The Effects of Microglia on Tauopathy Progression Can Be Quantified Using Nexopathy in Silico (Nexis) Models

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The effects of microglia on tauopathy progression can be quantified using Nexopathy *in silico* (Nexis) models

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ABSTRACT

The prion-like transsynaptic propagation of misfolded tau along the brain's connectome has previously been modeled using connectome-based network diffusion models. In addition to the connectome, interactions between the general neurological 'milieu' in the neurodegenerative brain and proteinopathic species can also contribute to pathology propagation. Such a molecular nexopathy framework posits that the distinct characteristics of neurodegenerative disorders stem from interactions between the network and surrounding molecular players. However, the effects of these modulators remain unquantified. Here, we present Nexopathy *in silico* ('Nexis'), a quantitative model of tau progression augmenting earlier models by including parameters of pathology propagation defined by the molecular modulators of connectome-based spread. Our Nexis:microglia model provides the first quantitative characterization of this effect on the whole brain by expanding previous models of neuropathology progression by incorporating microglial influence. We show that *Trem2*, but not microglial homeostasis genes, significantly improved the model's predictive power. *Trem2* appears to reduce tau accumulation rate while increasing its interregional spread from the hippocampal seed area, causing higher tau burden in the striatum, pallidum, and contralateral hippocampus. Nexis thus provides an improved understanding and quantification of microglial contribution to tau propagation and can be flexibly modified to include other modulators of progressive neurodegeneration.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that exhibits extracellular amyloid- β plaques and intracellular aggregates of hyperphosphorylated tau as its main neuropathological hallmarks; although the regional location and density of tau tangles correlate more with the degree of clinical symptoms than those of amyloid- β plaques^{1,2}. The stronger association between tau and regional atrophy, hypometabolism, and cognitive decline in AD^{3,4} makes it imperative to shift the long-standing focus from amyloid- β to tau and to further investigate effects that can modulate tau accumulation and spread.

Tau propagates in a stereotypical pattern in AD, starting from the entorhinal cortex to the hippocampus followed by the neocortex as described by the Braak staging scheme⁵. Experiments involving the injection of synthetic tau fibrils in the mouse brain have demonstrated that tau can propagate from the injection site to distant but connected regions⁶. Thus, tauopathy progression has often been described as a prion-like mechanism - templated and self-propagating⁷, with pre-existing aggregates acting as seeds to induce further misfolding. Since tau primarily migrates along the white matter tracts of the brain, it is reasonable to model its spatiotemporal dynamics in a first approximation as a diffusive process between connected brain regions. Prior work by our group has quantified pathology progression in humans and animals using such an approach via a graph-theory based system of differential equations called the Network Diffusion Model (NDM)⁸⁻¹⁰. In this framework, the connectome graph is the sole mediator of pathology spread along a networked representation of the brain.

However, despite the key mediatory role played by the connectome, there are many other interactions between cells, circuits, proteopathic species, and the general neurological 'milieu' present in the neurodegenerative brain that remains outside of the connectome-centric view of these diseases. These factors were collectively called the Molecular Nexopathy by Warren *et al*¹¹, based on their recognition of the fact that pathological tau protein transits with a different mechanism in a disorder-dependent manner^{11,12}. Evidences for this notion have only become stronger over years, suggesting the existence of important condition-altering differences in pathological tau species between degenerative conditions. For instance, tau displays differential 3R and 4R isoforms¹³ and sites of tau hyperphosphorylation¹⁴ in different tauopathic conditions. Gross tangle structure is also different

across diseases, with AD tau forming neurofibrillary tangles (NFTs) and pre-helical formations (PHFs), Pick's disease tau forming Pick bodies, and progressive supranuclear palsy, corticobasal degeneration, and frontotemporal dementia oligomers presenting more abundant tangles¹⁵. To restate the Nexopathy paradigm: *divergent spatiotemporal patterns of development between different tauopathic disorders may result from biochemical differences between tau species and the neural cells in the brain that interact with tau as well as the connectome-based mechanism of transsynaptic transmission*.

In this study we attempt to capture this nexopathy paradigm using mathematical *in silico* modeling, which we refer to as Nexopathy *in silico* or 'Nexis'. In order to do so, we formulate from first principles the process by which misfolding tau must nucleate, aggregate, and transit along neural connections. Although these principles are qualitatively well-established from preclinical *in vivo* and *in vitro* studies, the unique benefit of formulating them in a mathematical model is that the model then becomes a means for testing interesting hypotheses and becomes a *testbed* for novel hypothesis generation.

A particularly important set of nexopathic effects are wielded by the brain's resident immune cells, and tau dynamics is well known to be influenced by the action of such cells often found in the vicinity of pathological aggregates. Recent studies of postmortem AD brains have revealed that microglia co-localize with tau and activate themselves after internalizing tau aggregates¹⁶. Tau-containing neurons can also activate nearby passive microglia causing them to form junctions with other neurons as conduits to transfer tau and propagate pathology¹⁷. Using mouse models of rapid tau propagation, Asai *et al*¹⁸ and others have demonstrated that microglia markedly contribute to the clearance, recruitment, and spread of pathology. Microglial activation and neuroinflammation accompany tau tangle formation and importantly, parallel AD clinical symptoms¹⁹. Specifically, activated microglia express the triggering receptor expressed on myeloid cells 2 (TREM2). *Trem2* is a well-known AD risk-gene, with loss-of-function mutations increasing the susceptibility to late-onset AD²⁰ and being related to the rate of cognitive decline²¹. *In vivo*, the over-expression of *Trem2* in pathological conditions is related to the recruitment of microglia to pathological aggregates. Empirical clinical and preclinical data thus suggest that the modulation of tau propagation by the action of microglia^{18,22,23} is plausible and germane to the study of progressive tauopathies.

Owing to the microglial contributions, quantitative estimates based solely on connectivity and regional pathology levels, e.g. those from the prior Network Diffusion model (NDM)⁸, cannot recapitulate all aspects of pathology spread patterns in AD. Therefore in this paper, we apply the novel Nexis framework to interrogate the modulatory effect of microglia on tauopathy progression, by introducing microglial signatures within the Nexis model. We explore microglial influence under two potential modes, whereby microglia alter: (1) the rate of intra-regional tau accumulation and (2) the rate of inter-regional spread of tau. These effects are not mutually exclusive but have different biological and mathematical implications. We first apply the non-microglia-influenced Nexis model of tau aggregation and spread ('Nexis:global') characterized solely by tau accumulation/clearance rate in addition to its diffusion along the connectome. This model is further extended into a microglia-based 'Nexis:microglia' model to incorporate the effect of these cell types on tauopathy progression. We apply these models to data from mouse models of tauopathy²⁴ using a 426 x 426 region mouse connectome²⁵. By utilizing microglial homeostasis genes (their first principal component; see Methods) and an activated microglial signature (*Trem2*) gene²⁶, we show that the inclusion of microglia in the model quantitatively improves its predictive power, with *Trem2* inclusion largely outperforming that of homeostasis genes. We developed an optimization algorithm to infer model parameters. Results indicate that *Trem2* may reduce tau burden and increase tau transmissibility to regions ipsi- and contralateral to the hippocampal seed area. We also explored *Apoe*, another putative modulator of tau propagation, and found that it does not contribute significantly to tau progression in the current datasets used. Overall, the Nexis:microglia model provides a more complete model of tau propagation. This study brings together empirical mouse tauopathy models²⁴, the mouse mesoscale connectome²⁵, and gene expression atlas²⁶ to achieve the first quantitative study of microglial involvement in tauopathy propagation developed and validated at the whole-brain level.

Results

Association between spatiotemporal tau pathology and microglial spatial distribution

We demonstrated an association between tau pathology spread in a sample dataset (DS9) and baseline expression of microglial genes (depicted in Fig. 1). Correlations between tau pathology (Fig. 1(a)) and microglial homeostasis genes (Fig. 1(b-top and c)) as well as with *Trem2* (Fig. 1(b-bottom and d)) were significant (all *p* values less than 0.001), although modest. These data form a solid basis on which to undertake a more detailed and model-based exploration of the effects of microglia on tauopathy progression. Since homeostasis genes and *Trem2* have distinct functional roles and display distinct baseline distributions, we chose to model them separately with two models following the Nexis:microglia framework: Nexis:homeostasis for the microglial homeostasis genes and Nexis:*Trem2* for *Trem2*.

Model parameterization depends on tau strain

The Nexis:microglia models include four parameters: the global accumulation/clearance rate of pathology independent of any modulators, α ; the global diffusivity rate constant denoting the diffusion of pathology between regions, independent of

any other modulators, β ; accumulation/clearance of pathology as modulated by microglia, p ; and pathology diffusion rate as modulated by microglia, b . *Nexis:global* is the non-microglial model with $p = b = 0$. The tau strains of the selected datasets were derived from mutually distinct sources: DS4 mice were injected with human AD-derived tau isolates, whereas DS6 and DS9 mice were injected with tau derived from a P301S mouse and recombinant tau fibrils, respectively²⁴. The datasets demonstrated distinct tau bio-activity characterized by varying capacities to form and spread tau aggregates (seeding and spreading potential), widespread induction of pathology, and capacity to induce microgliosis²⁴. We implemented a robust strategy for parameter estimation using a 100 iteration bootstrapping approach on 80% resampled data (see Methods), allowing us to acquire 95% confidence intervals around the parameter means and to assess reproducibility. Tables 1, 2, and 3 summarize the inferred parameters (with a 95% confidence range) and quality-of-fit metrics for all datasets and models. Non-zero p and b values demonstrate microglial influence on tau accumulation and spread above and beyond *Nexis:global*. When *Trem2* microglial effects were included via *Nexis:microglia*, the datasets DS6 and DS9 indicated enhanced clearance of tau (since p less than 0) but increased inter-regional spread of tau (b greater than 0). Homeostatic microglial effects, however, led to lower inter-regional spread (since the corresponding b is less than 0; Tables 2, 3). DS4 demonstrated increased tau accumulation as well as spread due to *Trem2* compared to homeostatic microglia (Table 1).

Inclusion of microglia improves the predictive power of *Nexis*

As shown in Tables 1, 2, and 3 the NDM performed weakly compared to *Nexis* models for all datasets. *Nexis:global* performed weakly for DS4 ($R^2 = 0.17$) but well for DS6 and DS9 ($R^2 = 0.47$ and 0.44 , respectively), successfully recapitulating the spatiotemporal profile of empirical tau pathology. This suggests that passive network diffusion is insufficient, and additional aggregation processes enhance the model's predictive power.

For all three datasets, the inclusion of microglial gene expression demonstrated higher predictive power (as assessed by R^2) compared to the *Nexis:global*. The improvement was statistically significant (per Fisher's-R-to-Z transform p value and AIC) for DS6 and DS9. Further, *Trem2* imparted significantly higher predictive power compared to microglial homeostasis genes. It is important to note that across all datasets, AIC decreased upon the inclusion of microglia, indicating that the better fits achieved by the *Nexis:microglia* were not simply due to the introduction of additional model parameters. Instead, these results reflect reduction in uncertainty in the global parameters, α and β , as indicated by their narrower 95% confidence intervals.

Effect of *Trem2* on tau burden and spread

Since the fitting results highlight *Trem2* as by far the more significant mediator in comparison to homeostatic genes, the remainder of our results will focus on the former. Figure 2 shows the results for dataset DS9 in more detail; they are very similar for DS6. For dataset DS4, while the *Nexis* models were significantly better than the passive NDM, improvement from *Nexis:global* to *Nexis:microglia* was not statistically significant (Table 1). While Fig. 2 panel (a) depicts empirical spatiotemporal tau pathology in DS9²⁴, panels (b), (c), and (d) depict the spatial distribution of tau under the NDM, *Nexis:global* and *Nexis:Trem2* frameworks, respectively. Both *Nexis*-modeled distributions differ significantly from the NDM-modeled pathology distribution. While both *Nexis*-modeled distributions look similar visually, there are clear differences between them that begin to become apparent at later time points, especially in striatal and entorhinal areas. The scatter plots in panels (f) and (g) of Fig. 2 show that while both *Nexis* models produce excellent recapitulation of empirical tau burden at all time points, the *Nexis:Trem2* model produces by far the better fits. Pearson r values are reported for each scatter plot. Model fits in all cases improve over time, as may be expected from a ramifying pathological process. Note from Tables 1, 2, and 3 that the *Nexis:Trem2* fits incurred an increase in the microglia-dependent spread parameter b ; hence *Trem2* appears to allow more pathology to efflux from the hippocampal seed regions at early time points – this notion is confirmed visually in panels (c) and (d) of Fig. 2.

We next assessed, visually, the effect that microglia are imparting on the spread of tau within the model. Flow-maps depicting major pathology spreading directions were constructed using Brainframe, our in-house Matlab-based function. Figure 3 highlights differences in tau burden and spread as modeled by the *Nexis:global* and *Nexis:Trem2* for dataset DS9. Blue arrows depict *Nexis:global*-modeled tau flow without the influence of *Trem2* while magenta arrows show tau-flow mediated by *Trem2*, above and beyond that modeled by *Nexis:global*. A prevalence of magenta arrows (Fig. 3(b)) indicates that *Trem2* allowed more widespread propagation of tau (also reflected in the higher b values in Table 3). Although modeled tau propagated from the hippocampal seed area to connected regions, *Trem2* appeared to mediate more spread of tau to the striatal, entorhinal, pallidal, and contralateral hippocampal regions. Similarly, a prevalence of green spheres in the striatal, pallidal, and hippocampal areas indicates that these regions evidence higher tau burden due to *Trem2* than would otherwise be expected. Tau burden was unaffected by the presence of *Trem2* in the parasubiculum (red sphere at all time points). The results of the parameter estimation using the bootstrapping approach for dataset DS9 are presented in Fig. 4 which includes the mean and 95% confidence intervals for the model fit as well as global and microglial parameters for *Nexis:global* and *Nexis:Trem2* of DS9.

Negligible effect of *Apoe* on tau burden and spread

Apoe is a strongly implicated AD-risk gene expressed both in microglial cells and in astrocytes. Adding its gene signature as a modulator of pathology spread in *Nexis:microglia* did not outperform *Nexis:global* in any of the datasets (*Nexis:Apoe* $R^2 = 0.18, 0.50, 0.46$ for DS4, DS6, and DS9 respectively). Since *Trem2* and *Apoe* also work in concert in AD²⁷, we conducted a combined *Nexis:microglia* analysis including both the genes, but results for all three datasets matched those produced by *Nexis:Trem2* alone. (See Supplementary Tables S1, S2, and S3 for results of *Nexis:Apoe* fitting in datasets DS4, DS6, and DS9; and Supplementary Fig. S1 for depiction of bootstrapped fitting results for *Nexis:global* and *Nexis:Apoe* for dataset DS9).

Discussion

The spread of hyperphosphorylated tau in AD occurs along the brain's connectome. The presented modeling effort was inspired by and expand upon the 'molecular nexopathy' paradigm¹¹ in that a central role is noted for network-mediated spread, but also includes other sources of protein aggregation and mediators of trans-neuronal spread, especially microglia. Microglia co-localize with tau inclusions, taking up and releasing bioactive seed-competent tau, and thus emerging as key mediators of AD pathophysiology propagation along with connectome-mediated spread^{17,28}. Phagocytic microglia, instead of degrading tau, may package it into exosomes¹⁸ and form aberrant junctions with surrounding neurons through which tau might be transmitted between cells^{17,23}. While prior studies^{8–10} have successfully quantified key aspects of pathology progression along the connectome, they do not constitute a true Nexopathy model, since they did not account for the influence of non-neuronal modulators such as microglia.

In this study we presented a mathematical model from first principles, called *Nexis*, that attempts to incorporate diverse effects including misfolded protein seeding, aggregation, connectome-mediated spread, as well as the modulation of all the above processes by external agents residing within the brain's neurological 'milieu'. The presented model in particular augments prior quantitative models⁸ to include microglial modulation via two key processes: local tau accumulation and its enhanced trans-neuronal transmission.

Our results quantify by how much the inclusion of microglia improves the predictive power of the model and reduces the uncertainty in the accumulation and diffusion parameter estimates (see AIC and narrower confidence intervals around model parameters, Tables 1, 2, 3). The full model recapitulates empirical data with R^2 values up to 0.59 (as observed in the case of the DS6 dataset). *Trem2* distribution (Fig. 1(b), bottom) did not spatially correlate with regions with higher *Trem2*-mediated tau burden (Fig. 2(d)), suggesting that microglial effects are not strictly local. Instead, as shown by differences in fitted model parameters, *Trem2* appears to mobilize tau, increasing its network transmissibility and burden in susceptible regions.

Microglial modulation is strongly dependent on the tau strain used in the specific dataset. Datasets DS6 and DS9 have medium to high seeding capacity and are characterized by tau from a P301S tauopathy mouse model and recombinant fibrils, respectively. For these sets, *Trem2* was associated with decreased tau accumulation rate (p) and increased spread (b) to regions ipsi- and contralateral to the hippocampal seed area (Tables 2, 3). We hypothesize that *Trem2* renders tau more mobile, thus increasing its brain-wide spread but reducing its accumulation rate. *Trem2*-mediated high tau efflux with slower accumulation may reflect clearance and possible mitigation of pathology. Mouse studies have indeed demonstrated *Trem2*-mediated decrease in tau accumulation in early disease stages^{29,30}, as well as *Trem2* loss resulting in enhanced tau seeding and exacerbated pathology²¹. Microglia can internalize and release seed-competent tau, but also potentially degrade its seeding activity²⁸. Our model parameters here suggest that indeed the decreased accumulation rate (parameter p) may support the empirically observed reduced seeding activity²⁸, whilst still maintaining tau spreading potential (parameter b). Intriguingly, our results posit this effect of microglia to be specific to *Trem2*, and not to homeostatic microglial genes.

Neither *Nexis* framework recapitulates one of the datasets, DS4, particularly well (R^2 : 0.17-0.20) (Table 1). DS4, characterized by the human AD-brain derived tau isolate, demonstrated elevated rates of both tau accumulation (p) and spread (b) in the presence of either *Trem2* or homeostatic microglia; however, neither *Nexis:homeostasis* nor *Nexis:Trem2* was significantly better than *Nexis:global*. As observed by Kaufman *et al*²⁴, DS4 was a low-tangle tau strain with lower seeding capacity than DS6 and DS9. This may explain its comparatively weaker *Nexis:global* model fit, with a failure of any additional improvements to its model fit with the inclusion of microglial genes (Tables 1). We also speculate that since DS4 was characterized by amyloid-comorbid tau from a human-AD brain it evinced higher values of accumulation and spread rates. AD derived amyloid-comorbid tau can acquire more pathogenic conformations leading to its aggregation and accumulation at a faster rate³¹. Our results thus point to the importance of quantifying the heterogeneous effects that tau has on neuropathology progression, importantly because tau strain diversity based on differences in bioactivity (seeding and spreading) may explain the myriad of AD clinical outcomes³².

Apoe is a canonical risk-gene implicated in AD, and along with being a strong influencer of amyloid pathology, it is also suggested to influence microglial function and tau pathology³³. We thus explored the effects of *Apoe* via *Nexis:microglia* model alone and in conjunction with *Trem2* as these two genes appear to act in concert²⁷. The lack of improvement in both *Nexis:Apoe* and *Nexis:Trem2:Apoe* (See Supplementary Tables S1, S2, S3 in SI) can be related to the fact that *Apoe* acts downstream of

*Trem2*²⁷ (in disease conditions). Therefore, the key mediatory role manifested by *Trem2* can be expected to encompass the contribution of *ApoE*.

In conclusion, our *in silico* Nexis:microglia model quantifies the contribution of microglia to tau neuropathology progression. It recapitulates the characteristics of observed tau pathology progression, providing distinct results specific to the tau species employed in the mouse models we studied. Although prior bench studies have suggested a modulatory role for microglia, the present study is the first to demonstrate this *quantitatively* on the whole mouse brain, and for specific microglial genes and specific tau conformations. In particular, our results provide quantitative support to the observations made by Asai *et al* in that, microglia aid increased propagation of tau across brain regions¹⁸. The Nexis:microglia framework can greatly aid experimentalists in further hypothesis generation, validation, and quantification. It may be readily adapted to interrogate the effects of other putative modulators of pathology progression such as astrocytes and oligodendrocytes.

Limitations and future directions

The datasets (DS4, DS6, and DS9) monitored the levels of exogenous tau at 4, 8, and 12 weeks post hippocampal seeding. Additional time points may enable a more thorough understanding of the spatiotemporal behavior of tau progression. The current monitoring over time was also not longitudinal in the real sense since measurements did not track the same animal. Instead, tau progression was observed in groups of tau-injected mice sacrificed at different time-points post-injection. Further, the gene expression data used as a signature of activated and homeostatic microglia were from a healthy mouse brain and thus can infer only about the effects of baseline microglial density. However, interestingly, a recent clinical study indeed supports the effect of baseline microglia, rather than the brain's anatomic network, on tau propagation²³. Last, *Trem2* assumes opposing roles (protective vs. detrimental) in AD pathology depending on the stage of the disease. Such disease-stage dependent dual roles of *Trem2* have been observed with respect to amyloid β plaque pathology³⁴. Our current *in silico* investigation of the role of *Trem2* in neuropathology progression is restricted to tau propagation and is conducted on data acquired within three months of exogenous tau seeding in mice. Future goals would be to apply this approach to data collected at extended time points post tau-seeding (depending on data availability), as well as to include amyloid pathology in the model since amyloid β has been speculated to potentiate microglial influence on tauopathy propagation²³. Another possibility, that the seed region where tau was initially injected may confer specific molecular vulnerability to tau, remains outside the scope of the current study design. Our Nexis approach itself is fully capable of testing for site-specific effects but paucity of experimental data to date makes this challenging.

We note finally that the nexopathy paradigm enumerates a far more diverse set of potential mechanisms by which molecular dysfunction might interact with the neural architecture than is possible to explore in a single study. Gene expression in different tissues can change with age, stress and environment, such as epigenetic effects like DNA methylation³⁵. There can be other sources of regional variability, e.g. oxidative stress, vascular effects, etc³⁶. Epigenetic or post-translational modifications caused by these regionally variable causes will produce gene expression in diseased groups that is different from healthy mouse gene expression studied here, and therefore are not possible to study using the current approach. However, the primary goal of uncovering the molecular versus network correlates of AD topography may be largely unaffected by these additional factors, since they are either unlikely to have spatial gradients (e.g. age, environment) or have non-AD-like spatial patterning (oxidative/vascular stress).

Conclusion

Current models of neuropathology progression along the brain's anatomic network fail to consider other modulators thereof. Microglial activation is a key feature of tauopathies such as Alzheimer's, but its effects on tau burden and spread lack quantification. We introduce a nexopathy model, Nexopathy *in silico* or Nexis, that quantifies the influence of microglia on network spread of tau and captures pathology progression across the whole brain. This novel approach contrasts with previously published network diffusion models and provides the first *in silico* quantification of microglial contribution to progressive neurodegeneration. By applying Nexis models with microglial homeostatic and activation (represented by *Trem2*) gene signatures, we disentangled their effects, showing that *Trem2* reduces tau accumulation rate but increases its transmissibility across brain regions. Importantly, the model parameters depend on the strain of tau. Our Nexis framework is flexible and can be employed to model the effect of other modulators of neuropathology progression.

Methods

Study Design

Empirical spatiotemporal tau pathology data were obtained from a recent study on PS19 mice expressing 4R1N P301S with human tau, exogenously injected with distinct strains of pathological tau²⁴. Regional levels of tau burden were extracted from

heat maps displaying semi-quantified pathology anatomically following the approach from Mezias *et al*³⁷. The Kaufman datasets used in this study are DS4 (containing tau isolate derived from the human AD-brain), DS6 (contained tau injectate from a P301S tauopathy mouse), and DS9 (characterized by recombinant tau fibrils). Our 426×426 region mouse connectome is from the Allen Brain Atlas (ABA)²⁵, with 213 region bi-hemispheric symmetry. The microglial baseline spatial gene expression maps employed as signatures of baseline homeostatic and phagocytic microglia are from the ABA gene expression atlas of healthy 56-day old male C57BL/6J mice²⁶. Note that these microglial signatures represent quiescent rather than activated states and may not be considered disease-activated microglia (DAM). Regional homeostatic microglial abundance was defined as the first principal component of the expression levels of *P2ry12*, *Cx3cr1*, *Fcrls*, *Olfml3*, *Hexb*, *Siglech*, *Sox5*, and *Jun*³⁸. We tested separately the influence of *Trem2*, *Cd33*, and *Apoe*, activated microglial surface-markers and regulators of microglia-mediated phagocytosis strongly implicated in AD pathogenesis³⁹. *Cd33*'s influence was negligible compared to *Trem2* given its smaller effect size compared to *Trem2*⁴⁰, and thus, is not included in the results.

Nexopathy *in silico* Model (Nexis)

The Nexis model is derived from the Network Diffusion Model (NDM)⁸ - a mathematical model that describes pathology spread as a diffusive process between connected brain regions. Let c_{12} denote the inter-region connection strength between two brain regions (1,2) as inferred from tractography data, let $x_1(t)$ and $x_2(t)$ be the pathology concentration at two brain regions at time t as inferred from semi-quantitative immunohistochemistry analysis from the specific reference study. Following the NDM⁸, we impose first-order diffusive dynamics:

$$\frac{dx_1}{dt} = \beta c_{12}(x_2 - x_1), \quad (1)$$

where β is the global diffusivity rate that controls how fast pathology can flow from the more concentrated region to the less concentrated one independent of any other modulators.

Nexis:global adds another parameter to the NDM, αx_1 , a linear accumulation or clearance term depending on its sign.

$$\frac{dx_1}{dt} = \beta c_{12}(x_2 - x_1) + \alpha x_1, \quad (2)$$

This pair-wise relationship can be extended to the entire brain connectivity network using vector $\vec{x} \in \mathbb{R}^n$ over all n ROIs, to give $\frac{d\vec{x}}{dt} = -\beta L\vec{x}$, where L is the graph Laplacian matrix (when $\alpha = 0$)⁸.

Nexis:microglia

The Nexis:microglia framework incorporates the potential effects that different modulators, for instance cell-types such as microglia, may have on neuropathology progression, particularly in the context of AD and other tauopathies. It has two additional cell-type specific parameters p and b , which indicate the effect of the chosen cell type, u , on pathology accumulation/clearance and spread, respectively. Letting u_1 and u_2 indicate the local gene expression in regions 1 and 2, the extended pairwise relationship in (2) becomes

$$\frac{dx_1}{dt} = \beta c_{12}[(1 + u_2 b)x_2 - (1 + u_1 b)x_1] + \alpha x_1 + u_1 p x_1. \quad (3)$$

Equation [3] reduces to NDM equation [1] if $b = p = \alpha = 0$, and can similarly be extended brain-wide via pathology $\vec{x}$ and gene $\vec{u}$ vectors to $\frac{d\vec{x}}{dt} = [\Lambda(\vec{u}) - \beta \tilde{L}(\vec{u})]\vec{x}$, where Λ is a diagonal matrix and $\tilde{L}$ is a new Laplacian, both now depending on gene expression u . See the Supplementary Information for a detailed explanation.

For convenience of reading, in the remainder of the document the model name 'Nexis:microglia' will be replaced by Nexis:homeostasis in the case of homeostatic microglia or Nexis:(microglial gene name) in the case of a particular microglial gene being investigated.

Model validation at the whole-brain level.

We validated the Nexis models at the whole-brain level on mouse tauopathy datasets DS4, DS6, and DS9 from Kaufman *et al*²⁴. Figure 5 (a-c) schematizes the network representation of the mouse brain for which we extract the connectivity matrix C . This connectome is the central (sole) mediator of tau spread in NDM (see simulations in Fig. 5(d)). For Nexis, the analytical framework is augmented to account for the influence of tau accumulation/clearance rate (as in Nexis:global) followed by the influence of microglia (as in Nexis:microglia) (see simulations in Fig. 5(e)).

Seed scaling and model initialization

Both NDM and Nexis require a seed-rescale parameter γ . It denotes the scaling factor for initial pathology at the site of injection of pathology (tau in the current case). This parameter needs to be quantified since although we know the site of the injection (hippocampus for instance), we do not know the amount of pathology being injected. Thus, the pathology at $t = 0$ is represented as a binary vector multiplied by γ and is used to initialize the model.

Robust strategy for parameter estimation

We implemented a two-step, robust strategy for estimating parameters for the NDM and Nexis models. First, we fit the spread parameter (β) for the NDM, followed by the accumulation and spread parameters (α and β) for Nexis:global via grid search and random initialization. This was then followed by 100 iterations of bootstrapping with 80% re-sampling. Next, for the Nexis:microglia model, we initialized the values of α and β to those obtained from Nexis:global while also fitting (without initialization) the microglia-mediated accumulation and spread parameters (p and b). Anchoring the parameters shared by both Nexis frameworks within a reasonable range from each in the optimization routines prevents the algorithm from reaching local minima that yield better fits but have overall biologically-implausible parameter values. This also allowed us to assess reproducibility by acquiring the 95% confidence intervals around the mean estimates of the model fit as well as global and microglial parameters (see simulations in Fig. 4 for Nexis:global and Nexis:Trem2 for example dataset DS9). Importantly, model parameters were fit using all available quantified regions and all time points; prior formulations only allowed fitting to single time points^{9,10}.

Statistics and Reproducibility

The statistical significance of the differences in model fits between the NDM and the Nexis frameworks was determined by carrying out Fisher's-R-to-Z transformation. The p value of significance was obtained by the Student's t test on the Fisher-transformed Z scores. The model fits presented in Tables 1, 2, and 3, were determined by averaging the parameters across 100 iterations of bootstrapping with 80% re-sampling of the regions quantified by Kaufman, *et al*²⁴. When fitting the Nexis:microglia models we used the mean and 95% confidence intervals determined for the Nexis:global parameters α , β , and γ for each dataset as the initial guesses and bounds for those parameters, respectively; this helps to minimize the risk of finding degenerate solutions. (See Supplementary Information for details on parameter fitting). The R^2 values of the full model are within the range of the bootstrapped iterations of the 80% re-sampled data (Fig. 4(c)), thus confirming reproducibility. All analyses were conducted in Matlab R2020b.

Availability of data and materials

The source data on the mouse models, gene expression, and mesoscale mouse connectome as well as the computer code to run the models are available at our repository at <https://github.com/Raj-Lab-UCSF/Nexis>.

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Author contributions statement

A.R. conceptualized the study and developed the original network diffusion model (NDM, 2012). C.A. performed the literature search and helped in the conceptualization of the study. P.M., J.T., and C.M. expanded the NDM and optimized the *Nexis* framework. J.T. validated the reproducibility of the model by bootstrapping. C.M. curated and processed the tauopathy data and developed a Matlab-based 'Brainframe' toolbox for pathology depiction on glass brains. C.A. conducted the study, analyzed the results, and generated the figures. C.A. and P.M. co-wrote the manuscript. All authors reviewed and edited the manuscript.

Additional information

Competing interests. The authors declare that they have no competing financial or other interests.

Ethics approval and consent to participate. All data were obtained from publicly available datasets and published papers. No human subjects, live animals, patient or animal derived tissue, cell lines, or biological material were directly used in this research and thus, no consent or ethics approval was required.

Consent for publication. No human subjects were used in this research, nor were any materials derived from human subjects. Thus, no consent for publication was required.

Figures

Figure 1. Correlation between spatiotemporal empirical tau pathology at 4, 8, and 12 weeks post hippocampal tau injection and baseline microglial gene spatial distribution for DS9. Panel a: Empirical tau pathology in DS9 dataset post hippocampal seeding of exogenous tau²⁴. Panel b: Baseline spatial distribution of first principal component of microglial homeostasis genes (See Methods) and *Trem2* from a healthy adult mouse²⁶. Panels c and d: Correlation between spatiotemporal tau pathology progression and baseline expression levels of microglial homeostasis genes as well as *Trem2*. Sphere sizes in A correspond to the degree of tau burden in specific brain regions. (HIP: hippocampus, EC: entorhinal cortex, PAL: pallidum, STR: striatum.)

Figure 2. NDM, Nexis:global, and Nexis:Trem2 model fits for dataset DS9. Panel a depicts empirical pathology observed in DS9²⁴. Panels b, c, and d depict NDM, Nexis:global, and Nexis:Trem2 modeled tau pathology progression, respectively, in DS9 dataset at 4, 8, and 12 weeks post hippocampal injection. Panels e, f, and g are scatter-plots depicting the correlation between empirical and ND-modeled (e), Nexis:global-modeled (f), as well as Nexis:Trem2-modeled (g) tau pathology, respectively, at 4, 8, and 12 weeks post hippocampal tau seeding. (HIP: hippocampal area, EC: entorhinal cortex, PAL: pallidum, STR: striatum.)

Figure 3. Flow-map representations of tau accumulation and spread in DS9 dataset post hippocampal seeding. Tau burden and spread differences were evaluated between Nexis:global and Nexis:Trem2 modeled tau pathology. Blue arrows of tau spread (a) indicate a positive difference (tau spread: Nexis:global > Nexis:Trem2). Magenta arrows of tau spread (b) indicate a negative difference (tau spread: Nexis:global < Nexis:Trem2). Red and green spheres represent higher tau burden as modeled by Nexis:global and Nexis:Trem2, respectively. Sphere sizes correspond to the degree of tau burden. (HIP: hippocampal area, contra. HIP: contralateral hippocampal area, lateral EC: lateral entorhinal cortex, PAL: pallidal area, PAS: parasubiculum, STR: striatal area.)

Figure 4. Mean and 95% confidence intervals for the global and microglial parameters as well as model fit for dataset DS9. Panels a and b depict the 100-iteration 80% resampled bootstrapped values of the Nexis:global and Nexis:Trem2 model parameters. Panel c depicts the comparison between model fits of the 80% resampled bootstrapped dataset with the full dataset. Global parameters: γ (seed rescale), α (intra-regional tau accumulation rate), and β (inter-regional tau spread rate). Microglial parameters: p (microglia-mediated intra-regional tau accumulation rate) and b (microglia-mediated inter-regional tau spread rate). Model fit is depicted by R^2 .

Figure 5. Modeling of tau propagation and the hypothesized influence of microglia. Panel a: Representation of the brain as a network, with each ball indicating the center of mass of a region and the line between any pair of regions indicating a connection between them. Panel b: Connectivity matrix of unilateral 213 cortical regions of the adult mouse brain. Panel c: Graphic of the whole brain connectivity network with nodes (i, j, k) representing brain regions and connection strengths (C_{ij}, C_{jk}) representing mesoscale connectivity. Panel d: Illustration of Nexis-modeled progression of tau across time with each ball representing a region and ball-size indicating the degree of pathology burden. Panel e: Per-region baseline microglial density is depicted in blue on the graphic of the sagittal mouse brain. Tau pathology at 4 and 12 weeks post hippocampal tau seeding is depicted in red on the mouse brains. The black arrow indicates the hippocampal injection site. Panels a, c, and d are adapted from Mezias *et al*¹⁰. (AMY: amygdala, CRB: cerebellar region, CORT: cortical subplate, HPC: hippocampal formation, HYPO: hypothalamus, ISOCTX: isocortex, MED: medulla, MIDBR: midbrain, OLF: olfactory areas, PAL: pallidum, PONS: pons, STR: striatum, THAL: thalamic areas.)

Tables

Table 1. Comparison of model fits for NDM, Nexis:global, and Nexis:microglia for dataset DS4. All Nexis model fits were significantly better than those of the NDM ($p < 0.005$). Nexis:microglia model fits are compared to the Nexis:global model fit (significance indicated by *). Significance of model fit comparisons is confirmed using the Fisher's R-to-Z test. NDM: Network Diffusion Model, Nexis: Nexopathy *in silico*, AIC: Akaike's Information Criteria, BIC: Bayesian Information Criteria, R^2 : model fit parameter, α : intra-regional tau accumulation rate; β : inter-regional tau spread rate; p : microglia-mediated intra-regional tau accumulation rate; b : microglia-mediated inter-regional tau spread rate. The parameters are presented as mean with 95% confidence intervals. *** $p = 0.000$, ** $p < 0.005$.

Dataset	DS4			
Models compared	NDM	Nexis:global	Nexis:homeostasis	Nexis:Trem2
Model fit indices				
R^2	0.06	0.17	0.19	0.20
AIC	-2956	-3030	-3036	-3047
BIC	-2943	-3012	-3009	-3021
Fitted parameters				
α	-	0.25 [0.05 - 0.30]	0.17 [0.17 - 0.18]	0.18 [0.17 - 0.21]
β	1.26 [0.00 - 4.49]	1.89 [0.05 - 3.84]	2.46 [2.46 - 2.46]	1.41 [1.32 - 2.46]
p	-	-	0.23 [0.18 - 0.28]	0.75 [0.38 - 0.89]
b	-	-	-0.60 [-0.90 - -0.36]	4.30 [0.72 - 5.93]

Table 2. Comparison of model fits for NDM, Nexis:global, and Nexis:microglia for dataset DS6. All Nexis model fits were significantly better than those of the NDM ($p < 0.005$). Nexis:microglia model fits are compared to the Nexis:global model fit (significance indicated by *). Significance of model fit comparisons is confirmed using the Fisher's R-to-Z test. NDM: Network Diffusion Model, Nexis: Nexopathy *in silico*, AIC: Akaike's Information Criteria, BIC: Bayesian Information Criteria, R^2 : model fit parameter, α : intra-regional tau accumulation rate; β : inter-regional tau spread rate; p : microglia-mediated intra-regional tau accumulation rate; b : microglia-mediated inter-regional tau spread rate. The parameters are presented as mean with 95% confidence intervals. *** $p = 0.000$, ** $p < 0.005$.

Dataset	DS6			
Models compared	NDM	Nexis:global	Nexis:homeostasis	Nexis:Trem2
Model fit indices				
R^2	0.32	0.47	0.48	0.59***
AIC	-3110	-3250	-3262	-3405
BIC	-3093	-3232	-3236	-3379
Fitted parameters				
α	-	0.21 [0.14 - 0.23]	0.27 [0.27 - 0.27]	0.26 [0.22 - 0.27]
β	0.53 [0.00 - 2.24]	2.09 [0.29 - 2.61]	2.37 [1.46 - 2.71]	1.46 [1.46 - 1.46]
p	-	-	-0.28 [-0.37 - -0.18]	-0.35 [-0.49 - -0.06]
b	-	-	-1.14 [-1.79 - -0.42]	0.57 [-0.24 - 1.66]

Table 3. Comparison of model fits for NDM, Nexis:global, and Nexis:microglia for dataset DS9. All Nexis model fits were significantly better than those of the NDM ($p < 0.005$). Nexis:microglia model fits are compared to the Nexis:global model fit (significance indicated by *). Significance of model fit comparisons is confirmed using the Fisher's R-to-Z test. NDM: Network Diffusion Model, Nexis: Nexopathy *in silico*, AIC: Akaike's Information Criteria, BIC: Bayesian Information Criteria, R^2 : model fit parameter, α : intra-regional tau accumulation rate; β : inter-regional tau spread rate; p : microglia-mediated intra-regional tau accumulation rate; b : microglia-mediated inter-regional tau spread rate. The parameters are presented as mean with 95% confidence intervals. *** $p = 0.000$, ** $p < 0.005$.

Dataset	DS9			
Models compared	NDM	Nexis:global	Nexis:homeostasis	Nexis:Trem2
Model fit indices				
R^2	0.25	0.44	0.45	0.52**
AIC	-3726	-3899	-3910	-3981
BIC	-3709	-3881	-3884	-3954
Fitted parameters				
α	-	0.19 [0.16 - 0.20]	0.24 [0.24 - 0.24]	0.23 [0.19 - 0.24]
β	1.01 [0.09 - 3.32]	3.01 [2.19 - 3.35]	3.91 [3.91 - 3.91]	2.10 [2.10 - 2.10]
p	-	-	-0.22 [-0.28 - -0.18]	-0.31 [-0.45 - -0.11]
b	-	-	-0.85 [-1.25 - -0.68]	0.41 [-0.21 - 0.92]

Supplementary Files

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