

Supplementary Materials: Antidepressant-Like Properties of Intrastriatal Botulinum Neurotoxin-A Injection in a Unilateral 6-OHDA Rat Model of Parkinson's Disease

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Table S1. Overview of the preclinical studies investigating the depressive- and anxiety-like behavior after unilateral lesion into the MFB in rats.

Study	Rat Strain Male	Unilateral Injection into Medial Forebrain Bundle (MFB)	Depression/Test/Parameter/ Results	Anxiety/Test/Parameter/Results
Carvalho et al. 2013 [1]	Wistar-Han	Right MFB	Sucrose preference test: the preference for a sweet solution was shown to be significantly affected by unilateral 6-OHDA lesion of the MFB in 6-OHDA groups compare to Sham-lesioned group.	<p>OFT: Total distance traveled and number of ambulatory episodes.</p> <p>6-OHDA-lesioned group displaying a significantly lower motor activity when compare to Sham-operated rats (decreased total distance travelled) and overall reduction in movement initiation (decreased ambulatory episodes) caused by 6-OHDA lesion.</p> <p>EPM: The ration between time in open arms and in closed arms was measured.</p> <p>No significant differences were found between the 6-OHDA and Sham groups regarding the ratio between</p>

				time spent on the open arm and time spent on closed arms.
				OFT: Locomotor activity.
Delaville et al. 2012 [2]	Wistar	Right MFB	FST: Immobility: 6-OHDA depletion alone did not produce any changes in “depressive-like” behavior compare to Sham-lesioned animals.	6-OHDA-lesioned group displaying a significantly lower locomotor activity than Sham animals.
			Sucrose preference test: 6-OHDA-lesioned rats did not show any changes in sucrose consumption compared to Sham-lesioned animals.	EPM: Time spent in the open and closed arms and number of entries into the open and closed arms. No significant differences were found between the 6-OHDA and Sham groups regarding the number of entries, as well as the time spent in open arms.
Eskow Jaunarajs et al. 2010 [3]	Sprague-Dawley	Left MFB	FST: Immobility; Climbing; Swimming.	Locomotor chambers (motor and anxiety-like behaviors): significantly reduced total distance traveled in hemi-PD group compare to Sham group by approx. 50%.
			Immobility: no significant increase of immobility (no significant changes). Climbing: a significant effect on climbing behavior (decrease). Swimming: no significant changes.	Social interaction test: reduction in frequency to approach the stimulus animal in DA-lesioned rats compare to Sham-lesioned group; significant decrease of anogenital sniffing, but not other sniffing, or flights.

			Unilateral DA lesion not alter depression-like behavior in DA-lesioned rats measured by the FST.	Unilateral DA lesion exerted anxiogenic effects on locomotor activity and social interaction.
Hui et al. 2014 [4]	Sprague-Dawley	Right MFB	<p>FST: Immobility time: there was a strong tendency for 6-OHDA-lesioned rats to be more immobile than Sham-operated rats.</p> <p>Sucrose preference test: rats showed the decreased sucrose consumption compared to Sham-operated rats.</p>	<p>OFT: Number of squares crossed (horizontal locomotion); number of rearings (vertical activity).</p> <p>Unilateral 6-OHDA lesion decreased the number of squares crossed (horizontal movement) and rearing (vertical movement) - striking impairments in horizontal and vertical activity.</p>
Liu et al. 2015 [5]	Wistar	Left MFB	<p>FST: Immobility time: there was a strong tendency for 6-OHDA-lesioned rats to be more immobile than Sham-operated rats.</p> <p>Sucrose preference test: unilateral 6-OHDA lesion of the MFB in rats significantly decreased sucrose consumption when compare to Sham-operated rats.</p>	<p>OFT: Number of squares crossed (horizontal locomotion); number of rearings (vertical activity).</p> <p>Unilateral 6-OHDA lesion decreased the number of squares crossed (horizontal movement) and rearing (vertical movement).</p>

				<p>OFT: The total distance traveled; time spent in center; the number of entries into the center and latency to enter the center.</p> <p>6-OHDA-lesioned group displaying a significantly lower locomotor activity (distance traveled) than Sham animals, but no significant differences were found between the 6-OHDA-lesioned animals and Sham-lesioned animals in time in center; center crossing; latency to enter the center.</p>
O'Connor et al. 2016 [6]	Sprague-Dawley	Right MFB	Not done	<p>EPM: Time spent in the open arm and number of open and closed arm entries.</p> <p>6-OHDA animals significantly spent less time in the open arm (open arms entries) than Sham rats or naïve rats. Also, 6-OHDA animals significantly spent less time in the closed arm (closed arms entries) than Sham rats but not naïve rats – implying that 6-OHDA animals have high baseline anxiety-like behavior and 6-OHDA lesioning is anxiogenic.</p>
Sun et al. 2015 [7]	Sprague-Dawley	Right MFB	Not done	<p>OFT: Number of squares crossed (horizontal locomotion); number of</p>

				rearing (vertical activity); the percent of time spent in the central area.
				Unilateral 6-OHDA lesion decreased the number of squares crossed (horizontal movement) and rearing (vertical movement), and decreased the percent of time spent in central area in the OFT.
				EPM: The percent of time spent in open arms and the percent of entries in open arms.
				Unilateral 6-OHDA lesion decreased the percent of open arm time and open arm entries.
Zhang et al. 2011 [8]	Sprague-Dawley	Right MFB	FST: Time immobile: a strong trend for unilaterally 6-OHDA-lesioned rats to be immobile compared to control group.	OFT: Horizontal activity: no significant differences between 6-OHDA and control group. Rearing: no significant differences between 6-OHDA and control group.
Zhang et al. 2015 [9]	Sprague-Dawley	Right MFB	FST: Immobility time: unilateral lesioning MFB increased immobility time when compare to Sham-operated rats. Sucrose preference test: rats showed the decreased	OFT: Number of squares crossed (horizontal locomotion); number of rearings (vertical activity). Unilateral lesion decreased the number of squares crossed (horizontal movement) and rearing (vertical

sucrose consumption compared to Sham-operated rats.	movement) - striking impairments in horizontal and vertical activity.
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Table S2. Spearman correlation table.

Treatment Group	FST Parameter	Anxiety-Related Parameter	rs	p	Number of Samples
6-OHDA + BoNT	struggling frequency	EPM %time on open arms	−0.116	0.638	18
6-OHDA + Sham	struggling frequency	EPM %time on open arms	0.201	0.559	10
Sham + Sham	struggling frequency	EPM %time on open arms	−0.237	0.49	10
6-OHDA + BoNT	struggling time	EPM %time on open arms	−0.174	0.482	18
6-OHDA + Sham	struggling time	EPM %time on open arms	−0.0788	0.811	10
Sham + Sham	struggling time	EPM %time on open arms	−0.127	0.707	10
6-OHDA + BoNT	immobility frequency	EPM %time on open arms	−0.0836	0.736	18
6-OHDA + Sham	immobility frequency	EPM %time on open arms	0.360	0.292	10
Sham + Sham	immobility frequency	EPM %time on open arms	−0.079	0.811	10
6-OHDA + BoNT	immobility time	EPM %time on open arms	−0.0175	0.941	18
6-OHDA + Sham	immobility time	EPM %time on open arms	0.382	0.258	10
Sham + Sham	immobility time	EPM %time on open arms	0.309	0.365	10
6-OHDA + BoNT	struggling frequency	OFT center/total time	0.180	0.466	18
6-OHDA + Sham	struggling frequency	OFT center/total time	0.0306	0.919	10
Sham + Sham	struggling frequency	OFT center/total time	−0.00617	0.973	10
6-OHDA + BoNT	struggling time	OFT center/total time	−0.108	0.662	18
6-OHDA + Sham	struggling time	OFT center/total time	−0.419	0.213	10
Sham + Sham	struggling time	OFT center/total time	0.0308	0.919	10
6-OHDA + BoNT	immobility frequency	OFT center/total time	0.159	0.519	18
6-OHDA + Sham	immobility frequency	OFT center/total time	−0.107	0.759	10
Sham + Sham	immobility frequency	OFT center/total time	0.198	0.559	10
6-OHDA + BoNT	immobility time	OFT center/total time	0.138	0.574	18
6-OHDA + Sham	immobility time	OFT center/total time	0.620	0.048	10
Sham + Sham	immobility time	OFT center/total time	0.135	0.681	10

Green colored *p* values indicate significant correlation.

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