# Recovery from Experimental Parkinsonism in Primates with $G_{M1}$ Ganglioside Treatment

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**A parkinsonian syndrome can be produced in nonhuman primates by administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Parkinsonian-like symptoms induced acutely by MPTP were ameliorated after treatment with $G_{M1}$ ganglioside, a substance shown to have neurotrophic effects on the damaged dopamine system in rodents. Treatment with $G_{M1}$ ganglioside also increased striatal dopamine and metabolite levels and enhanced the dopaminergic innervation of the striatum as demonstrated by tyrosine hydroxylase immunohistochemistry. These results suggest that $G_{M1}$ ganglioside may hold promise as a therapeutic agent for the treatment of Parkinson's disease.**

Parkinson's disease (PD) is a degenerative neurological disorder that generally affects individuals over 45 years of age (*1*). The symptoms of PD (akinesia, rigidity, bradykinesia, stooped posture, and shuffling gait) appear after at least 85% of the dopaminergic innervation of the striatum has been lost as a result of degeneration of dopamine-producing neurons in the substantia nigra (SN) pars compacta (*2*). Long-term treatment of this disease is difficult because of the progressive degeneration of the nigrostriatal dopamine system (*3*). Chronic dopamine replacement therapy (levodopa in combination with a peripheral dopa decarboxylase inhibitor) does not treat the underlying pathogenic disorder and may contribute to the further degeneration of the remaining elements of the dopaminergic system (*4*). The monoamine oxidase B inhibitor L-deprenyl may slow the progression of the degeneration in PD patients if administered early in the disease process, but conclusive evidence for such a protective effect is unavailable (*5*).

The $G_{M1}$ ganglioside may stabilize injured or dying SN dopamine neurons and perhaps stimulate sprouting of new dopaminergic fibers and terminals from remaining intact ventral mesencephalic neurons (*6*). A monosialoganglioside, $G_{M1}$ ganglioside modulates a number of cell surface and receptor activities as well as neuronal differentiation and development, protein phosphorylation, and synaptic function (*7*). Chronic treatment of rats with $G_{M1}$ ganglioside after various types of lesions to the central nervous system (*8*, *9*) has resulted in biochemical and behavioral recovery. These effects have been particularly impressive in the damaged dopamine system, although rodent lesion models do not result in spontaneous parkinsonian motor or behavioral deficits. We examined the effects of chronic $G_{M1}$ ganglioside treatment on monkeys [*Saimiri sciureus* (squirrel) and *Macaca fascicularis* (cynomolgus) monkeys] made overtly parkinsonian by systemic administration of the dopamine neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Deficits induced by MPTP in monkeys differ from those caused by idiopathic PD; deficits occur acutely after MPTP exposure, whereas symptoms of PD usually develop after many years of progressive degeneration of the dopamine system (*5*).

The behavior and neurologic function of 15 adult squirrel monkeys and 4 adult cynomolgus monkeys were rated in their home cages for 1 to 2 weeks before the start of the study. The rating scale assessed overall activity, climbing ability, locomotion and gait, gross upper and lower limb movements, fine motor skills, bradykinesia, dyskinesia, dystonia, posture, resting and intention tremor, balance, grooming ability, involuntary arrest during attempted movements, and eating ability. Cynomolgus monkeys were also assessed for changes in facial expression and defense reactions (*10*). Squirrel monkeys were tested on a simple motor function task (reaching for food in recessed wells) that allowed measurement of response initiation times as well as assessment of limb function (*11*). The cynomolgus monkeys were trained to perform an object retrieval task (*12*), which is a sensitive measure of both motor and cognitive function in MPTP-treated monkeys (*13*).

MPTP-HCl was dissolved in physiological saline and delivered to all monkeys either intravenously or intramuscularly (*14*) every second or third day until a distinct parkinsonian motor syndrome developed (*15*). This syndrome usually consisted of severe akinesia (lack of movement or cage climbing), stooped posture, rigidity, decreased self-initiated food and water consumption, and lack of grooming behavior. If these symptoms were observed for 48 to 60 hours after the last of the MPTP injections, animals were paired and randomly assigned to receive either daily $G_{M1}$ ganglioside injections [15 mg per kilogram of body weight for cynomolgus monkeys ($n$ = 2) and 30 mg per kilogram of body weight for squirrel monkeys ($n$ = 7), all injected intramuscularly] or saline vehicle injections for 6 weeks (squirrel monkeys; $n$ = 8) or 8 weeks (cynomolgus monkeys; $n$ = 2). Injections, observations, and testing were performed with a rater blind with respect to the treatment conditions. All animals, after MPTP treatment, required hand feeding and hydration (*16*) in order to maintain their health.

All monkeys initially developed comparably severe parkinsonism in response to the MPTP treatment (Fig. 1). Maximum disability scores for squirrel monkeys ($n$ = 15) and macaques ($n$ = 4) were 44 and 49, respectively; completely normal monkeys had a rating of 0 (*10*). All monkeys remained severely parkinsonian for approximately the first 3 weeks after MPTP treatment was stopped. The condition of the $G_{M1}$-treated monkeys improved, and after 5 to 6 weeks they were relatively normal. The mean Parkinson rating improved 86% (± 8) for squirrel monkeys ($P < 0.001$ compared to initial post-MPTP rating) and 89%

**Table 1.** Effects of MPTP and $G_{M1}$ ganglioside treatment on object retrieval performance (*12*) by cynomolgus monkeys. In object retrieval, the monkeys had to reach into a clear box with one open side to retrieve food. The open side of the box faced front, left, or right relative to the monkey. Normal monkeys ($n$ = 6) and MPTP-$G_{M1}$–treated monkeys ($n$ = 2, assessed at the conclusion of the study and essentially motor-asymptomatic) retrieved the food target on the first trial (success on first attempt) most of the time, eventually correctly completed the trial (correct responses) even if unsuccessful on the first attempt, and made no barrier reaches (reaches toward the closed side of the box). MPTP-asymptomatic monkeys (MPTP-A) [exposed to low doses of MPTP over 45 to 175 days as part of another study (*17*)] were also without significant gross motor impairment but did have difficulty with the cognitive aspects of the object retrieval task (as shown by the number of barrier reaches). MPTP-saline–treated monkeys could not be tested effectively for object retrieval performance because of the severe nature of their motor deficits. The SEM is in parentheses.

| | Success on first attempt (%) | Correct responses (%) | Barrier reaches ($n$) |
|---|---|---|---|
| Normal | 97.2(2.4) | 100.0(0.0) | 0.0(0.0) |
| MPTP + $G_{M1}$ | 98.3(1.7) | 100.0(0.0) | 0.0(0.0) |
| MPTP-A | 43.5(4.4) | 76.0(15.2) | 7.2(3.2) |

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(± 10) for cynomolgus monkeys (compared to initial post-MPTP rating) (Fig. 1, A and B). Monkeys that received saline treatment after MPTP exposure showed minimal improvement over the course of the study [squirrel monkeys = 15% (± 18) improvement; cynomolgus monkeys = 27% (± 15) improvement]. By 6 weeks (squirrel monkeys) or by 8 weeks (cynomolgus monkeys), neurological rating scores for saline-treated animals were not significantly improved from the initial post-MPTP ratings (Fig. 1, A and B). Differences in neurological ratings between saline- and $G_{M1}$-treated monkeys were statistically significant beginning at 3 to 4 weeks and remained so for 6 to 8 weeks (Fig. 1, A and B).

Saline- and $G_{M1}$-treated monkeys also differed on performance of motor function tests. All squirrel monkeys performed the food retrieval task similarly before MPTP treatment and all had similar performance deficits during the first 2 weeks of the post-MPTP period (Fig. 1C). Over the next 4 weeks, $G_{M1}$-treated monkeys ($n$ = 4) significantly decreased their task initiation times and after 6 weeks of treatment had significantly shorter response initiation times ($P < 0.002$) (Fig. 1C) and were more successful (Fig. 1D) in retrieving food from the wells than were their saline-treated counterparts ($n$ = 5).

Cynomolgus monkeys treated with $G_{M1}$ also differed significantly from saline-treated animals on object retrieval performance. Performance deficits on this task can be a result of motor or cognitive difficulties or a combination of these factors (*13*). In the initial post-MPTP period, none of the animals could perform the object retrieval task because of their severe motor impairment. However, after 5 to 6 weeks of $G_{M1}$ treatment, two monkeys again performed the task normally and retrieved food on their first attempt. Testing of saline-treated monkeys was difficult throughout the study because of their severe motor impairments. However, when they did respond, these monkeys had difficulty with both the motor and cognitive requirements of the task (Table 1).

The reversal of all but minor and occasional bradykinesia and gait disturbances in $G_{M1}$-treated monkeys was accompanied by an apparent lack of residual cognitive deficits as demonstrated by performance of the object retrieval task. Preliminary data (Table 1) suggest that motor-asymptomatic monkeys chronically exposed to low doses of MPTP have difficulty performing the object retrieval task (*17*), as do other MPTP-treated monkeys with no gross motor impairments (*13*). Other than $G_{M1}$ treatment, only implantation of fetal SN grafts into the striatum has resulted in improvement in cognitive performance (object retrieval) in MPTP-treated monkeys (*13*).

Postmortem neurochemical examina-

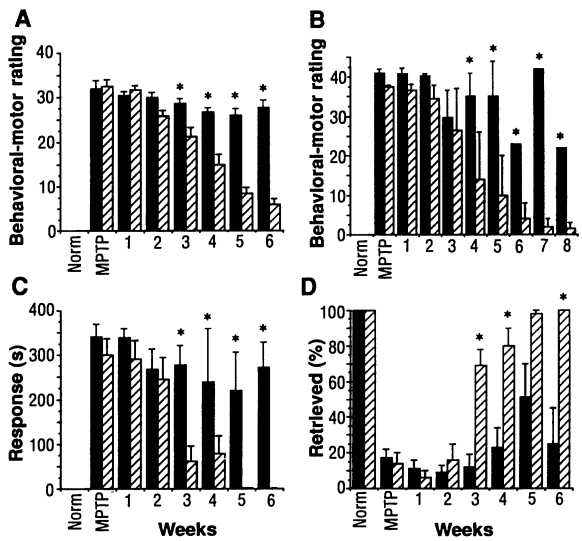


**Fig. 1.** Effects of MPTP and $G_{M1}$ ganglioside on motor behavior. Motor rating scores for squirrel monkeys (**A**) and cynomolgus monkeys (**B**). Bars represent mean rating scores; error bars represent SEM. Solid and hatched bars represent data from MPTP + saline–treated monkeys and MPTP + $G_{M1}$–treated monkeys, respectively. The rating of normal monkeys (Norm) was 0. Ratings labeled MPTP were obtained after the last MPTP injection but before $G_{M1}$ or saline administration. Squirrel monkeys received treatment for 6 weeks after MPTP, whereas cynomolgus monkeys received treatment for 8 weeks after MPTP. Squirrel monkey motor rating data were obtained from eight MPTP + saline–treated animals and seven MPTP + $G_{M1}$–treated animals. Cynomolgus monkey rating data were obtained from two monkeys in each condition. Asterisks represent significant differences [$P < 0.01$ in (A); $P < 0.05$ in (B), Mann-Whitney test] between MPTP + saline– and MPTP + $G_{M1}$–treated monkeys. (**C**) Response initiation times in seconds (s) and (**D**) percent rewards retrieved for squirrel monkeys performing a food retrieval task. Solid and hatched bars represent data from five MPTP + saline–treated monkeys and four MPTP + $G_{M1}$–treated monkeys, respectively. Normal monkeys (Norm) initiated a response immediately upon presentation of the food tray. Ratings labeled MPTP were obtained after the last MPTP injection but before $G_{M1}$ or saline administration. Asterisks represent significant differences [$P < 0.005$ in (C); $P < 0.05$ in (D), Mann-Whitney test] between MPTP + saline– and MPTP + $G_{M1}$–treated monkeys.

**Table 2.** Effects of MPTP and $G_{M1}$ ganglioside treatment on striatal dopamine and metabolite levels in squirrel and cynomolgus monkeys. Each value represents mean (± SEM) nanograms per milligram of protein. In seven squirrel monkeys studied, dopamine (DA), homovanillic acid (HVA), and 3,4-dihydroxyphenylacetic acid (DOPAC) amounts in all striatal regions sampled were higher than amounts measured in similar regions from eight saline-treated control animals. Similarly, in two cynomolgus monkeys studied, striatal DA and metabolite amounts were higher in animals given chronic $G_{M1}$ treatment (except in the dorsolateral putamen where there was not a significant increase) as compared to amounts measured in two saline-injected control animals. Normal values from three squirrel monkeys and one cynomolgus monkey are given for comparison. DLC, dorsolateral caudate; DLP, dorsolateral putamen; VMC, ventromedial caudate; VMP, ventromedial putamen. The SEM is in parentheses.

| Striatal region | MPTP/$G_{M1}$ | | | MPTP/saline | | | Normal | | |
|---|---|---|---|---|---|---|---|---|---|
| | DA | HVA | DOPAC | DA | HVA | DOPAC | DA | HVA | DOPAC |
| | | | | *Squirrel monkeys* | | | | | |
| DLC | 8.6(1.2)* | 46.2(6.5)* | 3.3(0.9)* | 2.4(0.6) | 16.4(2.7) | 0.5(1.5) | 161.7(12.3) | 117.3(17.3) | 15.1(0.9) |
| VMC | 37.0(4.2)* | 56.6(11.9)* | 12.7(3.6)* | 8.9(2.7) | 21.0(3.3) | 3.0(1.4) | 153.9(29.5) | 124.5(15.2) | 20.8(3.3) |
| DLP | 4.2(0.8)* | 45.9(6.9)* | 1.3(0.2)* | 1.4(0.4) | 11.9(1.4) | 0.5(0.2) | 188.8(45.9) | 144.5(4.5) | 19.0(6.1) |
| VMP | 21.9(6.1)* | 87.5(17.3)* | 5.9(1.6)* | 3.8(0.8) | 35.7(6.5) | 1.5(0.4) | 193.4(41.2) | 147.5(22.1) | 22.8(4.4) |
| | | | | *Cynomolgus monkeys* | | | | | |
| DLC | 4.4(0.6)* | 55.9(12.0)* | 2.7(0.3)* | 0.5(0.2) | 5.3(0.7) | 0.3(0.1) | 167.0 | 140.2 | 26.3 |
| VMC | 12.9(2.8)* | 60.9(6.9)* | 6.6(0.7)* | 1.2(0.7) | 15.5(1.8) | 1.7(0.6) | 141.5 | 131.5 | 29.0 |
| DLP | 3.7(0.9) | 51.9(9.4)* | 2.1(0.8)* | 0.9(0.22) | 7.4(1.7) | 0.3(0.2) | 160.8 | 154.3 | 24.3 |
| VMP | 11.2(4.1)* | 100.3(11.9)* | 7.1(3.2)* | 1.7(0.3) | 21.1(3.3) | 1.0(0.5) | 158.5 | 186.4 | 34.8 |

*$P < 0.05$, Mann-Whitney test.

tion of striatal tissue (*18*) revealed large dopamine (and metabolite) depletions in all MPTP-treated animals and increased dopamine and metabolite levels in most striatal subregions sampled from the $G_{M1}$-treated monkeys as compared to those from saline-treated control animals (Table 2). The MPTP caused greater dopamine depletion in dorsolateral (DL) striatal regions than in ventromedial (VM) regions in both squirrel and cynomolgus monkeys. Consequently, $G_{M1}$ treatment caused a greater overall increase in dopamine levels in the VM striatum than in the DL striatum.

All brains were examined for tyrosine hydroxylase (TH) immunohistochemistry, which revealed increased TH-positive staining in both DL and VM striatal regions in $G_{M1}$-treated animals in comparison to the same regions in saline-treated controls (Fig. 2), with a larger increase in the innervation of the VM striatum than that of the DL striatum. These findings were consistent in all cases. Increased striatal dopamine levels and TH immunohistochemical staining in the striatum of $G_{M1}$-treated monkeys may be a result of a sprouting effect (*19*), a $G_{M1}$-induced stabilization of MPTP-damaged neurons (*20*) and promotion of their recovery, or a combination of these factors. Treatment with $G_{M1}$ was initiated 48 to 60 hours after the last MPTP exposure, when an active degenerative process was still taking place. Neurons damaged by MPTP exposure but still viable may have been saved by $G_{M1}$ treatment and stimulated to repair themselves and perhaps sprout new processes. This result is supported by in vitro findings that after exposure to $MPP^+$, the toxic oxidation product of MPTP, surviving fetal dopaminergic neurons can be stimulated to recover normal soma morphology and sprout processes in the presence of $G_{M1}$ ganglioside (*21*).

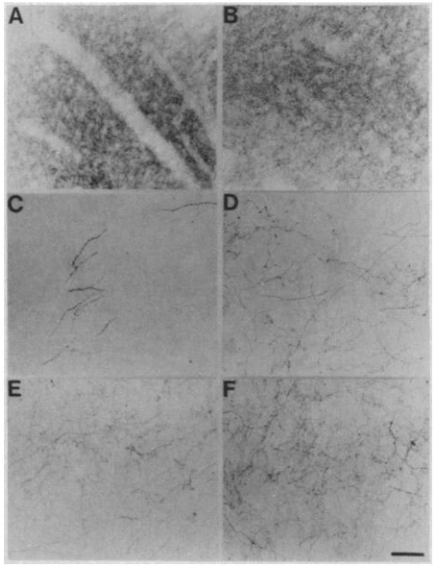


**Fig. 2.** Effects of MPTP and $G_{M1}$ ganglioside treatment on TH immunohistochemistry of the squirrel monkey putamen. TH staining of the (**A**) DL and (**B**) VM putamen in a normal monkey and TH staining in (**C**) DL and (**D**) VM putamen of a monkey that received MPTP and 6 weeks of saline treatment. Dopaminergic innervation of (**E**) DL and (**F**) VM putamen in an MPTP-treated monkey that received 6 weeks of $G_{M1}$ treatment. Bar represents 60 μm.

Although dopamine levels in the striata of MPTP-$G_{M1}$–treated monkeys were generally higher than those in MPTP-saline–treated animals, the absolute levels of tissue dopamine present appeared to be only a fraction of the normal dopamine content of these regions (*22*). Dopamine content of squirrel monkeys treated with MPTP and saline (and therefore severely parkinsonian) was 0.6 to 1.5% of that of normal monkeys in the dorsal striatum and 2 to 6% in the ventral striatum. Dopamine content in MPTP- and $G_{M1}$-treated monkeys (with relatively normal motor function) was approximately 3 to 6% in the dorsal striatum and 11 to 24% in the ventral striatum. These results were similar for cynomolgus monkeys. This finding supports earlier suggestions that only a small amount of the nigrostriatal dopamine system needs to be spared, or perhaps a small amount of functional sprouting may be sufficient to result in normal motor and cognitive function (*23*).

The mechanisms of action of $G_{M1}$ on the damaged dopamine system are presently unclear. Recent studies have shown, however, that after systemic administration, $G_{M1}$ is taken up by the brain, penetrates into neural cells, associates with plasma membranes and intracellular structures (such as the Golgi apparatus), and eventually undergoes extensive metabolic processing (*24*). $G_{M1}$ may potentiate a cell's response to endogenous trophic or injury-related factors by modulation of cell surface transduction events or specific factor-induced posttranslational events (*6*). It is possible that under the MPTP lesion condition, the monkeys may have had a propensity for spontaneous recovery, which was somehow accelerated by $G_{M1}$ ganglioside treatment. Such a phenomenon has been described in cats made parkinsonian by MPTP (*9*). Nonetheless, lesioned monkeys used as controls showed little or no evidence of spontaneous behavioral, neurochemical, or anatomical recovery over the 6 to 8 weeks of study.

These results suggest a potential new therapeutic strategy for the treatment of PD. Early in the disease process, administration of $G_{M1}$ ganglioside or other factors that provide trophic support for degenerating dopaminergic neurons or that stimulate sprouting of new fibers and terminals might slow or stop the disease's degenerative process.

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10. The rating scale used combines features of two previously published rating scales [J. S. Schneider and C. J. Kovelowski, Jr., *Brain Res.* **519**, 122 (1990); R. Kurlan, M. H. Kim, D. M. Gash, *Movement Disorders* **6**, 111 (1991)]. Behaviors and functions rated included overall activity, locomotion and gait, climbing ability, gross motor abilities of the upper and lower limbs including range of limb movements, fine motor control of the arm and hand, bradykinesia and akinesia, dystonia, balance, posture, involuntary arrest during movement, tremor, grooming ability, and eating behavior. Cynomolgus monkeys were also rated on facial expression and defense reactions. A score of 0 was normal for all features, and on most measures, a score of 1 signified mild disability, 2 was moderate, and 3 was severe. Some measures had a rating of 2 (posture, involuntary arrest, grooming, and overall activity) or 4 (bradykinesia and akinesia) as most severe.
11. Monkeys were trained to reach outside their cages to retrieve raisins from a Plexiglas platform that contained nine 5-mm-diameter wells. Time to initiate retrieval and the number of raisins retrieved within a maximum 6-min time limit were recorded. All testing was done before the first daily feeding.
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14. MPTP-HCl was dissolved in sterile saline and administered intramuscularly to squirrel monkeys and intravenously to cynomolgus monkeys after restraint anesthesia with ketamine HCl (5 mg per

kilogram of body weight, injected intramuscularly). Squirrel monkeys received 2 mg of MPTP per kilogram of body weight, whereas cynomolgus monkeys received a mean of 0.35 mg of MPTP per kilogram of body weight.

15. The MPTP was administered every third day until animals were akinetic, unresponsive to stimuli, unable to climb, and not eating effectively on their own. A rating score (for any given pair of animals) of at least 30 (*10*) needed to be achieved on three consecutive days before cessation of MPTP injections and random assignment to treatment groups. Administration of MPTP and degree of initial symptomatology were similar for animals chosen to receive saline or $G_{M1}$ ganglioside treatment.
16. During the first several weeks of the study, all animals required intensive hand feeding and hydration and sometimes received dopamine agonist intramuscularly (LY-171555; 0.1 mg per kilogram of body weight) to facilitate feeding. Animals were fed fruit, crushed chow, liquid diet (Primate Liquid Diet, BioServ, Frenchtown, NJ), and Gatorade. Occasionally, feedings needed to be supplemented with parenteral lactated Ringers solution (15 ml twice daily). Four to 5 weeks after MPTP was stopped, $G_{M1}$-treated monkeys resumed spontaneous eating and drinking while many of the saline-treated monkeys needed to be continued on some type of nutritional support for the 6 to 8 weeks of study. Animal care was administered in accordance with institutional guidelines.
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# Lateralization of Phonetic and Pitch Discrimination in Speech Processing

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**Cerebral activation was measured with positron emission tomography in ten human volunteers. The primary auditory cortex showed increased activity in response to noise bursts, whereas acoustically matched speech syllables activated secondary auditory cortices bilaterally. Instructions to make judgments about different attributes of the same speech signal resulted in activation of specific lateralized neural systems. Discrimination of phonetic structure led to increased activity in part of Broca's area of the left hemisphere, suggesting a role for articulatory recoding in phonetic perception. Processing changes in pitch produced activation of the right prefrontal cortex, consistent with the importance of right-hemisphere mechanisms in pitch perception.**

Extracting information from complex signals is one important function of the auditory nervous system. The cortex is crucial for many aspects of auditory cognition (*1*), although considerable subcortical neural processing occurs before information reaches the cortex. There is much evidence that specialized speech-decoding mechanisms rely on perisylvian areas in the left cerebral hemisphere (*2*), whereas certain aspects of pitch perception depend more on systems within the right hemisphere (*3*). Positron emission tomography (PET) studies have demonstrated activation of the primary auditory cortex with simple auditory stimuli (*4*) and bilateral activation of the superior temporal gyrus during passive word presentation (*5*, *6*), but the precise neural substrate for specialized linguistic and nonlinguistic processing mechanisms remains largely unknown.

We measured cerebral blood flow (CBF) changes with PET to examine several issues. First, we wished to clarify the role of primary versus secondary auditory regions in speech perception. We hypothesized that simple auditory stimulation should lead to activation of the primary cortex, whereas more complex signals should lead to activity in associative areas. Second, we tested the hypothesis that phonological processing depends on the left temporoparietal cortex (*5*) by using a phonetic discrimination task. Third, we attempted to dissociate linguistic from nonlinguistic processing by requiring judgments of pitch changes in the speech syllable, which we hypothesized to involve right-hemisphere mechanisms (*3*).

Ten adult volunteers (*7*) were given PET scans with the paired-image subtraction paradigm (*5*, *8*, *9*). Two types of stimuli were used: pairs of noise bursts (*10*) and pairs of consonant-vowel-consonant real speech syllables. The vowels in any given syllable pair were always different, but the final consonant differed in half of the pairs (Table 1); in addition, the second syllable had a higher fundamental frequency in half of the pairs and a lower frequency in the other half (*11*).

The study included five conditions (Table 1) arranged in a subtractive hierarchy (*5*). The first was a silent baseline; in the noise condition, subjects pressed a key to alternate pairs of noise bursts; in the passive speech condition, subjects listened to the syllables and pressed a key to alternate stim-

**Table 1.** Summary of paradigm. The five conditions are arranged hierarchically (*5*) so that subtractions may be performed holding constant all but the variables of interest. The last three conditions involved identical stimulation, but the expected response [key press (Y) or no response (N)] varied according to the instructions; the number of key presses expected was equal for all conditions. Accuracy and reaction time data, gathered on-line during scanning, substantiate that subjects were performing the intended judgments.

| Condition | Stimulus | Response | Example | Mean percent correct | Mean reaction time (ms) |
|---|---|---|---|---|---|
| Baseline | Silence | None | | | |
| Noise | Noise bursts | Alternating key press | | | |
| Passive speech | Speech syllables | Alternating key press | fat-tid (Y)<br>tig-lat (N)<br>bag-big (Y) | | 1382 |
| Phonetic | Speech syllables | Key press to same final consonant | fat-tid (N)<br>tig-lat (N)<br>bag-big (Y) | 97.9 | 1576 |
| Pitch | Speech syllables | Key press to rising pitch | fat-tid (N)<br>tig-lat (Y)<br>bag-big (N) | 88.5 | 1663 |

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