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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

HAGAI BERGMAN, THOMAS WICHMANN, MAHLON R. DELONG

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-

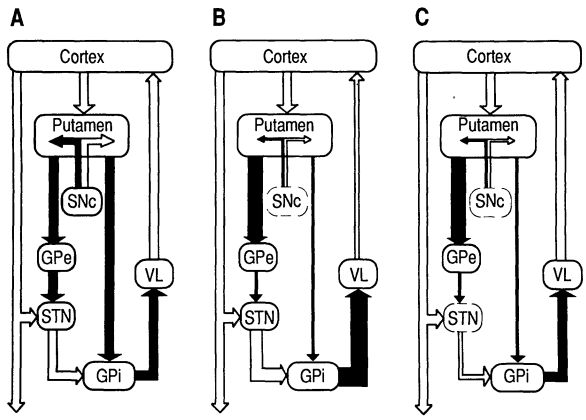

Fig. 1. Functional connectivity within the basal ganglia-thalamocortical circuit. (**A**) Normal. Open arrows, excitatory connections; filled arrows, inhibitory connections. SNc, substantia nigra pars compacta; VL, ventro lateral nucleus of the thalamus. The putamen (the "input" stage of the circuit) 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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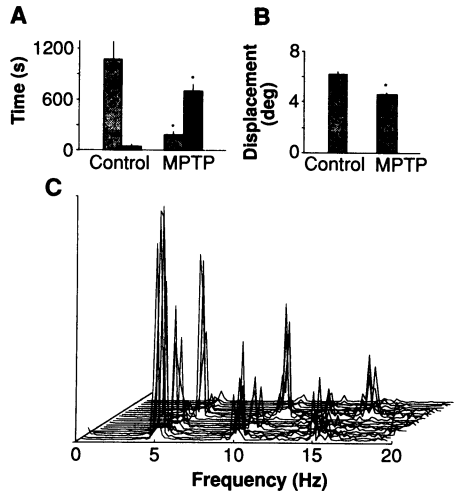

Fig. 2. Parkinsonian signs in MPTP-treated monkeys. (**A**) Akinesia and tremor. Bars represent the amount of time per 30 min (mean ± SEM) that in monkey D-32 movement (shaded bar) and tremor (solid bar) were present in the head, trunk, and limbs before (five observations) and after MPTP treatment (four observations). $*P < 0.01$; t test. (**B**) Rigidity. Bars represent the maximal forearm displacement (mean ± SEM) in monkey C-67, induced by elbow torque pulses, before (236 observations) and after MPTP treatment (108 observations). $*P < 0.01$; t test. (**C**) Tremor. An example of power spectra for successive trains of 1024 data points per train (z axis) of wrist tremor in monkey C-67. Ordinate, signal power (arbitrary units).

ments were markedly increased, such that the animals were again able to feed and groom themselves. However, some residual akinesia and clumsiness still remained in the contralateral limbs. Tremor was almost completely abolished contralaterally (Fig. 3A). Neurological examination and assessment of torque responses (Fig. 3B) showed that muscle tone was markedly reduced in the contralateral limbs as compared to the ipsilateral limbs. After lesioning, both animals developed transient dyskinesias of the contralateral arm and leg. In one monkey (C-67), dyskinesias appeared gradually after 24 hours, lessened within several days, and were no longer detectable after 1 week. In the other monkey (D-32), the dyskinesias appeared within minutes and then decreased gradually but had not fully disappeared even at the time the monkey was killed 3 weeks later.

Several weeks after lesioning, the monkeys were killed with an overdose of pentobarbital, and the brains were processed histologically. In both monkeys, tyrosine hydroxylase staining revealed an almost complete bilateral loss of dopaminergic cells in the substantia nigra with less damage in the ventral tegmental area. The lesioned STN showed marked gliosis and loss of neurons in cresyl violet stains (Fig. 3C). Lesions were confined to the STN, with no evidence of damage to nearby structures, such as the globus pallidus or the thalamus.

Our results support the postulated role of excessive STN activity in the pathophysiology of motor abnormalities in Parkinson's disease. Considering the traditional distinction between positive and negative parkinsonian signs, however, it was not expected that inactivation of the STN would improve all of the parkinsonian motor disturbances, in particular akinesia. Since previous attempts to ameliorate akinesia by lesions have been unsuccessful (*8, 9*), akinesia was classified as a negative sign. The reversal of akinesia by STN lesions suggests instead that akinesia may result in large part from excessive STN and GPi activity. As such, akinesia could now be viewed as a positive sign, because it too results from excessive activity of remaining neural systems and can be reversed by subsequent lesions.

The marked effect of STN lesions on tremor was also unexpected because parkinsonian tremor has generally been postulated to result from oscillatory activity of thalamocortical loops, independent of basal ganglia circuitry (*9, 10*). Our findings suggest that the basal ganglia may participate directly in tremor production in Parkinson's disease. Conceivably, tremor could result from enhanced pacemaker-like activity in certain populations of neurons in the basal ganglia or from oscillations of unstable cortico-basal ganglia circuits due to increased gain (*3, 4*). Alternatively, STN lesions may influence oscillatory activity in thalamocortical circuits indirectly by reducing excessive pallidal output to the thalamus.

The effects of STN lesions on rigidity are more readily understood than the effects on akinesia or tremor because rigidity is alleviated in parkinsonian patients by lesions of GPi (*11*) or its thalamic projection targets (*8, 9*). The effect of STN lesions on rigidity may result from removal of excessive tonic and phasic excitatory drive on GPi, with resultant lowering of GPi output to more normal levels.

The development of transient dyskinesias of contralateral limbs after lesions of the STN was also not surprising, because such dyskinesias are well documented both in experimental animals (*12*) and in man (*13*). Although the dyskinesias gradually decreased, the amelioration of parkinsonian signs remained unchanged.

Our results suggest a potential clinical application for surgical or pharmacological (*14*) inactivation of the STN as a treatment for Parkinson's disease. However, further studies in experimental animals are necessary to examine the long-term effects of STN lesions and possible interactions with drug treatment.

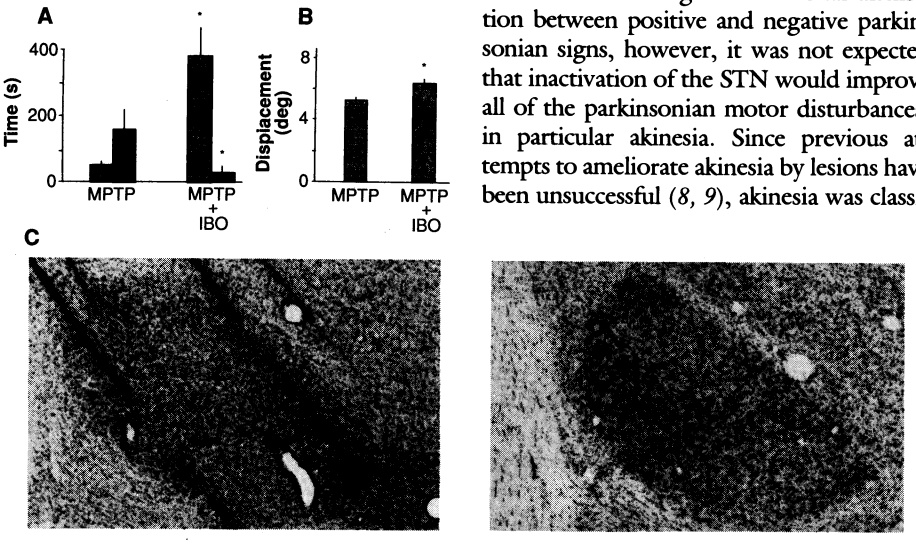

Fig. 3. Effects of IBO-lesions of the STN in parkinsonian monkeys. (**A**) Akinesia and tremor. Bars represent the amount of time per 30 min (mean ± SEM) before (8 observations) and after the lesion of the STN (16 observations), during which movement (shaded bar) and tremor (solid bar) were detected in the contralateral arm and leg. Pooled data from both monkeys; $*P < 0.01$; t test. (**B**) Rigidity. Bars represent the maximal displacement of the contralateral forearm (mean ± SEM), induced by elbow torque pulses before (151 observations) and after the lesion of the subthalamic nucleus (128 observations). Pooled data from both monkeys; $*P < 0.01$; t test. (**C**) Structural damage induced by the lesion. Cresyl violet stain of a sagittal section of the lesioned STN (left) and of the corresponding section from the unlesioned side (right) in monkey D-32. Note injection electrode tracts on the lesioned side.

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7. The behavior of the monkeys in their home cages was videotaped and the monkeys were neurologically examined. A computer-assisted method of behavioral assessment was used to quantify the amount of tremor and movement. An observer watched the monkeys in their home cages and pressed specific keys on a keyboard whenever movement or tremor occurred. The computer measured the amount of time given keys were pressed. This method was used during the MPTP- and IBO-treated stage in monkey C-67 and throughout all stages of the experiment in monkey D-32. An accelerometer (Entran Devices, Fairfield, NJ) attached to the wrists or heads of the monkeys was used to obtain power spectra of tremor. The output was amplified, filtered (0 to 50 Hz), digitally sampled at 200 Hz, and processed off-line. The forearm displacement evoked by application of elbow torque pulses yielded a measure of rigidity. For this, the monkeys were seated in a primate chair with one arm in a manipulandum through which flexion torque pulses (60 ms; 0.1 N-m), generated by a torque motor, were applied. The manipulandum was coupled to a potentiometer with an output, indicating the position of the handle, that was digitally sampled at 100 Hz and averaged over 5 to 15 trials to calculate maximal displacement values.
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15. We thank A. P. Georgopoulos and G. E. Alexander for reading the manuscript, C. A. Kitt for assistance with the histology, G. L. Wenk for help with IBO, and L. H. Rowland for technical assistance. Supported by NIH grant 5 R01 NS15417-09 and by a gift from the E. K. Dunn family. H.B. was a Chaim Weizman fellow.

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Xotch, the *Xenopus* Homolog of *Drosophila Notch*

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During the development of a vertebrate embryo, cell fate is determined by inductive signals passing between neighboring tissues. Such determinative interactions have been difficult to characterize fully without knowledge of the molecular mechanisms involved. Mutations of *Drosophila* and the nematode *Caenorhabditis elegans* have been isolated that define a family of related gene products involved in similar types of cellular inductions. One of these genes, the *Notch* gene from *Drosophila*, is involved with cell fate choices in the neurogenic region of the blastoderm, in the developing nervous system, and in the eye-antennal imaginal disc. Complementary DNA clones were isolated from *Xenopus* embryos with *Notch* DNA in order to investigate whether cell-cell interactions in vertebrate embryos also depend on *Notch*-like molecules. This approach identified a *Xenopus* molecule, *Xotch*, which is remarkably similar to *Drosophila Notch* in both structure and developmental expression.

Drosophila Notch IS A GENE REquired for local cell-cell interactions that specify cell fate in the fly embryo (*1*). Although the mechanisms underlying these interactions are not fully understood, the *Notch* gene product probably functions at the cell surface by permitting determinative interactions between cells. Because similar forms of cell interactions may also occur in vertebrate embryos, we probed an early neurula *Xenopus* cDNA library with *Notch* DNA using low stringency hybridization (*2–4*). This screening resulted in two overlapping cDNA clones that correspond to a 10-kb transcript and encode the amino acid sequence of *Xotch* (Fig. 1A). *Xotch* contains the three structural features by which *Notch* and the two nematode genes *lin-12* and *glp-1* are classified as a family of determinative, cell interaction molecules (Fig. 1B) (*2, 5–7*). Two of these features form a single extracellular domain consisting of multiple epidermal growth factor (EGF)–like repeats (10 to 36) and three *lin-12/Notch* repeats (defined by a region of similarity between these two genes) (*2, 6*). The third feature is within the intracellular domain and consists of six *cdc10/SWI6* repeats, a motif shared by molecules associated with cell-cycle regulation in yeast (*cdc10*, *SWI4*, and *SWI6*), human erythrocyte ankyrin, a sex-determining gene in *C. elegans* (*fem-1*), and a human proto-oncogene (*bcl-3*) (*8*). *Xotch*, like *Notch*, has exactly 36 EGF-like repeats. If the regions containing the EGF-like repeats in *Notch* and *Xotch* are aligned, 51% of the amino acids are identical. A similar alignment of *Xotch* to either *lin-12* or *glp-1* produces a lower match (36% and 39%, respectively) (*5–7*). Closer analysis of individual repeats shows that *Xotch* and *Notch* share amino acids in addition to the ones that make up the consensus sequence and that even irregular spacings are preserved between corresponding repeats. This conservation implies that the repeats are not interchangeable and that minor differences between each repeat are required for function. Such an interpretation is supported by genetic analysis of *Drosophila Notch* in which a single amino acid change within the repeat region can result in a mutant phenotype (*9, 10*). The portion of *Xotch* most highly conserved relative to *Notch* (70% identity) contains the cytoplasmic *cdc10/SWI6* repeats and downstream 30 amino acids. This degree of conservation further indicates the probable involvement of this region in mediating the intracellular functions of the *Xotch* and *Notch* proteins (*8*).

Some areas of similarity between *Notch* and *Xotch* are not shared by *lin-12* and *glp-1*. One area is a stretch of polyglutamine residues encoded by a sequence referred to in *Drosophila* as the *opa* or M-repeat (*11*). The *opa* repeat has no known function, but is a molecular feature found in many *Drosophila* genes. The presence of an apparent skeleton of the *opa* repeat in the *Xotch* sequence is further evidence of the relatedness of these molecules. Another area of similarity, at the extreme COOH-terminus, contains a cluster of proline, serine, and threonine residues referred to as a PEST sequence, some of which are putative sites of phosphorylation (*12, 13*). The PEST sequence may be involved in decreasing protein stability. The similarities between *Xotch* and *Notch* indicate that the two are homologs and suggest that both could have identical functions during development.

To analyze the functions of *Xotch*, we began by studying the expression of *Xotch* RNA during frog embryogenesis using a ribonuclease (RNase) protection assay (*14*). The pattern of *Xotch* expression was similar in many respects to the pattern of *Notch* expression that has been described for fly development (*9, 15*). *Xotch* RNA was pre-

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