# Fall propensity associated with multi-system, cholinergic-dopaminergic losses in PD

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## INTRODUCTION

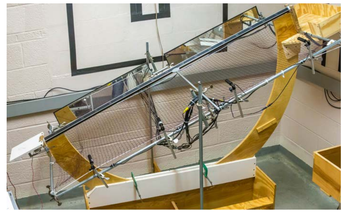
- In addition to the primary disease-defining symptoms that result from extensive loss of nigrostriatal dopamine neurons, Parkinson's disease (PD) patients often suffer from a cluster of related movement impairments such as postural instability, gait control deficits, and a propensity for falls. These levodopa-unresponsive symptoms are associated with losses of cholinergic neurons situated in the basal forebrain (BF) and in the brainstem pedunculopontine nucleus (PPN), and often co-occur with cognitive symptoms, particularly attentional deficits.
- We recently developed a test system (Michigan Complex Motor Control Task, MCMCT) for the assessment of complex movement and fall propensity in rats (Kucinski et al., 2013; 2015). The MCMCT was designed to tax the ability to rapidly correct movement errors while traversing dynamic surfaces (rotating square rods). Traversing these rods requires strict attentional control of gait, precise limb coordination, and carefully timed and placed steps. Rats were also tested for attentional capacity on an operant Sustained Attention Task (SAT).
- In a series of lesion experiments, we assessed the impact of different combinations of cholinergic and/or dopaminergic system losses on fall propsentiy and related movement impairments such as freezing of gait, as well relationships between attentional deficits and falling behavior.
- Fall propensity was aslo assessed in rats that are prone to attribute incentive motivational value ("incentive salience") to reward cues ("sign trackers;" STs) relative to others ("goal trackers;" GTs). STs were previously shown to exhibit poor SAT performance and low cholinergic neuromodulatory activity during task performance (Paolone et al, 2013). Here, using a modified MCMCT compatible for *in vivo* microdialysis, brain analytes were collected during traversals in ST and GTs and cholinergic neuromodulatory activity was assessed using HPLC-mass spectomtery.

## Lesion Experiments and Groups

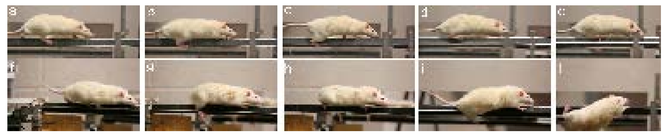
Rats were first trained to traverse MCMCT rods before receiving cholinergic and/or striatal dopaminergic lesions or sham lesions (see Table below for lesion groups). Some rats were also trained on the SAT. Following surgeries, rats were tested on 15-20 day MCMCT test batteries with increasingly complex traversal conditions, including trials in which the direction of the rod was alternated between successive trials and trials that included distractors, as well as SAT in some groups.

| **Labels, lesions and experimental group names** | |
|---|---|
| **Label** | **Lesions** |
| DA | bilateral infusions of 6-OHDA into dorsomedial striatum |
| BF/SAP | bilateral infusions of 192 IgG saporin into basal forebrain |
| PPN | bilateral infusions of anti-ChAT-SAP into the pedunculopontine nucleus |
| **Experimental group names and lesion combinations** | |
| Sham | vehicle infusions into PPN, BF, and striatum |
| PPN-DA | DA + PPN |
| PPN-BF | BF + PPN |
| DL | BF + DA |
| TL | BF + DA + PPN |

## Michigan Complex Motor Control Task (MCMCT)

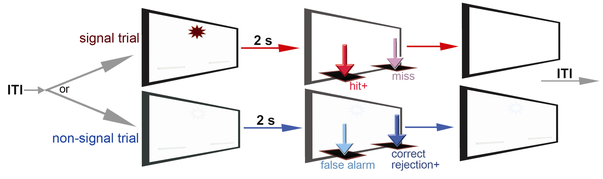


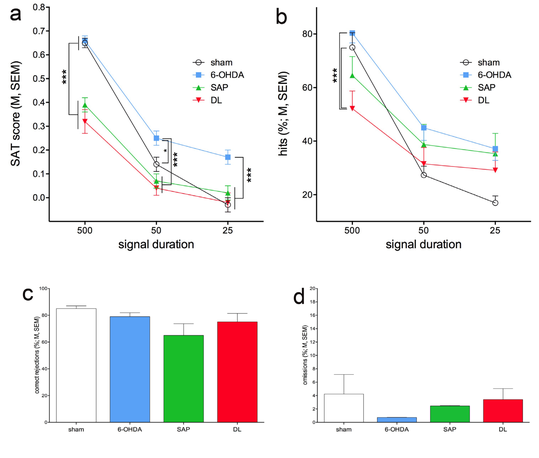
The MCMCT consists of various runways (plank, round and square rods, placed horizontally or, as shown in a, at inclines), and with rods rotating (10 rpm) while distractors were presented. The rods were capable of rotating in both directions. Cameras and mirrors generated a complete record of each trial.



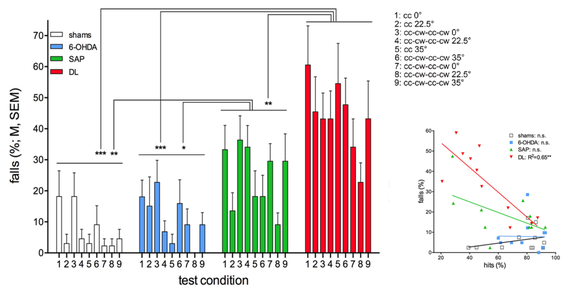

Sham-operated control (a-e) and a rat with BF and PPN cholinergic loss as well as striatal dopamine loss ("TL"; f-j) traversing a rotating rod (10 rpm) at 0° incline. Both slip (b,g,i), but the TL rat fails to evoke correcting steps and compensatory postural control after the second slip and falls (into a net).

## Sustained Attention Task (SAT)



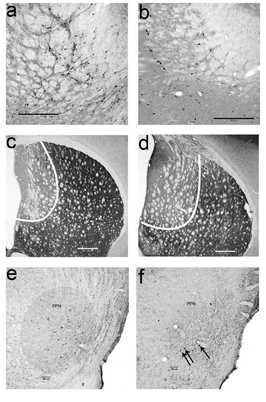




## High Rates of Falls and Correlation with SAT Performance in Dual Lesion (BF + Striatum) Rats




DL (dual lesion) ratsmaintained a high rate of falls across the rotating rod MCMCT test conditions. Rats with only BF cholinergic lesions (SAP) also fell more than the sham and DA lesion groups. SAT performance correlated with falls only in DL rats.

## Triple System Lesions: PPN, BF, and Striatum

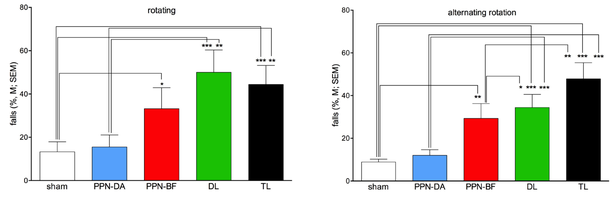



**BF, DA, and PPN lesions**.

**a** shows ChAT-immunoreactivity on a coronal section of the BF from a sham-lesioned rat. Infusions of 192-IgG saporin removed 70-90% of the cholinergic neurons in this region **(b)**.

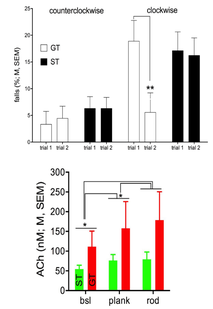
Infusions of 6-OHDA in the dorsomedial striatum removed dopaminergic afferents as visualized by TH-immunoreactivity **(c, d)**.

**e** shows cholinergic neurons in the intact PPN (shaded region; ChAT-immunoreactivity). Infusions of anti-ChAT-SAP lesions lesioned >90% of the neurons (**f**; arrows point to neurons spared by the toxin). Scales in a-d are 500 µm and in e and f are 100-µm.




The rotating rods (cc and alternating direction) elicited relatively high fall rates in PPN-BF, DL and in rats with triple system lesions (TLs), with DL and TL rats consistently exhibiting statistically similar fall rates. In general, PPN lesions did not further increase fall propensity.

## Sign Trackers: More Falls and Attenuated ACh Levels During MCMCT Performance




Modified MCMCT apparaus with an overhead sliding rig and motorized retractable walls for *in vivo* microdialysis (left). STs continued to fall at high rates when the direction of the rod was reversed from the familiar direction, whereas GTs rapidly adjusted by the second trial (upper right). Extracellular ACh levels in prefrontal cortex increased following traversals of rotating rods compared to plank and baseline conditions. Similar to ACh levels in STs performing an attention task, MCMCT performance failed to elevate ACh levels in these rats.

## CONCLUSIONS

- ❑ Rats with dual cholinergic-dopaminergic lesions (DL) exhibited a high rate of falls from the rotating rods, which correlated with SAT performance. Also, in DL rats, but not in rats with only dopaminergic lesions, the placement and size of dopaminergic lesion correlated significantly with fall rates.
- ❑ The results support the hypothesis that after dual cholinergic-dopaminergic lesions, attentional resources can no longer be recruited to compensate for diminished striatal control of complex movement, thereby "unmasking" impaired striatal control of complex movements and yielding falls.
- ❑ The performance of rats with losses in all three regions (PPN, BF, and DA) was not more severely impaired than following combined BF cholinergic and striatal DA lesions.
- ❑ STs fell more than GTs in more complex MCMCT trials and had attenuated ACh levels in the PFC during performance. Overall, the rotating rod increased ACh release during the rotating rod compared to during traversals of the plank.
- ❑ The MCMCT is suitable for assessing the potential of new treatments for falls and related movement errors, including freezing of gait. Preliminary evidence treating DL rats with nicotinic drugs (alpha4beta2* agonist) and a low dose of L-DOPA support the promise of such a treatment combination.

## References & Acknowledgements

Kucinski et al., Journal of Neuroscience (2013) 33:16522-39
Kucinski et al., Behavioral Neuroscience (2015) 129: 96-104
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