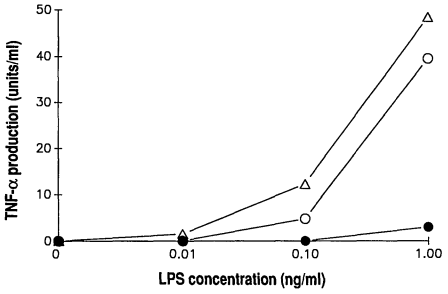

Fig. 4. Anti-CD14 MAb blocks the synthesis of TNF-α stimulated by LPS. Heparinized human blood was cultured with graded doses of LPS (type Re595) as described (○) (*26*). In parallel cultures, MAbs to CD14 (3C10, ●) and CD18 (IB4, △) were added to 10 μg/ml. After 16 hours at 37°C, TNF-α levels in the plasma were determined by the WEHI clone 13 assay (*27*). Parallel studies showed that all of the TNF-α activity in the supernatants could be neutralized by an antibody to TNF-α. Results are representative of four separate experiments, and similar results were also obtained after a shorter (4-hour) incubation of blood with LPS. The amount of TNF-α secreted could not be increased by raising LPS concentrations beyond the doses shown.

ger TNF-α release itself, but may serve to bring LBP-LPS complexes to the cell surface in such a way that other proteins (perhaps other subunits of a complex receptor) are stimulated. The latter possibility is supported by the observation that raising the concentration of LPS to 10 ng/ml causes TNF-α synthesis in the absence of either LBP (*4*) or CD14 (*11*). In either case, it is clear that the mechanism by which CD14 and LBP act represents an unanticipated departure from the mechanism by which eukaryotic cells respond to other agonists such as hormones or lymphokines. The binding of the agonist (LPS) is mediated by a soluble protein rather than a membrane-bound protein, and only after the formation of a soluble complex does the LPS interact with a membrane-bound "receptor."

CD14 is a well-known marker for monocytes and macrophages and is recognized by several monoclonal antibodies commonly used in flow cytometry studies (LeuM3, Mo2, and MY4). The genes for both human and murine CD14 have been cloned and sequenced (*14, 15*). The predicted protein sequence contains several copies of a leucine-rich 24-residue repeat also found in the serum protein, leucine-rich glycoprotein, in the *Drosophila* membrane proteins Chaoptin and Toll (*15*), and the lutropin-choriogonadotropin receptor (*16*). The gene for CD14 is located in a region of chromosome 5 known to encode several myeloid-specific growth factors or receptors, including IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), CSF-1, CSF-1 receptor, and the platelet-derived growth factor receptor (*17*). However, the function of CD14 has not been defined. Here we show that CD14 binds LBP-LPS complexes and thereby serves two important functions. LBP acts as an opsonin (*3*), and CD14 may thus function in the clearance of Gram-negative pathogens during infection. LBP also promotes the secretory responses of cells to LPS, and CD14 may thus function to heighten the sensitivity of the immune system to infection.

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Acceleration of Diabetes in Young NOD Mice with a CD4$^+$ Islet-Specific T Cell Clone

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Nonobese diabetic (NOD) mice develop an autoimmune form of diabetes, becoming hyperglycemic after 3 months of age. This process was accelerated by injecting young NOD mice with CD4$^+$ islet-specific T cell clones derived from NOD mice. Overt diabetes developed in 10 of 19 experimental animals by 7 weeks of age, with the remaining mice showing marked signs of the disease in progress. Control mice did not become diabetic and had no significant pancreatic infiltration. This work demonstrates that a CD4 T cell clone is sufficient to initiate the disease process in the diabetes-prone NOD mouse.

TYPE I DIABETES OR INSULIN-DEPENDENT diabetes mellitus (IDDM) is thought to be an autoimmune disease in humans. Two animal models for inherited autoimmune diabetes are the Biobreeding (BB) rat and the NOD mouse. A role for T cells in diabetes was implicated by studies with these diabetes-prone animals in which treatments that led to the depletion of T cells resulted in a decreased incidence of disease (*1*). More conclusive evidence was provided by transfer of disease into nondiabetic recipients with T cells from diabetic rats (*2*) or mice (*3*). Since depletion of either the CD4 or the CD8 T cell subsets from diabetic donor T cells can prevent transfer of disease, the consensus has been that both CD4 and CD8 T cells are needed for development of diabetes (*4*). To learn more about the T cells involved in the disease process, we isolated islet-specific T cell clones from NOD mice (*5, 6*) and used two of these clones to accelerate the diabetogenic process in unmanipulated, nondiabetic NOD recipients.

The islet-specific T cell clones in our panel (*6*) are all CD4$^+$, and they proliferate and produce interleukin-2 (IL-2) in response to whole islet cell antigen and irradiated NOD spleen cells as antigen-presenting cells (APC). The clones do not respond to islet antigen or APC alone, nor do they react in vitro with other cell types (thyroid, pituitary, or spleen) or in vivo with pituitary grafts. They are unlikely to be artifacts of in vitro immunization, as our attempts to pro-

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duce islet-specific clones from non-NOD or NOD F_1 mice were not successful. Our first disease transfer studies were carried out by injecting clones into (CBA × NOD)F_1 mice (*6, 7*) and resulted in some intraislet infiltrate (insulitis) and β cell degranulation, but the animals did not develop hyperglycemia. We used young NOD mice as recipients in the disease transfer experiments to check whether full development of disease requires a diabetes-prone environment. Insulitis is virtually undetectable in NOD mice less than 6 weeks of age and hyperglycemia typically develops only after 3 months; thus 2- to 3-week-old animals are suitable as nondiabetic recipients (*8*).

Data from five disease transfer experiments are shown in Table 1. Disease transfer experiments in young NOD mice were done with three T cell clones from the panel: BDC-2.5, BDC-6.9, and BDC-2.4, a nonislet-specific control clone (*9*). Before transfer clones were activated for 3 to 4 days by restimulation with antigen and APC, and then were expanded for 3 to 4 days with IL-2 alone. The first four experiments tested varying cell numbers in relation to transfer of disease. In experiment 1 we injected the BDC-6.9 clone into two 14-day-old NOD mice and retained two littermates as controls; the experimental animals received four injections of cells over a 2-week period. Both of the mice that had received BDC-6.9 injections became acutely diabetic in about 2 weeks. We next (experiment 2) tried a protocol with the three clones, using a single injection of each. The animals were normoglycemic in the 4-week period after injection; however, histological examination of pancreatic tissue revealed that all animals that received the islet-specific clones BDC-2.5 and BDC-6.9 had an advanced degree of insulitis and β cell degranulation, indicating that the disease process was under way. Islets from mice that received the control clone, BDC-2.4, were completely free of infiltrate and could not be distinguished from those of untreated controls. In experiment 3, the same protocol was repeated with two injections of cells 1 week apart. Three of the animals that received the islet-specific clones became diabetic 13 days after the first injection, at which time the experiment was terminated and all the animals were killed. In the pancreas of the one animal (*3–6*) that received an islet-specific clone, but was not yet hyperglycemic, there were islets with significant insulitis and β cell degranulation, but there were also islets that were apparently not yet affected by disease. Again, no differences were observed between the animals that received the BDC-2.4 nonislet-specific control clone and untreated controls. The mice in experiment 4 received only clone BDC-6.9; this experiment was ended when one of the disease-transfer animals became diabetic at 13 days.

To confirm previous results and to look at some variations from other experiments, we performed experiment 5 with a litter of nine mice who received the BDC-2.5 and BDC-6.9 clones. Animals in this experiment received injections on different start dates and in some cases were followed longer if they had not become diabetic by the same time as their littermates. Three mice received BDC-2.5 when 17 days old (group 1), two mice received BDC-6.9 at 18 days of age (group 2), and two mice received BDC-6.9 at 22 days of age (group 3). Two of the animals injected with BDC-2.5 received two injections of cells 5 days apart, and mouse 5-3 was injected a third time after another week. The latter animal became diabetic 3 days later and was killed along with one of the other mice that was not yet diabetic. Mouse 5-2 was retained to see if diabetes would eventually develop; although the animal

Table 1. Disease transfer with islet-specific T cell clones. Data from five separate experiments are presented. Experimental groups consisted of four to ten animals from a single litter, between 2 to 3 weeks of age. Two animals from each litter were retained as controls, receiving no treatment, and the remaining mice received intraperitoneal injections of T cell clones (2×10^6 to 6×10^6 cells in a volume of 100 to 150 μl). The animals were not irradiated before injection. Each experiment was done with a different NOD litter; the number of the experiment is the first number in the left-hand column, mouse number is second (for example, mouse 2–3 is the third mouse in experiment 2). Ages of mice are given at the start of the experiment (date of first injection) and at the time the experiment was terminated. Under the column headed "cells administered" are listed the name of the clone, the number of cells per injection, and the number of injections. Blood sugar concentrations were measured and experiments were terminated when animals became glycosuric or by 4 weeks after the first injection. Blood glucose values >10 (in bold type) indicate hyperglycemia. In the far right-hand column are histology scores for intraislet pathology: −, no infiltrate; +/−, small focal or scant infiltrate in a few islets, no evidence of β cell degranulation; +, significant infiltrate in some islets with some beta cell degranulation; ++, advanced insulitis and considerable beta cell degranulation; and +++, massive infiltration, total islet disruption, or both, and complete or nearly complete β cell degranulation. ND, not determined.

Mouse	Sex	Age (in days)		Cells administered			Blood glucose (mM)	Insulitis and β cell degranulation
		Start	End	Clone	Dose ($\times 10^6$)	Injections (no.)		
1–1	M	14	30	0	0	0	6.1	−
1–2	F	14	30	0	0	0	5.5	−
1–3	M	14	30	6.9	2	4	**22.2**	(no islets)
1–4	F	14	30	6.9	2	4	**22.2**	+++
2–1	F	20	49	0	0	0	5.9	−
2–2	M	20	49	0	0	0	5.1	−
2–3	F	20	49	2.4	6	1	5.2	+/−
2–4	M	20	49	2.4	6	1	6.3	−
2–5	F	20	49	2.5	6	1	5.9	++
2–6	F	20	49	2.5	6	1	5.7	+
2–7	M	20	49	6.9	6	1	6.0	+++
2–8	M	20	49	6.9	6	1	5.4	++
3–1	M	18	31	0	0	0	6.8	+/−
3–2	F	18	31	0	0	0	6.5	+/−
3–3	M	18	31	2.4	5	2	7.0	+/−
3–4	M	18	31	2.4	5	2	6.7	+
3–5	M	18	31	2.5	5	2	**12.2**	+++
3–6	M	18	31	2.5	5	2	7.0	++
3–7	M	18	31	6.9	5	2	**12.9**	+++
3–8	F	18	31	6.9	5	2	**22.2**	+++
4–1	ND	17	30	0	0	0	5.7	−
4–2	ND	17	30	0	0	0	6.5	−
4–3	ND	17	30	6.9	5	2	5.7	+++
4–4	ND	17	30	6.9	5	2	**>22.2**	+++
5–8	F	17	32	0	0	0	3.5	−
5–9	F	17	44	0	0	0	3.3	−
5–1	F	17	33	2.5	5	2	4.5	+
5–2	M	17	44	2.5	5	2	**11.7**	+++
5–3	M	17	33	2.5	5	3	**>22.2**	+++
5–4	F	18	32	6.9	5	2	3.0	+/−
5–5	F	18	32	6.9	5	2	**10.9**	+++
5–6	ND	22	44	6.9	5	2	**11.2**	+++
5–7	ND	22	44	6.9	5	2	3.2	++

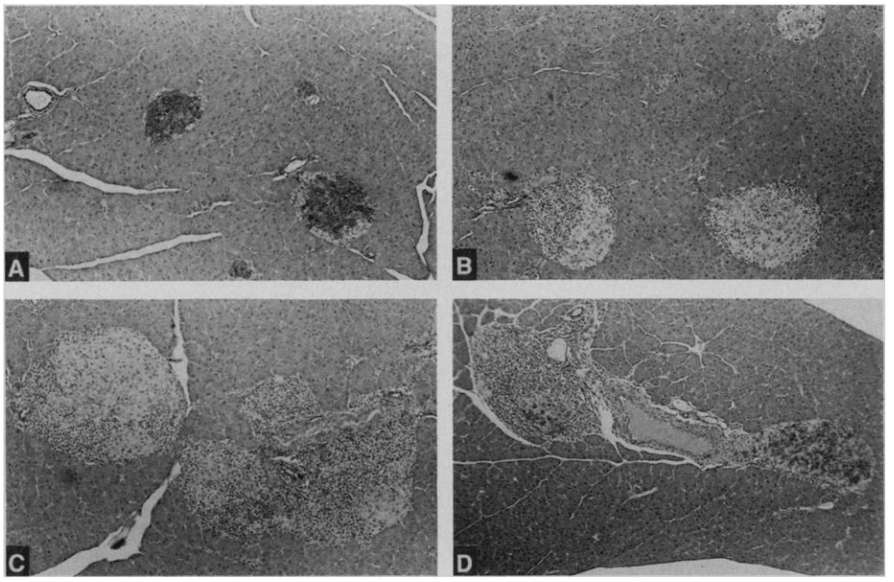

Fig. 1. Photographs of pancreatic sections from mice in experiment 5. Sections were stained with A/F. (**A**) Control mouse with typical normal islet structure and well-granulated β cell mass as indicated by the A/F-stained dark areas. (**B**) Section from mouse 5-2, treated with BDC-2.5. One islet is considerably infiltrated, whereas the other islets appear to have fewer infiltrating cells; the lack of A/F stain indicated that islets were completely degranulated. (**C**) Section from mouse 5-6, treated with BDC-6.9. Two large islet structures are heavily infiltrated and islet disruption and degranulation were extensive. (**D**) Section from mouse 5-7, also injected with BDC-6.9. This animal was not hyperglycemic; one islet is heavily infiltrated and almost completely degranulated whereas the other islet is only partially degranulated. Exocrine tissue in all sections appeared to be uninvolved.

seemed healthy at the termination of the experiment (when animals were 44 days old), it had become hyperglycemic. In group 2, one mouse became diabetic 2 weeks after the first injection; both animals were killed at this time. The group 3 animals were killed at 44 days, at which time one was found to be hyperglycemic. The results from this experiment, in which four of seven mice that received islet-specific clones became hyperglycemic, indicated that (i) there are probably individual variations between mice, apparently not sex-linked and present even among animals from a single litter, that cause some of the animals to develop overt signs of diabetes before others, and (ii) the time in which the disease develops may well be correlated with cell numbers and possibly the time at which transfers begin.

Histochemical analysis of the mice from experiment 5 revealed cellular infiltrates and degranulated islet tissue in all of the animals that received the islet-specific clones. Pancreatic sections from animals that were not yet overtly diabetic at the termination of the experiment contained some granulated β cells, but no granulated β cells could be found in sections from diabetic animals. In Fig. 1, sample sections shown are from a control mouse and from three mice (5-2, 5-6, and 5-7) treated with the islet-specific clones. These sections were stained with aldehyde fuchsin (A/F), a dye that detects the disulfide bonds in insulin and can thus serve as an indicator of islet function by revealing the extent of β cell granulation. Untreated control animals had well-defined islet structure, no cellular infiltrate, and well-granulated β cells (Fig. 1A). Islets in sections from animal 5-2, which had received the BDC-2.5 clone, showed various degrees of infiltrate (Fig. 1B); more extensive cellular infiltration was observed in mice 5-6 and 5-7 that received BDC-6.9 (Fig. 1, C and D). Animals 5-2 and 5-6 were hyperglycemic at the time of death and sections from both animals revealed completely degranulated islet tissue (Fig. 1, B and C), whereas the second BDC-6.9 mouse (5-7) was not hyperglycemic and its pancreas contained some islets that were not totally degranulated (Fig. 1D).

These data show that $CD4^+$ islet-specific clones can accelerate disease in young NOD mice. Of 19 animals from five different litters that received the islet-specific T cell clones, 10 became overtly diabetic, as indicated by elevated blood glucose values, compared to 0 of 14 animals that received no injections or the control clone BDC-2.4 (P by Fisher's exact test = 1×10^{-5}). Nearly all (18 of 19) of the mice that received BDC-2.5 or BDC-6.9 showed marked signs of disease in progress as indicated by the degree of insulitis and β cell degranulation in the islet tissue (histology score of ≥1+), versus 1 of 14 control mice (P by Fishers exact test = 3×10^{-7}). In the one experiment in which the recipient mice did not become diabetic (Table 1, experiment 2), the experimental animals received only one injection of cells, suggesting that the disease transfer is dose dependent.

The observation that a CD4 T cell alone can transfer diabetes is different from earlier studies in the NOD mouse in which induction of diabetes depended on the presence of both CD4 and CD8 T cells (*4, 10*). Our results do not rule out a role for CD8 T cells; they do show that a primed CD4 T cell can accelerate diabetes in a diabetes-prone environment in the absence of a specifically primed CD8 T cell, suggesting that the CD4 T cell initiates the disease process. Another difference in our experiments from other disease transfer studies is that our recipient animals were not irradiated, a condition that has been a prerequisite for effective transfer of disease in other studies (*3, 10*). Transfer of disease with a single cloned T cell line in unmanipulated animals provides a more physiological system in which to study the pathogenesis of diabetes. The roles of the diabetes-prone environment and that of CD8 T cells in the diabetic process remain undefined.

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7. The (CBA × NOD)F_1 mouse was chosen as the recipient animal in islet transplant studies and initial disease transfer efforts because this NOD F_1 mouse is not prone to diabetes and does not exhibit any pancreatic infiltrate.
8. The suitability of young NOD mice as nondiabetic recipients was indicated because histological examination of pancreatic sections from 19 mice (male and female) that were 4 to 6 weeks old revealed no animals with signs of intraislet infiltration (insulitis) or β cell degranulation, although approximately one-third of the animals showed scattered, minor foci of periductular or perivascular infiltrate, or both (histological grade of +/−, Table 1). Virtually no animals in our colony (NOD/Bdc) developed hyperglycemia before 3 months of age (M. McDuffie, unpublished observations).
9. BDC-2.5 and BDC-6.9 were chosen for disease transfer studies because they are the most extensively characterized islet-specific clones in our panel, both

in vitro and in vivo. BDC-2.5 reacts with islet cell antigen from various mouse strains, whereas BDC-6.9 responds only to NOD islet cells. In addition, the latter clone appeared to be particularly effective in previous in vivo studies. BDC-2.4, a clone from the same line as BDC-2.5, but not islet-specific because it responds to NOD APC in the absence of added antigen, provided an activated CD4 clone as a nonislet-specific control.

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Reversal of Experimental Parkinsonism by Lesions of the Subthalamic Nucleus

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Although it is known that Parkinson's disease results from a loss of dopaminergic neurons in the substantia nigra, the resulting alterations in activity in the basal ganglia responsible for parkinsonian motor deficits are still poorly characterized. Recently, increased activity in the subthalamic nucleus has been implicated in the motor abnormalities. To test this hypothesis, the effects of lesions of the subthalamic nucleus were evaluated in monkeys rendered parkinsonian by treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The lesions reduced all of the major motor disturbances in the contralateral limbs, including akinesia, rigidity, and tremor. This result supports the postulated role of excessive activity in the subthalamic nucleus in Parkinson's disease.

PARKINSON'S DISEASE IS CHARACTERIZED by akinesia (poverty of movement), muscular rigidity, and tremor. Traditionally, akinesia has been viewed as a "negative" sign, that is, a loss of function due to tissue damage per se that cannot be restored by subsequent lesions. In contrast, rigidity and tremor have been viewed as "positive" signs, resulting from excessive activity of the remaining neuronal systems (*1*).

Several lines of evidence (*2, 3*) suggest that loss of dopamine in Parkinson's disease ultimately results in an increased (inhibitory) output from the basal ganglia to the thalamus (Fig. 1, A and B). Because the net action of dopamine appears to be different on two subpopulations of striatal output neurons, dopamine depletion results also in different effects. Loss of striatal dopamine causes a decrease in the activity of (inhibitory) striatal neurons projecting directly to the internal division of the globus pallidus (GPi) and an increase in the activity of (inhibitory) striatal neurons projecting to the external division of the globus pallidus (GPe). Increased inhibition of the GPe allows more activity in the subthalamic nucleus (STN). The increased activity of STN neurons exerts an enhanced (excitatory) drive on neurons in GPi. The increased GPi output leads in turn to increased inhibition of the thalamus and thalamocortical neurons. The resulting reduction of cortical activation would then account for some of the parkinsonian signs.

In this model, excessive output from the STN is postulated to play a critical role in the pathophysiology of Parkinson's disease. The two lines of evidence needed to confirm this hypothesis are (i) direct measurement of increased activity of STN neurons in parkinsonian animals and (ii) a determination of the effects of lesions of the STN in such animals. These questions are best studied in monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a model of Parkinson's disease similar to human parkinsonism. In an initial study of such monkeys, the activity of STN neurons was increased over that in normal animals (*3*). Moreover, in the animals used in the present study we also observed increased tonic discharge rates of STN neurons after treatment with MPTP as compared to before treatment (*5*). We present here the behavioral effects of lesions of the STN in MPTP-treated monkeys (Fig. 1C).

In two African green monkeys (*Cercopithecus aethiops aethiops*; monkeys C-67 and D-32), recording chambers were attached to the skull to allow access to the subthalamic area (*6*). During all subsequent stages of the experiment, the behavior of the animals was objectively quantified (*7*). First, the animals were treated systemically with MPTP (*6*). The earliest behavioral effect was akinesia, which appeared 5 to 6 days after the first injection and increased until both monkeys sat largely motionless in their cages (*7*) (Fig. 2A). Several days after the appearance of akinesia, they developed muscular rigidity with cogwheeling (*7*) (Fig. 2B) and an intermittent 5-Hz postural tremor involving the proximal limb muscles and trunk (*7*) (Fig. 2C). Other parkinsonian signs such as postural instability and drooling were also observed.

After the animals had developed stable parkinsonian signs, a combined injection-recording device, which allowed simultaneous injection of drugs and recording of neuronal activity close to the injection site, was used to map the subthalamic area and to place injections of ibotenic acid (IBO) (*6*) in the STN under electrophysiological guidance. Within 1 min after the injection, both monkeys began to move the contralateral extremities (Fig. 3A). Purposeful move-

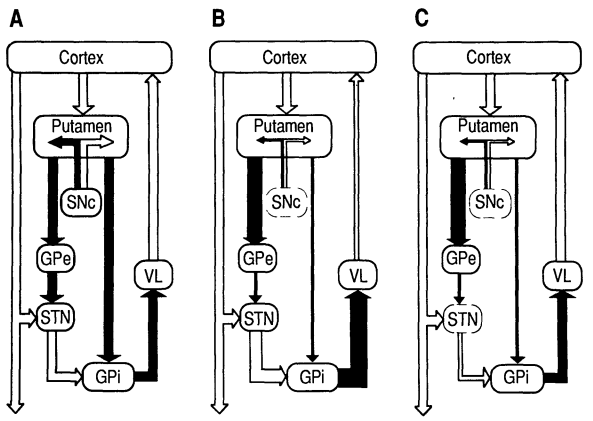

Fig. 1. Functional connectivity within the basal ganglia-thalamocortical circuit. (**A**) Normal. Open arrows, excitatory connections; filled arrows, inhibitory connections. SNc, substantia nigra pars compacta; VL, ventro lateral nucleus of the thalamus. The putamen (the "input" stage of the circuit) is connected with GPi (the "output" stage) by direct and indirect projections (via GPe and the STN). The postulated differential effects of dopamine on the two striatal output systems are indicated schematically. (**B**) MPTP-induced parkinsonism. After treatment with MPTP, the SNc is damaged. Resulting changes in the overall activity in individual projection systems are indicated by changes in the width of arrows. Inactivation of the nigroputaminal projection increases GPi activity, secondary to an increase in excitatory drive from the STN and a decrease in direct inhibitory input from the striatum. The resulting overinhibition of thalamocortical circuits may account for some of the parkinsonian motor signs. (**C**) Effect of STN lesions in parkinsonism. Inactivation of the STN reduces GPi output to the thalamus toward more normal levels, thus reducing parkinsonian motor signs.

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