# Glutathione Depletion Models PD in Mice

**21 Dec 2007**

Lowering the antioxidant defenses of dopaminergic neurons in mice leads to the mitochondrial dysfunction and age-related neurodegeneration characteristic of Parkinson disease, concludes a new study in the December 19 Journal of Neuroscience. The work, from Julie Andersen and colleagues at the Buck Institute for Age Research in Novato, California, suggests that deficiency of the antioxidant protein glutathione in the substantia nigra could cause PD, and provides a new mouse model for the disease.

There is much evidence linking oxidative stress to Parkinson disease, which features faltering activity of the mitochondrial complex I (CI) system and the death of the dopaminergic neurons in the substantia nigra (SN), among other areas. A deficiency in the reactive oxygen scavenger glutathione was observed long ago in SN dopaminergic neurons in postmortem brain from Parkinson patients (Sian et al., 1994), and there is a literature on glutathione and PD, but even so it has not been clear if the decrease is a cause or effect of the disease. The genetics of PD also point toward a role of glutathione in the disease, with alleles of human glutathione transferase genes influencing the onset of sporadic PD as well as Alzheimer disease (see PDGene entries #10, 180, and 181). Previously, the Andersen group had shown that depleting glutathione in dopaminergic neurons in culture led to the inhibition of CI by thiol oxidation of cysteine residues, killing the cells.

To find out if reducing glutathione levels in vivo has a similar effect, first author Shankar Chinta devised a way to induce depletion selectively in dopaminergic neurons. He used a tetracycline-responsive antisense construct to knock down the expression of γ-glutamyl cysteine ligase, the rate-limiting enzyme in the de-novo synthesis of glutathione. The investigators targeted the antisense to dopaminergic neurons by placing the system under control of the tyrosine hydroxylase promoter. In cultured primary mesencephalic neurons from the transgenic mice, treatment with the antibiotic doxycycline reduced glutathione levels, decreased cell viability, and caused shortened neuritic processes in tyrosine hydroxylase positive (TH+) dopaminergic cells.

Detecting a reduction of glutathione in TH+ neurons in vivo was more of a challenge. Since dopaminergic neurons make up only 1 to 5 percent of the cells in the substantia nigra, measuring glutathione in cell lysates was unlikely to reveal changes. Instead, the researchers isolated synaptosomes from the striatum, where the SN dopaminergic neuron projections terminate. In dopaminergic synaptosomes (purified with an anti-dopamine transporter

antibody), both the oxidized and reduced forms of glutathione were decreased by one-third after 3 months of doxycycline induction, and by 39 percent after 12 months. When doxycycline feeding was stopped, glutathione levels began to rise and reached near pretreatment levels after 3 weeks. As expected, the depletion was specific for dopamine-containing synaptosomes, and no change was seen in glutathione levels in GABAergic synaptosomes.

Together with a decrease in glutathione, there was a significant and specific decrease in complex I activity in the dopaminergic synaptosomes. No change was seen in other mitochondrial electron transport chain transport complexes II, III, or IV. This mimics what Andersen and others had reported previously in cultured cells, where they showed that glutathione depletion decreases the enzyme activity via a nitric oxide-mediated thio-oxidation of the complex I subunits. The same effect seemed to occur in vivo, where the depleted mice displayed an increase in NO and thio-nitrosylation of mitochondrial proteins, which was reversed when doxycycline was discontinued and glutathione levels recovered.

The decrease in glutathione and complex I activity was accompanied by neurodegeneration, but only in adult mice. When 12-month-old mice were fed doxycycline for 3 weeks, striatal dopamine levels declined and the number of TH+ neurons in the substantia nigra shrank by 20 percent. In young mice (3 months old), the same glutathione depletion occurred without consequences on dopamine levels or cell viability. “The results show that GSH deficiency alone is not sufficient to cause degeneration, but that the effect also has an aging component,” Andersen told ARF.

That could make the mice an apt model for Parkinson disease. “In terms of emulating the human disease, I think these mice are as good as any other,” Andersen said. “Glutathione depletion causes selective complex I inhibition, and complex I inhibition causes age-related neurodegeneration.” Preferential loss of midbrain dopaminergic neurons is one pathological hallmark of PD, which also features the deposition of aggregated synuclein protein as Lewy bodies. Andersen said her group is currently looking into synuclein pathology in the mice and has encouraging preliminary results.

“There is a general acceptance in the field that depletion of glutathione occurs in PD, but it is still controversial how or if that contributes to disease,” Andersen said. “Our results make the connection between early depletion of glutathione and later, specific mitochondrial deficits that are seen in PD.” The reduction in glutathione seems quite selective to dopamine neurons in the brains of PD patients, and it is still a mystery what causes that. Nonetheless, Andersen says, “Our model shows that if you emulate that depletion, you see important characteristics of the disease.”

Glutathione reductions are also seen in other neurodegenerative diseases, such as Alzheimer disease, and polymorphisms in the glutathione transferase gene have been linked to age of onset and rate of decline in late-onset AD (Spalletta et al., 2007; Hong et al., 2007). Reduction in the overall antioxidant capacity of cells, to which glutathione contributes just one portion, leads to

oxidative damage, which occurs in AD and other neurodegenerative diseases. However, Andersen opines that the special oxidizing properties of dopamine and the toxicity of its oxidized products may make dopaminergic neurons particularly susceptible to loss of antioxidant defenses.

Glutathione depletion has also been linked to exacerbated neurotoxicity of metals including copper (Du et al., 2008), and accumulation of iron is another change seen in PD brain. Andersen says this, too, may be a result of glutathione depletion. The mice are yielding evidence that loss of glutathione causes oxidative stress that affects the ability of the cell to regulate iron stores, she said.

If maintaining glutathione levels is critical for protecting aging dopaminergic SN neurons from neurodegeneration, its replacement may be a possible therapeutic avenue for PD and other related disorders, the study suggests. This echoes earlier work in a fly model of PD (see ARF related news story) and harkens back a decade to attempts to treat PD patents with infusions of glutathione (Sechi et al., 1996). Effectively deploying such a strategy will require a way to detect the disease much earlier, Andersen stressed. "By the time somebody is diagnosed, they've lost 60 percent of their midbrain DA neurons. That means we really need an earlier diagnostic marker. We could develop means to deliver glutathione to the brain, but we need to know who to give it to."—Pat McCaffrey

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## REFERENCES

### News Citations

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### External Citations

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## FURTHER READING

### Papers

Sayre LM, Perry G, Smith MA. Oxidative stress and neurotoxicity. *Chem Res Toxicol*. 2008 Jan;21(1):172-88. PubMed.

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## PRIMARY PAPERS

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