cage for 1 h (10:00–11:00 am) with the cage bottom covered with a fitted clean white filter paper (Bio–Rad, Hercules, CA, USA, cat# 1650962) as shown in Fig. 1a. Filter papers were collected, labeled, and allowed to dry. Urine spots illuminated with UV light allowed spots to be categorized as small ($\leq 0.2\,cm^2$) or large ($> 0.2\,cm^2$) and counted by individuals blinded to genotypes, using established methods (Birder et al., 2002; Bjorling et al., 2015; Hamill et al., 2012; Hodges et al., 2008; Studeny et al., 2008; Yu et al., 2014). If overlapping urine spots were detected, they were not included in the counts. An image of a UV illuminated filter shows large and small urine spots that can be easily differentiated (Fig. 1b).

2.2.3. Water intake

(N = 10, 5 WT and 5 KO). Mice were individually housed in cages with water delivered from a 50 mL conical tube sealed with a #7 single hole rubber stopper containing a double-ball water sipper. Tubes were weighed before testing and the next morning in order to calculate water intake.

2.3. Bladder volumes

(N = 15, 8 WT and 7 KO). Mice were anesthetized with isoflurane and abdomens were shaved with electric clippers. Post void bladder volumes were measured by ultrasonography using a moveable Real Time 4D Volume probe with data collected by associated imaging software (Voluson® VE8/VE8 Expert General Electric® Health Care; Wauwatosa, Wisconsin, USA). Briefly, the transducer was coated with ultrasound gel (McKesson Ultrasound Gel PINK; McKesson Medical – Surgical, Richmond, VA, USA), placed at a 45° degree angle on the abdomen and moved until the bladder image appeared on the monitor. Bladder volumes were calculated by measuring bladder length and

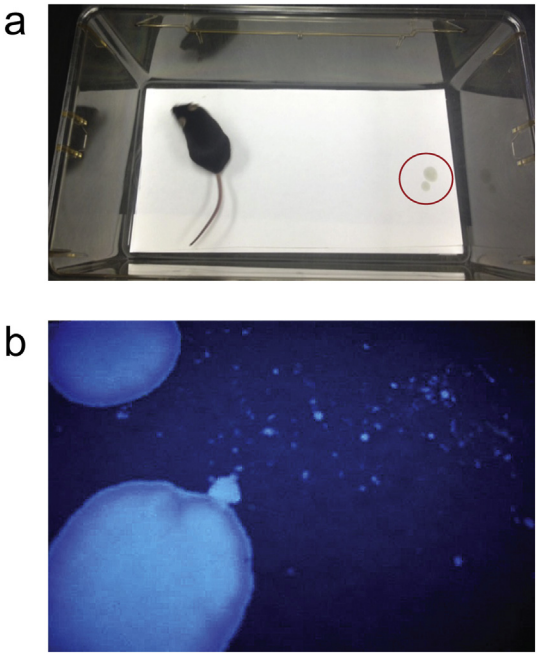

Fig. 1. Void spot method to assess urinary patterns in GM2 Synthase KO and WT mice. (a) A GM2 Synthase KO mouse is shown in a cage with a filter paper covering the entire bottom surface. Damp urine spots, circled in red, can be seen on the filter. (b) A representative image of two large and many small void spots on a dried filter illuminated by UV light, on which spot sizes were easily discernable for measurement and counting using established methods. (N = 26, 15 WT and 11 KO). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

depth according to established methods (Al-Izki et al., 2009).

2.4. Tissue collection and sample preparation

Mice were euthanized by CO_2 inhalation followed by decapitation. Bladder and brain tissues were dissected and prepared as described below.

2.4.1. Immunohistochemistry

Brains (N = 6 WT, 6 KO) were collected and post-fixed in 4% formaldehyde/sucrose overnight. Tissues were immunostained free-floating using antibodies for aSyn (C20, sc-7011-R, Santa Cruz, CA, 1:100) and tyrosine hydroxylase (TH; chicken anti-TH, Aves Labs, Tigard, OR, 1:200–1:250) followed by secondary antibodies, then evaluated by confocal microscopy using the Olympus FluoView 1000 with quantification as previously described (Alerte et al., 2008; Farrell et al., 2014; Vidal-Martinez et al., 2016) or with the EVOS Cell Imaging System (Thermo-Fisher, USA) and ImageJ Software (Rueden et al., 2017).

2.4.2. Transmission electron microscopy

(N = 2 WT, 2 KO). We followed traditional protocols for fixing and embedding tissues. Briefly, bladders with the bladder base and major pelvic ganglia intact, were fixed in 4% paraformaldehyde overnight at 4 °C then washed with 10 mL of 0.1 M imidazole-HCl buffer at pH 7.2 and fixed in 2.5% glutaraldehyde in 0.1 M imidazole buffer at room temperature. Epoxy resin embedded-tissue samples were trimmed and regions of interest were surveyed in semi-thin sections stained with a methylene blue dye mixture (Richardson et al., 1960) on glass microscope slides with a bright field optical microscope. Thin sections of tissue regions, including selected bundles of nerve fibers and nerve cells, were cut with a diamond knife and mounted on 100 mesh copper grids, followed by staining with 2% uranyl acetate solution and lead citrate. Grids were examined at 80 kV accelerating voltage on a model H-7650 transmission electron microscope (Hitachi High Technologies, Dallas, TX) with digital images collected using a mid-mount CCD camera system and acquisition software (Advanced Microscopy Techniques, Woburn, MA).

2.4.3. Immunoblots

For standard immunoblots, tissues were homogenized in 8 volumes (w/v) ice-cold buffer containing 1 mM AEBSF, 5 μM aprotinin and 5 μM leupeptin using a Bullet Blender (Next Advance, Inc., Averill Park, NY, USA). Samples were then sonicated (Sonic Dismembrator Model 100, Thermo-Fisher, USA) and spun at 14,000 ×g, 10 min at 4 °C to remove particulates.

2.4.4. Sequential protein extraction

To separate soluble from insoluble proteins we used a series of buffers and pellet re-extractions performed with ultracentrifugation as previously described (Vidal-Martinez et al., 2016; Waxman and Giasson, 2008; Wu et al., 2012b). (N = 7, 1 A53T, 3 WT, and 3 KO). Protein concentrations were determined by bicinchoninic acid assay (Thermo-Fisher, USA). Gels, loaded with 50 μg total protein per sample, were transferred to nitrocellulose, blocked in 5% non-fat milk containing buffer and incubated in primary antibodies overnight at 4 °C. Immunoblot antibodies included aSyn (C20, sc-7011-R, Santa Cruz, CA), proNGF (H-20, sc-548, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and β-actin (4970S, Cells Signaling Technology, Danvers, MA, USA). Secondary antibodies IRDye 800CW (green) or 680RD (red) produced infrared fluorescence for capture using the Li-Cor Odyssey system (Lincoln, NE, USA).

2.5. Statistical analysis

Data from WT and GM2 Synthase KO mice were analyzed by independent sample t-tests or two way ANOVA, as appropriate to the data, using Prism 6 or InStat (GraphPad Software Inc., San Diego, CA, USA), with significance levels set to $p < .05$ and variability shown as the standard error of the mean.

3. Results

3.1. Total movement and water intake are similar in GM2 Synthase KO and WT mice

As previously shown by others (Chiavegatto et al., 2000; Sheikh et al., 1999), we also noted that GM2 Synthase KO mice developed coordination and balance problems as they aged (not shown). Though GM2 Synthase KO mice became uncoordinated, they still efficiently moved around the cage during urinary pattern tests. This was confirmed by measuring total movement in an open field for WT and GM2 Synthase KO mice at 9, 12, 16, and 19 months of age. We noted that though GM2 Synthase KO mice tended to move slightly less than WT mice, all mice had similar total movement at all time points (Fig. 2). We also assessed overnight water intake for GM2 Synthase KO and WT mice and confirmed equivalent hydration of the mice for our study (WT, 4.08 ± 0.3 mL; KO, 4.07 ± 0.4 mL; p = .52). These data show that the GM2 Synthase KO and WT mice had similar baselines for measuring potential differences in urinary patterns using the void spot test.

3.2. GM2 Synthase KO mice initially show urinary frequency that diminishes with age

We noted that male mice of both genotypes consistently produced more large and small void spots than female mice. Thus, we compared mouse urination patterns according to genotype and sex. Regarding small spots at 9–12 months, GM2 Synthase KO males tended to have more small spots than WT males, however, at 16 months the KO males

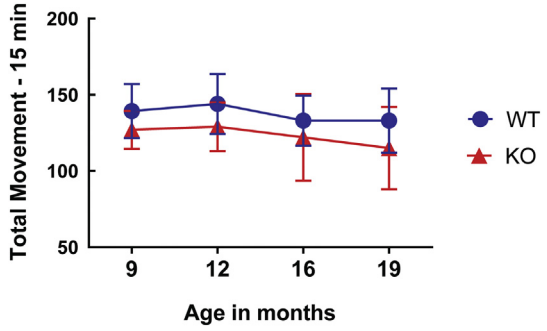

Fig. 2. Total movements are similar in WT and GM2 Synthase KO mice. The movement of WT and KO mice across the floor plane in an open field during 15 min trials was evaluated at 9–19 months. Both KO and WT mice have similar total movement at all ages. (N = 20, 9 WT and 11 KO).

tended to have fewer small spots than WT males. Yet, small spot differences were not statistically significant for males (Fig. 3a). With regard to large spots, KO males initially showed a tendency to produce more large spots, but at 16–19 months KO males had significantly fewer large spots than WT males (Fig. 3b). Regarding female small spots, KO females had significantly more small spots at 12–16 months than WT females (Fig. 3c), as can be better appreciated in the inset graph. With regard to large spots, KO females tended to have more large spots than WT females at 9–12 months, but then tended to have fewer large spots produced at 16–19 months though not significantly (Fig. 3d). The finding that all KO mice initially had more void spots that reduced with age suggested that KO mice initially had bladder hyperreflexia and then developed hyporeflexia. This led us to suspect that GM2 Synthase KO mice developed urine retention that can manifest as bladder enlargement, which we quantitatively tested by ultrasound.

3.3. GM2 Synthase KO mice exhibit significant bladder enlargement

We noted urine retention and bladder enlargement in a GM2 Synthase KO mouse that had to be euthanized (Fig. 4a). We later obtained ultrasound equipment which allowed us to evaluate bladders of live mice. Using ultrasonography, we measured bladder volumes of 19–20 month old GM2 Synthase KO and WT male and female mice after all urination pattern testing was complete. Bladders (volume–cm^3) of WT mice were smaller than those of GM2 Synthase KO mice. In GM2 Synthase KO males, bladders were two times larger (0.18 ± 0.03 cm^3) than were the bladders of age matched WT males (0.09 ± 0.01 cm^3) (Fig. 4b). For KO females, their bladders were ~ 6 times larger (0.61 ± 0.16 cm^3) than the bladders of age matched WT females (0.09 ± 0.03 cm^3) (Fig. 4c). This strongly suggested that the reduction in void spots seen for aging GM2 Synthase KO mice paralleled an increase in urine retention that had produced bladder enlargement.

3.4. Tyrosine hydroxylase differences in WT and GM2 Synthase KO brain micturition centers

In addition to the PAG micturition center, dopamine signaling related to VTA significantly modulates micturition (Hashimoto et al., 2003). Moreover, accumulating data show that VTA dopaminergic cell loss is relatively common in patients with PD (Alberico et al., 2015; Dragicevic et al., 2015; Harrison et al., 2016; Rinne et al., 1990) as are problems with urinary bladder function (Dubow, 2007; Ginsberg, 2013; Hall et al., 2012; Lemack et al., 2000). Thus, we assessed tyrosine hydroxylase (TH) immunohistochemistry in PAG and VTA of WT and GM2 Synthase KO mice. There is a significant loss of TH in the VTA of GM2 KO mice compared to WT mice (Fig. 5a) and a trend toward reduced TH in GM2 KO PAG compared to WT littermates (Fig. 5b). This provides new evidence that dopaminergic damage in brain micturition centers can impact voiding in association with a loss in mature gangliosides.

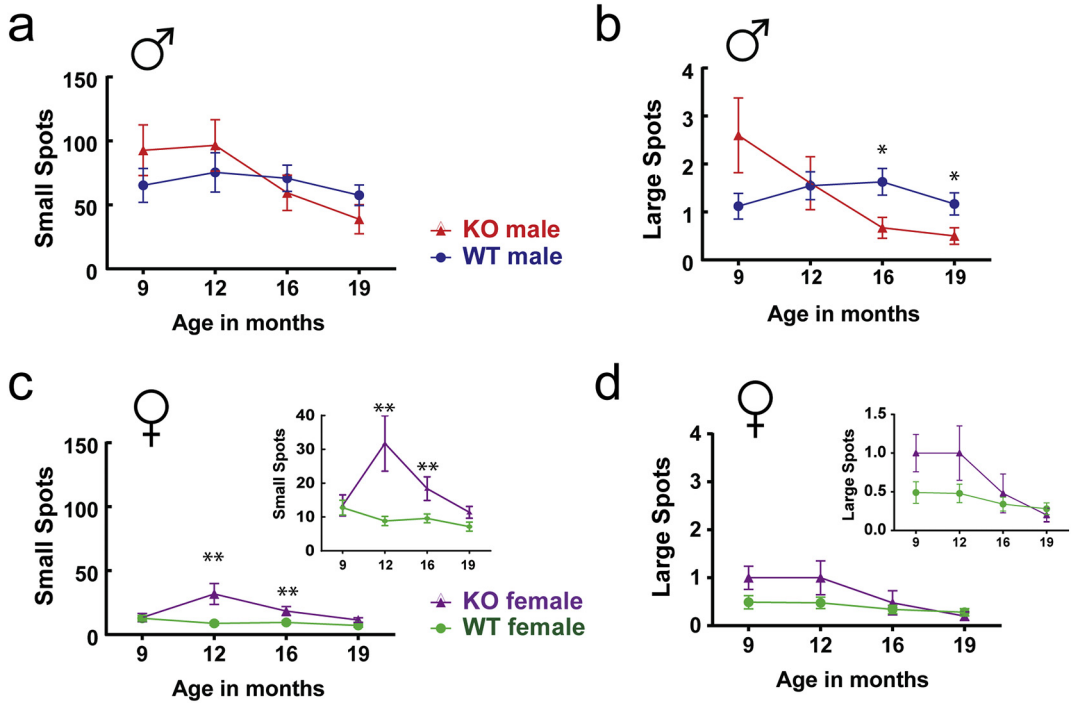

Fig. 3. Urination patterns of GM2 Synthase KO and WT mice from 9 to 19 months. Due to sex differences data for male (♂) and female (♀) mice are plotted separately. (a) KO males tend to have more small spots than WT males at 9–12 months, but fewer small spots at 16–19 months, though differences are not significant. (b) KO males tend to have more large spots than WT males at 9 months, though not significantly different. However, by 16–19 months, KO males had significantly fewer large spots than WT males. (c) At 9 months WT and KO females had similar numbers of small spots. From 12 to 16 months, KO females had significantly more small spots than WT females, as is better appreciated in the inset graph with a different Y axis. Though not significantly different, KO females show a trend toward more large spots at 9–12 months that decrease at 16–19 months, (N = 26, 15 WT and 11 KO), * $p < .05$; ** $p < .01$.

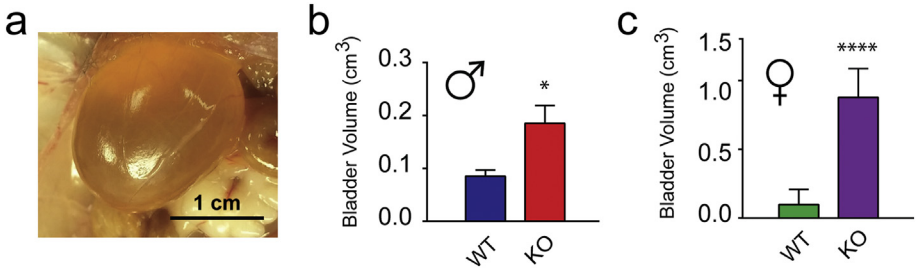

Fig. 4. Bladder enlargement in aging GM2 Synthase KO mice. Due to sex differences, quantitative data for male (♂) and female (♀) mice are plotted separately. (a) A representative enlarged bladder from a 12 month old mouse collected before initiating ultrasound analyses. Data in (b) and (c) are from bladder ultrasounds of 20 month old mice. (b) Male KO bladders (volume–cm^3) are significantly enlarged compared to bladders of WT males. (c) Female KO bladders are also significantly enlarged compared to WT female bladders. These increases paralleled a decrease in the number of void spots produced by both sexes of KO mice as they aged. (N = 15, 8 WT and 7 KO), * p < .05; **** p < .0001.

Moreover, SNc dopaminergic neurons also contribute to micturition based on data from 6-hydroxydopamine lesioned rats (Soler et al., 2011; Yoshimura et al., 2003). Thus, we also evaluated TH immunohistochemistry which reconfirmed SNc dopaminergic loss in our GM2 Synthase KO mice (Fig. 5c). We also noted that aSyn levels were somewhat increased in the GM2 Synthase KO brain.

3.5. Assessment of aSyn in WT and GM2 Synthase KO bladders

As GM2 Synthase KO mice had significant micturition problems, we measured insoluble aSyn in bladder using an established sequential protein extraction and immunoblot method as before (Lou et al., 2010; Vidal-Martinez et al., 2016; Waxman et al., 2008; Wu et al., 2012b) (Fig. 6). With this method, soluble proteins are released by the first three buffers used for sequential extraction of tissue: high salt (HS), high salt triton (HST), and the radio immunoprecipitation assay buffer (RIPA). Insoluble, *aggregated*, proteins are extracted in the final SDS-Urea buffer. As a positive control we used a bladder from an A53T aSyn transgenic mouse model that is known to develop age-onset bladder dysfunction (Hamill et al., 2012). Monomeric 17–19 kDa aSyn was present in soluble extracts of A53T Tg, WT, and GM2 KO bladders, however, insoluble high molecular weight (HMW) aSyn was found only in A53T bladder (at arrow, Fig. 6, left side) but not in WT or GM2 Synthase KO bladders (at arrows, Fig. 6, right side). These findings revealed that aSyn did not aggregate in GM2 Synthase KO bladders. We then evaluated other possible mechanisms known to be associated with bladder dysfunction.

3.6. ProNGF levels are elevated in GM2 Synthase KO bladder

As bladder NGF is a proposed neurogenic biomarker, we evaluated NGF levels in whole bladders from WT and GM2 Synthase KO mice (N = 4 WT, 5 KO). We noted that there was no mature NGF in the bladders of either WT or GM2 KO mice (not shown). However, proNGF levels were significantly increased in GM2 KO as compared to WT bladders (Fig. 7a). Quantification of bladder proNGF levels in 12–23 month old GM2 KO and WT mice confirmed a significant increase in proNGF protein levels only in GM2 KO bladders (Fig. 7b, WT 155.9 ± 28.23; KO 555.4 ± 183.9, t-test, p = .049). Others recently confirmed that bladder proNGF is detrimental to normal bladder function (Ryu et al., 2018). Because trophic factors can also modulate innervation, we next evaluated bladder innervation by transmission electron microscopy.

3.7. GM2 Synthase KO mice have abnormal myelination in the bladder innervation

Transmission electron micrographs revealed differences in bladder innervation in the major pelvic ganglion of 17 month old WT and KO Synthase GM2 mice. Large and small nerve fibers, as well as neurons in and around the ganglia were examined with cross-sections of nerve fibers from GM2 Synthase KO and WT mice compared. In WT samples, nerve fibers appeared uniformly filled with cytoplasm containing profiles of neurotubules and neurofilaments along with profiles of mitochondria and small vesicles, surrounded by myelin sheaths of uniform thickness and electron-density (Fig. 8a). In contrast, the nerve fibers of the GM2 Synthase KO samples included large irregular vacuoles at the interface between the axolemma and the myelin sheath, which also contained many smaller electron-lucent pockets (Fig. 8b, at red arrows). Moreover, the distribution of fibers (collagen) in the extracellular space of the nerve fiber bundles in WT mice were more uniform (Fig. 8a) than in corresponding areas from GM2 Synthase KO mice, where large spaces between nerve fibers appeared to be devoid of visible extracellular material (Fig. 8b).

The discovery of abnormal bladder innervation in GM2 Synthase KO mice parallels the myelination abnormalities previously described in the optic and sciatic nerves in this mouse model (Chiavegatto et al., 2000; Schnaar and Lopez, 2009; Sheikh et al., 1999). Moreover, our findings provide new insight into the urinary dysfunction found in aging GM2 Synthase KO mice. Collectively, the data suggest that in male and female GM2 Synthase KO mice the loss of mature gangliosides reduced dopaminergic function in brain micturition centers and increased bladder proNGF levels in a manner to impair bladder innervation and detrusor function without inducing bladder aSyn aggregation.

4. Discussion

4.1. Role of mature gangliosides in humans with PD and in PD mouse models

PD patients have lower GM1 ganglioside levels in brain (Hadaczek et al., 2015; Wu et al., 2012a) and GM1 administration to parkinsonian rodents induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or 6-hydroxydopamine, enhances nigral regeneration and reduces their motor impairment (Mocchetti, 2005). PD patients also respond favorably to GM1 treatment by showing significant improvement in motor function (Schneider, 1998; Schneider et al., 2013; Schneider et al., 1995; Schneider et al., 2010). GM1 is able to stimulate signaling through the NGF, BDNF, and GDNF receptors which may underlie improvements previously seen in response to GM1 treatments (Hadaczek et al., 2015; Mocchetti, 2005; Mocchetti et al., 2007; Posse de Chaves and Sipione, 2010) or a GM1 analog, LIGA-20 (Wu et al., 2011). Treating diabetic rats with mixed brain gangliosides containing 20% GM1 reversed their urinary dysfunction (Paro et al., 1994), establishing a role for mature gangliosides in normal micturition function. While the above findings provide evidence for a role for GM1 in normal motor and urinary function, as we did not treat our GM2 Synthase KO mice with GM1, it is possible that a complete loss of GM1, GM2 or other mature gangliosides may have produced the changes found in this model.

4.2. Urinary dysfunction and aSyn pathology in humans and in PD mouse models

PD patients often present with age-onset urinary dysfunction (Araki et al., 2000; Martignoni et al., 1995; VanderHorst et al., 2015) which

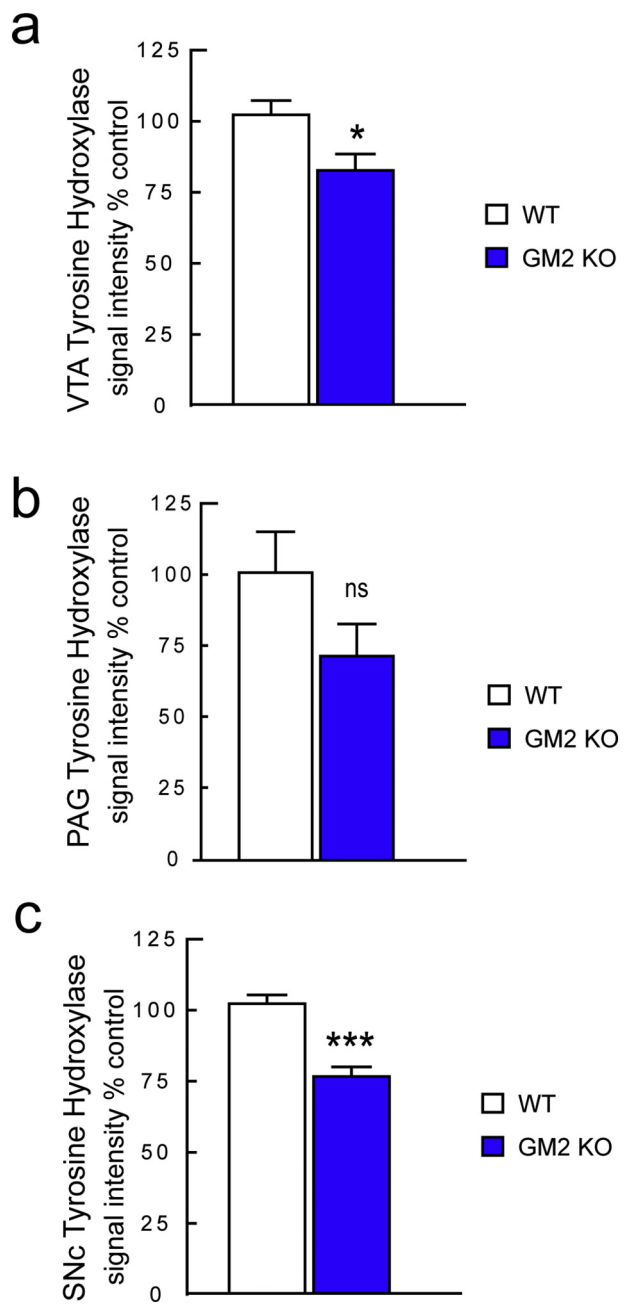

Fig. 5. Ventral tegmental area, periaqueductal gray, and nigral micturition centers have diminished tyrosine hydroxylase staining in 19 month old GM2 Synthase KO mouse brain. (a) VTA dopaminergic TH signal intensity reveals a significant loss in KO VTA compared to WT VTA. (b) Similar analysis of the PAG micturition center shows a trend toward a loss in dopaminergic TH signal intensity in KO as compared to WT mice, but that difference is not statistically significant. (c) SNc dopaminergic TH signal intensity reveals a significant loss in KO compared to WT SNc. (N = 12, 6 WT and 6 KO), * p < .05, *** p < .005, ns = not significant.

has been shown to correlate with aSyn accumulation in neurons of the spinal cord, paraspinal sympathetic ganglia, and vagal nerve (Braak et al., 2003). Bladder aSyn pathology has been reported only in one mouse model of multiple system atrophy and in one patient with Lewy Body dementia (Beach et al., 2010; Boudes et al., 2013). aSyn pathology was also identified in a region of human PAG that regulates arousal and wakefulness in patients with Lewy body dementia and multiple system atrophy (Benarroch et al., 2009).

4.3. GM2 Synthase KO mice as a parkinsonian neurogenic bladder model

The above findings suggested that the GM2 Synthase KO mice, which completely lack all mature gangliosides, would develop age-onset autonomic problems in addition to parkinsonian abnormalities related to motor pathways as previously reported for both the GM2 Synthase KO mice and GM2 Synthase Heterozygous mice (Wu et al., 2011; Wu et al., 2012a). While original reports on the KO mice showed Wallerian degeneration, demyelination of the optic and sciatic nerves, and development of movement problems (Chiavegatto et al., 2000; Sheikh et al., 1999), more recent studies suggest that KO mice nicely model parkinsonism (Forsayeth and Hadaczek, 2018; Hadaczek et al., 2015). Yet, it is worth noting that humans that entirely lack GM2 Synthase expression do not develop parkinsonism but rather spastic paraplegia (Harlalka et al., 2013). Still the data suggest that loss of mature gangliosides in the GM2 Synthase KO mice may have impacted their urinary function.

To test for this, we evaluated GM2 KO mice for PD-like urinary problems as well as their bladder biochemistry and innervation. By measuring void spot patterns from 9 to 19 months in GM2 Synthase KO and WT mice we obtained data suggesting that our hypothesis was at least partially correct: overall GM2 Synthase KO mice had more void spots than WT mice, especially at younger ages. This suggested that GM2 Synthase KO mice initially suffered bladder hyperreflexia of the detrusor muscle causing them to have urinary frequency at 9–12 months in males and 9–16 months in females.

As they aged more, both male and female GM2 Synthase KO mice showed a decrease in the numbers of void spots produced in urinary pattern tests. By 19–20 months, GM2 Synthase KO mice also developed bladder enlargement that paralleled the decrease in void spots, which is consistent with hyporeflexia and/or urinary retention due to detrusor underactivity (Cockayne et al., 2000) a dysfunction that is common in patients with PD (Kadow et al., 2015). We had anticipated that such dysfunction may have resulted from PD-like aggregation of aSyn and Lewy body/Lewy neurite formation in neurons in the brain micturition centers or in bladder. Remarkably, we saw no evidence of aSyn aggregation in the brains of GM2 KO mice. As a positive control, we used bladder from an A53T aSyn Tg mouse which had abundant aSyn aggregation consistent with the known urinary problems in this model (Hamill et al., 2012). However, GM2 Synthase KO mice did not accumulate insoluble aSyn in bladder, suggesting that their urinary problems were not due to aSyn pathology in bladder tissue. Yet, the finding of TH loss in both VTA and SNc of GM2 Synthase KO mice revealed a loss of dopaminergic function that appears to have contributed to the dysregulation of micturition in these mice.

Denervation of the bladder can produce voiding abnormalities ranging from overactivity to urinary retention. Neurological bladder damage can also cause a loss of detrusor movement, or areflexia, that results in incomplete bladder emptying (Yoshimura and Chancellor, 2004). In the rat, the major pelvic ganglion supplies efferent neurons to regulate bladder function (Birder et al., 2010). Using electron microscopic analyses, we assessed myelinated axons running within the pelvic nerve and major pelvic ganglion of KO and WT mice, and detected atypical myelination and dysmorphic axonal structures only in KO mice. Interestingly, unmyelinated axons in the base of the bladder appeared to have larger diameters in KO compared to WT GM2 Synthase mice (not shown), consistent with prior findings for unmyelinated axons in the KO optic nerve (Sheikh et al., 1999). It has previously been demonstrated that impaired bladder innervation can affect bladder trophic factor levels.

4.4. Contribution of trophic factors to bladder dysfunction

Denervation can increase NGF levels in bladder smooth muscle (Steers and Tuttle, 2006; Tanner et al., 2000), and elevated levels of NGF have been associated with bladder dysfunction in A53T aSyn Tg

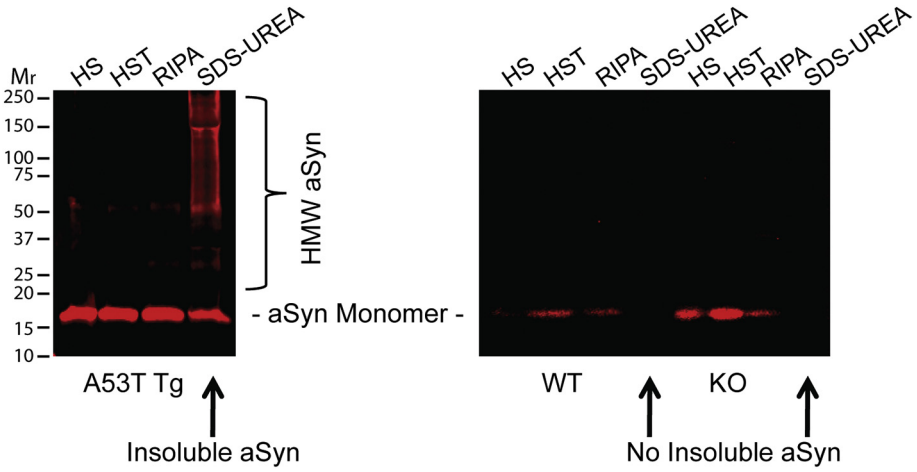

Fig. 6. A positive control bladder from an A53T aSyn transgenic (Tg) mouse shows robust insoluble aSyn in the SDS-Urea sample in contrast to SDS-Urea samples from WT and GM2 Synthase KO mice that lack insoluble aSyn. Bladders were assessed using a method to isolate soluble from insoluble proteins in tissue as detailed in Methods. Soluble aSyn appears as a single band of 17–19 kDa in samples extracted with High Salt (HS) buffer, High Salt Triton (HST) buffer, and RIPA buffer from bladders. Insoluble high molecular weight (HMW) aSyn is seen only in the A53T Tg SDS-Urea sample that was used as a positive control, and not in SDS-Urea samples from 20 to 23 month WT or GM2 Synthase KO bladders. ($N = 7$, 1 A53T, 3 WT, and 3 KO). (Red signal was produced using C20, Cat # sc-7011-R antibody, Santa Cruz, CA, 1:100; IRDye 680 anti-Rabbit Secondary antibody, 1:10,000 using the LiCor Odyssey system). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

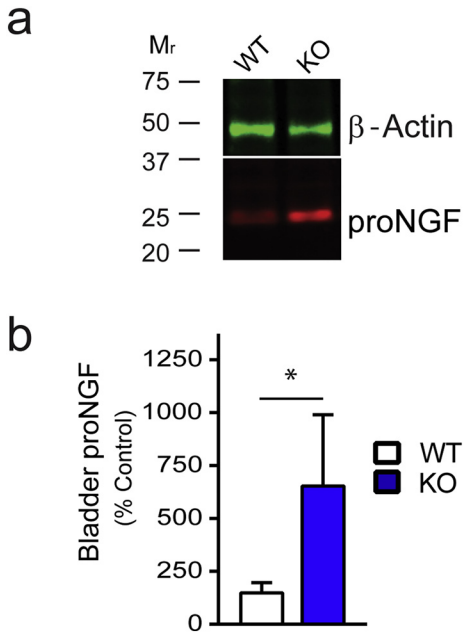

Fig. 7. Levels of the precursor form of Nerve Growth Factor, proNGF, are elevated in GM2 Synthase KO bladder as compared to WT bladder. (a) Representative immunoblot from 20 month old GM2 Synthase KO and WT mice shows much more proNGF in KO than in WT bladder, probed with antibody for NGF (H-20, Cat # sc-548, Santa Cruz Biotechnology, Santa Cruz, CA, USA), with β-actin (4970S, Cell Signaling Technology, Danvers, MA, USA) used as an internal control. (b) Quantification of proNGF normalized to β-actin from WT and KO bladders ($N = 9$, 4 WT and 5 KO) confirms significant increase in bladder proNGF in GM2 KO bladders. * $p < .05$.

parkinsonian mice and other rodents (Hamill et al., 2012; Vizzard, 2000). However, we did not see changes in bladder NGF in the GM2 Synthase KO mice, perhaps because NGF levels are typically so low in the adult nervous system (Al-Shawi et al., 2007). We did however find high levels of bladder proNGF, the precursor form of NGF, in GM2 Synthase KO mouse bladder. ProNGF has been implicated in bladder dysfunction in rodents after spinal cord injury (Ryu et al., 2018) and has also been implicated in neurodegeneration of peripheral and central nervous system neurons in adult humans and rodents, as well as in cell models (Al-Shawi et al., 2008; Fahnestock et al., 2001; Ioannou and Fahnestock, 2017; Sobottka et al., 2008).

5. Conclusions

Cumulatively, the findings suggest that loss of mature gangliosides in the GM2 Synthase KO mice is associated with dopaminergic cell loss in brain micturition centers, increased proNGF expression in bladder, and abnormal myelination associated with the major pelvic ganglion leading to neurogenic bladder symptoms. Studying animals like the GM2 Synthase KO mice, which model motor and non-motor PD symptoms, may help assess emerging PD therapies. Also, as urinary dysfunction can manifest before motor symptoms in PD (Sakakibara et al., 2012; Takahashi et al., 2014), careful evaluation of patients above 45 years of age may help identify those who could benefit most from neuroprotective therapies that are in development as therapeutics for PD and related disorders. In addition, if such therapies also reverse urinary problems they may benefit not only parkinsonian patients but anyone with urinary dysfunction, regardless of the cause.

Competing interests

The authors declare no conflict of interest.

Author contributions

CAR and RGP conceived of the project and conducted pilot studies; CGT and GVM extended analyses with ISU, DM, GVH, SAC and SMM; CGT and DM performed immunohistochemistry; CGT and JVM did biochemical analyses; GVM did mouse breeding and genotyping with CGT, ISU, SAC and GVH; CGT and RGP analyzed data and wrote the paper with editorial assistance from GVM, CAR, and JVM.

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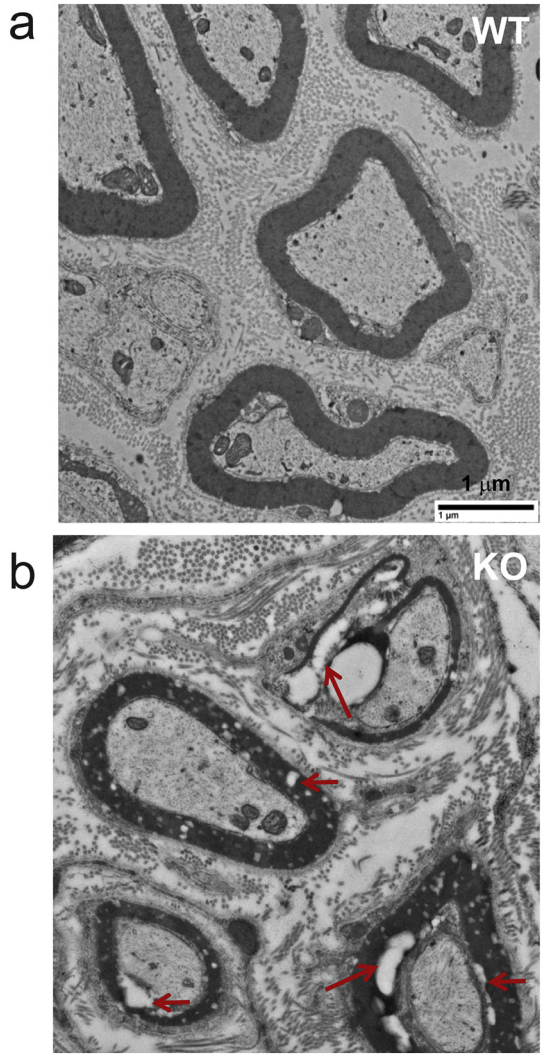

Fig. 8. Ultrastructural differences in axons within the major pelvic ganglion at the base of the bladder of 17 month old WT and GM2 Synthase KO mice. (a) Representative transmission electron micrograph of myelinated fibers found coursing through the major pelvic ganglion of a WT mouse shows uniform myelin sheaths surrounding axons with uniform morphologies. (b) Transmission electron micrograph of myelinated fibers coursing through the major pelvic ganglion of GM2 Synthase KO mouse shows large and small vacuoles in the myelin sheaths (at red arrows) surrounding axons of variable sizes and morphologies. (N = 4, 2 WT and 2 KO). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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References

Alberico, S.L., Cassell, M.D., Narayanan, N.S., 2015. The Vulnerable Ventral Tegmental Area in Parkinson's Disease. Basal ganglia 5, 51–55.
Alerte, T.N., Akinfolarin, A.A., Friedrich, E.E., Mader, S.A., Hong, C.S., Perez, R.G., 2008. Alpha-synuclein aggregation alters tyrosine hydroxylase phosphorylation and immunoreactivity: lessons from viral transduction of knockout mice. Neurosci. Lett. 435, 24–29.
Al-Izki, S., Pryce, G., Giovannoni, G., Baker, D., 2009. Evaluating potential therapies for bladder dysfunction in a mouse model of multiple sclerosis with high-resolution ultrasonography. Mult. Scler. 15, 795–801.
Al-Shawi, R., Hafner, A., Chun, S., Raza, S., Crutcher, K., Thrasivoulou, C., Simons, P., Cowen, T., 2007. ProNGF, sortilin, and age-related neurodegeneration. Ann. N. Y. Acad. Sci. 1119, 208–215.
Al-Shawi, R., Hafner, A., Olsen, J., Chun, S., Raza, S., Thrasivoulou, C., Lovestone, S., Killick, R., Simons, P., Cowen, T., 2008. Neurotoxic and neurotrophic roles of proNGF and the receptor sortilin in the adult and ageing nervous system. Eur. J. Neurosci. 27, 2103–2114.
Araki, I., Kitahara, M., Oida, T., Kuno, S., 2000. Voiding dysfunction and Parkinson's disease: urodynamic abnormalities and urinary symptoms. J. Urol. 164, 1640–1643.
Beach, T.G., Adler, C.H., Sue, L.I., Vedders, L., Lue, L., White Iii, C.L., Akiyama, H., Caviness, J.N., Shill, H.A., Sabbagh, M.N., Walker, D.G., 2010. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. Acta Neuropathol. 119, 689–702.
Benarroch, E.E., Schmeichel, A.M., Dugger, B.N., Sandroni, P., Parisi, J.E., Low, P.A., 2009. Dopamine cell loss in the periaqueductal gray in multiple system atrophy and Lewy body dementia. Neurology 73, 106–112.
Birder, L.A., Nakamura, Y., Kiss, S., Nealen, M.L., Barrick, S., Kanai, A.J., Wang, E., Ruiz, G., De Groat, W.C., Apodaca, G., Watkins, S., Caterina, M.J., 2002. Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. Nat. Neurosci. 5, 856–860.
Birder, L., de Groat, W., Mills, I., Morrison, J., Thor, K., Drake, M., 2010. Neural Control of the lower Urinary Tract: Peripheral and Spinal Mechanisms. Neurourol. Urodyn. 29, 128–139.
Bjorling, D.E., Wang, Z., Vezina, C.M., Ricke, W.A., Keil, K.P., Yu, W., Guo, L., Zeidel, M.L., Hill, W.G., 2015. Evaluation of voiding assays in mice: impact of genetic strains and sex. Am. J. Physiol. Ren. Physiol. 308, F1369–F1378.
Boudes, M., Uvin, P., Pinto, S., Voets, T., Fowler, C.J., Wenning, G.K., De Ridder, D., Stefanova, N., 2013. Bladder dysfunction in a transgenic mouse model of multiple system atrophy. Movement Disorders 28, 347–355.
Braak, H., Del Tredici, K., Rub, U., de Vos, R.A., Jansen Steur, E.N., Braak, E., 2003. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol. Aging 24, 197–211.
Campos-Sousa, R.N., Quagliato, E., da Silva, B.B., de Carvalho, R.M., Ribeiro, S.C., de Carvalho, D.F., 2003. Urinary symptoms in Parkinson's disease: prevalence and associated factors. Arq. Neuropsiquiatr. 61, 359–363.
Chan, R.B., Perotte, A.J., Zhou, B., Liong, C., Shorr, E.J., Marder, K.S., Kang, U.J., Waters, C.H., Levy, O.A., Xu, Y., Shim, H.B., Pe'er, I., Di Paolo, G., Alcalay, R.N., 2017. Elevated GM3 plasma concentration in idiopathic Parkinson's disease: a lipidomic analysis. PLoS One 12, e0172348.
Chiavegatto, S., Sun, J., Nelson, R.J., Schnaar, R.L., 2000. A functional role for complex gangliosides: motor deficits in GM2/GD2 synthase knockout mice. Exp. Neurol. 166, 227–234.
Cockayne, D.A., Hamilton, S.G., Zhu, Q.M., Dunn, P.M., Zhong, Y., Novakovic, S., Malmberg, A.B., Cain, G., Berson, A., Kassotakis, L., Hedley, L., Lachnit, W.G., Burnstock, G., McMahon, S.B., Ford, A.P., 2000. Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. Nature 407, 1011–1015.
Cuello, A.C., 2012. Gangliosides, NGF, brain aging and disease: a mini-review with personal reflections. Neurochem. Res. 37, 1256–1260.
Dragicevic, E., Schiemann, J., Liss, B., 2015. Dopamine midbrain neurons in health and Parkinson's disease: emerging roles of voltage-gated calcium channels and ATP-sensitive potassium channels. Neuroscience 284, 798–814.
Dubow, J.S., 2007. Autonomic dysfunction in Parkinson's disease. Disease-a-month 53, 265–274.
Fahnestock, M., Michalski, B., Xu, B., Coughlin, M.D., 2001. The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease. Mol. Cell. Neurosci. 18, 210–220.
Farrell, K.F., Krishnamachari, S., Villanueva, E., Lou, H., Alerte, T.N., Peet, E., Drolet, R.E., Perez, R.G., 2014. Non-motor parkinsonian pathology in aging A53T alpha-synuclein mice is associated with progressive synucleinopathy and altered enzymatic function. J. Neurochem. 128, 536–546.
Forsayeth, J., Hadaczek, P., 2018. Ganglioside Metabolism and Parkinson's Disease. Front. Neurosci. 12.
Ginsberg, D., 2013. Optimizing therapy and management of neurogenic bladder. Am. J. Manag. Care 19, s197–s204.
Hadaczek, P., Wu, G., Sharma, N., Ciesielska, A., Bankiewicz, K., Davidow, A.L., Lu, Z.H., Forsayeth, J., Ledeen, R.W., 2015. GDNF signaling implemented by GM1 ganglioside; failure in Parkinson's disease and GM1-deficient murine model. Exp. Neurol. 263, 177–189.
Hall, S.A., Curto, T.M., Onyenwenyi, A., Lemack, G.E., Tennstedt, S.L., Link, C.L., McKinlay, J.B., 2012. Characteristics of persons with overactive bladder of presumed neurologic origin: results from the Boston Area Community Health (BACH) survey. Neurourol. Urodyn. 31, 1149–1155.
Hamill, R.W., Tompkins, J.D., Girard, B.M., Kershen, R.T., Parsons, R.L., Vizzard, M.A., 2012. Autonomic dysfunction and plasticity in micturition reflexes in human alpha-synuclein mice. Developmental Neurobiology 72, 918–936.
Harlalka, G.V., Lehman, A., Chioza, B., Baple, E.L., Maroofian, R., Cross, H., Sreekantan-Nair, A., Priestman, D.A., Al-Turki, S., McEntagart, M.E., Proukakis, C., Royle, L., Kozak, R.P., Bastaki, L., Patton, M., Wagner, K., Coblentz, R., Price, J., Mezei, M., Schlade-Bartusiak, K., Platt, F.M., Hurles, M.E., Crosby, A.H., 2013. Mutations in B4GALNT1 (GM2 synthase) underlie a new disorder of ganglioside biosynthesis. Brain 136, 3618–3624.
Harrison, I.F., Anis, H.K., Dexter, D.T., 2016. Associated degeneration of ventral tegmental area dopaminergic neurons in the rat nigrostriatal lactacystin model of parkinsonism and their neuroprotection by valproate. Neurosci. Lett. 614, 16–23.
Hashimoto, K., Oyama, T., Sugiyama, T., Park, Y.C., Kurita, T., 2003. Neuronal excitation in the ventral tegmental area modulates the micturition reflex mediated via the dopamine D1 and D2 receptors in rats. J. Pharmacol. Sci. 92, 143–148.

Hodges, S.J., Zhou, G., Deng, F.M., Aboushwareb, T., Turner, C., Andersson, K.E., Santago, P., Case, D., Sun, T.T., Christ, G.J., 2008. Voiding pattern analysis as a surrogate for cystometric evaluation in uroplakin II knockout mice. J. Urol. 179, 2046–2051.
Ioannou, M.S., Fahnestock, M., 2017. ProNGF, but not NGF, switches from neurotrophic to apoptotic activity in response to reductions in TrkA receptor levels. Int. J. Mol. Sci. 18, 599.
Kadow, B.T., Tyagi, P., Chermansky, C.J., 2015. Neurogenic Causes of Detrusor Underactivity. Current Bladder Dysfunction Reports 10, 325–331.
Kitta, T., Matsumoto, M., Tanaka, H., Mitsui, T., Yoshioka, M., Nonomura, K., 2008. GABAergic mechanism mediated via D receptors in the rat periaqueductal gray participates in the micturition reflex: an in vivo microdialysis study. Eur. J. Neurosci. 27, 3216–3225.
Lemack, G.E., Dewey Jr., R.B., Roehrborn, C.G., O'Suilleabhain, P.E., Zimmern, P.E., 2000. Questionnaire-based assessment of bladder dysfunction in patients with mild to moderate Parkinson's disease. Urology 56, 250–254.
Lou, H., Montoya, S.E., Alerte, T.N., Wang, J., Wu, J., Peng, X., Hong, C.S., Friedrich, E.E., Mader, S.A., Pedersen, C.J., Marcus, B.S., McCormack, A.L., Di Monte, D.A., Daubner, S.C., Perez, R.G., 2010. Serine 129 phosphorylation reduces the ability of alpha-synuclein to regulate tyrosine hydroxylase and protein phosphatase 2A in vitro and in vivo. J. Biol. Chem. 285, 17648–17661.
Martignoni, E., Pacchetti, C., Godi, L., Micieli, G., Nappi, G., 1995. Autonomic disorders in Parkinson's disease. J. Neural Transm. Suppl. 45, 11–19.
Mocchetti, I., 2005. Exogenous gangliosides, neuronal plasticity and repair, and the neurotrophins. Cellular and Molecular Life Sciences CMLS 62, 2283–2294.
Mocchetti, I., Bachis, A., Nosheny, R.L., Tanda, G., 2007. Brain-derived neurotrophic factor expression in the substantia nigra does not change after lesions of dopaminergic neurons. Neurotox. Res. 12, 135–143.
Ohmi, Y., Ohkawa, Y., Tajima, O., Sugiura, Y., Furukawa, K., Furukawa, K., 2014. Ganglioside deficiency causes inflammation and neurodegeneration via the activation of complement system in the spinal cord. J. Neuroinflammation 11, 61.
Palmano, K., Rowan, A., Guillermo, R., Guan, J., Mc Jarrow, P., 2015. The role of gangliosides in neurodevelopment. Nutrients 7, 3891–3913.
Paro, M., Prashar, A., Prosdocimi, M., Cherian, P.V., Fiori, M.G., Sima, A.A., 1994. Urinary bladder dysfunction in the BB/W diabetic rat: effect of ganglioside treatment on functional and structural alterations. J. Urol. 151, 781–786.
Posse De Chaves, E., Sipione, S., 2010. Sphingolipids and gangliosides of the nervous system in membrane function and dysfunction. FEBS Lett. 584, 1748–1759.
Richardson, K.C., Jarett, L., Finke, E.H., 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain. Technol. 35, 313–323.
Rinne, J.O., Paljärvi, L., Rummukainen, J., Röyttä, M., Rinne, U.K., 1990. Neuronal loss in the substantia nigra and ventral tegmental area in parkinson's disease and alzheimer's disease. In: Nagatsu, T., Fisher, A., Yoshida, M. (Eds.), Basic, Clinical, and Therapeutic Aspects of Alzheimer's and Parkinson's Diseases. Springer, Boston, pp. 377–380.
Rueden, C.T., Schindelin, J., Hiner, M.C., Dezonia, B.E., Walter, A.E., Arena, E.T., Eliceiri, K.W., 2017. ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics 18, 529.
Ryu, J.C., Tooke, K., Malley, S.E., Soulas, A., Weiss, T., Ganesh, N., Saidi, N., Daugherty, S., Saragovi, U., Ikeda, Y., Zabbarova, I., Kanai, A.J., Yoshiyama, M., Farhadi, H.F., de Groat, W.C., Vizzard, M.A., Yoon, S.O., 2018. Role of proNGF/p75 signaling in bladder dysfunction after spinal cord injury. J. Clin. Invest. 128, 1772–1786.
Sakakibara, R., Tateno, F., Kishi, M., Tsuyuzaki, Y., Uchiyama, T., Yamamoto, T., 2012. Pathophysiology of bladder dysfunction in Parkinson's disease. Neurobiol. Dis. 46, 565–571.
Schnaar, R.L., 2010. Brain gangliosides in axon-myelin stability and axon regeneration. FEBS Lett. 584, 1741–1747.
Schnaar, R.L., Lopez, P.H., 2009. Myelin-associated glycoprotein and its axonal receptors. J. Neurosci. Res. 87, 3267–3276.
Schneider, J.S., 1998. GM1 ganglioside in the treatment of Parkinson's disease. Ann. N. Y. Acad. Sci. 845, 363–373.
Schneider, J.S., Roeltgen, D.P., Rothblat, D.S., Chapas-Crilly, J., Seraydarian, L., Rao, J., 1995. GM1 ganglioside treatment of Parkinson's disease: an open pilot study of safety and efficacy. Neurology 45, 1149–1154.
Schneider, J.S., Roeltgen, D.P., Mancall, E.L., Chapas-Crilly, J., Rothblat, D.S., Tatarian, G.T., 1998. Parkinson's disease: improved function with GM1 ganglioside treatment in a randomized placebo-controlled study. Neurology 50, 1630–1636.
Schneider, J.S., Sendek, S., Daskalakis, C., Cambi, F., 2010. GM1 ganglioside in Parkinson's disease: results of a five year open study. J. Neurol. Sci. 292, 45–51.
Schneider, J.S., Gollomp, S.M., Sendek, S., Colcher, A., Cambi, F., Du, W., 2013. A randomized, controlled, delayed start trial of gm1 ganglioside in treated Parkinson's disease patients. J. Neurol. Sci. 324, 140–148.
Seth, J.H., Sahai, A., Khan, M.S., van der Aa, F., de Ridder, D., Panicker, J.N., Dasgupta, P., Fowler, C.J., 2013. Nerve growth factor (NGF): a potential urinary biomarker for overactive bladder syndrome (OAB)? BJU Int. 111, 372–380.
Sheikh, K.A., Sun, J., Liu, Y., Kawai, H., Crawford, T.O., Proia, R.L., Griffin, J.W., Schnaar, R.L., 1999. Mice lacking complex gangliosides develop Wallerian degeneration and myelination defects. Proc. Natl. Acad. Sci. U. S. A. 96, 7532–7537.
Shulman, J.M., De Jager, P.L., Feany, M.B., 2011. Parkinson's disease: genetics and pathogenesis. Annu. Rev. Pathol. 6, 193–222.
Sobottka, B., Reinhardt, D., Brockhaus, M., Jacobsen, H., Metzger, F., 2008. ProNGF inhibits NGF-mediated TrkA activation in PC12 cells. J. Neurochem. 107, 1294–1303.
Soler, R., Fullhase, C., Santos, C., Andersson, K.E., 2011. Development of bladder dysfunction in a rat model of dopaminergic brain lesion. Neurourol. Urodyn. 30, 188–193.
Steers, W.D., Tuttle, J.B., 2006. Mechanisms of Disease: the role of nerve growth factor in the pathophysiology of bladder disorders. Nat. Clin. Pract. Urol. 3, 101–110.
Studeny, S., Cheppudira, B.P., Meyers, S., Balestreire, E.M., Apodaca, G., Birder, L.A., Braas, K.M., Waschek, J.A., May, V., Vizzard, M.A., 2008. Urinary bladder function and somatic sensitivity in vasoactive intestinal polypeptide (VIP) −/− mice. Journal of Molecular Neuroscience 36, 175–187.
Sturgill, E.R., Aoki, K., Lopez, P.H., Colacurcio, D., Vajn, K., Lorenzini, I., Majić, S., Yang, W.H., Heffer, M., Tiemeyer, M., Marth, J.D., Schnaar, R.L., 2012. Biosynthesis of the major brain gangliosides GD1a and GT1b. Glycobiology 22, 1289–1301.
Takahashi, O., Sakakibara, R., Tateno, F., Kishi, M., Tsuyusaki, Y., Aiba, Y., Yano, H., Sugiyama, M., Yamamoto, T., Yamanishi, T., Uchiyama, T., Shibata, C., Tomaru, T., 2014. Overactive bladder may precede motor disorder in Parkinson's disease: a urodynamic study. Parkinsonism Relat. Disord. 20, 1030–1032.
Tanner, R., Chambers, P., Khadra, M.H., Gillespie, J.I., 2000. The production of nerve growth factor by human bladder smooth muscle cells in vivo and in vitro. BJU Int. 85, 1115–1119.
Vanderhorst, V.G., Samardzic, T., Saper, C.B., Anderson, M.P., Nag, S., Schneider, J.A., Bennett, D.A., Buchman, A.S., 2015. Alpha-Synuclein pathology accumulates in sacral spinal visceral sensory pathways. Ann. Neurol. 78, 142–149.
Vidal-Martinez, G., Vargas-Medrano, J., Gil-Tommee, C., Medina, D., Garza, N.T., Yang, B., Segura-Ulate, I., Dominguez, S.J., Perez, R.G., 2016. FTY720/Fingolimod reduces synucleinopathy and improves gut motility in A53T mice: Contributions of pro-brain-derived neurotrophic factor (pro-BDNF) and mature BDNF. J. Biol. Chem. 291, 20811–20821.
Vizzard, M.A., 2000. Changes in urinary bladder neurotrophic factor mRNA and NGF protein following urinary bladder dysfunction. Exp. Neurol. 161, 273–284.
Waxman, E.A., Giasson, B.I., 2008. Specificity and regulation of casein kinase-mediated phosphorylation of alpha-synuclein. J. Neuropathol. Exp. Neurol. 67, 402–416.
Waxman, E.A., Duda, J.E., Giasson, B.I., 2008. Characterization of antibodies that selectively detect alpha-synuclein in pathological inclusions. Acta Neuropathol. 116, 37–46.
Winge, K., Skau, A.M., Stimpel, H., Nielsen, K.K., Werdelin, L., 2006. Prevalence of bladder dysfunction in Parkinsons disease. Neurourol. Urodyn. 25, 116–122.
Wu, G., Xie, X., Lu, Z.H., Ledeen, R.W., 2001. Cerebellar neurons lacking complex gangliosides degenerate in the presence of depolarizing levels of potassium. Proc. Natl. Acad. Sci. U. S. A. 98, 307–312.
Wu, G., Lu, Z.H., Kulkarni, N., Amin, R., Ledeen, R.W., 2011. Mice lacking major brain gangliosides develop parkinsonism. Neurochem. Res. 36, 1706–1714.
Wu, G., Lu, Z.H., Kulkarni, N., Ledeen, R.W., 2012a. Deficiency of ganglioside GM1 correlates with Parkinson's disease in mice and humans. J. Neurosci. Res. 90, 1997–2008.
Wu, J., Lou, H., Alerte, T.N., Stachowski, E.K., Chen, J., Singleton, A.B., Hamilton, R.L., Perez, R.G., 2012b. Lewy-like aggregation of alpha-synuclein reduces protein phosphatase 2A activity in vitro and in vivo. Neuroscience 207, 288–297.
Yoshimura, N., Chancellor, M.B., 2004. Differential diagnosis and treatment of impaired bladder emptying. Reviews in Urology 6 (Suppl. 1), S24–S31.
Yoshimura, N., Kuno, S., Chancellor, M.B., De Groat, W.C., Seki, S., 2003. Dopaminergic mechanisms underlying bladder hyperactivity in rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal pathway. Br. J. Pharmacol. 139, 1425–1432.
Yu, W., Ackert-Bicknell, C., Larigakis, J.D., MacIver, B., Steers, W.D., Churchill, G.A., Hill, W.G., Zeidel, M.L., 2014. Spontaneous voiding by mice reveals strain-specific lower urinary tract function to be a quantitative genetic trait. Am. J. Physiol. Ren. Physiol. 306, F1296–F1307.