Published in final edited form as:

Mov Disord. 2016 August ; 31(8): 1134–1138. doi:10.1002/mds.26711.

***Nocardia asteroides*-induced movement abnormalities in mice: relevance for Parkinson's disease?**

David A. Loeffler, Ph.D., D.V.M.¹, Peter A. LeWitt, M.D.^{2,3}, and Dianne M. Camp, Ph.D.⁴

¹Department of Neurology, Beaumont Hospital-Royal Oak, Beaumont Health, Royal Oak, MI, USA

²Department of Neurology, Henry Ford West Bloomfield Hospital, West Bloomfield, MI

³Department of Neurology, Wayne State University School of Medicine, Detroit, MI, USA

⁴National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health, Bethesda, MD, USA

Keywords

Animal models; motor abnormalities; mouse; *Nocardia*; Parkinson's disease

Evidence for genetic causes is lacking in most Parkinson's disease (PD) cases,¹ so alternative explanations have been sought.^{2,3} Because the disease was first described two centuries ago, it cannot be due to modern chemical or dietary exposures. Epidemiological studies suggest that most cases of PD may be due to interactions of genes and environmental factors.² The possibility that PD might be due to an infectious agent was suggested by the epidemic-like spreading of post-encephalitic Parkinsonism.⁴ Additional support for this hypothesis comes from cases of viral encephalitis in which Parkinsonism has developed,⁵ and reports of conjugal Parkinsonism⁶ (which could be due to common environmental exposure). In 1991 Drs. Shunro Kohbata and Blaine Beaman from the University of California at Davis

Corresponding author: David A. Loeffler, D.V.M., Ph.D., Neurology Research Laboratory, Department of Neurology, Beaumont Hospital-Royal Oak, Beaumont Health, 3601 West Thirteen Mile Road, Royal Oak, MI 48073, USA; DLoeffler@beaumont.edu; phone: (248) 551-3170; fax: (248) 551-5069.

Conflict of Interest concerning research related to manuscript, regardless of date: David A. Loeffler declares no potential conflicts of interest. Peter A. LeWitt declares no potential conflicts of interest. Dianne M. Camp has no potentially competing financial interests to declare.

Documentation of Author Roles: 1) Research project: A. Conception: DAL, DMC, and PAL, B. Organization: DAL, DMC, and PAL, C. Execution: DAL, DMC, and PAL; 2) Statistical Analysis: not applicable; 3) Manuscript: A. Writing of first draft: DAL and PAL, B. Revising of manuscript and final approval of version to be published: DAL, DMC, and PAL.

Disclaimer: This article was edited by Dianne M. Camp, Ph.D. in her private capacity.

The opinions expressed in this article are the author's own and do not reflect the view of the National Institutes of Health, the Department of Health and Human Services, or the United States government.

Movement abnormalities in mice experimentally infected with *N. asteroides* strain GUH-2, compared to movements of uninfected age-matched "control" mice: The control mouse displays rapid and coordinated exploratory movements, whereas movements of the *Nocardia*-infected mouse (beginning at 49 seconds on the recording) reveal dystonic posturing, slowed and halting gait, and spontaneous head extension and shaking. When suspended by the tail, the control mouse (beginning at 2:28 on the recording) maintains normal postural control, without spinning, whereas the infected mouse (beginning at 2:41 on the recording) spins rapidly. The control mouse swims easily (2:49 on the recording) with its head held up and coordinated use of its limbs and tail during sustained swimming, whereas the infected mouse (3:29 on the recording) is unable to keep its head up, and sinks within a few seconds. Continued open field observation of the infected mouse (3:46 on the recording, which was made in a darkened home cage at night) reveals immobility and slowed movements, spontaneous retropulsion, and distended tail posture.

described motor abnormalities which developed in BALB/c mice which they infected with a Gram positive, acid-fast bacterium named *Nocardia asteroides* strain GUH-2⁷ (since re-named *N. cyriacigeorgica*). We subsequently collaborated with Dr. Beaman to investigate this model further. This Viewpoint will review our findings and those of Dr. Beaman, and will address the relevance of this model for PD.

The model described by Kohbata and Beaman had some interesting clinical and neuropathological similarities to PD. The animals developed movement abnormalities (spinning when suspended by the tail (referred to hereafter as “tail spinning”), head shaking, slowed movements, and flexed axial posture with tail positioning), nigrostriatal dopamine loss, and Lewy body-like intraneuronal inclusions (Figure 1). The accompanying video shows these movement abnormalities. The head shaking could be stopped for up to 2 hours by administration of levodopa/carbidopa.⁷ The inclusions were circular or oval-shaped. H & E staining produced a hematoxylin-stained inner core and eosin-stained outer structure. Neurochemical studies found striatal dopamine (DA) to be decreased by 72% on day 29,⁸ returning to control levels by one year post-infection despite persistence of the movement abnormalities. No changes in striatal serotonin (5-HT) or norepinephrine (NE) were found on post-infection days 7, 16, or 29.

We investigated whether *Nocardia* secreted a dopamine-depleting toxin. We incubated rat pheochromocytoma PC12 cells with strain GUH-2 sterile culture filtrate for 24 hours and found a 65% decrease in intracellular DA levels, at which time there was minimal loss of cell viability⁹ (which did decrease at later time points). In a later study we localized this DA-depleting activity to a protease-resistant, non-lipid, low molecular weight fraction.¹⁰ Our finding that *Nocardia* secreted a DA-depleting toxin suggested that this toxin could have caused the striatal DA depletion.

Dr. Beaman performed an *in situ* hybridization study looking for evidence of *Nocardia* in Lewy body-containing human brain specimens.¹¹ The positive control brain specimens (cerebral cortex, thalamus, caudate, and putamen) for that study were from a cynomolgus monkey who was administered strain GUH-2 and euthanized after 48 hours. Two other monkeys were initially administered *N. asteroides* strain I-38-SYN (a mutant strain of GUH-2) and 1.5 months later, because no neurological abnormalities had developed, they were then administered GUH-2. These monkeys became listless on day 5. After treatment with trimethoprim-sulfamethoxazole, they recovered but one developed a tremor. These monkeys were euthanized 3.5 months after GUH-2 infection. *Nocardia* was cultured from brain specimens from both of the 3.5 month-infected monkeys, and intraneuronal inclusions, some of which stained for α -synuclein and ubiquitin, were detected in cortical specimens from both animals (Figure 2). Some of these inclusions reacted with a *Nocardia*-specific 16S ribosomal RNA (rRNA) hybridization probe, whose binding was then examined on 35 human formalin-fixed SN specimens (normal controls, n = 5; neurodegenerative disorders without Lewy bodies, n = 6; and neurodegenerative disorders with Lewy bodies, n = 24, including PD, dementia with Lewy bodies (DLB), and combined diagnoses of PD and Alzheimer's disease [AD]). The reverse sequence of the probe was used as a negative control. Nine of the specimens, all containing Lewy bodies, hybridized to the probe.

We subsequently attempted to replicate the *in situ* hybridization study in our laboratory.¹² We examined 125 SN specimens (28 PD, 21 DLB, 32 other neurodegenerative disorders, and 44 controls). Some of the specimens had been used in Dr. Beaman's study. We performed Gram staining and Dr. Beaman's *in situ* hybridization protocol¹¹ on the specimens, as well as polymerase chain reaction (PCR) on some of them. We found only three specimens (one case each of PD, DLB, and AD) which hybridized to the nocardial probe, and none of the specimens showed evidence for *Nocardia* by PCR or Gram staining. Our results were unable to confirm Dr. Beaman's finding of evidence for nocardial infection in brain specimens from individuals with Lewy body-containing disorders.

We were interested in comparing the movement abnormalities in *Nocardia*-infected mice to those in well-established rodent models of PD, namely 6-hydroxydopamine (6-OHDA)-hemilesioned rats and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice. Rats with extensive unilateral 6-OHDA lesions display spontaneous and drug-induced rotational behavior.¹³ Borlongan and Sanberg¹⁴ developed an "elevated body swing test" in which they suspended an animal by its tail and counted the number and direction of its swings for 30 seconds. The results of the test correlated with apomorphine-induced rotations in 6-OHDA-hemilesioned rats. Although this "swing test" behavior and apomorphine-induced rotations were associated with DA deficits resulting from unilateral nigrostriatal lesioning, of relevance to the movement abnormalities in *Nocardia*-infected mice is that Borlongan and Sanberg suggested that stress might induce swinging behavior when an animal was suspended by its tail. So although *Nocardia*-infected mice do not have hemispherical dopamine imbalance, the stress of being suspended by the tail could have triggered tail spinning due to their abnormal dopamine neurotransmission. Alternatively, tail spinning could have represented the efforts of the mice to right themselves in the presence of a balance problem caused by inner ear pathology, as will be discussed below.

Motor deficits in MPTP-treated mice typically have been assessed by open-field activity monitoring, swim test, pole test, treadmill running, and rotarod testing.¹⁵ Only open-field monitoring and swimming were performed on the *Nocardia*-infected mice. Although the striatal dopamine deficit in the chronic MPTP mouse model of PD is greater than reported for the *Nocardia* mice, head shaking and tail spinning have not been reported in MPTP-treated mice. This again raises the question of whether these behaviors in the *Nocardia*-infected mice were due to striatal DA depletion.

Detection of Lewy body-like inclusions in *Nocardia*-infected mice is noteworthy because such inclusions have rarely been reported in rodent PD models. Administering MPTP to mice acutely or subacutely does not produce these inclusions^{16,17} although inclusions were induced in mouse SN by chronic MPTP treatment.¹⁸ 6-OHDA does not induce Lewy bodies in rats.¹⁹ Rotenone, administered chronically as a low dose to rats, induces α -synuclein-immunoreactive inclusions,²⁰ and paraquat produces similar inclusions in mice.²¹ McNaught et al.²² generated Lewy body-like inclusions in rats by administering proteasome inhibitors. These inhibitors are produced by strain GUH-2,²³ suggesting that the inclusions in *Nocardia*-infected mice could have been produced by this mechanism.

Despite the similarities of the *Nocardia*-infected mouse model to PD, we have several concerns about the relevance of this model to PD:

Our major concern is the relationship between striatal DA concentration and the movement abnormalities in the *Nocardia*-infected mice. Tail spinning was reported as early as post-infection day 4-5,⁷ yet the striatal DA loss, which was first measured on day 7, was 55%.⁸ This may not have been sufficient to cause movement abnormalities; in humans, striatal loss of 60-80% of dopamine is considered to be the threshold for manifestation of Parkinsonian symptoms.²⁴ Inner ear pathology could have caused the spinning behavior. There is a literature predating Dr. Beaman's studies that describes head tilt, tail spinning (referred to as "spinning disease"), and circling in mice experimentally infected with *Nocardia*.²⁵⁻²⁷ Postmortem examinations of mice with spinning disease found inner ear inflammation ranging from necrotic lesions to *Nocardia*-containing granulomas.²⁷ A similar syndrome was also reported in mice infected with *Pseudomonas*²⁸ or *Mycobacteria*.²⁹ Whether the mice in Dr. Beaman's studies had inner ear pathology, and if so, whether it caused (or contributed to) the movement disorders he described, is unknown.

A second concern regards the relationship, in *Nocardia*-infected mice, between the reduced neuron immunoreactivity for TH in the SN and the decrease in striatal DA. In the 1991 Kohbata and Beaman study⁷ a marked decrease in staining intensity of TH⁺ neurons was observed in the SN on day 14. Figure 5 in that study shows neurons with "significantly reduced tyrosine hydroxylase." We would expect this reduction in TH staining to have caused greater striatal DA loss than the 66% decrease reported for day 16.⁸ In the MPTP/probenecid model, for example, in which up to 70% of SN TH⁺ neurons are lost by 3 weeks, > 90% striatal DA loss occurs.³⁰ Further, we suspect that the decreased TH staining of SN neurons in the GUH-2-infected mice might have been reversible, because the striatal DA deficit normalized by one year post-infection. The possibility that neuronal TH immunoreactivity could have recovered by one year post-infection was apparently not examined by Dr. Beaman. A similar scenario occurs during recovery of mice from MPTP toxicity, which reversibly blocks TH gene expression.³¹ Alternatively, sprouting of surviving striatal dopaminergic axons might have occurred, although this would be unlikely to have completely reversed the striatal dopamine deficit.

Our third concern is the extent to which the neurochemical abnormalities in *Nocardia*-infected mice match those in PD. Although the main neuronal loss in the PD brain is of nigrostriatal dopaminergic neurons, dopaminergic neurons are also lost in the ventral tegmental area, and less severe losses of noradrenergic and cholinergic neurons occur in other brain regions. The neurochemical study of brain specimens from *Nocardia*-infected mice⁸ examined only striatum and cerebellum so the neurotransmitter alterations, if any, in other brain regions are unknown.

Despite these concerns, the *Nocardia* model of PD makes an important contribution by raising the issue of whether a similar organism could be a cause of the disease. PD has been hypothesized to originate in the gut.³² The colonic microbiome differs between PD subjects and healthy controls,³³ suggesting that some alterations in gut microflora might increase the risk for PD. This is relevant to an organism like *Nocardia* which can enter the circulation

through breaks in gut mucosa.³⁴ Other organisms which can reside in the gut, including *Helicobacter pylori*³⁵ and *Mycobacterium paratuberculosis*,³⁶ have also been proposed as possible causes of PD. The *Nocardia* model also offers some insights as to how a bacterium might initiate PD pathology while remaining undetected. After encountering activated macrophages, *Nocardia* can survive as cell wall-deficient L-forms³⁷ which are difficult to detect. Without typical signs of CNS involvement, persisting L-forms in the brain would not be likely to raise suspicions of infection.

Human exposure to *Nocardia* frequently occurs.³⁸ If PD's etiology might be due to such an organism, then why is the prevalence of the disease so low? Again, a clue might come from the nocardial model. Persistence of a low-grade bacterial infection in the CNS might require a specific set of virulence factors, and localization of the organism to the SN, plus secretion of a DA-depleting toxin, might be required for the organism to reduce striatal DA to clinically relevant (movement abnormality-inducing) levels. Genetically-determined resistance factors might be part of the susceptibility picture, together with some degree of immune impairment and/or other environmental or genetic factors.

Conclusion

An unexpected pathogenic role for a bacterium is shown by the discovery that *Helicobacter pylori* causes peptic ulcer disease.³⁹ Despite our reservations about the nocardial mouse model of Parkinsonism, it serves as a reminder that a similar agent might be a cause of PD.

Acknowledgments

The authors dedicate this review to the memory of Blaine Beaman, Ph.D., former chairperson of the Department of Microbiology and Immunology, University of California, Davis. A world authority on *Nocardia* and a passionate researcher and educator, he never missed an opportunity to search each stone in his path to learn what hid underneath. This study was supported by a grant from the National Institute of Environmental Health Sciences (R01-ES-010793, to P.A.L.), a National Parkinson Foundation Center of Excellence grant (to P.A.L.), and a generous donation to the Beaumont Foundation (Beaumont Health) from Ms. Marilyn Bishop to support Parkinson's disease research in Beaumont's Neurology Research Laboratory.

Financial disclosure: David A. Loeffler's support for his *Nocardia* research reported in this manuscript came from PHS grant R01-ES010793 (P. LeWitt, P.I.) and a National Parkinson Foundation Center of Excellence grant (to P.A.L.). He serves without compensation on the editorial board of the Journal of Neuroinflammation. Peter A. LeWitt's *Nocardia* research reported in this manuscript was supported by PHS grant R01-ES010793 and a National Parkinson Foundation Center of Excellence grant. In the past year, he served as a consultant or advisor for Acorda, Biogen Idec, Britannia, Insightec, Intec, Kyowa Hakko, Lundbeck, Luye, NeuroDerm, Pfizer, ProStrakan, Prothena, SynAgile, Teva, UCB Pharma, and USWorldMeds, and received speaker honoraria from Acadia, The International Parkinson's and Movement Disorders Society, Lundbeck, and USWorldMeds. He is compensated for services as Editor-in-Chief of Clinical Neuropharmacology and serves without compensation on the editorial boards of Journal of Neural Transmission, Translational Neurodegeneration, Journal of Parkinson's Disease, and Neurology Reviews. The Parkinson's Disease and Movement Disorders Program that Dr. LeWitt directs has received clinical research grant support (for conducting clinical trials and other research) from AbbVie, Acorda, Adamas, Biotie, Kyowa Hakko, The Michael J. Fox Foundation for Parkinson's Research, the Parkinson Study Group, Pharma 2B, and USWorldMeds.

Funding sources for study: David Loeffler's work on this manuscript was supported by a generous donation from Ms. Marilyn Bishop to the Beaumont Foundation (Beaumont Health) to support Parkinson's disease research in Beaumont's Neurology Research Laboratory.

References

1. Houlden H, Singleton AB. The genetics and neuropathology of Parkinson's disease. *Acta Neuropathol.* 2012; 124:325–338. [PubMed: 22806825]
2. Tanner CM. Advances in environmental epidemiology. *Mov Disord.* 2010; 25(Suppl 1):S58–62. [PubMed: 20187243]
3. Muangpaisan W, Hori H, Brayne C. Systematic review of the prevalence and incidence of Parkinson's disease in Asia. *J Epidemiol.* 2009; 19:281–293. [PubMed: 19801887]
4. Rail D, Scholtz C, Swash M. Post-encephalitic Parkinsonism: current experience. *J Neurol Neurosurg Psychiatry.* 1981; 44:670–676. [PubMed: 7299406]
5. Jang H, Boltz DA, Webster RG, Smeyne RJ. Viral parkinsonism. *Biochim Biophys Acta.* 2009; 1792:714–721. [PubMed: 18760350]
6. Willis AW, Sterling C, Racette BA. Conjugal Parkinsonism and Parkinson disease: a case series with environmental risk factor analysis. *Parkinsonism Relat Disord.* 2010; 16:163–166. [PubMed: 19818671]
7. Kohbata S, Beaman BL. L-DOPA-responsive movement disorder caused by *Nocardia asteroides* localized in the brains of mice. *Infect Immun.* 1991; 59:181–191. [PubMed: 1670928]
8. Hyland K, Beaman BL, LeWitt PA, DeMaggio AJ. Monoamine changes in the brain of BALB/c mice following sub-lethal infection with *Nocardia asteroides* (GUH-2). *Neurochem Res.* 2000; 25:443–448. [PubMed: 10823576]
9. Camp DM, Loeffler DA, Razoky BA, Tam S, Beaman BL, LeWitt PA. *Nocardia asteroides* culture filtrates cause dopamine depletion and cytotoxicity in PC12 cells. *Neurochem Res.* 2003; 28:1359–1367. [PubMed: 12938858]
10. Loeffler DA, Camp DM, Qu S, Beaman BL, LeWitt PA. Characterization of dopamine-depleting activity of *Nocardia asteroides* strain GUH-2 culture filtrate on PC12 cells. *Microb Pathog.* 2004; 37:73–85. [PubMed: 15312847]
11. Chapman G, Beaman BL, Loeffler DA, et al. In situ hybridization for detection of nocardial 16S rRNA: reactivity within intracellular inclusions in experimentally infected cynomolgus monkeys – and in Lewy body-containing human brain specimens. *Exp Neurol.* 2003; 184:715–725. [PubMed: 14769363]
12. Lu L, Camp DM, Loeffler DA, LeWitt PA. Lack of evidence for *Nocardia asteroides* in brain specimens from Lewy body-containing disorders. *Microb Pathog.* 2005; 39:205–211. [PubMed: 16257505]
13. Dunnett SB, Iversen SD. Spontaneous and drug-induced rotation following localized 6-hydroxydopamine and kainic acid-induced lesions of the neostriatum. *Neuropharmacology.* 1982; 21:899–908. [PubMed: 6815549]
14. Borlongan CV, Sanberg PR. Elevated body swing test: a new behavioral parameter for rats with 6-hydroxydopamine-induced hemiparkinsonism. *J Neurosci.* 1995; 15:5372–5378. [PubMed: 7623159]
15. Jakowec MW, Petzinger GM. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned model of parkinson's disease, with emphasis on mice and nonhuman primates. *Comp Med.* 2004; 54:497–513. [PubMed: 15575363]
16. Heikkilä RE, Hess A, Duvoisin RC. Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine in mice. *Science.* 1984; 224:1451–1453. [PubMed: 6610213]
17. Vila M, Vukosavic S, Jackson-Lewis V, Neystat M, Jakowec M, Przedborski S. Alpha-synuclein up-regulation in substantia nigra dopaminergic neurons following administration of the parkinsonian toxin MPTP. *J Neurochem.* 2000; 74:721–729. [PubMed: 10646524]
18. Fornai F, Schlüter OM, Lenzi P, Gesi M, Ruffoli R, Ferrucci M, Lazzeri G, Busceti CL, Pontarelli F, Battaglia G, Pellegrini A, Nicoletti F, Ruggieri S, Paparelli A, Südhof TC. Parkinson-like syndrome induced by continuous MPTP infusion: convergent roles of the ubiquitin-proteasome system and alpha-synuclein. *Proc Natl Acad Sci U S A.* 2005; 102:3413–3418. [PubMed: 15716361]
19. Tieu K. A guide to neurotoxic animal models of Parkinson's disease. *Cold Spring Harb Perspect Med.* 2011; 1:a009316. [PubMed: 22229125]

20. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci.* 2000; 3:1301–1306. [PubMed: 11100151]
21. Manning-Bog AB, McCormack AL, Li J, Uversky VN, Fink AL, Di Monte DA. The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha-synuclein. *J Biol Chem.* 2002; 277:1641–1644. [PubMed: 11707429]
22. McNaught KS, Perl DP, Brownell AL, Olanow CW. Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. *Ann Neurol.* 2004; 56:149–162. [PubMed: 15236415]
23. Barry DP, Beaman BL. *Nocardia asteroides* strain GUH-2 induces proteasome inhibition and apoptotic death of cultured cells. *Res Microbiol.* 2007; 158:86–96. [PubMed: 17258894]
24. Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F. Brain dopamine and the syndromes of Parkinson and Huntington. *J Neurol Sci.* 1973; 20:415–455. [PubMed: 4272516]
25. Gorrill RH, Heptinstall RH. The animal pathogenicity of *Nocardia seaborum* nov. spec *J Pathol Bacteriol.* 1954; 68:387–393. [PubMed: 14354544]
26. Gorrill RH. Spinning disease of mice. *J Pathol Bacteriol.* 1956; 71:353–358. [PubMed: 13398880]
27. Smith IM, Hayward AH. *Nocardia caviae* and *Nocardia asteroides*: comparative bacteriological and mouse pathogenicity studies. *J Comp Pathol.* 1971; 81:79–87. [PubMed: 5090889]
28. Gorrill RH. Bacterial localisation in the kidney with particular reference to *Pseudomonas pyocyanea*. *J Pathol Bacteriol.* 1952; 64:857–864. [PubMed: 13000597]
29. Saito H, Tasaka H. Comparison of the pathogenicity for mice of *Mycobacterium fortuitum* and *Mycobacterium abscessus*. *J Bacteriol.* 1969; 99:851–855. [PubMed: 5370281]
30. Meredith GE, Rademacher DJ. MPTP mouse models of Parkinson's disease: an update. *J Parkinsons Dis.* 2011; 1:19–33. [PubMed: 23275799]
31. Xu Z, Cawthon D, McCastlain KA, Slikker W Jr, Ali SF. Selective alterations of gene expression in mice induced by MPTP. *Synapse.* 2005; 55:45–51. [PubMed: 15499605]
32. Braak H, Rüb U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J Neural Transm (Vienna).* 2003; 110:517–536. [PubMed: 12721813]
33. Scheperjans F, Aho V, Pereira PA, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord.* 2015; 30:350–358. [PubMed: 25476529]
34. Tsushima K, Koizumi T, Aoki H, Furuta K, Fujimoto K, Kubo K. A case of acute respiratory distress syndrome caused by systemic nocardiosis. *Respiration.* 2000; 67:591–592. [PubMed: 11070472]
35. Dobbs RJ, Dobbs SM, Weller C, et al. Helicobacter hypothesis for idiopathic parkinsonism: before and beyond. *Helicobacter.* 2008; 13:309–322. [PubMed: 19250506]
36. Dow CT. M. paratuberculosis and Parkinson's disease--is this a trigger. *Med Hypotheses.* 2014; 83:709–712. [PubMed: 25459140]
37. Filice GA, Beaman BL, Remington JS. Effects of activated macrophages on *Nocardia asteroides*. *Infect Immun.* 1980; 27:643–649. [PubMed: 6991421]
38. Hubble JP, Cao T, Kjelstrom JA, Koller WC, Beaman BL. *Nocardia* species as an etiologic agent in Parkinson's disease: serological testing in a case-control study. *J Clin Microbiol.* 1995; 33:2768–2769. [PubMed: 8567923]
39. Peterson WL. *Helicobacter pylori* and peptic ulcer disease. *N Engl J Med.* 1991; 324:1043–1048. [PubMed: 2005942]

Figure 1.

Light micrographs of H & E-stained brain specimens from mice experimentally infected with *N. asteroides* strain GUH-2. (A) Thalamus from a mouse with head shaking 6 weeks after infection with *N. asteroides*. The arrow indicates a hyaline, Lewy body-like intraneuronal inclusion. (B) Substantia nigra from a mouse with head shaking 8 months after infection with *N. asteroides*. The arrow indicates an intraneuronal inclusion. (Bars = 10 μ m) (From: Kohbata S and Beaman BL, "L-Dopa-Responsive Movement Disorder Caused by

Nocardia asteroides Localized in the Brains of Mice.” Infect. Immun. 1991; 59:181-191.
Used with permission.)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2.

Inclusion bodies which developed in brain (cortex) of a cynomolgus monkey at 3.5 months after infection with *N. asteroides* strain GUH-2. (A) H & E stained cortex; arrow indicates an eosinophilic inclusion α-synuclein staining. (B) Cortical specimen from an individual with dementia with Lewy bodies (DLB), α-synuclein staining; arrow indicates an immunoreactive Lewy body (scale bar = 10 μm). (C) 3.5-month-infected monkey, α-synuclein staining of cortex; arrow points to an immunoreactive inclusion (scale bar = 10 μm). (D) Same brain (cortical) specimen, with ubiquitin staining. Arrow indicates

immunoreactive inclusion (scale bar = 10 μ m). (E) Caudate from same monkey, electron micrograph. Microfilaments similar to those present in Lewy bodies are indicated by the arrow (scale bar = 1 μ m). (From: Chapman et al., "In situ hybridization for detection of nocardial 16S rRNA: reactivity within intracellular inclusions in experimentally infected cynomolgus monkeys – and in Lewy body-containing human brain specimens." Exp. Neurol. 2003; 184: 715-725. Used with permission.)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript