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### Introduction/Background

#### Parkinson's Disease

- Parkinson's disease (PD) is a neurodegenerative disease affecting over one million Americans.
- In PD, a brain area known as the substantia nigra, that provides a neurotransmitter dopamine, is severely damaged causing impaired movement and abnormal motor function.
- It is characterized by such symptoms as akinesia, bradykinesia, resting tremors, and muscular rigidity.

#### Trouble With Treatment

- Traditional treatment for the disease is twice-daily oral intake of levodopa, a direct precursor to dopamine which works well initially in conferring symptomatic relief.
- After two to five years of chronic treatment, however, the drug becomes less effective and severe disabling motor complications appear.
- These are termed motor response complications (MRCs) and include shortening of the effects of levodopa treatment and appearance of abnormal involuntary movements (AIMs) or dyskinesias.

#### Cellular Mechanisms

- Much of the cellular mechanisms underlying MRCs are not clearly understood.
- Supersensitization of striatal kinases PKA and CaMKII are present after chronic levodopa treatment.
- PKA and CaMKII Kinase inhibitions have shown to decrease corticostriatal activity and the AMPA and NMDA receptor channel function resulting in attenuated motor response complications.
- Cycline-dependent kinase 5 (CDK5) is an inhibitory kinase to PKA that regulates dopamine signaling (via DARPP32 and PPI), and may contribute to striatal neuroadaptive mechanisms associated with MRCs.

### Research Question

Because CDK5 has an inhibitory role in dopamine neuronal signaling and PKA-mediated hyperphosphorylation of corticostriatal glutamatergic receptors, inhibiting it further should exacerbate motor response complications.

Using silencing RNA (siRNA) technique, CDK5 was inhibited in the striatum in order to investigate its role in the expression of MRCs in hemiparkinsonian rats chronically treated with levodopa.

### Methods

#### Preparing the Animal Model

- Rats were surgically rendered parkinsonian by injection of 6-Hydroxydopamine (6OHDA), a neurotoxin, into the medial forebrain bundles.
- Following three weeks of recovery, a rotational screening using apomorphine selected eligible rats to begin twice daily injections of levodopa for the following three weeks.
- Weekly assessments allowed for progressive comparison of PD and MRC symptoms.
- Over the 21 days of levodopa treatment, the parkinsonian rats exhibited similar symptoms as humans: shortened duration of treatment effect and AIMs, corresponding to motor response fluctuations of wearing-off type and dyskinesias in humans, respectively.

#### Testing the Variable

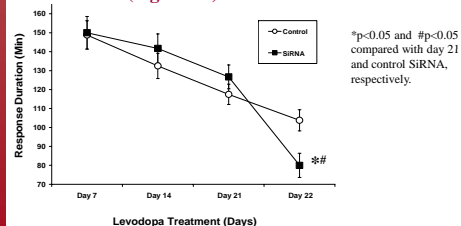
- A dendrimer compound with FITC fluorescence was injected intracranially into the control group while the same compound with an addition of a Silencing RNA strand attached was injected in the treatment group.
- 12 hours after injections, acute levodopa treatment was administered and behavioral effects were assessed.
- Following the final levodopa injection and behavioral assessment, rats were sacrificed and their brains prepared for cellular observations.

#### Analysis of Data

- Behavioral data was compared for both AIMs and duration of rotational response using repeated-measures ANOVA.
- Comparisons were made to show differences between treatment groups (control vs. siRNA) and before and after treatment.

### Results

#### Effects of Cdk5 SiRNA on Duration of Levodopa-Induced (high dose) Contralateral Rotations



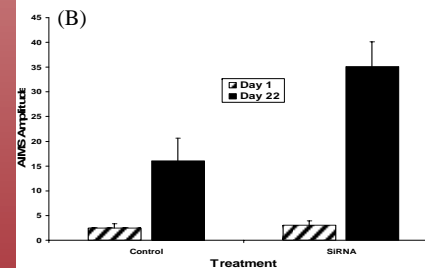
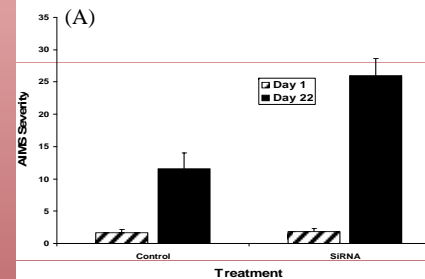
### Results (continued)

(Previous figure)

The duration of the rotational response is compared on levodopa day 1 (baseline), day 7, day 14, day 21, and day 22 + local injections (control, SiRNA). On day 22, Cdk5 SiRNA was given 12 hours prior to levodopa challenge. On day 22, response duration was worsened by Cdk5 treatment.

#### Exacerbating Effects of Cdk5 SiRNA on Chronic Levodopa-Induced AIMs Severity and Amplitude

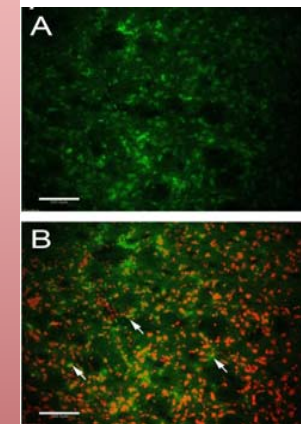
- (A) AIMs severity (B) AIMs amplitude in 6-OHDA lesioned rats.



Levodopa given twice daily for 21 days increased AIMs amplitude and severity scores in response to acute levodopa challenge on day 22. On day 22 of levodopa treatment, local injection of Cdk5 SiRNA (2µg per injection, intrastriatal; n=5) 12 hours prior to acute levodopa challenge worsened (increased) both amplitude and severity AIMs scores compared with those animals that received control SiRNA (n=5). The AIMs amplitude increase in SiRNA treated animals was 105% more than the control. The severity increase in SiRNA treated animals was 132% more than the control.

### Results (continued)

#### Fluorescence Staining: Tracking the Dendrimer CDK5 siRNA Complex



FITC-labeled dendrimer plus Cdk5 siRNA (FITC green; Panel A) co-stained with propidium iodide nuclear staining (Red; Panel B [Co-label]) in dyskinetic hemiparkinsonian rats 12 Hours after SiRNA gene silencing with dendrimer

conjugated with Cdk5 siRNA. Arrows indicate striatal neuronal profile co-labeled with Cdk5 siRNA in cytoplasm (green) and propidium staining in nucleus (red) produced by dendrimer-mediated siRNA gene transfer.

### Conclusion

- The current results support the view that Cdk5 provides striatal neuroadaptive mechanisms in response to chronic levodopa treatment.
- Cdk5 agonists may thus confer therapeutic benefits to patients with Parkinson's disease.
- It is possible that Cdk5 agonists may represent safer and more effective ways to manage both the primary symptoms of PD and the motor response complications that develop with chronic levodopa treatment in advanced PD

### Acknowledgements

A special thanks to Central Michigan University McNair Scholars Program, Dr. Justin Oh-Lee, and Carol Stevens.