Robust synapse loss in striatal spiny projection neurons from transgenic mice overexpressing human wild-type alpha synuclein

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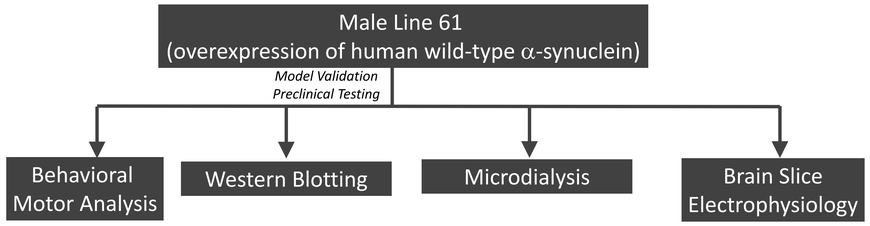
Abstract

Alpha-synuclein is a central component of Lewy bodies present in Parkinson's disease (PD) patients. The consequences of synucleopathy in neuronal function remains unclear and preclinical therapy testing targeting alpha-synuclein are needed. To help identify biomarkers, we utilized whole-cell patch clamp in brain slices to evaluate alterations in synaptic properties in the dorsal striatum from Line 61 transgenic mice overexpressing human wild-type alpha synuclein. Brain slices were prepared from Line 61 mice at ages where they display significant impaired motor behavior and synuclein pathology. Analysis of striatal spiny projection neurons (SPNs) showed a robust decrease in the frequency of miniature excitatory synaptic events in Line 61 compared to wild-type littermates. This phenotype was the same between 2-month and 6-month old Line 61 mice, suggesting a stable readout throughout aging. Analysis of paired-pulse ratio in 6-month old Line 61 corticostriatal synapses revealed no changes in the probability of release, indicating a decrease in excitatory drive in Line 61 is related to synapse loss. The reduction in the frequency of synaptic events was accompanied by a significant increase in the amplitude of synaptic events. This suggests that striatal SPNs from Line 61 underwent synaptic scaling to compensate for the loss of synapses. To further support the synapse loss and scaling phenotype in Line 61, alterations in levels of different synaptic protein markers in the striatum from Line 61 mice will be analyzed using western blotting. In addition, Line 61 mice displayed impaired dopamine release in the striatum in vivo in response to a single intraperitoneal injection of amphetamine. Altogether, we have a stable and robust synaptic deficit, and an impairment in dopamine release in the Line 61 model that we can offer for preclinical testing, supporting and translatable to the loss of dendritic spines reported in PD patients and rodent models.



Methods

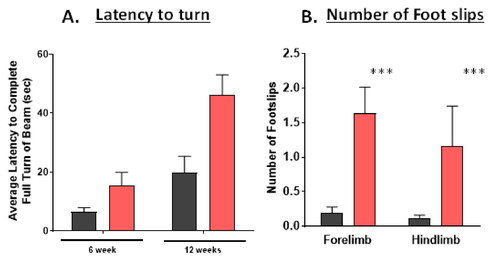
Animals: Breeding pairs from Masliah's laboratory were bred to generate the experimental animals which received unique identification numbers and housed in polycarbonate cages with filter tops in groups of 7-8 animals. All animals were examined, manipulated and weighed prior to initiation of the study to assure adequate health and suitability and to minimize non-specific stress associated with manipulation. During the course of the study, 12/12 light/dark cycles will be maintained. The room temperature was maintained between 20 and 23°C with a relative humidity maintained around 50%. Chow and water were provided ad libidum for the duration of the study. Wet chow was placed on the cage floor and was changed daily. The tests were performed during the animal's light cycle phase unless otherwise specified.

Motor paradigms: Tapered Balance Beam test consisted of a beam angled and elevated from the floor. At the opposite side of the balance beam ('end' portion) there was a goal box which rests on the aforementioned support stand. Following habituation to the testing room, mice were place on the 'starting' end of the balance beam. Mice (n=12/genotype) received 2-8 trials per day, with an ITI of at least 60 sec, and returned to the home cage between trials. The number of trials and timing was optimized for each animal model tested on the apparatus, but the maximum number of trials per day didn't exceed 8 trials. Latency to traverse the beam (sec), and number of foot slips (left / right; fore / hind) were recorded. All tests were also recorded using a video camera for aid in scoring. For longitudinal studies that monitor disease onset and progression, mice were tested weekly, but weren't tested if they were unable to walk. Testing time points: 6 and 12 weeks of age. **Wire Hang:** The four limb hanging test (SOP: DMD_M.2.1.005) from the TREAT-NMD Neuromuscular network was employed. Briefly, mice (n=12/genotype) were placed on top of a steel grid cage lid which was then inverted over a 35cm high circular Plexiglas cylinder. Mice were allowed to grip the steel grid for as long as possible with no maximum testing time cut-off. Mice were given three trials with an ITI of 2-3minutes Testing time point: 10 weeks of age**. NeuroCube®** system is a platform that employs computer vision to detect changes in gait geometry and gait dynamics. Mice were tested for 5minutes in a rectangular Neurocube® chamber where mice were allowed move freely back and forth through the rectangular walkway. Complex bioinformatics algorithms are employed to subtle phenotypes related to gait. Data was analyzed via multi-factorial analyses of variance (ANOVA) with a Tukey Post-Hoc.

Electrophysiology: Brain slices were prepared from 2 or 6-month male Line 61 and wild-type littermate controls . The brain was rapidly removed and cooled in ice-cold oxygenated sucrose-ACSF (in mM): Sucrose 220; KCl 2.5; CaCl2 0.5, MgSO4 3, NaH2PO4 1.2, NaHCO3 26, glucose 5. Para-horizontal slices (300 micron) were prepared in ice-cold oxygenated ACSF then warmed to 36ºC for 30min, allowed to cool to room temperature, and transferred as needed to a submerged slice chamber mounted on the stage of an upright microscope, perfused at 2 ml/min with oxygenated normal ACSF (in mM): NaCl 124; KCl 3.5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 26, glucose 11. For mEPSC recording, the pipettes were filled with a cesium-based internal solution containing (in mM): Cs-methansulfonate 110, EGTA 10, HEPES 10, TEA-Cl 10, NaCl 10, $CaCl_2$ 1, Mg-ATP 5, Na_2GTP 0.5, Qx314-Cl 5, pH 7.2. Miniature excitatory postsynaptic currents (mEPSCs) were isolated by including 0.5uM TTX and 40µM picrotoxin in the ACSF solution, recordings were filtered at 1KHz and collected continuously for 5 minutes at room temperature from each cell at a holding potential of -80mV. Data was excluded if either access resistance or input resistance changed by more than 30%.

Microdialysis: Briefly, the animals were administered isoflurane anesthesia while guide cannulas were stereotactically implanted into the striatum. The guide was fixed using three anchoring bone screws and binary dental cement. After a 7-day recovery from surgery, the rats were placed in standard microdialysis cages and 2 mm microdialysis probes were inserted through the guide (CMA7 2mm membrane). Fitted with fluorinated ethylene propylene (FEP)-tubing (Microbiotech/se AB, Stockholm, Sweden) the probe inlets were connected to a microinjection pump while the outlets were connected to refrigerated fraction collector. Microdialysis perfusion buffer is artificial CSF (aCSF) consisting of (in mM): NaCl (122), KCl (3), CaCl2 (1.3), MgSO4 (1.2), NaHCO3 (25), KH2PO4 (0.4) pH 7.4. After the insertion of the probes flow rate was kept constant on 1.0 µL/min and samples were collected in 20 min intervals. To obtain stable baselines, the mice were allowed to run in the microdialysis setup overnight before collection of baseline samples the next morning. Samples were collected every 20 min for 1hour 20 minutes baseline and amphetamine i.p. for another 2 hours 40 min at 20 min interval. All samples were snap frozen and stored at -80ºC until the analysis of neurotransmitters levels. Microdialysates were combined with an internal standard solution (d4-dopamine in water) 1:1 and then injected on a UPLC-MS/MS system for determination of dopamine levels.

Impaired motor coordination in 3-month old Line 61 mice



D. NeuroCube® analysis	7 weeks	11 weeks
Overall	87% (p<0.001)	99% (p<0.0001)
Gait measures	62% (p>0.24)	99% (p<0.0001)
Paw Positioning	89% (p<0.0001)	95% (p<0.0001)

Figure 1. Analysis of motor behavior in Line 61 mice: A progressive increment was observed in the latency to turn and to traverse the beam in SCNA Het animals from 6 to 12 weeks of age (A). Number of footslips from the hindlimb or forelimb is higher in SCNA Het at the two testing time points (B). Reduced hanging time in Line 61 mice compared to WT littermates in the Wire Hang test at 10 weeks of age (C.).

Decreased frequency of excitatory synaptic events striatal SPNs from 2 and 6-month old Line 61 mice

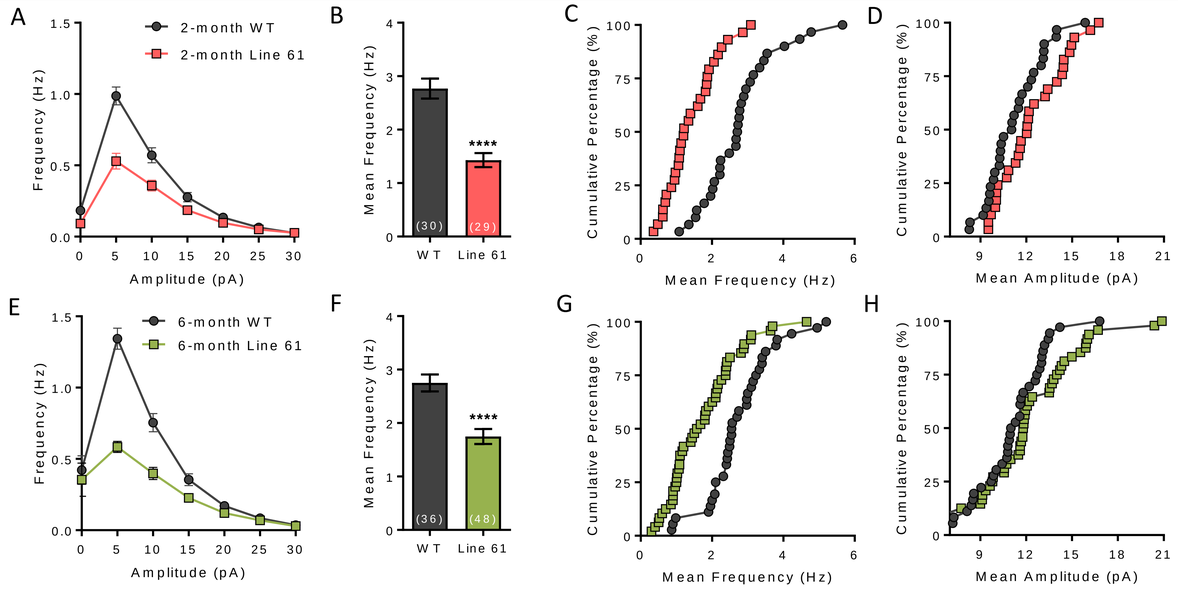


Figure 2. Reduction in the frequency of miniature excitatory postsynaptic currents in striatal SPNs from 2-month and 6-month old Line 61 mice. (A-C) Frequency histogram, mean frequency, and cumulative distribution of mean frequencies showing reduced frequency of excitatory synaptic events in 2-month old and 6-month old Line 61 (E-G) (Two-way ANOVA with Bonferroni's post hoc test, p<0.001 for genotype). (D and H) Slight increase in the mean amplitude of excitatory synaptic events as a result of the robust reduction in small amplitude events between 5-15 pA. . (Number in parenthesis represents number of neurons from 6 mice per genotype)

Unaltered corticostriatal release probability in 6-month old Line 61 mice

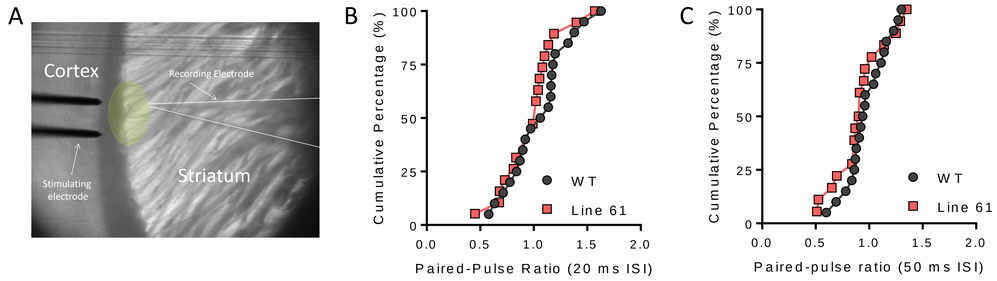


Figure 3. Corticostriatal release probability assessed by paired pulse stimulation of cortical afferents was unaltered in 6-month old Line 61 mice. Paired pulse stimulation was performed by stimulation of callosal fibers at inter-stimulus intervals (ISI) of 20 and 50 milliseconds using a 2-lead matrix stimulation electrode. The green circle represents the area in the striatum for patch clamping SPNs with respect to the positioning of the stimulating electrode. (B-C) Cumulative percentage graphs showing complete overlap of paired-pulse ratios between wild-type and Line 61 for 20 and 50 ms ISI. (n=20 neurons from 3 mice per genotype)

Impaired evoked striatal dopamine release in 9-month old Line 61 mice in response to amphetamine challenge

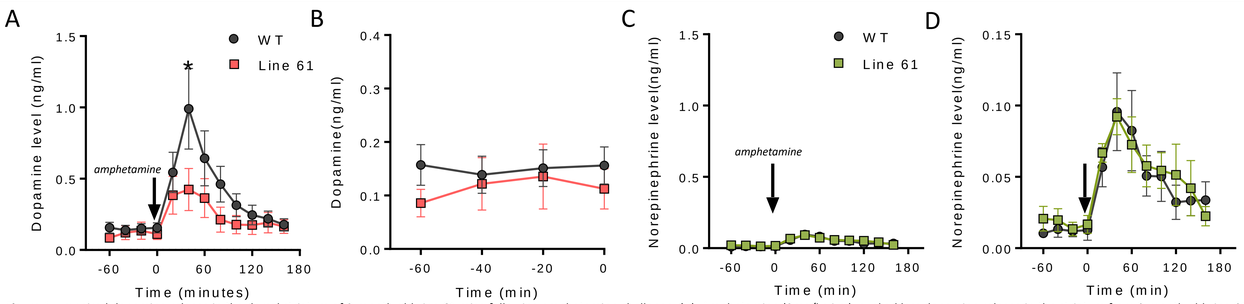


Figure 4. Impaired dopamine release in the dorsal striatum of 9-month old Line 61 mice following amphetamine challenge. (A) Amphetamine (2mg/kg i.p.) evoked less dopamine release in the striatum from 9-month old Line 61 mice relative to wild-type controls (p<0.05 at time point 40 min post amphetamine, Two-Way ANOVA with Bonferroni's post hoc multiple comparison test (n=7 WT, and n=4 Line 61). (B) Basal levels of dopamine were not affected in Line 61 mice. (C-D) Unaltered changes in evoked norepinephrine in Line 61 mice. Amphetamine evokes a larger increase in dopamine than norepinephrine in the dorsal striatum, suggesting the dorsal striatum is innervated primarily by dopaminergic terminals rather than noradrenergic terminals.

SUMMARY

Analysis of Line 61 mice revealed major synaptic abnormalities in the striatum of Line 61 mice that correlate with impaired motor coordination. These deficits were not related to alterations in the striatal protein levels of synaptophysin, PSD95, vesicular glutamate 1 and 2 transporters (Vglut1 and Vglut2) (data not shown, western blotting from protein homogenates and synaptosomes from striatal tissue 6-month old Line 61). In addition, we have now preliminary evidence demonstrating that dopamine release is impaired in Line 61 mice. The reduction in the frequency of synaptic events might reflect loss of specific type of synapses in response to alpha-synuclein overexpression in Line 61 and is consistent with published work (Wu, N. et al (2010) *J. Neuroscience Research*. In addition, alpha-synuclein overexpression can be impairing dopaminergic terminals to properly release dopamine in the striatum. Future experiments using immunhistochemistry and imaging are required to assess whether synapse loss in the striatum is driven by reduced cortical/thalamic afferents, and reduction in the number of specific types of dendritic spines in spiny projection neurons. Finally, the robust and stable change in synaptic deficits in the striatum can be a potential readout that can be used for preclinical testing in the Line 61 model.