In vivo electrophysiology of nigral and thalamic neurons in alpha-synuclein-overexpressing mice highlights differences from toxin-based models of parkinsonism

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Lobb CJ, Zaheer AK, Smith Y, Jaeger D. In vivo electrophysiology of nigral and thalamic neurons in alpha-synuclein-overexpressing mice highlights differences from toxin-based models of parkinsonism. J Neurophysiol 110: 2792–2805, 2013. First published September 25, 2013; doi:10.1152/jn.00441.2013.—Numerous studies have suggested that alpha-synuclein plays a prominent role in both familial and idiopathic Parkinson’s disease (PD). Mice in which human alpha-synuclein is overexpressed (ASO) display progressive motor deficits and many nonmotor features of PD. However, it is unclear what in vivo pathophysiological mechanisms drive these motor deficits. It is also unknown whether previously proposed pathophysiological features (i.e., increased beta oscillations, bursting, and synchronization) described in toxin-based, nigrostriatal dopamine-depletion models are also present in ASO mice. To address these issues, we first confirmed that 5- to 6-mo-old ASO mice have robust motor dysfunction, despite the absence of significant nigrostriatal dopamine degeneration. In the same animals, we then recorded simultaneous single units and local field potentials (LFPs) in the substantia nigra pars reticulata (SNpr), the main basal ganglia output nucleus, and one of its main thalamic targets, the ventromedial nucleus, as well as LFPs in the primary motor cortex in anesthetized ASO mice and their age-matched, wild-type littermates. Neural activity was examined during slow wave activity and desynchronized cortical states, as previously described in 6-hydroxydopamine-lesioned rats. In contrast to toxin-based models, we found a small decrease, rather than an increase, in beta oscillations in the desynchronized state. Similarly, synchronized burst firing of nigral neurons observed in toxin-based models was not observed in ASO mice. Instead, we found more subtle changes in pauses of SNpr firing compared with wild-type control mice. Our results suggest that the pathophysiology underlying motor dysfunction in ASO mice is distinctly different from striatal dopamine-depletion models of parkinsonism.

basal ganglia; mouse; Parkinson’s disease; recording; thalamus

ELECTROPHYSIOLOGICAL EXPERIMENTS in 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine (MPTP)-treated monkeys and 6-hydroxydopamine (OHDA)-lesioned rats have been important in furthering our understanding of Parkinson’s disease (PD) pathophysiology. Although the pathology of these animal models is mainly restricted to lesions of the nigrostriatal dopaminergic system, they exhibit pathophysiological changes in basal ganglia activity similar to PD patients (Eusebio and Brown 2007; Wichmann and Dostrovsky 2011). However, it is well recognized that PD pathology extends far beyond dopaminergic neuron loss (Lang and Obeso 2004) and that genetic factors play an important role (Klein and Westenberger 2012).

The alpha-synuclein gene, SNCA, was the first gene implicated in familial PD (Polymeropoulos et al. 1997) and is the strongest risk factor for idiopathic PD (Nalls et al. 2011). Alpha-synuclein aggregates are a major constituent of Lewy bodies, a prominent histological feature of idiopathic PD (Spillantini et al. 1997). It is known that either mutations (Kruger et al. 1998; Polymeropoulos et al. 1997; Zarranz et al. 2004) or overexpression of normal (Chartier-Harlin et al. 2004; Farrer et al. 2004; Singleton et al. 2003) alpha-synuclein can cause familial PD.

A genetic mouse model based on normal human alpha-synuclein overexpression (ASO) (Masliah “line 61”; Rockenstein et al. 2002) shows progressive motor deficits (Fleming et al. 2004) as well as nonmotor features of PD despite the absence of significant loss of nigrostriatal dopamine (Chesselet et al. 2012). Specific neurochemical and electrophysiological changes have been reported in the striatum of these mice. For instance, spontaneous excitatory postsynaptic currents in medium spiny neurons are decreased at 3 mo (Lam et al. 2011; Wu et al. 2010), consistent with a role of alpha-synuclein in inhibition of synaptic transmission (Sulzer 2010), while extracellular levels of striatal dopamine are transiently increased at 6 mo (Lam et al. 2011). Apart from these striatal abnormalities, the pathophysiology underlying motor dysfunction in ASO mice is unknown.

The aim of this study was to determine whether the pathophysiological mechanisms of dopamine-depletion models (i.e., increased beta oscillations, bursting, and synchronization) are present in the major basal ganglia output nucleus (the substantia nigra pars reticulata, SNpr) of ASO mice and, if so, whether these mechanisms disrupt neural processing in the downstream ventromedial (VM) thalamic nucleus. Simultaneous recordings of local field potentials (LFPs) in the primary motor cortex combined with LFPs and single-unit recordings in the SNpr and VM were obtained in anesthetized ASO mice and their wild-type littermates. The anesthetized state has been widely used to study changes of neural activity in toxin-based rodent models of PD (Magill et al. 2001; Mallet et al. 2008a, 2008b, 2012; Parr-Brownlie et al. 2009). Our findings confirm that ASO mice display robust motor deficits despite the absence of significant loss of striatal dopamine innervation. However, neither increased beta oscillations in motor cortex nor striking changes in SNpr or VM neuron activity, commonly seen in dopamine-depleted animals, were found. Our results suggest...
that the pathophysiology underlying motor dysfunction in ASO mice is different from that associated with nigrostriatal dopamine-depletion models of PD, most likely involving changes in neural networks outside of the basal ganglia.

METHODS

A total of 21 male mice graciously donated by the Chesselet/Levine laboratory at UCLA were used in the following experiments [12 wild-type (WT), 9 ASO; Masliah line 61 backcrossed to B6 background]. All experiments were done in accordance with protocols reviewed and approved by the Emory University Institutional Animal Care and Use Committee (IACUC). Both wild-type and ASO mice were housed together in an isolated cubicle under a reverse light cycle (9 AM lights off, 9 PM lights on). Mice were socially housed with WT and ASO mice intermingled. After a 2-wk accommodation period in their new environment, mice underwent a battery of motor behavioral tests that have been shown previously to identify motor deficits in ASO mice (in a mixed C57BL/6-DBA/2 background; Fleming et al. 2004). There was no difference in the age of WT and ASO mice at the time of the behavioral testing \( P > 0.05 \), Mann-Whitney U-test; WT \((n = 12): 129 \pm 9.3 \) (SE) days, range 93–190 days, ASO \((n = 9): 119 \pm 9.2 \) days, range 93–179 days]. Several days to a few weeks after behavioral testing, electrophysiological recordings were obtained from each of these animals in a single session under anesthesia. Again, at the time of recording there was no difference in the age of WT and ASO mice on the recording/perfusion day \( P > 0.05 \), Mann-Whitney U-test; WT: 171 \pm 5.1 \) days, range 143–201 days; ASO: 170 \pm 3.9 \) days, range 144–186 days); however, WT mice weighed significantly more \( P < 0.05 \), Mann-Whitney U-test; WT: 30.1 \pm 0.84 \) g; ASO: 26.8 \pm 0.62 \) g). At the end of the recording session, mice were perfused with 4% paraformaldehyde and sucrose for histological processing (see below). The identity of genotyped ASO mice was further confirmed by immunostaining for human alpha-synuclein, and the extent of striatal dopamine denervation was assessed by densitometry measurements of tyrosine hydroxylase (TH) immunoreactivity.

Behavioral Testing

All behavioral tests were performed during the dark cycle, starting around 10 AM with minimal white light. To reduce experimental bias, all behavioral testing and scoring was done with the experimenter blind to the genotype of the mice. Mann-Whitney U-tests were used for groupwise comparisons for each test.

Spontaneous activity. Mice were placed in a cylindrical glass beaker (15.5-cm diameter) positioned on a pane of glass bridging two empty rat cages that were placed on a table. A video camera was placed underneath the glass pane and beaker to record footprints. Two additional video cameras and a mirror were used to ensure that a 360° view was seen. Activity was recorded for 10 min. The number of steps taken, rears, and grooming episodes in the first 3 min were counted off-line with the recorded videos.

Challenging beam test. Mice were trained for two sessions (1 session/day) to walk across a tapering plastic beam toward their home cage (with gentle nudging, if necessary) until they were able to traverse the entire length of the beam unassisted. On the third day (test day), a mesh grid was placed over the beam to increase difficulty. Each mouse had five trials. Beam traversal was videotaped. The total time taken to traverse the beam and the number of errors made during beam traversal were analyzed. The first trial was excluded.

Pole test. Mice were placed just below the top of a vertical pole facing upward. The metal pole was wrapped in wire to facilitate walking. The base of the pole was placed in the mouse’s home cage. Mice received 2 days of training (5 trials per session) and 1 test day (5 trials). The time to turn completely around and reorient as well as the time taken to climb down the pole once reoriented were measured. Trials in which the mouse fell from the pole or descended the pole without complete reorientation were counted as error trials and analyzed separately.

Behavioral Motor Deficits Score

Mice were scored according to their behavioral results on the cylinder (total steps, total rears), challenging beam (errors, traversal time), and pole (time to turn around, number of falls) tests. Each measurement was normalized to the maximum score of the entire cohort (WT and ASO) to yield a score between 0 and 1, with 1 being the most affected. For total steps and rears in the cylinder, a mouse was given a severity score of 1 minus the total steps (or rears) divided by the maximum total steps (or rears) taken by any mouse (WT or ASO). For all other tests the severity score was calculated by the behavioral measurement divided by the respective maximum measurement for all mice. Thus an individual mouse received a score between 0 and 1 for each of the six tests. The average severity score was determined by dividing the sum of each of its severity scores by the number of tests (usually 6; could be less if there were technical errors during a particular test).

Electrophysiological Recordings

Mice were anesthetized with urethane alone (1.7 g/kg ip) or a lower dose of urethane (1.3 g/kg ip) in conjunction with supplemental doses of a cocktail of ketamine (30 mg/kg ip) and xylazine (3 mg/kg ip). This combination has been used extensively in previous studies in the 6-OHDA rat model of PD (Mallet et al. 2008a, 2008b). Mice were placed into a stereotaxic frame (ASI Instruments) in which a mouse gas anesthesia head holder (Kopf Instruments 923-B) was installed. Oxygen flowed through the nose cone via a regulator. Nonpuncture ear bars were further used to secure the head. Body temperature was maintained at 36.7°C with a homeothermic heating pad and a rectal probe. After surgical exposure of the skull, burr holes were drilled for electrode insertion. A depth LFP electrode (100–200 μm; Epoxyfilet-6001 insulated no. 000 stainless steel insect pin) was implanted in the primary motor cortex \([-1.1 \text{ mm AP (from bregma), } 1.2 \text{ mm ML, } 0.8 \text{ mm DV}] \) and secured with light-curable dental cement (Kerr Maxcem Elite). In some experiments, a second depth LFP electrode was implanted 1–1.2 mm caudal (caudal end of the primary motor cortex) in a similar manner for bipolar recordings. Next, burr holes were drilled over recording sites in the motor thalamus \([-1.4 \text{ mm AP (from bregma), } 0.7 \text{ mm ML, } 3.7–4.4 \text{ DV}] \) and substantia nigra \([\text{on a diagonal starting at } -3.3 \text{ mm AP (from bregma), } 1.6 \text{ ML, } 3.8 \text{ DV} \text{ and ending at } -3.2 \text{ mm AP, } 1.6 \text{ ML, } 4.4 \text{ DV}] \). An indifferent ground was placed underneath the scalp above the contralateral temporal musculature. Glass micropipettes filled with 1 M NaCl and 1% pontamine sky blue (2- to 5-μm tip diameter) or tetrodes (1–2 MHz quartz-platinum/tungsten; Thomas Recording) were lowered into the motor thalamus and/or SNpr for monopolar recordings. Raw signals (0.1–10 kHz band-pass filtered) were acquired at 10 kHz, amplified (A-M Systems model 1700 Extracellular Amplifier), and digitized with custom LabVIEW-based acquisition software for off-line data analysis.

Previous results in 6-OHDA-treated rats described increased power in beta frequencies during the desynchronized or activated state (Mallet et al. 2008a, 2008b). In our mice, this state occurred spontaneously or could be evoked with a brief (<10 s) and mild pinch by the experimenter to the toe or tail of the mouse. These periods typically lasted about a minute. This state could be more reliably evoked in mice anesthetized with urethane and ketamine-xylazine anesthesia than in higher-dose urethane-only anesthesia.

At the end of each successful penetration (usually 1–2 per animal), a blue dot was made by iontophoretic ejection of pontamine sky blue (-10 to -20 μA for 25 min; WPI A365 Stimulus Isolator). When
tetrodes were used, the sites of recordings were approximately marked by withdrawing the tetrode and lowering a glass electrode to the target depth. The locations of the blue dots were determined histologically.

**Data Analysis and Statistics of Electrophysiology Data**

Data analysis was performed in MATLAB (MathWorks). Linear correlations were done in Prism 5.0 (GraphPad). All other statistical analysis was done in MATLAB.

LFP recordings were generally obtained in a monopolar configuration against ground. In 13 of the 21 total mice two monopolar cortical LFPs were recorded, and digital subtraction of these two recordings (rostal M1 – caudal M1) yielded a bipolar LFP. Power spectra for both monopolar and bipolar recordings were computed similarly. Raw signals (0.1–10 kHz) were first low-pass filtered and downsampled to 200 Hz. Welch’s method was then used (NFFT = 2,048 pts, Hamming window, 50% overlap) on 100-s segments of data. Statistical testing of total power was done on these values. Power spectra plots are shown in decibels.

Single-unit spiking was filtered out of the raw signal with a 300 Hz Chebyshev Type II high-pass digital filter with zero-phase forward and reverse filtering (MATLAB function: ‘filtfilt’). Spike times were determined with a threshold-based approach in custom MATLAB software or in Offline Sorter (version 3, Plexon). Spikes were assigned to units with either custom MATLAB software or Offline Sorter. The basic descriptive spike train statistics [mean interspike interval (ISI), coefficient of variation (CV), and CV2] were computed for each neuron. The mean firing rate was calculated to be the reciprocal of the coefficient of variation (CV).

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**Histological Verification and Immunohistochemistry**

After perfusion, fixed brain tissue was cut into 50-μm-thick coronal sections on a freezing microtome. Sections containing blue dots at the levels of the VM and SNpr were Nissl-stained with Neutral Red or cresyl violet to distinguish between cell groups. The anatomical location of each recorded cell was extrapolated from these blue dots with a mouse brain atlas (Franklin and Paxinos 2008).

A series of sections at the level of the striatum and the substantia nigra were immunostained to localize TH or alpha-synuclein with the immunoperoxidase avidin-biotin-peroxidase (ABC) method. Both the TH (Millipore AB152) and alpha-synuclein (Millipore AB5334) antibodies have been extensively used and shown to be highly specific for their corresponding antigens (Azeredo da Silveira et al. 2009; Gaugler et al. 2012; Papachroni et al. 2005). The processing of tissue sections was the same for both series of incubations, except for changes in the primary and secondary antibodies. In brief, after sodium borohydride treatment, sections were incubated for 1 h at room temperature (RT) in phosphate-buffered saline (PBS, 0.01 M, pH 7.4) containing 1% nonimmune normal serum [normal goat serum (NGS) or TH, normal rabbit serum (NRS) for alpha-synuclein], 1% bovine serum albumin (BSA), and 0.3% Triton X-100, followed by the primary antibody solution containing 1% NGS or NRS, 1% BSA, and 0.3% Triton X-100 in PBS for 24 h at RT. After three rinses in PBS, sections were incubated in secondary biotinylated goat anti-rabbit (for TH) or rabbit anti-sheep (for synuclein) IgGs at a concentration of 1:200 (Vector Laboratories, Burlingame, CA) for 90 min. The sections were rinsed again in PBS and then incubated for another 90 min with the ABC at a dilution of 1:100 (Vector Laboratories). Finally, the sections were washed in PBS and Tris buffer (50 mM: pH 7.6) and transferred to a solution containing 0.025% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO), 10 mM imidazole, and 0.005% hydrogen peroxide in Tris buffer for 10 min. Sections were rinsed in PBS, mounted onto gelatin-coated slides, dehydrated, and then coverslipped with Permunt. Tissue was scanned, and areas of interest photographed, with a Scanscope (Aperio Technologies, Vista, CA).

**Densitometry Analysis of TH Immunostaining**

To quantitatively change sparseness in striatal TH innervation between ASO and WT mice, densitometry measurements of TH immunostaining were performed in different dorsoventral and mediolateral quadrants (dorsolateral, dorsomedial, ventrolateral, ventromedial) of the striatum at three different rostrocaudal levels (precommissural, commissural, postcommissural). The choice of these regions was based on the functional compartmentation of the rodent striatum (McGeorge and Faull 1989). After image inversion, regions of interest were outlined and the integrated optical density was calculated (ImageJ, National Institutes of Health). The optical density of the internal capsule was subtracted from optical densities from striatal regions of interest to control for differences in background staining (Galvan et al. 2011). A two-way repeated-measures ANOVA with post hoc tests...
(Bonferroni) was used to determine differences in optical density measurements.

RESULTS

Motor Deficits in ASO Mice

To characterize the behavioral phenotype of B6-backcrossed ASO mice, we performed behavioral testing of these mice (n = 9) and their age-matched WT (n = 12) littermates (see Methods). Both groups of mice underwent three tests: the cylinder test, the challenging beam test, and the pole test. To minimize bias, the experimenter was blinded to the genotype of each mouse until after scoring was complete.

ASO mice in the cylinder were hypokinetically, displaying a significant reduction in spontaneous activity (Fig. 1A; P < 0.01, Mann-Whitney U-tests; WT: 838 ± 56.0 steps, ASO 529 ± 39.6 steps). There was a significant reduction in the total number of steps of each individual paw in ASO mice (P < 0.05; left forepaw: WT 251 ± 13.7 steps, ASO 203 ± 10.6 steps; right forepaw: WT 248 ± 13.2, ASO 208 ± 12.9; left hindpaw: WT 156 ± 16.3, ASO 59.1 ± 10.9; right hindpaw: WT 183 ± 17.4, ASO 59.8 ± 14.8). There was also a significant reduction in the number of rears and grooming episodes during the first 3 min in ASO mice (Fig. 1, B and C; P < 0.05; rears: WT 28 ± 3.8, ASO 15 ± 3.4; groomings: WT 2.8 ± 0.3, ASO 1.6 ± 0.2). In the challenging beam test (Fig. 1, D and E), ASO mice took a significantly longer time to traverse the beam (P < 0.01, Mann-Whitney U-test; WT: 12.1 ± 1.12 s, ASO: 21.2 ± 2.82 s) and made significantly more errors while traversing the beam (P < 0.001; WT: 2.98 ± 0.36, ASO: 11.1 ± 0.84) than their WT littermates.

In the pole test (Fig. 1,F–H), ASO mice took a longer time to turn and orient (P < 0.001; WT: 1.56 ± 0.18 s, ASO: 8.74 ± 1.60 s) than their WT littermates. There was no difference in the total time to descend the pole after orientation between groups (P > 0.05; WT: 5.36 ± 0.54 s, ASO: 6.70 ± 0.51 s). ASO mice made a greater number of error trials (trials in which the mouse fell off the pole or did not fully turn around to descend the pole) than their WT littermates (P < 0.05; WT: 0.1 ± 0.1, ASO: 2.2 ± 0.5).

A behavioral motor deficits score was calculated for the 21 mice (higher score indicates more impairments, see Methods). The distributions of WT and ASO scores were nonoverlapping (WT: 0.21 ± 0.02, ASO: 0.59 ± 0.03). There was no significant correlation between age and severity score for either WT (P = 0.37) or ASO (P = 0.91) mice. However, it is noteworthy that the range of ages examined was relatively narrow (WT range 93–190 days; ASO range 93–179 days) and that previous work has noted little motor deficit progression during this age range (Fleming et al. 2004). Together, these results confirm previously published data in C57BL/6-DA/2 ASO mice (Fleming et al. 2004) and demonstrate that ASO mice displayed significant motor dysfunction at the time they were used in electrophysiological studies.

Histological Analysis of ASO Mice

Immunohistochemistry was used to confirm the genotype of ASO mice and to determine the state of degeneration of the dopaminergic nigrostriatal system (Fig. 2). In line with previous reports (Masliah et al. 2000; Rockenstein et al. 2002), ASO mice displayed a substantial increase in alpha-synuclein staining throughout the whole brain compared with WT mice (compare Fig. 2, A1–A3, with Fig. 2, B1–B3). In addition to an increased level of immunoreactivity, the pattern of labeling in some brain regions was also different. In the SNPr of WT mice a moderate level of neuropil immunoreactivity was found (Fig.

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![Fig. 1. Motor deficits in human alpha-synuclein-overexpressing (ASO) mice. A–C: cylinder test. The number of steps taken (A), grooming episodes (B) and the number of rears (C) in 3 min were counted after introduction of the mouse into the cylinder. ASO mice were hypokinetically, as they made fewer steps (P < 0.01) and had fewer grooming episodes (P < 0.05) and fewer rears (P < 0.05) than their wild-type (WT) littermates. D and E: challenging beam traversal test. ASO mice took a longer time to traverse the beam (D, P < 0.01) and made more errors during traversal (E, P < 0.001) than WT mice. F–H: pole test. ASO mice took longer to turn around (F, P < 0.001) but, once oriented, descended the pole in a time equal to WT mice (G, P > 0.05). ASO mice fell off the pole more often than WT mice (H, P < 0.05). Mann-Whitney U-tests were performed for all statistical analysis. Means ± SE are shown. *Statistical differences between WT and ASO mice.](http://jn.physiology.org/)

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Fig. 2. Alpha-synuclein and tyrosine hydroxylase (TH) staining of WT and ASO mouse brain tissue. A and B: there was a large increase in the overall alpha-synuclein (αSyn) staining in the brain of ASO (B1–B3) mice compared with WT (A1–A3) mice. Higher-magnification pictures of the labeling in substantia nigra pars reticulata (SNpr) of WT mice are shown in A2 and A3, while B2 and B3 illustrate higher-power views of SNpr labeling in ASO mice. Within the SNpr of ASO mice, but not WT mice, several alpha-synuclein-immunoreactive aggregates were observed (A5 vs. B3). C: similar levels of TH immunostaining were observed in the striatum (STR) of WT (C1) and ASO (C2) mice. MGN, medial geniculate nucleus; Hip, hippocampus; AC, anterior commissure. Scale bars: 1 mm (A1, B1, C1, C2), 0.5 mm (A2, B2), or 0.2 mm (A3, B3).

Analysis of Local Field Potential Frequency Changes in ASO Mice

Electrophysiological experiments were performed with both WT and ASO mice to determine whether the pathophysiological mechanisms of dopamine depletion models (i.e., increased beta oscillations, bursting, and synchronization) were present in the SNpr and VM of ASO mice. Simultaneous recordings of LFPs in the primary motor cortex combined with LFPs and single-unit recordings in the SNpr and VM were obtained in anesthetized ASO mice and their age-matched WT littermates (see METHODS).

Previous studies have shown an increase in beta (15–30 Hz) frequencies within the basal ganglia and primary motor cortex of anesthetized 6-OHDA-lesioned rats (Mallet et al. 2008a, 2008b). To determine whether such a change is also present in ASO mice, we analyzed LFPs recorded in the SNpr, VM, and primary motor cortex for increased beta oscillations during slow wave activity (SWA) and in pinch-induced desynchronized (DS) states (Fig. 3). Because it was difficult to induce desynchronized states in mice anesthetized with high-dose urethane, a combination of low-dose urethane with ketamine-xylazine was used as anesthetic agent in a subset of mice (WT: n = 4, ASO: n = 4), as previously employed in 6-OHDA-lesioned rat models of PD (Mallet et al. 2008a, 2008b). Power spectra of the LFP recorded in primary motor cortex of DS states in both groups displayed a significant reduction in low-frequency power (0.1–1.5 Hz) compared with SWA (Fig. 3B; P < 0.05, Mann-Whitney U-tests). No significant change in beta frequencies was seen during SWA between groups (P > 0.05, Mann-Whitney U-tests). Unexpectedly, a small but significant decrease in beta frequencies was seen in ASO mice in DS states (Fig. 3C; P < 0.05, Mann-Whitney U-tests). There was no significant difference in the total power (0.1–100 Hz) in either the SWA or DS state (P > 0.05, Mann-Whitney U-tests) between ASO and WT mice. A lack of a clear beta peak with a trend toward a decrease in beta frequencies was also seen in LFPs recorded in the SNpr and MTH and in bipolar recordings (see METHODS) within the primary motor cortex (data not shown).

Thus, in contrast with data obtained from the 6-OHDA-lesioned rat model of PD (Mallet et al. 2008a, 2008b), our results indicate that anesthetized ASO mice do not display increased beta frequencies, thereby suggesting that increased human alpha-synuclein alone, without any loss of striatal dopamine innervation, cannot induce pathophysiological changes in the oscillatory activity of basal ganglia neurons commonly associated with PD.
a strong preference to fire when cortical neurons go into the UP state in anesthetized rats (Belluscio et al. 2003; Sanderson et al. 1986; Walters et al. 2007). To determine whether these changes in neural activity can be induced by the overexpression of alpha-synuclein, we also recorded the activity of individual SNpr neurons in anesthetized WT and ASO mice under urethane-only (U) or low-dose urethane with ketamine-xylazine (uKZ) anesthesia (Fig. 4). Thus SNpr neurons were recorded in four groups: 1) WT U, 2) WT uKZ, 3) ASO U, and 4) ASO uKZ. Detailed descriptive and inferential statistics for each group are given in Tables 1 and 2, respectively.

SNpr neurons in all four groups fired in a regular, single-spike firing pattern (Fig. 4A, Table 1). There was no interaction or main effect of genotype or anesthesia type on the mean ISI. The pooled mean ISI of all four groups was 37.8 ± 1.24 ms (n = 53 neurons). Even though there was no difference in firing rate between groups, there were significant effects on spike train irregularity. We employed two measures of irregularity: CV and CV2 (Holt et al. 1996). The former is a more global measure of irregularity determined by the ratio of the standard deviation of the ISI distribution to the mean ISI. The latter is a more local measure of irregularity calculated from neighboring ISIs. There was a moderate increase in CV in uKZ anesthesia compared with U anesthesia [pooled U (n = 33): 0.34 ± 0.02, pooled uKZ (n = 20): 0.47 ± 0.04] but no interaction or genotype effects. There was a significant genotype × anesthesia interaction on CV2, with ASO uKZ SNpr neurons displaying the greatest CV2 values. In effect, SNpr neurons in ASO mice under U anesthesia were firing more regularly than in WT mice, but this difference disappeared in uKZ anesthesia. Because a high degree of regularity indicates a lack of external input, these data suggest that the SNpr in ASO mice receives less external input than in WT, but the stronger cortical state transitions elicited by ketamine-xylazine anesthesia may mask this effect.

Although neurons were largely regular in firing, we found statistically significant bursts and pauses, which are defined as transient increases or decreases in firing rate, respectively, using the method of Robust Gaussian Surprise (Ko et al. 2012) (Table 1). No genotype × anesthesia interaction effects were found for either burst or pause data. While no main effect of genotype was seen for bursting, ASO mice displayed an increased pause rate (no. of pauses/total duration) and an increased mean intrapause ISI. Therefore ASO mice showed

Fig. 4. SNpr neurons in WT and ASO mice. A: representative 1-s traces from histologically confirmed SNpr neurons from a WT (top) and an ASO (bottom) mouse. Both neurons were recorded under urethane-ketamine-xylazine (uKZ) anesthesia. The WT neuron fired at 29.6 Hz with a coefficient of variation (CV) of 0.36. The ASO neuron fired at 29.6 Hz with a CV of 0.57. There was no difference in firing rate across groups (P > 0.05). B: there was a significant main effect of anesthesia [uKZ vs. urethane only (U)] on CV (*P < 0.05) on SNpr neurons. C: burst rate was not significantly different across groups (P > 0.05). D: a significant effect of genotype (**P < 0.01) on pause rate was seen.

**ELECTROPHYSIOLOGY OF ASO MICE**

Previous studies using the 6-OHDA-treated rodent model of PD have shown that SNpr neurons on the lesioned side have a reduced firing rate and change their firing pattern, developing
Table 1. Descriptive statistics of SNpr neuron spike trains in all four groups

<table>
<thead>
<tr>
<th></th>
<th>WT U (n = 17)</th>
<th>WT uKZ (n = 5)</th>
<th>ASO U (n = 16)</th>
<th>ASO uKZ (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ISI, ms</td>
<td>39.9± 2.49</td>
<td>40.1± 3.15</td>
<td>35.8± 1.89</td>
<td>35.4± 2.44</td>
</tr>
<tr>
<td>CV</td>
<td>0.371± 0.029</td>
<td>0.413± 0.043</td>
<td>0.326± 0.036</td>
<td>0.513± 0.051</td>
</tr>
<tr>
<td>CV2</td>
<td>0.344± 0.016</td>
<td>0.336± 0.025</td>
<td>0.275± 0.028</td>
<td>0.365± 0.021</td>
</tr>
<tr>
<td>Bursts/s</td>
<td>0.612± 0.117</td>
<td>0.556± 0.132</td>
<td>0.652± 0.164</td>
<td>0.557± 0.085</td>
</tr>
<tr>
<td>Mean intraburst ISI, ms</td>
<td>18.0± 1.04</td>
<td>18.6± 1.12</td>
<td>19.4± 0.82</td>
<td>16.9± 0.85</td>
</tr>
<tr>
<td>No. of spikes/burst</td>
<td>3.40± 0.28</td>
<td>4.65± 0.22</td>
<td>3.33± 0.19</td>
<td>3.67± 0.26</td>
</tr>
<tr>
<td>Pauses/s</td>
<td>0.419± 0.11</td>
<td>0.294± 0.09</td>
<td>0.399± 0.17</td>
<td>0.890± 0.12</td>
</tr>
<tr>
<td>Mean intrapause ISI, ms</td>
<td>96.5± 7.1</td>
<td>105± 10.3</td>
<td>73.5± 5.3</td>
<td>89.1± 7.9</td>
</tr>
<tr>
<td>No. of spikes/pause</td>
<td>2.67± 0.18</td>
<td>3.51± 0.65</td>
<td>2.65± 0.14</td>
<td>3.11± 0.35</td>
</tr>
</tbody>
</table>

Means ± SE of each dependent variable are given for each group: 1) wild-type (WT) substantia nigra pars reticulata (SNpr) neurons recorded under urethane only (WT U), 2) WT SNpr neurons recorded under low-dose urethane with supplemental ketamine-xylazine (WT uKZ), 3) human alpha-synuclein-overexpressing (ASO) SNpr neurons recorded under urethane only (ASO U), and 4) ASO SNpr neurons recorded under low-dose urethane with supplemental ketamine-xylazine (ASO uKZ). CV, coefficient of variation; ISI, interspike interval.

significantly more and stronger pauses in spike activity than control mice. However, even SNpr spike pauses in ASO mice should be considered moderate, given their rare occurrence and short duration (Fig. 4, Table 1). There was also no significant correlation between pause rate (P = 0.75) or pause mean ISI (P = 0.88) and behavioral severity in ASO mice. The significant increase in the number of spikes in a burst and the increase in the number of spikes in a pause found in uKZ over U anesthesia is suggestive of a stronger, possibly more synchronized input.

To summarize, spike trains from SNpr neurons in ASO mice are largely similar to those from WT mice. These results demonstrate that SNpr neurons in ASO mice do not exhibit the feature of increased bursting that has been observed in uKZ-anesthetized 6-OHDA-lesioned rats (Belluscio et al. 2003; Sanderson et al. 1986; Walters et al. 2007). In contrast, significant effects of genotype were noted for pauses. Curiously, while pause rate and mean pause ISI were different between control and ASO mice, the type of anesthesia had the strongest effect on the number of spikes in a burst or pause. However, the observed pauses were weak, without a complete cessation of spiking and occurring at a rate of <1/s.

Next, we analyzed whether there was any phase of SWA activity of the cortical field potential in which SNpr neurons preferred to fire. A strong switch of SNpr phase preference in uKZ-anesthetized 6-OHDA-lesioned rats has been observed previously (Belluscio et al. 2003; Sanderson et al. 1986; Walters et al. 2007). For each neuron, the distribution of Hilbert-transformed instantaneous SWA phase was computed (see METHODS). Similar results were obtained with U and uKZ anesthesia, and thus the data were pooled. During SWA, the cortical field potential is dominated by a low-frequency (~1 Hz) oscillation. Cortical neurons tend to fire during the UP state, which corresponds to the negative phase of the depth LFP electrode (Contreras and Steriade 1995; Steriade et al. 1993b; Volgushev et al. 2006). The peak of the cortical negativity had a Hilbert phase of ±π (Fig. 5A). In WT mice, 12 of 25 neurons (48%) had a weak but significant phase preferences (an example is shown in Fig. 5B; P < 0.05, Rayleigh test). There was a significant overall mean direction for the entire sample (n = 25) of WT SNpr neurons of 194° (Fig. 5C; P < 0.05, Moore’s test). Thus WT SNpr neurons had a weak preference to fire in phase with cortical cells during SWA. In ASO mice, 8 of 26 neurons (31%) had similarly weak but significant phase preferences (Fig. 5C; P < 0.05, Rayleigh test). In contrast to WT recordings, there was no significant overall mean direction for the phase preference of ASO SNpr neurons (P > 0.05, Moore’s test).

To determine whether bursts and pauses tended to occur at a particular phase of the cortical LFP, we performed a similar analysis on the onsets of each burst and pause. Burst onsets of both WT and ASO SNpr neurons had a significant phase preference to fire in phase with cortical cells (Fig. 5D; P < 0.05, Moore’s test; WT: 176.6°, ASO: 171.1°). There was no significant difference in the preferred direction between the onsets of bursts in WT and ASO SNpr neurons (P > 0.05; Mardia’s test). These results suggest that bursts are caused by the transition of the cortex into the UP state during SWA. In contrast, pause onsets of neither WT nor ASO SNpr neurons

Table 2. Inferential statistics of SNpr neuron spike trains

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Genotype (WT vs. ASO)</th>
<th>Anesthesia (U vs. uKZ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ISI, ms</td>
<td>F1,49 = 0.02, P = 0.89</td>
<td>F1,49 = 1.97, P = 0.17</td>
</tr>
<tr>
<td>CV</td>
<td>F1,49 = 2.23, P = 0.14</td>
<td>F1,49 = 0.07, P = 0.79</td>
</tr>
<tr>
<td>CV2</td>
<td>F1,49 = 5.34, P = 0.025</td>
<td>F1,49 = 4.27, P = 0.044</td>
</tr>
<tr>
<td>Bursts/s</td>
<td>F1,49 = 0.02, P = 0.90</td>
<td>F1,49 = 0.02, P = 0.90</td>
</tr>
<tr>
<td>Mean intraburst ISI, ms</td>
<td>F1,49 = 1.85, P = 0.18</td>
<td>F1,49 = 0.03, P = 0.86</td>
</tr>
<tr>
<td>No. of spikes/burst</td>
<td>F1,49 = 2.31, P = 0.14</td>
<td>F1,49 = 3.08, P = 0.086</td>
</tr>
<tr>
<td>Pauses/s</td>
<td>F1,49 = 0.44, P = 0.51</td>
<td>F1,49 = 8.91, P = 0.0044</td>
</tr>
<tr>
<td>Mean intrapause ISI, ms</td>
<td>F1,49 = 0.18, P = 0.67</td>
<td>F1,49 = 5.36, P = 0.025</td>
</tr>
<tr>
<td>No. of spikes/pause</td>
<td>F1,49 = 0.38, P = 0.54</td>
<td>F1,49 = 0.49, P = 0.42</td>
</tr>
</tbody>
</table>

A 2 × 2 ANOVA was performed for each dependent variable. The F statistic (with its respective degrees of freedom) and P value are reported for main effects of genotype and anesthesia as well as an interaction effect (genotype × anesthesia). Statistical significance: —P > 0.05; *P < = 0.05; **P < = 0.01.
had a significant phase preference (Fig. 5E; \( P > 0.05 \), Moore’s test).

Finally, we analyzed simultaneously recorded pairs of SNpr neurons for spike synchronization to determine whether there were any changes in the degree of synchronized activity in the SNpr between WT and ASO mice (Fig. 6), a feature that has been commonly reported in 6-OHDA-lesioned rats (Belluscio et al. 2003; Walters et al. 2007). Cross-correlograms were constructed from all spikes of simultaneously recorded WT (\( n = 7 \); 5 same electrode, 2 different tetrode channels) and ASO (\( n = 10 \); 4 same electrode, 6 different tetrode channels) SNpr neuron pairs. Cross-correlations of all but one WT pair were flat (\( n = 7 \); data not shown), suggesting that the overall SNpr activity is uncorrelated in the normal state. On the other hand, cross-correlations of 4 of 10 ASO pairs (2 U, 2 uKZ) contained one or more peaks indicative of correlated activity. A representative example is shown in Fig. 6, top. Auto-correlograms for both example neurons are shown in Fig. 6, bottom. Cross-correlations in the other three neurons were qualitatively similar. However, the proportion of cells exhibiting significant cross-correlations was not significantly different between WT and ASO groups (\( P = 0.28 \), 1-sided Fisher’s exact test), which may be due to the small available sample of paired recordings. Note that our tetrode spike discrimination procedure did not allow finding precisely synchronously occurring spikes from two neurons, resulting in a steep trough at time 0.

Unit Activity of VM Neurons

To determine whether alpha-synuclein overexpression has any impact on the activity of basal ganglia-receiving neurons in the motor thalamus, we recorded from neurons in the VM nucleus, which receives strong input from the basal ganglia via the SNpr in rodents (Kuramoto et al. 2011; Nakamura et al. 2012). VM neurons typically had a bursty firing pattern in both WT and ASO mice (Fig. 7, A and B), and bursts fit the stereotypic form of low-threshold spike (LTS) bursts described to be T-type calcium channel dependent in many previous publications (Llinas et al. 1984; Lu et al. 1992; Nakamura et al.
2012; Parr-Brownlie et al. 2009). In fact, on average 49% of all recorded spikes was part of such bursts, with no significant interaction effect or main effects of anesthesia or genotype. Since VM neurons tended to have a bursty firing pattern and thalamic bursting activity is hypothesized to increase in Parkinsonism (Brazhnik et al. 2012; Hadipour Niktarash 2003; Pare et al. 1990), we investigated whether LTS-type bursting was increased in VM neurons of ASO mice. The detailed descriptors and values from a two-way ANOVA are given in Tables 3 and 4, respectively. There were no main genotype effects on LTS burst rate (Fig. 7C) or spikes/LTS burst. However, LTS bursts were strongly affected by anesthesia type. Under uKZ anesthesia, bursts occurred at a significantly higher rate (Fig. 7C) with a significantly increased number of spikes per burst (2-way ANOVA, Tables 3 and 4). Overall, these findings indicate that the exact regime of anesthesia used can have an important influence on spike frequency and pattern, but no significant change in burst dynamics is seen in ASO mice under either anesthetic regime.

Next, we looked at whether thalamic neuron spiking and bursting tended to occur at a particular phase of the cortical LFP, because previous studies have demonstrated that thalamic neurons typically fire single spikes and LTS bursts in phase with the cortex during SWA (Nakamura et al. 2012; Parr-Brownlie et al. 2009). There was no main effect of genotype or anesthesia or interaction effect of genotype × anesthesia on low-frequency power in SWA (P > 0.05, 2-way ANOVA, <1.5 Hz). Most thalamic neurons showed a strong firing preference at a particular phase of the LFP (Fig. 7B and D). Almost all WT VM neurons preferred to fire during the cortical negativity. Unexpectedly, VM U neurons had a phase preference significantly different from that of VM uKZ neurons, with VM U neurons firing later during the cortical negativity (P < 0.05, Mardia’s test; WT U grand mean: 97.1°, WT uKZ grand mean: 172°). The SWA phase preference of VM neurons in the ASO condition was a little less clear-cut, as only a relatively small sample was available. However, bursts in the VM of ASO mice occurred with a phase preference very similar to that of WT mice (Fig. 7D). The phase preference for the majority of both WT and ASO VM neurons was confined to the later parts of the cortical negativity (Fig. 7D), consistently later than bursts in the SNpr, which occurred about ¼π earlier in respect to the Hilbert transform of SWA (Fig. 5D).

In some cases, multiple VM neurons were recorded simultaneously (data not shown). Cross-correlations of these pairs sometimes had significant peaks, which corresponded to their difference in preferred phases with the cortical LFP.
To determine whether inhibition from the SNpr affected thalamic activity, we simultaneously recorded individual neurons in the SNpr and VM thalamus. Cross-correlations were flat in 14 of 15 cases (WT U, n = 10; WT uKZ, n = 1; ASO U, n = 2; ASO uKZ, n = 2) suggesting that thalamic activity is not shaped by SNpr inhibition at these faster timescales in a synchronized fashion across large neural populations.

**DISCUSSION**

Our results support previous findings that 5- to 6-mo-old ASO mice display robust motor dysfunction despite the lack of significant nigrostriaal dopamine denervation. Our goal was to determine whether the pathophysiological mechanisms of ASO mice with respect to changes in basal ganglia network dynamics are similar to those previously found in striatal dopamine-depletion models of PD. In contrast to previous findings in dopamine-depletion models, we found that ASO mice do not display increased beta oscillations but rather show a small, but significant, decrease in this oscillatory activity. The activity of SNpr and VM neurons in ASO mice was overall similar to WT, with significant changes mainly given by an increase in SNpr pauses of firing. Our results suggest that the motor deficits in young adult ASO mice are due to neural mechanisms different from those in dopamine-depletion models of PD but may involve basal ganglia circuits to some degree. Because of the widespread overexpression of alpha-synuclein, however, the exact circuit causing these symptoms cannot be assessed with recording sites in basal ganglia circuits alone.

**Effects of ASO on Neural Activity**

Here we investigated the electrophysiological changes in vivo in SNpr neurons and VM motor thalamic neurons in anesthetized WT and ASO mice at 5–6 mo of age. We found that ASO SNpr neurons fired in a single spiking mode and did not develop a strong oscillatory burst pattern, as previously reported in 6-OHDA-lesioned rats (Belluscio et al. 2003; Sanderson et al. 1986; Walters et al. 2007). However, SNpr neurons had a tendency to pause more frequently in ASO mice but without relation to any particular phase of cortical SWA. Our results suggest that the basal ganglia-mediated inhibition onto motor thalamic neurons in the VM is largely unaffected by overexpression of alpha-synuclein at 5–6 mo of age. While these results from anesthetized mice cannot address whether there are changes in behaviorally related activity of SNpr with alpha-synuclein overexpression, they clearly indicate that SNpr activity did not undergo the electrophysiological changes observed in rodent dopamine-depletion models of PD.

VM thalamic neurons in both WT and ASO mice had a strong LTS-type burst firing pattern during SWA and burst spikes comprising nearly 50% of all spikes in both genotypes. A majority of WT and ASO VM neurons showed a very strong phase preference with respect to the cortical LFP. Interestingly, the phase preference of LTS bursts in VM occurred later in the putative cortical UP state (as defined by the negative LFP depth recording) than the phase preference of SNpr bursts, which occurred early in the UP state. This difference in SWA phase relationship is consistent with the early SNpr activity aiding in suppressing VM spiking during the early phase of cortical UP states, and perhaps a combined rebound from the strongest period of SNpr inhibition in conjunction with maintained cortical input leading to the LTS burst later in the UP state. However, SNpr spiking did not cease during this late phase of the cortical UP state, so a shift toward excitement is more likely the best explanation for an LTS burst trigger as opposed to a pure postinhibitory rebound. Intracellular thalamic recordings would be required to more directly address the question of relative input balances triggering LTS bursts.

**Effects of Anesthesia on Neural Activity**

We found that significant changes in neural activity could result from the choice of anesthetic. In the above studies, we chose to use two seemingly similar means for anesthesia: high-dose urethane (1.7 g/kg) or a cocktail of low-dose urethane (1.3 g/kg) combined with ketamine-xylazine. Thalamic neurons were the most strongly affected by anesthesia, displaying a large increase in burst activity and a significant change in phase preference with uKZ. An increase in thalamic activity with the addition of ketamine has been noted previously (Steriade et al. 1993b). The change in phase preference may be due to the action of xylazine, which was found to promote neuronal synchronization (Amzica and Steriade 1995; Steriade et al. 1993a). Thus our results suggest that the thalamic
network dynamics in urethane-alone and urethane-ketamine-xylazine anesthesia are not equivalent. These changes were not likely due to an influence of anesthetic on inhibitory inputs to VM from SNPr, as we found only a very modest influence of anesthetic in SNPr, which was given by a mildly increased CV of spiking in uKZ.

Comparison with Dopamine-Depleted Rodent Models of PD

A wide range of neurotoxin-based (e.g., 6-OHDA, MPTP, rotenone) and genetic-based (e.g., alpha-synuclein, LRRK2, DJ1, Parkin) rodent models of PD are currently available. One of the fundamental questions related to these models is whether the pathophysiology of neural networks underlying motor dysfunctions in these animal models is similar to that seen in PD patients. While this question has been partly addressed for the rodent 6-OHDA and primate MPTP models, the genetic models remain far understudied electrophysiologically. This is a serious gap in our knowledge, as genetic models have taken the forefront of rodent PD research in recent years and are often compared on the basis of the progressive development of PD-like motor dysfunctions. Thus, to determine whether the motor dysfunctions seen in these various animal models are associated with a common or different pathophysiological substrate, it is important to investigate whether the pathophysiology of the basal ganglia-thalamocortical networks described in striatal dopamine-depleted animals and in PD patients is also seen in behaviorally deficient genetic mouse models of PD. This issue was directly addressed in the present study by specifically collecting data about electrophysiological parameters previously obtained in anesthetized 6-OHDA-lesioned rats (Mallet et al. 2008a, 2008b; Walters et al. 2007) in ASO mice.

One of the current hypotheses of the pathophysiological process underlying motor dysfunction involves increased beta oscillations in the motor cortex and basal ganglia. Increased beta oscillations have been reported in the lesioned hemisphere in the desynchronized/activated state of the EEG in anesthetized (Mallet et al. 2008a, 2008b) and awake (Avila et al. 2010; Brazhnik et al. 2012; Sharott et al. 2005) 6-OHDA-treated rats as well as in in PD patients (Brown et al. 2001; Gage et al. 2006; Levy et al. 2002), although others have suggested that this may occur only at later stages of the disease (Degos et al. 2009; Leblois et al. 2007; Quiroga-Varela et al. 2013). In contrast, we found a small, but significant, decrease in LFP beta frequencies in the desynchronized state in anesthetized ASO mice in the absence of any large beta peaks under any anesthetized condition (Fig. 3). It is interesting that an increase in extracellular dopamine has been found in the striatum of ASO mice at ages similar to those used here (Lam et al. 2011), which is consistent with the hypothesis that increased beta oscillations in PD patients result from striatal dopamine depletion and that increased dopamine levels can depress beta oscillations. Such an interaction is plausible because striatum is a likely source for beta oscillations in PD and has been strongly linked to cholinergic transmission (McCarthy et al. 2011), which itself is dependent on dopamine (Aosaki et al. 1994; Ding et al. 2010). Because ASO mice display dopamine loss at 14 mo of age, enhanced beta oscillations might be seen at this later age in this animal model. Our experiments indicate, however, that increased beta oscillations do not underlie motor dysfunction in 5- to 6-mo-old ASO mice.

Another pathophysiological sign described in 6-OHDA-treated rats is changes in the preferred phase of firing with respect to the cortical LFP during SWA. Subthalamic nucleus (STN) neurons tend to fire in phase with the cortex in both normal and 6-OHDA-treated rats, while globus pallidus (GP) neurons fire in phase or out of phase with the cortex (Magill et al. 2001; Zold et al. 2007) depending on their efferent target (Mallet et al. 2012). Regularly firing SNpr neurons develop a strong STN-mediated ~1-Hz oscillatory burst firing pattern in phase with the cortex (MacLeod et al. 1990; Murer et al. 1997; Sanderson et al. 1986; Walters et al. 2007). As more neurons become entrained to the SWA oscillation, SNpr neurons become more synchronized. In contrast, in the 5- to 6-mo-old ASO mice used in the present study SNpr neurons did not develop a strong SWA oscillation but rather continued to fire in a fairly regular manner (Fig. 4). On the other hand, ASO SNpr neurons displayed a significant increase in pauses, a feature of spike trains that is not typically measured in PD research. The development of new methods such as the Robust Gaussian Surprise method (Ko et al. 2012) will hopefully facilitate the use of this metric in order to determine whether pause increases in the firing activity of basal ganglia output neurons, which could result in thalamic disinhibition and rebound bursting, are a cardinal feature of PD pathophysiology.

Motor Dysfunction of ASO mice and Relevance for PD

Parkinsonism can be caused by mutations or overexpression (duplication, triplication) of SNCA (Klein and Westenberger 2012). Motor symptoms occur much earlier than in classical forms of PD (<50 yr of age at onset), and early motor deficits are also present in the ASO mouse model (Fleming et al. 2004). However, a major divergence of the ASO model from human SNCA parkinsonism is with respect to L-DOPA responsiveness. Early motor symptoms of these patients are responsive to administration of L-DOPA, unlike young adult ASO mice, in which symptoms are exacerbated (Fleming et al. 2006). This is particularly interesting considering that both forms (L-DOPA-responsive and L-DOPA-unresponsive motor symptoms in humans and mice, respectively) are caused by SNCA overexpression. Alpha-synuclein pathology in the nervous system is widespread in both humans with SNCA duplication (Ikeuchi et al. 2008; Obi et al. 2008) or triplication (Farrer et al. 2004; Gwinn-Hardy et al. 2000) and ASO mice (Chesselet et al. 2012; Rockenstein et al. 2002). However, humans initially exhibit a predominantly dopaminergic phenotype and the Masliah (line 61) ASO mice (Rockenstein et al. 2002) initially a nondopaminergic phenotype. Only toward the end of their life span at 12–15 mo of age do these ASO mice develop dopaminergic pathology (Chesselet et al. 2012; Lam et al. 2011). Thus the motor deficits of the young adult ASO mouse are not due to striatal dopamine depletion but rather are a consequence of alpha-synuclein overexpression. The dissociation of dopamine-dependent and -independent motor symptoms presents a challenge for the rodent PD community as the behavioral tests commonly used show deficits in ASO mice as well as dopamine-depletion models (Hwang et al. 2005; Matsura et al. 1997; Ogawa et al. 1985; Sedelis et al. 2001; Tillerson et al. 2002).
On one hand, the ASO mouse model does not fully capture several relevant and desirable features for a mouse model of PD, namely, that the early motor deficits seen in these animals are not dependent on striatal dopamine denervation nor are they likely to be caused by previously observed pathophysiological basal ganglia mechanisms for PD such as bursting or synchrony. However, it is well recognized that PD pathophysiology extends far beyond dopaminergic neuron loss (Lang and Obeso 2004), as some of the typical motor symptoms in idiopathic PD (e.g., gait and postural instability) and some of the multifarious motor symptoms of human SNCA PD are not responsive to administration of L-DOPA. It is unclear whether some of these L-DOPA-resistant motor symptoms in humans are caused by alpha-synuclein pathology, and which brain areas are primarily dysfunctional. Therefore, while the ASO mouse does not display the dopamine-dependent motor symptoms of PD, the young adult ASO mouse model may present a valuable opportunity to study the pathophysiology underlying synuclein-mediated, L-DOPA-resistant motor deficits in the absence of dopamine depletion. Our results suggest that these deficits are not primarily caused by basal ganglia dysfunction, but future recordings in a number of other potentially involved brain areas (e.g., motor cortex, cerebellum, brain stem, spinal cord) are needed to further a better understanding of the neural mechanisms underlying nondopaminergic motor symptoms in parkinsonism. If common mechanisms of dysfunction for non-L-DOPA-responsive motor deficits can be identified in mice and humans, this would increase our chances to use the mouse model to assess neuroprotective treatment for alpha-synuclein pathology using mice by monitoring the effect on the identified circuits.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.J.L. and D.J. conception and design of research; C.J.L., A.K.Z., and Y.S. performed experiments; C.J.L. and A.K.Z. analyzed data; C.J.L., Y.S., and D.J. interpreted results of experiments; C.J.L. prepared figures; C.J.L. drafted manuscript; C.J.L., Y.S., and D.J. edited and revised manuscript; C.J.L., A.K.Z., Y.S., and D.J. approved final version of manuscript.

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