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L-Dopa-Responsive Movement Disorder Caused by *Nocardia asteroides* Localized in the Brains of Mice

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Nocardia asteroides **can cause infections in the brain of humans and a variety of animals. In mice, invasion of the central nervous system results in specific neurologic signs. Following intravenous injection of various doses of log-phase *N. asteroides* GUH-2 into female BALB/c mice, localization and growth of nocardial cells within the brains were determined, histopathological sections were prepared, and Nissl substance and tyrosine hydroxylase immunoreactivity were observed. Mice were monitored for the development of neurologic signs, and their responsiveness to L-dopa was determined. It was shown that nocardial cells became localized within specific regions of the brain and then underwent rapid growth followed by a delayed clearance, and there was no inflammatory response at the site of invasion for 24 h. Mice that received a subclinical dose of nocardiae developed specific neurologic signs that emerged following the elimination of nocardial cells from the brain. On the basis of the specific signs, mice could be divided into distinct groups. One group consisted of animals that had a form of hemiparesis that did not respond to L-dopa. They expressed a deviation of the head and a tendency to roll, and when suspended by the tail they would spin rapidly. The second group of mice developed a rhythmic, uncontrolled vertical shake of the head (four to five times per s) with tremulous movement, stooped posture, restlessness, and no signs of hemiparesis. The head shakes were temporarily stopped by treatment with L-dopa. Mice that expressed head shakes had a loss of Nissl substance and tyrosine hydroxylase immunoreactivity in the neurons of the substantia nigra and ventral tegmental areas of the brain. Hyaline inclusion bodies that resembled Lewy bodies were found in the neurons of mice with head shake 1 month after infection. Therefore, mice infected with *N. asteroides* may serve as a model for studying parkinsonian signs and other degenerative diseases involving extrapyramidal and pyramidal systems.**

Nocardia is a genus of aerobic gram-positive bacteria belonging to the actinomycetes (30). Pathogenic species of *Nocardia* are commonly found in soils worldwide, and infection in humans and other animals may occur following inhalation of the microorganisms into the respiratory tract or by traumatic inoculation (6). Persistent localization of nocardiae in the lungs without apparent clinical findings and hematogenous dissemination of the nocardiae to the central nervous system (CNS) does occur and may be relatively common (2, 39). Cerebral nocardiosis often has an insidious onset and usually presents as a severe form of brain abscess (4). However, because there are no reliable immunologic tests routinely used for diagnosing nocardial infections, less-severe CNS infections caused by *Nocardia* spp. may not be recognized or may be attributed to unknown or incorrect etiologic agents (2, 5, 6, 39).

During experiments investigating the mechanisms of host resistance to pathogenic nocardiae, it was observed that several strains of mice and rats experimentally infected with sublethal doses of different strains of *Nocardia* spp. developed a variety of neurologic signs which included a rhythmic vertical head shake, accompanied by tremulous movements, affecting groups of animals. It was suggested that similarities between these movement disorders in the experimental animals and those observed with Parkinson's disease (PD) in humans might involve similar mechanisms.

A review of the literature revealed that, in humans, *N. asteroides* can cause encephalitis with parkinsonian features (35). Richter et al. (35) reported on the following case. A 39-year-old male developed fever, myalgia, and arthralgia. Five days later, he experienced visual hallucinations and severe tremors developed. After 6 weeks of illness, his face was masklike, his speech was dysarthric, his movement was tremulous, and his muscle tone was increased. Generalized hyperreflexia with bilateral patellar and ankle clonus was noted. The patient experienced coarse and irregular tremors while at rest in all extremities including the head. *N. asteroides* was recovered from the cerebrospinal fluid, and the patient improved upon therapy with sulfadiazine (35). Furthermore, there are numerous reports in the literature describing involuntary, neurological manifestations in humans with brain infections caused by *N. asteroides* (1, 17, 26, 34, 35, 38, 40, 41). The clinical signs and symptoms of these patients with cerebral nocardiosis indicate a variety of neurological manifestations and an involvement of *N. asteroides* in extrapyramidal disorders.

N. asteroides GUH-2 has been studied extensively, and it infects the murine brain following intravenous injection (7). Mice that recover from a near-lethal dose of *N. asteroides* demonstrate numerous neurological signs, and these mice can be separated into well-defined groups based upon the specific set of signs that are present. By utilizing a nonlethal dose of *N. asteroides* GUH-2 in BALB/c mice, an experimental model for studying the subclinical form of cerebral

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nocardiosis was established. The purposes of this study were to localize the nocardiae within the brain, to determine the pathological basis for the vertical head shake and movement disorders that developed in some of these mice, and to determine whether similarities exist between this animal model and neurodegenerative diseases such as PD.

MATERIALS AND METHODS

Microorganism. *N. asteroides* GUH-2 was isolated from a fatal human infection at Georgetown University Hospital, Washington, D.C., and its pathogenesis has been studied extensively (7). In order to obtain a homogeneous single-cell suspension of *N. asteroides*, the organism was grown as previously described (7). Briefly, a stock culture was prepared by adding 1 drop of a 5-day broth culture of a frozen sample (−70°C) of the original human isolate into 50 ml of brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Mich.) in a 250-ml Erlenmeyer flask and incubated at 37°C with mild rotational agitation (150 rpm) in a New Brunswick Psychrotherm Environmental incubator. After 5 days, the culture was collected and centrifuged for 30 min at 3,600 × *g* in a Sorval RC5C centrifuge. The bacterial pellet was washed twice with sterile BHI broth in order to remove nocardial cell debris, and the washed pellets were resuspended in the original volume of fresh, sterile BHI broth and centrifuged for 15 min at 1,300 × *g*. The supernatants were collected and recentrifuged at 1,300 × *g* for 15 min. The supernatant of the second centrifugation contained a homogeneous single-cell suspension of coccoid cells of *N. asteroides*. These were transferred in 5-ml amounts to sterile plastic tubes and stored at −70°C to be used as the stock culture for all subsequent experiments.

Inoculum. Five milliliters of the stock culture was transferred into 50 ml of BHI broth and incubated for 16 h at 37°C with rotational agitation (150 rpm). The bacterial culture was passed through a sterile millipore filter (0.22-μM pore size; Millipore Corp.), and the filter was washed with an additional 50-ml volume of BHI broth. The nocardial cells on the filter were resuspended into 10 ml of sterile BHI broth and transferred into a centrifuge tube. The bacterial suspension was centrifuged twice for 5 min at 50 × *g*. The supernatant contained a homogeneous suspension of single filamentous log-phase bacteria that were then adjusted to a cell density of 0.02 by using a Spectronic 20 spectrophotometer (560 nm). Dilutions of this bacterial suspension were injected into mice. At the same time, the number of bacteria were quantitated by plating serial dilutions in tryptic soy agar.

Animals. Female BALB/c mice (specific pathogen free) averaging 18 to 20 g were obtained from Simonsens (Gilroy, Calif.). Following infection, these mice were placed in a special animal room supplied with filtered air, fed with Purina Laboratory Chow, and provided water ad libitum. They were maintained by the Animal Resource Service at the University of California, Davis. Sentinel mice from the same groups were randomly monitored for a variety of bacterial, viral, and parasitic infections. These mice were free of detectable mouse hepatitis virus (MHV) and other pathogenic agents.

Infection schedule. A single-cell suspension of log-phase cells of *N. asteroides* GUH-2 was injected into the tail veins (0.1 ml per mouse) of groups of mice. At 2 h and 1, 2, 5, 7, 10, and 13 days after infection, the mice (5 mice per group) were given 0.1 to 0.2 ml of dilute (10-fold dilution of stock solution) nembutal (Abbott Laboratories), and the brains were removed, placed in preweighed tubes containing 3.0 ml of sterile saline, and homogenized as described previously (7). The number of bacteria in each brain was quantitated by plating serial dilutions in tryptic soy agar.

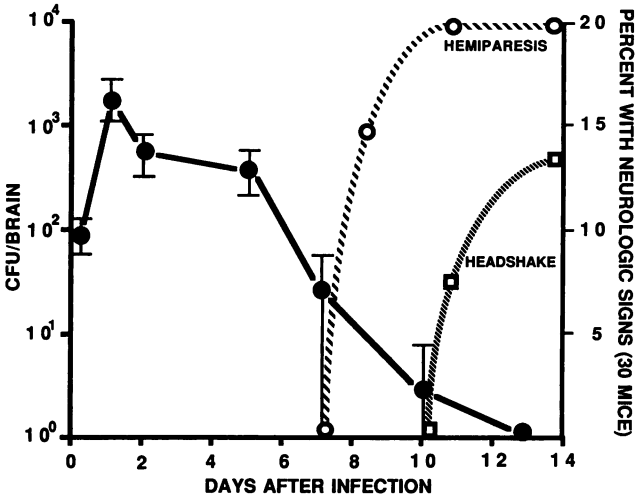


FIG. 1. The growth and clearance of *N. asteroides* GUH-2 in the brains of mice (●) contrasted with the development of neurologic signs such as "hemiparesis" (○) or frequent head shake (□). Each mouse was injected intravenously with 2.7 × 10^5 CFU. The bars represent the standard deviation of mean values per group of 5 mice. The percentage of neurologic signs is calculated by using 30 mice.

Localization of bacteria in the brain. In order to facilitate microscopic visualization of the localization of nocardiae in the brain, 10^6 CFU of nocardiae per mouse (100% lethal dose) was injected into the tail vein of several mice. After 24 h, three mice were injected with nembutal and perfused with modified Karnovsky's fixative (27); the brain was removed from the skull and embedded in paraffin, 5-μm serial sections were placed on glass slides, and the sections were stained either with hematoxylin and eosin or by the Brown and Brenn modification of the Gram stain (32).

Neurological signs following infection. Several groups of mice, varying in numbers from 16 to 60 (more than 200 mice studied), were injected intravenously with doses of *N. asteroides* GUH-2 varying from 2.7 × 10^5 to 4.4 × 10^5 CFU per mouse. These mice were monitored daily for the emergence of specific neurological signs, and the specific signs and date of appearance for each mouse were recorded. The mice were placed into groups based on the specific neurological manifestations that developed. Some of these mice had been observed for more than 9 months after infection.

Response of mice to L-dopa. L-Dopa (Sigma) dissolved in sterile saline was administered intraperitoneally (20 mg/kg of body weight) in combination with carbidopa (5 mg/kg; Sigma). Groups of uninfected controls with no signs and infected mice that demonstrated a variety of neurological signs were treated, and their responses were observed for several hours.

Histochemical and immunochemical staining. Fourteen days after the injection of 2.7 to 4.4 × 10^5 CFU per mouse, the brains of mice that developed neurological signs as well as of uninfected, age-matched control mice were fixed and embedded as described above. Serial coronal sections 10 μm thick were cut, placed on glass slides and stained either for

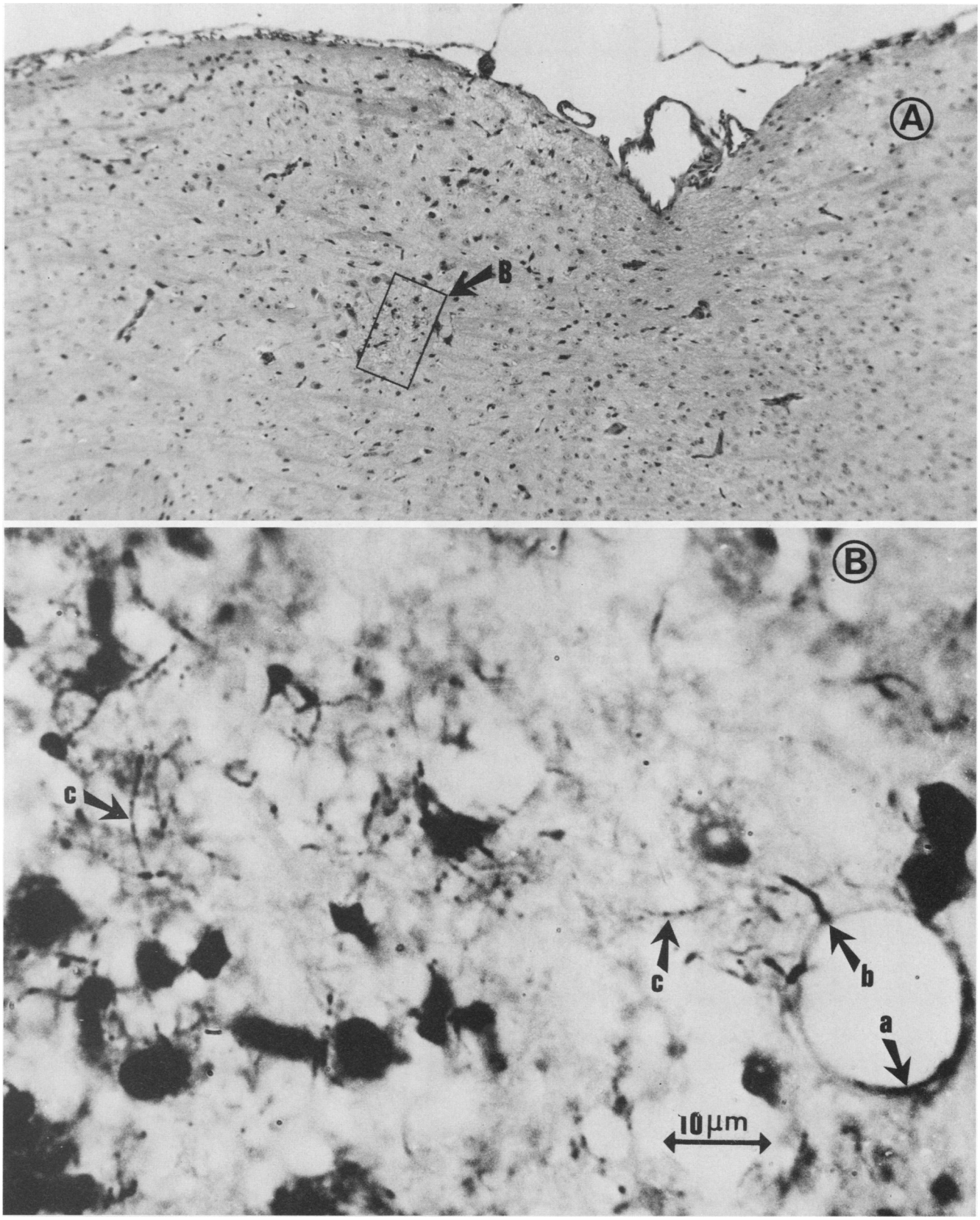
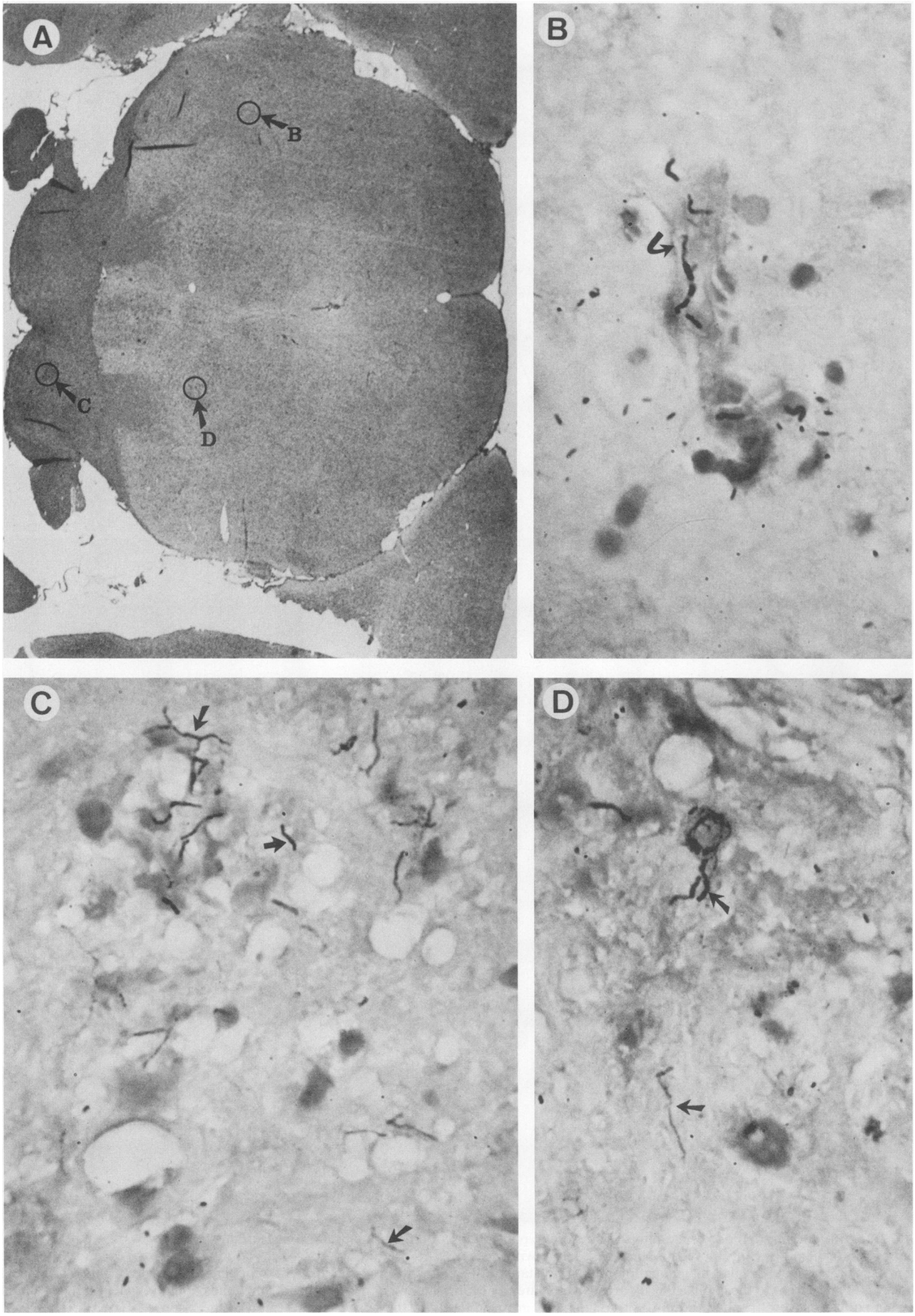


FIG. 2. (A) Light micrographs of paraffin sections of the pons region of the murine brain 24 h after injection of *N. asteroides* GUH-2. Low magnification of hematoxylin- and eosin-stained sections with insert B showing an area of nocardial invasion in the brain. Note a lack of inflammatory response. (B) High magnification of a Gram stain of the adjacent serial section shown in panel A. Arrow a points to a nocardial filament tightly adherent to the capillary wall; arrow b indicates a nocardial filament penetrating through the capillary wall into the brain tissue; Arrows c point to several beaded, gram-positive filaments growing freely in the perivascular region of the brain.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Nissl substance with cresyl violet (32) or for dopaminergic neurons with anti-tyrosine hydroxylase (Eugene Tech International, Allendale, N.J.). To visualize the antibody against tyrosine hydroxylase, the procedure described by Mason et al. (33) was followed. Briefly, rabbit antiserum to tyrosine hydroxylase (Eugene Tech) was used at a dilution of 1:200. The incubation was carried out for 18 h at room temperature, and visualization of bound primary antibody was carried out with the peroxidase-antiperoxidase complex (DAKO Corp., Santa Barbara, Calif.) by using the manufacturer's instruction. 3,3'-Diaminobenzidine was used as the chromogen (33).

Visualization of inclusion bodies in the brain. Mice that expressed rhythmic head shakes and control mice were sacrificed and perfused with fixative, and the brains were embedded in paraffin as described above. Serial coronal sections 5 to 10 μm thick were cut and placed on glass slides, and the sections were stained with hematoxylin and eosin. Each section was studied microscopically in a stepwise fashion, and all inclusion bodies within neurons were critically evaluated as to location, number, size, shape, and appearance.

RESULTS

Growth of *N. asteroides* in the brain. During the first 24 to 48 h after intravenous injection of *N. asteroides*, there was a rapid increase of viable nocardiae within the brain (Fig. 1). With a lethal dose (10^6 CFU per mouse), this increase continued until the animal died. However, when the mice were infected with a sublethal dose (2.7×10^5 CFU per mouse), the number of bacteria in the brain remained relatively constant from 48 through 120 h postinfection, followed by a gradual decrease so that at 13 days the brains of these infected mice appeared to be sterile (Fig. 1). Microscopic examination of serial sections of the brains revealed both large and small foci of bacteria in the upper portion of the pons of all brains studied (Fig. 2 and 3). In addition, medium-sized foci were observed in the region of the interpeduncular nucleus, the red nucleus, the area of the thalamus, and the substantia nigra pars compacta in some of the mice. In one brain, for example, bacteria were found growing within the neurons in the pons (Fig. 3C), red nucleus (Fig. 3D), and substantia nigra pars compacta (Fig. 3B). In all of these areas, the nocardiae appeared as filamentous cells less than 1 μm in width and 10 to 20 μm long, and in the hematoxylin- and eosin-stained sections they were difficult to distinguish from the fine neural fibrils located between glia cells and neurons. However, they were readily visualized and distinguishable from the neural filaments by using the Gram stain, by which the bacteria appeared as gram-positive beaded filaments typical of *Nocardia* spp. (Fig. 2B and 3B, C, and D; arrows).

Twenty-four hours after infection, nocardiae were observed to adhere to the capillary wall (Fig. 2B; arrow a), where they grew through the capillary (Fig. 2B; arrow b) to invade the brain tissue (Fig. 2B; arrow c). At this time there was extensive nocardial growth within the brain and there was no evidence of an acute inflammatory host response (Fig. 2 and Fig. 3).

Occurrence of neurological signs. Following the decline of bacteria in the brain, mice began to express a variety of neurological signs (Fig. 1). Approximately 30% of the mice injected with 5×10^5 CFU of *N. asteroides* GUH-2 began to display clinical signs characterized by a hemiparesislike syndrome with deviation of the head 4 to 5 days after infection. These mice with "hemiparesis" had a tendency to roll in response to stimuli, and when held by the tail they spun in a rapid circular motion. At 8 days postinfection, one of the mice developed tremors, generalized twitching, seizures, and jumpiness and it died. Ten days after infection, several mice that did not express "hemiparesis" began to show an abnormal behavior characterized by a rhythmic vertical head shake while the mice were quiescent. The mice with vertical head shake expressed a rapid, uncontrolled vertical jerk of the head four to five times per second. Furthermore, these mice had stooped posture and tremulous movement, often with the dragging of the limbs, and they expressed a restlessness that manifested itself by the mice continuously rocking their bodies forward, backward, and side to side. These mice could be divided into two groups; the mice in the first group were hypoactive, whereas the mice in the second group were hyperactive. Among these signs, "hemiparesis" and vertical head shake were easily monitored and could be reliably measured. Following the injection of 40 mice with a partially lethal dose of 5×10^5 CFU, four mice demonstrated a rhythmic vertical head shake without previous neurological signs while 13 developed "hemiparesis." Several days later, in eight of the mice, the "hemiparesis" began to subside, followed by an expression of vertical head shake, and one mouse died.

In a subsequent series of several experiments, a total of 216 mice were injected with nonlethal doses of *N. asteroides* GUH-2, and the development of neurological signs was monitored (Table 1). In these experiments, the mice appeared healthy until 7 days after infection wherein 25.4% of the mice (55 of 216) developed "hemiparesis." Fourteen days after infection, 11.1% of the mice that had no evidence of previous neurological damage and no "hemiparesis" had developed head shakes (24 of 216; Fig. 1 and Table 1). Approximately 4% of the mice that had previously exhibited evidence of "hemiparesis" later developed head shake signs. Therefore, about 40% of the infected mice developed one of these two measurable clinical signs that persisted for several months. The mice with rhythmic head shakes expressed these signs for the remainder of their lives (greater than 9 months for some mice). On the basis of these observations, the mice could be divided into the following groups: (i) mice that had no visible neurological signs following sublethal infection, (ii) mice that developed "hemiparesis" following sublethal infection with no development of head shake, (iii) mice that developed a rhythmic head shake with no "hemiparesis," and (iv) mice that developed a rhythmic head shake after the "hemiparesis" had subsided (a small percentage of mice [<4%] was in this group).

FIG. 3. Light micrographs of coronal sections of the murine brain 24 h after injection of *N. asteroides* GUH-2. (A) Low magnification of hematoxylin-and-eosin-stained sections with inserts B, C, and D showing areas of nocardial invasion in the brain. Note a lack of inflammatory response. (B, C, and D) High-magnification micrographs of a Gram stain of the adjacent serial section shown in panel A. Arrows point to nocardial filaments growing within each region of the brain. (B) Nocardial growth in the substantia nigra pars compacta region. (C) Extensive nocardial growth in the pons region. (D) Nocardial growth in the red nucleus region. (The upper arrow notes nocardial cells inside a probable neuron.)

TABLE 1. Neurological signs induced in mice 2 weeks after intravenous injection of sublethal doses of log-phase cells of *N. asteroides* GUH-2

Expt	Dose (10^5 CFU/mouse)	No. of mice	No. (%) of mice with neurological signs		
			"Hemiparesis"	Head shake	Hemiparesis and head shake
1	2.7	30	6 (20)	3 (10)	1 (3.3)
2	3.0	20	5 (25)	2 (10)	3 (15)
3	3.3	50	13 (26)	4 (8)	1 (2)
4	3.4	40	5 (12.5)	6 (15)	1 (2.5)
5	4.1	16	3 (18.7)	2 (12.5)	0 (0)
6	4.4	60	23 (38.3)	7 (11.7)	3 (5)
Total		216	55 (23.4 ± 8.8)[a]	24 (11.2 ± 2.4)	9 (4.6 ± 5.3)

[a] Mean percent of six experiments ± standard deviation.

Response of neurological signs to L-dopa. Groups of mice that expressed either rhythmic vertical head shakes or "hemiparesis" and normal controls were injected with L-dopa. Within 4 min after administration of L-dopa, the frequent vertical head shakes were completely inhibited in 10 of 10 mice (100%). The tremulous movements and restlessness were reduced, and most of these mice (8 of 10) were indistinguishable from normal mice with the ability to move smoothly. The effects of the L-dopa lasted for 2 h, wherein the neurological signs gradually reappeared to the same level as before treatment. In contrast, L-dopa had no effect on the signs of the mice with "hemiparesis."

Effect on Nissl substance and tyrosine hydroxylase activity. Examination of the Nissl-stained sections revealed a loss of Nissl substance in the neurons of the substantia nigra pars compacta region of the brains of mice with head shake but not in any of the uninfected, control mice (Fig. 4). In addition, coronal serial sections stained for tyrosine hydroxylase showed that mice with vertical head shakes and restlessness had a loss of immunoreactivity to tyrosine hydroxylase in the neurons of the substantia nigra pars compacta and in the ventral tegmental area of the brain (Fig. 5).

Cytoplasmic inclusion bodies in neurons. Microscopic examination revealed hyalinelike cytoplasmic inclusion bodies localized in the neurons in the substantia nigra, thalamus, periaqueductal region, and the ventral tegmental area of the brains of mice with rhythmic head shakes at 6 weeks postinfection (Fig. 6). These inclusion bodies consisted of a hyalinized, poorly stained peripheral region with a targetlike core (Fig. 6). They varied in size from 5 to 10 μm in diameter and were ovoid to round in shape, and the central cores were hematoxylinophilic, whereas the peripheral zones were usually weakly eosinophilic. No neurofibrillary tangles were observed in any of the brain sections studied. Therefore, these inclusions were Lewy-like bodies. Inclusion bodies were not observed either in the brains of control mice or in mice prior to 1 month after infection (i.e., prior to 2 weeks after the onset of rhythmic head shake).

DISCUSSION

PD is a slowly progressive neurologic disorder that has an insidious onset. It is characterized pathologically by degenerative changes in the substantia nigra region of the brain in humans resulting in a striatal dopamine deficiency (22, 24). There are several disorders recognized in humans that must be differentiated from idiopathic PD (true PD). These include postencephalitic parkinsonian syndrome (encephalitis lethargia), drug-induced parkinsonism, and motor neuron disease and parkinsonian syndrome (16, 21, 25, 31). True PD comprises four cardinal signs; rhythmic tremor at rest, muscular rigidity, bradykinesia, and postural instability (21, 31). In addition, there is usually a unilateral onset and 70 to 100% of the patients respond to L-dopa (8, 14, 21, 24). Pathological diagnosis depends upon nigral changes with a cell loss in the substantia nigra and the presence of Lewy bodies (21). Gibb and Lees (21) state that "(idiopathic) Parkinson's disease should be used synonymously with Lewy body-Parkinson's disease." Furthermore, non-Lewy-body PD can masquerade as true PD (21). The various parkinsonian syndromes can be differentiated from true PD by utilizing exclusion criteria which include the following: remitting course, history of definite encephalitis lethargia, supranuclear down gaze, cerebellar signs, pyramidal signs of unidentified cause, ataxic gait, stroke, negative response to large doses of L-dopa, and the absence of Lewy bodies (3, 10, 16, 21, 25).

The most important pathologic feature diagnostic for true PD disease appears to be the presence of Lewy bodies in the substantia nigra and associated regions of the brain (21). The Lewy body is defined as a hyaline, round or ovoid acidophilic inclusion in the cytoplasm of nerve cells or nerve cell processes. They are variable in size and have an eosinophilic, targetlike core (21). In postencephalitic and drug-induced parkinsonism, neurofibrillary changes resulting in neurofibrillary tangles occur; however, Lewy bodies are rarely observed in these conditions (21).

The etiology of PD remains unknown. On the basis of reports of postencephalitic parkinsonism (16, 21) and 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP)-induced parkinsonism (29), environmental factors such as infectious agents and toxic substances have been suggested as the etiology of PD. Even though viral infections of the CNS can lead to a progressive parkinsonian syndrome, there is no conclusive evidence that viruses cause PD (15, 25). MPTP-induced parkinsonism is distinguishable from PD (10, 18), and at the present time, there are no reports that implicate bacterial infections as the etiology of this disorder (11). Currently, many investigators believe that PD is not a single entity but instead a complex resulting from the interplay of many different etiologic factors (3). It has been proposed that the initial etiologic factor may be an infectious agent that may have disappeared before the appearance of the symptoms (12), and that a series of clinically silent CNS infections may produce a cumulative effect on vulnerable regions of the brain (13).

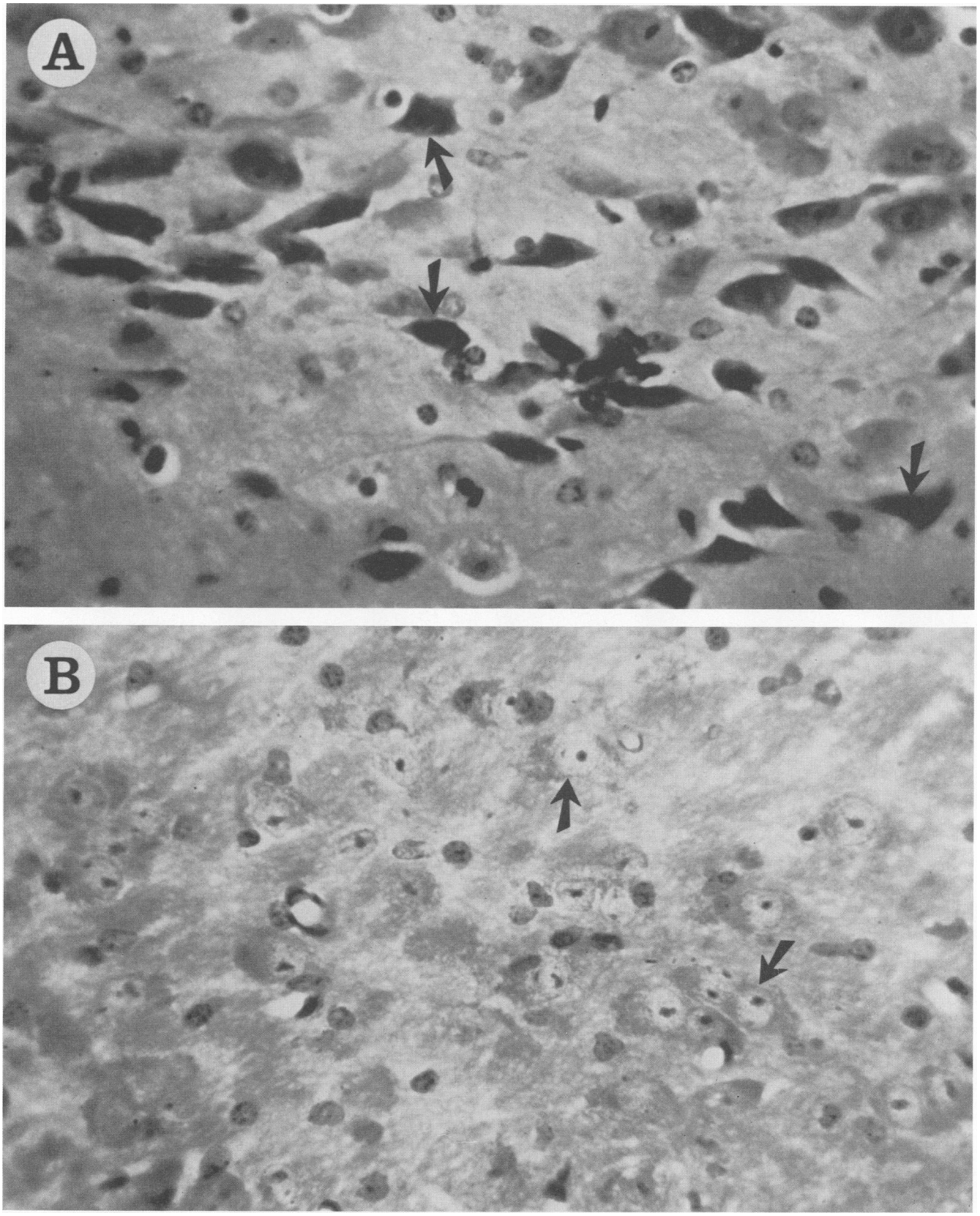
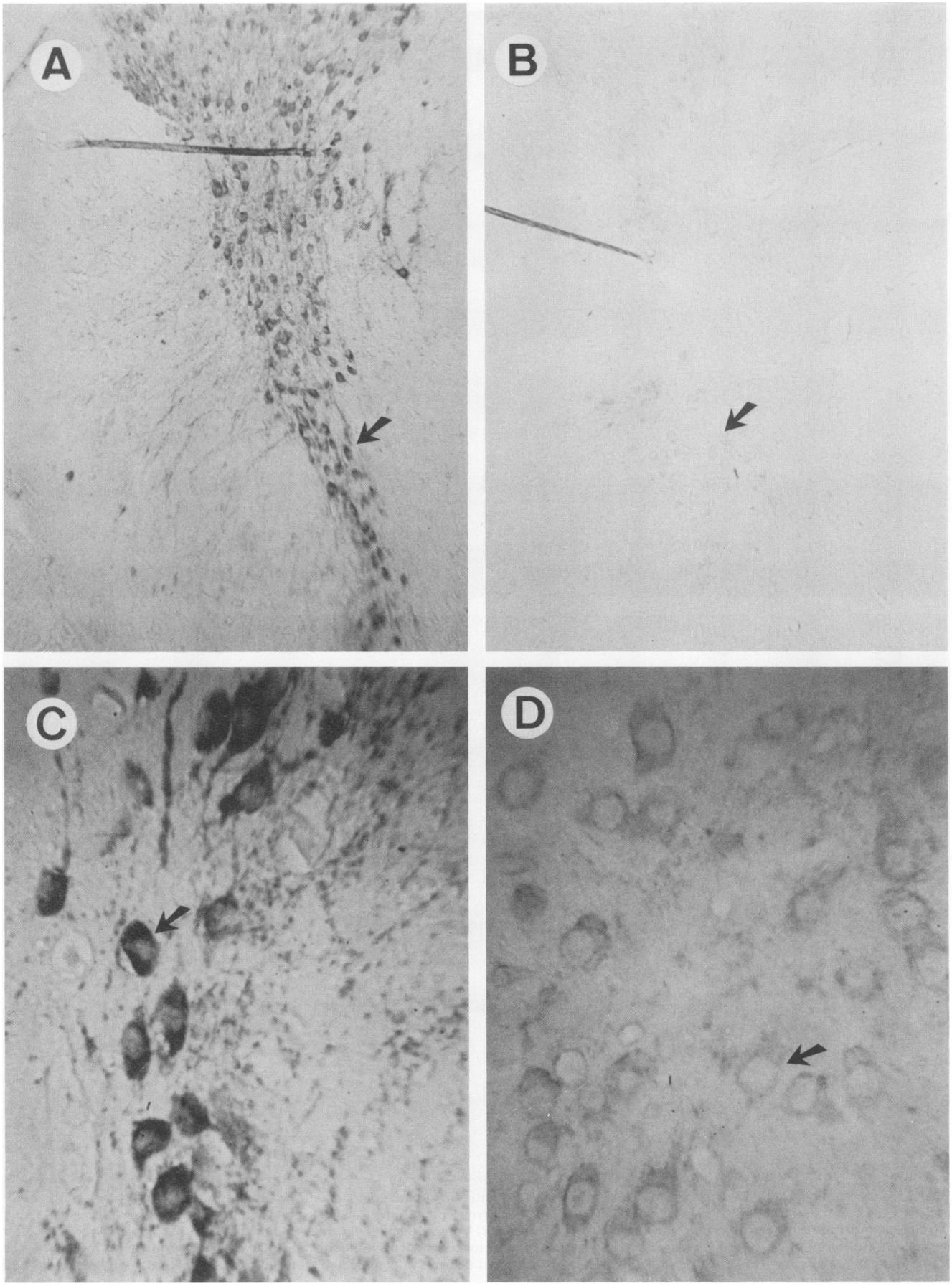


FIG. 4. Photomicrographs of Nissl-stained sections of the substantia nigra pars compacta of the murine brain. (A) Coronal section of an age-matched control mouse that was not exposed to *N. asteroides*. Arrows point to neuron cells which stained dark with the Nissl stain. Note the large number of neurons with prominently stained Nissl bodies. (B) Coronal section of an age-matched mouse (as in panel A) 14 days after intravenous injection of *N. asteroides* GUH-2. This mouse had developed frequent vertical head shakes. The sections in both A and B are at approximately the same level and orientation within the brain. Most neurons in the injected mouse do not show Nissl granules, but instead remain unstained and appear swollen (arrows; compare A and B).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In humans, *N. asteroides* can induce aneurysms of the brain artery (23), which suggests that the nocardial cells have a high affinity for the endothelial cells of the brain capillary. In mice, log-phase cells of *N. asteroides* became localized within specific regions of the brain such as the pons, interpeduncular nucleus, red nucleus, thalamus, and substantia nigra. As shown in Fig. 2B (arrow a), the nocardial cells became bound to the surface of capillary endothelial cells and initiated growth that extended through the capillary wall into the brain tissue. This high affinity of nocardial cells for the brain capillary suggests the presence of surface adherence receptors and may explain the predilection of *Nocardia* spp. for the CNS.

As the nocardiae invaded the brain tissue and grew within neurons as shown in Fig. 3D, there was little or no tissue damage because an inflammatory response did not ensue. At low doses of nocardiae, this localization, growth, and persistence of nocardial cells within the brains of mice for 9 to 13 days did not result in overt illness, and mice remained healthy until the specific neurologic manifestations appeared. A similar, silent infectious process in humans would not be recognized. In fact, even progressive nocardial brain abscess in humans may not be recognized because a fever or other indications of an infectious process are not always present (5, 20).

After localization and growth in the brain, the number of nocardial cells decreased so that at 9 to 13 days, the brains became sterile. Coincident with this disappearance of nocardia, there was emergence of a variety of neurological signs. Mice could be divided into well-defined groups based upon the specific signs that were expressed. This investigation focused on the group of mice that remained healthy until the onset of rhythmic head shakes, tremulous movement, stooped posture, and hypoactivity. These signs in this group of mice were temporarily stopped following treatment with L-dopa (complete elimination of head shakes in 100% of the mice for more than 2 h). Mice that had L-dopa-responsive, rhythmic head shake were found to have a loss of Nissl-staining bodies in the nerve cells of the substantia nigra. The Nissl staining procedure stains the endoplasmic reticulum of nerve cells, and it reacts primarily with ribonucleoprotein. Therefore, the loss of these stained areas within the neurons suggested blockage of protein synthesis (28, 42). Furthermore, a stain for tyrosine hydroxylase indicated a loss of enzyme immunoreactivity in the dopaminergic neurons of the substantia nigra and ventral tegmental area of the brains of these mice. These data indicate a blockage of the dopaminergic pathway within the brains of mice that developed the L-dopa-responsive head-shake signs.

Microscopic analysis of coronal sections of the brains from head-shake mice revealed the presence of hyaline inclusion bodies in neurons, and these inclusions resembled Lewy bodies. Lewy bodies are diagnostic for idiopathic PD in humans (21), and inclusions observed in the brains either of MPTP-treated mice (18) or aging mice (19) are distinct from Lewy bodies seen in PD. Therefore, Lewy bodies have never been reported in mice. The Lewy-like inclusions induced in the brains of mice 6 weeks after infection with *N. asteroides* appeared to be very different from the inclusions seen in MPTP-treated mice (18) or in aging mice (19). By light microscopy, they were different from true Lewy bodies seen in PD in humans only in that the central cores were hematoxylinophilic whereas the cores in true Lewy bodies are eosinophilic (21); otherwise the two types of inclusions appeared to be similar (Fig. 6).

Bojinov (9) described several patients diagnosed as having encephalitis with a parkinsonian syndrome. The patients had bilateral inflammatory necrosis of the substantia nigra caused by an unknown etiology. Pathologic examination of the brain in four fatal cases revealed a severe inflammatory necrosis of the substantia nigra. In two of the patients, there were scattered filamentous, rod-shaped structures with a beaded appearance among glial cells in the area of the substantia nigra. These beaded filaments were 1 to 2 μm in diameter, varied from 30 to 80 μm in length, and displayed a positive Fe-staining reaction (9). Bojinov described these filamentous structures as axon fragments or dystrophic astrocytic fibers; however, they appear to be very similar to the morphology of the beaded cells typical for *Nocardia* spp. (Fig. 2B, arrow c). Furthermore, the course of the illness described in these patients was similar to that reported in several cases of cerebral nocardiosis that present with parkinsonian features (35). In order to diagnose cerebral nocardiosis, isolation of the organism is essential. Because of the difficulty in establishing a diagnosis in cerebral nocardiosis, numerous cases of nocardial infections in the brain are misdiagnosed or may be attributed to an unknown etiology (1, 17, 21, 26, 35, 38, 40, 41, 43).

N. asteroides is capable of causing a silent, persistent infection in the brains of mice resulting in a dopamine deficiency, loss of Nissl-staining bodies and tyrosine hydroxylase immunoreactivity, the formation of hyaline inclusion bodies in the neurons of the substantia nigra, and the induction of parkinsonian signs. In healthy humans, nocardiae may localize in the respiratory tract without clinical evidence of disease (2, 43) or they may cause a self-limited pulmonary infection resulting in nonprogressive pneumonia or a flulike illness (20, 37). Transient bacteremia from the nocardial focus may occur (36), resulting in nocardiae penetrating the blood-brain barrier and invading the CNS. However, primary cerebral nocardiosis without evidence of prior pulmonary infection can also occur (4, 5). In the compromised patient this usually leads to progressive brain abscess (4), but in the noncompromised host the nocardial invasion of the brain may remain silent and cause a self-limited infection. These silent infections may cause damage to vulnerable regions of the brain such as the substantia nigra, as seen in the animal model presented above. On the basis of these observations combined with the clinical features described for cerebral nocardiosis, it is tempting to speculate that *N. asteroides* may be one of the causative agents for PD. However, these data show that mice infected

FIG. 5. Photomicrographs of tyrosine hydroxylase-stained sections of the substantia nigra pars compacta region of the brain. (A) Low magnification of a coronal section of an age-matched control mouse that was not exposed to *N. asteroides*. The arrow points to the heavily labeled neurons in the substantia nigra pars compacta region. (B) Low magnification of a coronal section of an age-matched mouse (as in panel A) 14 days after intravenous injection of *N. asteroides* GUH-2. This mouse had developed rapid vertical head shakes. Both sections A and B are at approximately the same level and orientation within the brain. Arrow points to neurons of the substantia nigra pars compacta region (note the reduction of tyrosine hydroxylase). (C) A high magnification of panel A (control). The arrow points to heavily labelled neuron cells. (D) A high magnification of panel B (nocardia-infected). The arrow points to neurons with significantly reduced tyrosine hydroxylase.

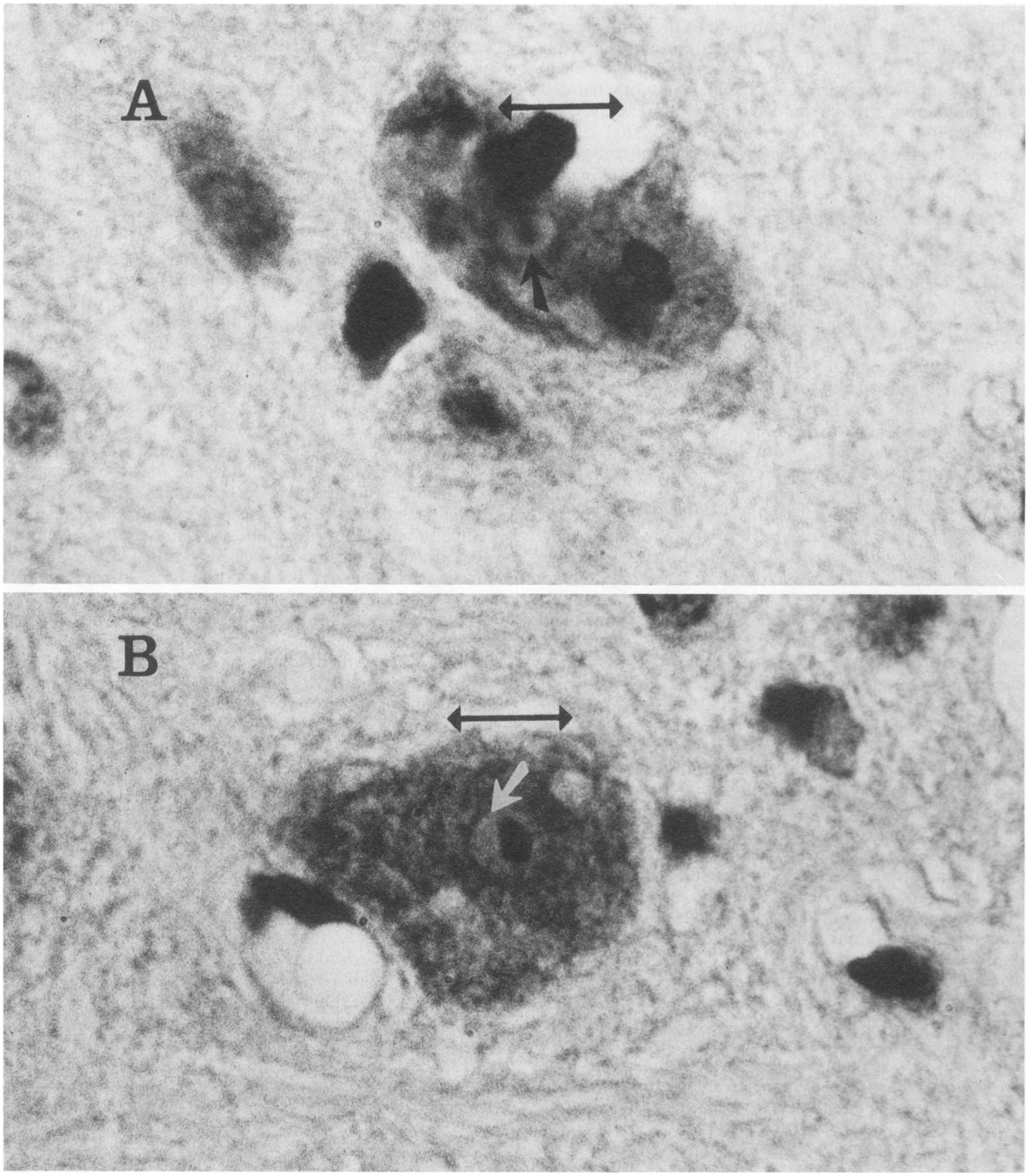

FIG. 6. Light micrographs of hematoxylin-and-eosin-stained coronal sections of the murine brain after injection of *N. asteroides* GUH-2. Bars, 10 μm. (A) High magnification of thalamus region of the brain of a mouse with rhythmic head shake 6 weeks after infection with *N. asteroides*. Arrow points to a hyaline, Lewy-like inclusion in a neuron. (B) High magnification of the substantia nigra region of the brain of a mouse with rhythmic head shake 8 months after infection with *N. asteroides*. Arrow points to a hyaline, Lewy-like inclusion in a neuron.

with *Nocardia* spp. may serve as a model for studying parkinsonian signs, as well as for studying other neurodegenerative diseases involving both pyramidal and extrapyramidal regions of the brain.

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