Pathological α-Synuclein Transmission Initiates Parkinson-like Neurodegeneration in Nontransgenic Mice
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A proportion of the costs would be shared between the species and site targets considered here. Establishing protection and managing sites made up to 55% of the total costs for sampled bird species. Discounting this proportion from the total cost of species conservation across all taxa, a combined cost needed to meet both species and site CBD targets may be in the order of U.S. $78.1 billion annually (Fig. 2). It is also highly likely that actions to meet these two targets will contribute to other targets in the CBD strategic plan, which are critical to delivering sustainable development and the safeguarding of global biodiversity in the long term (4).

Even with increased investment, careful prioritization will continue to be necessary to inform decisions about which areas to protect and which actions to undertake for species, e.g., using approaches that optimize returns on investment, given fixed budgets and defined objectives, for approaches that optimize returns on investment, decisions about which areas to protect and which prioritization will continue to be necessary to inform sustainable development and the safeguarding of global biodiversity and the greater will be the difficulty of success.

Highly likely that actions to meet these two targets may be in the order of $78.1 billion annually (Fig.2). It is also made up 50 to 55% of the total costs for sampled species conservation across all ecosystems that are lost per year (estimated to 1 to 4% of the estimated net value of ecosystem services that are lost per year [estimated at $2 to $6.6 trillion ($2–6.6 trillion would be shared between the species and site targets considered here. Establishing protection and managing sites made up to 55% of the total costs for sampled bird species. Discounting this proportion from the total cost of species conservation across all taxa, a combined cost needed to meet both species and site CBD targets may be in the order of U.S. $78.1 billion annually (Fig. 2). It is also highly likely that actions to meet these two targets will contribute to other targets in the CBD strategic plan, which are critical to delivering sustainable development and the safeguarding of global biodiversity in the long term (4).

Even with increased investment, careful prioritization will continue to be necessary to inform decisions about which areas to protect and which actions to undertake for species, e.g., using approaches that optimize returns on investment, given fixed budgets and defined objectives, for sites (27), species (7, 8), and management actions (22). Our finding that species facing higher categories of extinction risk require less investment for downlisting than do those in lower categories suggests that in many cases such analyses will prioritize actions for the most threatened species first. We also note that there is considerable global spatial variation in costs and the number of threatened species per unit area (Fig. 3). Although the shortfalls in higher-income countries are substantial, the greatest gains per dollar will be in lower-income countries (23).

Despite the limitations of the available data, the shortfalls we have identified clearly highlight the need to increase investment in biodiversity conservation by at least an order of magnitude, especially given the small, but growing, body of evidence linking spending and effectiveness (24, 25). Nevertheless, the total costs are small relative to the value of the potential goods and services that biodiversity provides (26), e.g., equivalent to 1 to 4% of the estimated net value of ecosystem services that are lost per year [estimated at $2 to $6.6 trillion (27–29)]. More prosaically, the total required is less than 20% of annual global consumer spending on soft drinks (30).

These results should inform discussions among governments on the magnitude of the financing needs for implementing the CBD Strategic Plan for Biodiversity 2011–2020. A particular challenge will be how to address the current mismatch between the greater resources available in richer countries and the higher potential conservation gains in financially poor, biodiversity-rich countries (31, 32). Resolving the ongoing conservation funding crisis is urgent; it is likely that, the longer that investments in conservation are delayed, the more the costs will grow (23, 33), and the greater will be the difficulty of successfully meeting the targets (6, 34).

References and Notes
4. Further information is available as supplementary materials on Science Online.

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Supplementary Materials
www.sciencemag.org/cgi/content/full/science.1229803/DC1 Materials and Methods Supplementary Text Figs. S1 to S2 Tables S2 to S6 References (35–126)

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Pathological α-Synuclein Transmission Initiates Parkinson-like Neurodegeneration in Nontransgenic Mice
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Parkinson’s disease is characterized by abundant α-synuclein (α-Syn) neuronal inclusions, known as Lewy bodies and Lewy neurites, and the massive loss of midbrain dopamine neurons. However, a cause-and-effect relationship between Lewy inclusion formation and neurodegeneration remains unclear. Here, we found that in wild-type nontransgenic mice, a single intrastriatal inoculation of synthetic α-Syn fibrils led to the cell-to-cell transmission of pathologic α-Syn and Parkinson’s-like Lewy pathology in anatomically interconnected regions. Lewy pathology accumulation resulted in progressive loss of dopamine neurons in the substantia nigra pars compacta, but not in the adjacent ventral tegmental area, and was accompanied by reduced dopamine levels culminating in motor deficits. This recapitulation of a neurodegenerative cascade thus establishes a mechanistic link between transmission of pathologic α-Syn and the cardinal features of Parkinson’s disease.

The etiology of these processes remains unclear, although in familial PD, autosomal dominant α-Syn gene mutations or amplifications directly

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link α-Syn dysfunction to disease causation (2, 3). Moreover, despite the observation that α-Syn pathology is present in virtually all sporadic and familial PD patients (4) and that its distribution correlates with clinical symptoms (5–7), the precise relationship between α-Syn misfolding and DA neuron loss remains controversial because neither transgenic nor neurotoxin-based animal models of PD fully recapitulate the DA neuron degeneration, motor deficits, and the LB/LN-like α-Syn pathology seen in PD (8, 9).

Converging lines of evidence indicate that misfolded fibrillar forms of neurodegenerative disease–related proteins self-propagate and spread between interconnected central nervous system (CNS) regions, suggesting that cell-to-cell transmission of pathological proteins contributes to disease progression (10–15). Indeed, the pattern of LB/LN accumulation in human PD is highly stereotypical, consistent with propagation of disease over functional networks, suggesting that the transmission of pathologic α-Syn and its corruption of endogenous protein plays a central role in PD pathogenesis and progression (16, 17). Moreover, reports that embryonic mesencephalic neuron grafts in PD patients develop LBs many years after grafting (18, 19) further support the transmissibility of pathologic α-Syn. Consistent with this hypothesis, intracerebral inoculation of α-Syn preformed fibrils (PFFs) composed of recombinant protein accelerates the onset of neurological symptoms and death in transgenic mice overexpressing human A53T mutant α-Syn by enhancing the conversion of endogenous α-Syn into pathological LB-like inclusions (20). Although these data implicate α-Syn misfolding and fibrillization as triggering events upstream of neuronal dysfunction/death, minimal DA cell loss was observed, possibly reflecting alterations in the pattern of α-Syn transgene expression and the rapid demise of the inoculated mice.

Because synthetic α-Syn PFFs efficiently seed the aggregation and fibrillization of soluble endogenous α-Syn in primary neuronal cultures generated from wild-type (WT) mice (21), we asked whether transmission of LB/LN pathology might occur in WT mice in vivo. To answer this, we first determined whether PFFs assembled from recombinant mouse α-Syn initiate pathological α-Syn conversion when introduced into young WT (C57BL6/C3H) mice by stereotoxic injections targeting the dorsal striatum (fig. S1), a region receptive to PFF uptake (20) and interconnected with multiple CNS nuclei, including midbrain DA neurons. Deposits of hyperphosphorylated α-Syn (pSyn), a marker of human LBs/LNs (22), were visible at the injection site within 30 days of a single unilateral PFF inoculation (Fig. 1, A, B, and G). Intraneuronal α-Syn accumulations, primarily diffuse LN- and LB-like inclusions, were also present in several areas directly interconnected to the striatum that accumulate LBs in human synucleinopathies, most prominently in cortical layers IV and V and the olfactory bulb (Fig. 1, C and D), confirming the pathological conversion.

Fig. 1. Intrastratal inoculation of synthetic mouse α-Syn PFFs seed the aggregation of endogenous mouse Syn in WT mice. Pathology in brains of C57BL6/C3H F1 mice after a single unilateral injection of mouse α-Syn PFFs into the dorsal striatum. (A to D) Accumulation of α-Syn in neuritic processes or as pale cytoplasmic inclusions in striatum (Str) and neocortex (Ctx), and olfactory mitral neurons (OB) ipsilateral to the injection at 30 dpi. Black arrows highlight pathology revealed by immunostaining using antibodies to pSyn ([A] to [C]) or Syn506 ([D]). (E and F) LB-like inclusions in striatum and contralateral neocortex at 180 days postinjection with PFFs. (G) CNS distribution of pSyn accumulations of mice that received a single inoculation of PFFs in the dorsal striatum (indicated by gray circles). Representative maps of LB/LN-like pathology (red dots and stippled, respectively) in the PFF-injected hemisphere are shown for mice sacrificed at 30, 90, or 180 dpi. (H) α-Syn pathology in amygdala (Amyg) and (I) in frontal cortex (Fr). (J) pSyn staining in ipsilateral striatum 160 dpi with monomeric recombinant α-Syn. (K) Double immunostaining for pSyn (red) and TH (green) in a PFF-injected animal sacrificed at 180 dpi showing LB-like α-Syn pathology in ipsilateral SNpc. (L) High magnification revealing colocalization of pSyn inclusions to DA neurons (white arrows) and reduced TH immunoreactivity compared with unaffected DA neurons (white arrowheads). Images are representative of 3 to 7 mice examined per group (see table S1). Scale bars: 10 μm ([A] to [C], ([E] and [F]), and ([H] to [L]); 25 μm (D).
of endogenous mouse α-Syn in WT mice. At 30 days postinjection (dpi), pSyn-positive LB-like accumulations were exclusively ipsilateral to the injection site, with the exception of the amygdala, to which the striatum connects bilaterally (23), suggesting that cell-to-cell transmission followed interneuronal connectivity.

LB/LN pathology within affected regions showed markedly increased pSyn immunoreactivity in mice examined 90 and 180 dpi (Fig. 1, E, F, and G). Additional populations, such as the contralateral neocortex, also developed LBs/LNs indicative of progressive spread to CNS regions (Fig. 1, F to I, and table S1). Mapping of pSyn pathology in mice at 30, 90, and 180 dpi (Fig. 1G) revealed a time-dependent dissemination of LBs/LNs between 30 and 180 dpi, with sequential involvement of populations initially unaffected at 30 dpi, including ventral striatum, thalamus, and occipital cortex, along with commissural and brainstem fibers. Although inclusions were absent from multiple regions, including hippocampus, septum, and cerebellum (Fig. 1G and table S1), stereotoxic inoculation of equal quantities of PFFs into the hippocampus resulted in massive accumulation of α-Syn pathology (fig. S2), indicating that neurons in this region also are susceptible to transmitted pathologic α-Syn. Thus, propagation of LBs/LNs is connectivity-dependent, and this provides an explanation for the nonuniform distribution of LBs/LNs observed in α-Syn PFF-seeded animals. In contrast, monomeric α-Syn or phosphate-buffered saline (PBS) injections did not result in α-Syn pathology at any time point examined (Fig. 1J and fig. S2).

Striatal PFF inoculation led to abundant α-Syn pathology in tyrosine hydroxylase (TH)-positive SNpc DA neurons (Fig. 1, K and L) that project to medium spiny neurons of the dorsal striatum and whose selective degeneration is a major contributor to motor deficits in PD (24). Furthermore, α-Syn pathology in PFF-inoculated mice colocalized with key markers of LBs, including ubiquitin, heat-shock protein 90, and the amyloid-binding dye thioflavin S, indicating that they share common properties with authentic LBs/LNs in human PD (fig. S3).

SNpc α-Syn pathology developed progressively after PFF injection, evolving from pale cytoplasmic accumulations at 30 dpi to dense perinuclear LB-like inclusions by 90 and 180 dpi, particularly among ventromedial SNpc populations (Fig. 2, A to C). Because the nigrostriatal pathway is exclusively unilateral, LB/LN pathology in the SNpc was confined to the injected hemisphere at all time points examined (Figs. 1G and 2, A to D). In addition to being absent in ventral tegmental (VTA) neurons, α-Syn pathology was not observed in DA-expressing olfactory glomerular interneurons and arcuate cells of the hypothalamus (fig. S4). Similarly, noradrenergic (locus coeruleus) and serotonergic (Raphe nucleus) populations also remained unaffected at 180 dpi, suggesting that monoaminergic neurons are not equally susceptible to pathological α-Syn spread.

α-Synuclein pathology in the SNpc was accompanied by the gradual and unilateral loss of TH immunoreactivity and neurons (Fig. 2, F to I), suggesting that intraneuronal α-Syn inclusions lead to DA neuron loss. A total of 27.7% of SNpc DA neurons colocalized with pSyn pathology at 30 dpi (Fig. 2K), a stage at which SNpc and VTA DA neuron number were similar in both hemispheres (Fig. 2, L and M). SNpc, but not VTA DA, neurons developed LBs/LNs, confirming transmission of misfolded α-Syn through network projections and suggesting that connectivity is a determinant of susceptibility. The proportion of inclusion-bearing SNpc DA neurons did not increase further but instead declined over

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**Fig. 2.** Seeded α-Syn pathology leads to progressive DA system degeneration. (A to D) pSyn immunostaining in SNpc of mice sacrificed at 30, 90, or 180 days after striatal PFF injection. (A) Diffuse perinuclear pSyn inclusions (black arrowheads) at 30 dpi. (B and C) Dense LB-like inclusions (black arrows) at 90 and 180 dpi. Absence of pSyn pathology in SNpc contralateral to the PFF injection site (D) and in ipsilateral SNpc of PBS-injected control mice (E) at 180 dpi. (F to I) TH immunostaining of SNpc at 30, 90, and 180 days after inoculation with Syn PFFs. Arrowheads in (G) indicate neurons with reduced TH staining in the ipsilateral SNpc at 90 dpi. (H and I) Ventral SNpc, ipsilateral and contralateral to the site of PFF injection at 180 dpi. Arrows point to areas of DA neuron loss. (J) PBS-injected control at 180 dpi. (K) Percentage of SNpc TH neurons containing pSyn-immunoreactive inclusions for each treatment group. Data for treated ipsilateral (black) and contralateral (gray) hemispheres are shown. *P < 0.001, paired t test (N = 3 to 5 mice per group). (L and M) Quantification of TH-immunoreactive neurons in the SNpc and VTA of mice after intrastriatal PFF, monomer or PBS injection. Data represent mean number of cells per region ± SEM (N = 3 to 4 mice per group). *P < 0.05, one-way analysis of variance (ANOVA). Scale bars: 25 μm [(A) to (D)]; 50 μm [(E) to (J)].
time. There was a concomitant decrease of SNpc DA neurons by 15 and 35% at 90 and 180 dpi, respectively, suggesting that LBs/LNs form before SNpc DA neuron loss. Moreover, DA neurons in the contralateral SNpc and the VTA of both hemispheres (where inclusions were present in <0.2% of neurons) (Fig. 2K) were indistinguishable from those in age-matched PBS- or monomer-injected controls and PFF-injected Snca−/− mice, all of which did not develop α-Syn deposits (Fig. 2, D and E, and K to M). Thus, accumulation of pathologic α-Syn appears to be upstream of and directly linked to SNpc DA neuron loss.

Concomitant with the progressive loss of SNpc neurons, striatal concentrations of DA and its metabolites in the injected hemisphere showed progressive reductions after PFF inclusions, whereas contralateral striatal DA levels remain unaltered and noradrenaline (NA) levels were unchanged bilaterally (Fig. 3A and B, and fig. S5A). Immunoblot analyses confirmed reductions in TH and dopamine transporter (DAT) expression in the ipsilateral striatum (Fig. 3C), consistent with the loss of nigrostriatal DA innervation. Despite the loss of DA terminals, D1 and D2 dopamine receptor levels remained constant (Fig. 3C and fig. S5C), suggesting the preservation of striatal medium spiny projection neurons.

Given the PD-like Lewy pathology, SNpc neuron loss, and reduced striatal DA levels, PFF-inoculated WT mice might be expected to exhibit altered motor function, as in PD. Although no gross motor or behavioral abnormalities were observed up to 180 dpi, rotarod testing revealed significant and progressive performance deterioration in PFF-injected animals, indicative of impaired motor coordination and balance (Fig. 3D, left). Furthermore, mice with α-Syn pathology performed poorly on the wire-hang test, a complementary measure of motor strength and coordination, declining 53% from baseline at 30 dpi and 81% at 180 dpi (Fig. 3D, middle). However, open-field activity remained unchanged, indicating that overall motor activity was not significantly altered (Fig. 3D, right).

As previously noted, intrastratal PFF-inoculated mice did not develop hippocampal pathology (Fig. 1G), and no changes in memory function were observed as assessed using the Y maze (Fig. 3E). This corroborates our interpretation that declining neuronal function is correlated with increasing α-Syn pathology in affected neurons. Moreover, in keeping with the relative sparing of mesolimbic projections, no significant differences were found between PFF-treated and control mice in the tail-suspension test (Fig. 3E, right) a measure of depression-like behavior. The correlation between α-Syn pathology and neuronal deficits was also seen in striatal PFF-inoculated Snca−/− mice, where at 180 dpi both SNpc α-Syn pathology and motor impairments were attenuated relative to WT animals (Figs. 3, D and E, and fig. S6A). Finally, striatal inoculation into WT CD1 (fig. S6B) and C57BL6/SJL mice (table S1) also resulted in transmission of α-Syn pathology, suggesting that cell-to-cell spread of LBs/LNs is independent of mouse genetic background.

In summary, we demonstrate that a single intrastratal injection of synthetic misfolded α-Syn seeds into WT mice initiates a neurodegenerative cascade characterized by the accumulation of intracellular LB/LN pathology, selective loss of SNpc DA neurons, and impaired

Fig. 3. Decreased striatal DA and motor deficits in mouse α-Syn PFF-injected WT mice. DA (A) and NA (B) concentrations in dorsal striata of PFF-injected WT mice and PBS-treated controls measured at the indicated time points. Means ± SD are shown (N = 3 to 6 mice per group. *P < 0.01; **P < 0.001, one-way ANOVA. (C) Immunoblot analysis of striata from PFF- and PBS-treated animals, using antibodies against TH, DAT, D1 dopamine receptor (D1DR), and D2 dopamine receptor (D2DR). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is shown as a loading control. Mean intensity values are shown for TH and DAT (N = 3 to 4 striata per marker). Black and gray bars denote ipsilateral and contralateral regions, respectively. *P < 0.05; **P < 0.001, one-way ANOVA. (D) and (E) Behavioral assessment of WT mice 30, 90, or 180 dpi (N = 6, 9, and 17 mice, respectively) after a single unilateral inoculation of α-Syn PFFs into the striatum. PFF-injected Snca−/− mice (N = 4), as well as age-matched noninjected (N = 20) and PBS-injected (N = 8) WT mice are also shown. Results of animals on the rotarod test (left panel) and wire-hang test (right panel, middle panel) show progressive deficits in PFF-injected but not control mice. (E) Performance on the Y-maze and tail-suspension test. Data are mean values ±SD. Differences were established using one-way ANOVA (P = 0.0012) with Tukey post-hoc test. *P < 0.05; **P < 0.01.
The orbitofrontal cortex is thus fundamental for accessing model-based representations of the information. We found that the orbitofrontal cortex is critical for both value-based and associative model-based representations, particularly neuroeconomics does not attend to changes in value when value must be inferred or derived from model-based representations. We tested this hypothesis in rats using sensory preconditioning and blocking.

In sensory preconditioning, a subject is taught a pairing between two cues (e.g., white noise and tone) and later learns that one of these cues predicts a biologically meaningful outcome (e.g., food) (18). Thereafter, the subject will exhibit a strong conditioned response to both the reward-paired cue and the preconditioned cue. The response to the preconditioned cue differs from the response to the reward-paired cue, in that it cannot be based on a cached value; rather, it must reflect the subject’s ability to infer value by virtue of a knowledge of the associative structure of the task (see supplementary discussion for further details). If the OFC is required only for behavior that requires inferred value, then inactivating it at the time of this test should prevent behavior driven by this preconditioned cue, while leaving unimpaired behavior driven by the reward-paired cue.

Cannulae were implanted bilaterally in the OFC of rats (19 controls and 16 inactivated (OCFs)) at coordinates used previously (12, 19) (Fig. 1, A and B). After recovery from surgery, these rats were deprived of food and then trained in a sensory preconditioning task (Fig. 1) (see materials and methods).

In preconditioning, rats were taught to associate two pairs of unrelated auditory cues (A→B and C→D; clicker, white noise, tone, siren; counterbalanced). Food cup responding was measured during presentation of each cue versus baseline guided decisions per se, but rather in behaviors that require a new value to be estimated after little or no direct experience (7–14). Further, the OFC is often involved in a behavior that depends on whether learning is required (10, 15, 16), even when that learning does not involve changes in value (17). These data seem to require the OFC to perform one function—anticipating outcomes, in some settings—whereas it performs another, calculating economic value, in others. However, an alternative hypothesis is that the OFC performs the same function in all settings and specifically contributes to value-guided behavior and learning when value must be inferred or derived from model-based representations. We tested this hypothesis in rats using sensory preconditioning and blocking.

Computational and learning theory models propose that behavioral control reflects value that is both cached (computed and stored during previous experience) and inferred (estimated on the fly on the basis of knowledge of the causal structure of the environment). The latter is thought to depend on the orbitofrontal cortex. Yet some accounts propose that the orbitofrontal cortex is critical for both value-based behavior and learning when value must be inferred but not when a cached value is sufficient. The orbitofrontal cortex is thus fundamental for accessing model-based representations of the environment to compute value rather than for signaling value per se.

References and Notes

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Orbitofrontal Cortex Supports Behavior and Learning Using Inferred But Not Cached Values

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