Inflammation in Mild Cognitive Impairment due to Parkinson's disease, Lewy Body disease and Alzheimer's disease

[1]Eleanor King, [1]John O'Brien, [1]Paul Donaghy, [2]Caroline H. Williams-Gray, [1]Rachael A. Lawson,

[1]Christopher M. Morris, [1]Nicola Barnett, [1]Kirsty Olsen, [1]Carmen Martin-Ruiz, [1]David Burn, [1]Alison J. Yarnall, [1]John-Paul Taylor, [3]Gordan Duncan, [4]Tien K. Khoo and [1]Alan Thomas

[1]Institute of Neuroscience, Campus for Aging and Vitality, Newcastle upon Tyne, United Kingdom

[2]John Van Geest Centre for Brain Repair, Department of Clinical Neurosciences, Cambridge University, United Kingdom

[3]Department of Medicine for the Elderly, Western General Hospital, Edinburgh, United Kingdom

[4]Menzies Institute of Health, Queensland, Australia

Corresponding author: Dr Eleanor King

        3rd Floor Biomedical Research Building

        Campus for Ageing and Vitality

        Newcastle University

        Newcastle upon Tyne

        NE4 5PL

        0191 208 1318

        0191 208 1301

        Eleanor.king@newcastle.ac.uk

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Shared Data Declaration

The data that support the findings of tis study are available from the corresponding author upon reasonable request.

Abstract

Background

Inflammation appears to play a role in the progression of neurodegenerative diseases. However, little is known about inflammation during early stages of cognitive decline or whether this differs in different disease groups. We sought to investigate this by assessing the inflammatory profile in patients with Parkinson's disease with the early stages of cognitive impairment (PD-MCI), patients with prodromal Alzheimer's disease (MCI-AD), prodromal Lewy Body disease (MCI-LB) and controls.

Methods

We obtained venous blood samples from participants with PD-MCI (n = 44), PD-normal cognition (n = 112) MCI-LB (n = 38), MCI-AD (n = 21) and controls (n = 84). We measured 10 cytokines using Meso Scale Discovery V-Plex Plus including interferon-gamma, interleukin (IL)-10, IL-12p70, IL-13, IL-1beta, IL-2, IL-4, IL-6, IL-8 and tumour necrosis factor alpha. High-sensitivity C–reactive protein was measured.

Results

There was a higher level of inflammation in patients with MCI-AD and MCI-LB compared with controls. PD non-cognitively impaired had higher inflammatory markers than controls but there was no difference between PD-MCI and controls. There was a decrease in inflammatory markers with increasing motor severity based on the Unified Parkinson's Disease Rating Scale.

Conclusions

Inflammation may be involved in the onset of cognitive decline in patients with MCI-AD and MCI-LB but appears to be less prominent PD-MCI albeit in a small data set. This suggests that anti-inflammatory medications may have most benefit at the earliest stages of neurodegenerative diseases. For PD cases, this might be in advance of the development of MCI.

Key Points

- There is higher inflammation in patients with MCI-LB and MCI-AD than controls

- The level of inflammation in patients with PD-MCI is similar to controls

- There is an association between decreasing inflammation and worsening motor severity

- Anti-inflammatory medications may be useful in patients with MCI-LB and MCI-AD

- Anti-inflammatory medication may be less useful in patients with PD-MCI

Introduction

There is a well-established association between neuroinflammation and neurodegenerative diseases [1]. Microglia in the brain of patients with Alzheimer's disease (AD) co-localize with beta-amyloid (Aβ) [2], and are better predictors of synaptic loss than Aβ [3]. In Parkinson's disease (PD) microglia are particularly overactive in areas of the brain affected by dopaminergic loss early in disease [4], and inflammation may trigger this selective neuronal loss [5]. Furthermore, there appears to be an element of inflammation in Parkinson's disease dementia (PDD), with more widespread microglial activation in PDD [6].

Increased levels of peripheral immune activation has also been repeatedly found in patients with AD and PD, suggesting that there may be an element of inter-play between the immune activation in the periphery and central neuroinflammation [7, 8]. There have been few studies investigating inflammatory markers in PDD, but the reported findings have been mixed [9, 10].

Questions still arise as to the specific role that inflammation plays in the pathogenesis of neurodegenerative diseases. In order to gain a better understanding of this, we must understand the time point in disease in which inflammation occurs.

Peripheral cytokine levels in AD reach their peak in early disease [11], and this may precede clinical symptoms [12]. Genetic studies have found an association between the Human Leukocyte Antigen and increased susceptibility of PD, suggesting that immune mediators may be involved in triggering disease onset. Whilst anti-inflammatory agents have no benefit when used in patients with established AD [13] observational studies report a reduced risk of AD [14] and PD [15] in people using anti-inflammatory medications, implying that their beneficial effects may be at the pre-symptomatic stage.

We found increased inflammation in patients with dementia with Lewy bodies (DLB) at the mild cognitive impairment (MCI) stage [16], and recently patients with PD have been found to have increased inflammation peripherally compared to controls [17]. PD with mild cognitive impairment (PD-MCI) is a particularly relevant sub-group for investigating inflammation in neurodegenerative disease; whilst this group have established Parkinson's disease, they are also at higher risk of dementia. Studying their inflammatory profile may give us a clearer understanding of whether inflammation plays a role in the

development of dementia in people with PD. We therefore compared the systemic inflammatory profiles of patients with MCI from three different subgroups; MCI with AD subtype (MCI-AD), MCI with DLB subtype (MCI-LB), and MCI with PD subtype (PD-MCI).

Hypothesis

As previous studies have shown that inflammation occurs early in both Alzheimer's disease and Parkinson's disease, we hypothesised that patients with MCI-AD, MCI-LB and PD-MCI would have higher levels of inflammation compared with controls, and that the three MCI groups would all have similar levels of inflammation.

Methods

Participants

MCI-AD and MCI-LB patients were recruited from memory clinics and dementia services in the North East of England as part of the National Institute for Health Research (NIHR) LewyPro study [16]. All participants were over the age of 60. Participants all met the National Institute on Ageing and Alzheimer's Association (NIA-AA) MCI criteria [18]. MCI-LB was diagnosed if two or more of the four consensus criteria for DLB were present, or patients had one of these and abnormal dopaminergic imaging [19]. MCI-AD was diagnosed if none of the core symptoms for DLB were met, they had a normal dopaminergic scan, and evidence of cognitive decline as is characteristically seen in AD with no evidence of another aetiology. The 'one year rule' was applied so that no subjects had shown evidence of Parkinsonism for more than a year before the onset of cognitive decline. An expert consensus panel (AJT, PD, JPT) reviewed all clinical assessment data to confirm subjects met NIA-AA MCI criteria, and they rated the presence or absence of each of the four core clinical Lewy Body symptoms characteristic of DLB. These assessments were based on taking a clinical history and neurological examination, as well as the use of various assessment tools as stated below. Clinicians were blinded to the results of the other clinicians and to scan results. Participants were excluded if they had a diagnosis of dementia, and if they had severe physical, neurological or psychiatric illness, a past history of alcohol excess or use of psychotropic drugs.

PD-MCI patients were participants in the Incidence of Cognitive Impairment in Cohorts with Longitudinal Evaluation in Parkinson's Disease study (ICICLE-PD), and we selected those who were aged over 60 [20]. Participants with newly diagnosed PD, diagnosed by a movement disorder specialist according to the Queen's Square Brain Bank criteria [21], were recruited from the community and outpatient clinics in Newcastle upon Tyne/Gateshead and Cambridgeshire, UK. Participants were classified as having PD-MCI at baseline according to the Movement Disorder Society (MDS) PD-MCI modified level 2 criteria [22], if they were impaired on two tests in one cognitive domain or on one test in 2 different domains, whereby an impairment was classified as being 2 standard deviations below normative means [20]. Participants were excluded if they had an MMSE < 24 or an existing diagnosis of dementia. We also included the participants with Parkinson's disease (all those over 60 years old) but without cognitive impairment in order to investigate any differences in cytokine levels between patients with PD with normal cognition and PD-MCI.

Healthy controls were recruited from community settings. They were all over 60 years old, group-matched by age either to the LewyPro participants or the ICICLE-PD participants.

All participants provided informed written consent to take part in the study.

Assessments

Clinical assessments included an assessment of parkinsonism using the Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III (MDS UPDRS III). Illness burden was also assessed using the Cumulative Illness Rating Scale (CIRS). An array of neuropsychological and clinical scales were performed, dependant on the study [20]) including a Mini Mental State Examination (MMSE).

Measurement of inflammatory markers

Venous blood samples were taken from all participants and processed in line with their study protocols. Serum was removed for ICICLE-PD participants and plasma was removed for LewyPro participants, and samples were stored at -80 degrees until assays were performed. Cytokine assays were measured using the Meso Scale Discovery (MSD) V-PLEX Plus Pro-inflammatory Panel 1, which measured Tumour Necrosis Factor (TNF)-alpha, Interleukin (IL)-1beta, IL-2, IL-4, IL-6, IL-8, IL-10, IL-

12p70, IL-13 and Interferon (IFN)-gamma. High sensitivity C – reactive protein (hs-CRP) was measured separately, using V-PLEX CRP for ICICLE-PD samples and using Roche Cobas c702 for LewyPro samples. Samples were measured in duplicate for ICICLE-PD samples, and in triplicate for LewyPro samples.

Although it is stated in the MSD manufacturer's information that there is very little difference between using serum and plasma to measure these cytokines [23] we corrected for this by having two separate control groups; one matched to the ICICLE-PD cohort using serum and the other matched to the LewyPro cohort using plasma.

Analysis

All statistical analysis was carried out using IBM SPSS statistics 23. Data distributions were assessed for normality using Shapiro-Wilk test. For any non-normally distributed data, where possible log transformations were attempted using log(x+1) in order to normalise any skewed data. Associations were sought between inflammatory markers and patient demographics to assess for confounding variables. Comparisons were made between groups using Analysis of Covariance (ANCOVA) to adjust for age and gender. A significant difference was $p < 0.05$, and Bonferroni correction was applied to adjust for multiple variables. Comparisons were made between groups using Analysis of Covariance (ANCOVA) to adjust for age and gender. A significant difference was $p < 0.05$, and Bonferroni correction was applied to adjust for multiple comparisons. Associations were also sought between inflammatory markers and clinical markers of PD motor severity (MDS-UPDRS III) and cognitive severity (MMSE) using Pearson's correlation.

Results

Demographics

In total 187 subjects were included; 38 had a diagnosis of MCI-LB, 21 of MCI-AD, 44 of PD-MCI and 112 of PD with normal cognition. There were 20 controls matched to the participants in the MCI-AD and MCI-LB groups, and 64 controls matched to the participants in the PD-MCI group. Participants in the MCI-AD and MCI-LB group were older than those in the PD-MCI group, but all groups were age-matched to their respective control (table 1). There were differences in gender between groups; all groups had more males than females

apart from the MCI-AD group which had more females than males. As expected the MMSE score was higher in the control groups than the MCI groups, and MDS UPDRS score was highest in both of the groups with Lewy body disease. There was no difference in CIRS score between disease groups and their control groups.

45% of the MCI-LB group were using anti-dementia medications, in comparison to 24% of the MCI-AD group and none of the PD-MCI group (table 1). Between 0% and 36% of the participants in each group were using anti-inflammatory medications, not including aspirin 75mg. Of these 24 people were using Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and the remaining 35 were using other anti-inflammatory medications such steroids and 5-aminosalicylic acid.

Cytokine and CRP levels

IL-13 and IL-1beta were removed from further analysis as more than 50% of the samples had non-detectable cytokine levels. IL-12p70 was also removed as there was high inter-assay variability. We compared the LewyPro participants against LewyPro controls, and we compared the ICICLE-PD patients against the ICICLE-PD controls.

The MCI-AD and MCI-LB groups had significantly higher levels of IL-10, IL-2 and IL-4 than their control group (table 2). TNF-alpha was the only cytokine which was significantly lower in MCI-AD and MCI-LB than their controls. There was no significant differences in any of the inflammatory markers measured between PD-MCI and their control group.

As an exploratory analysis we investigated whether there were any differences between the MCI groups. We found higher levels of IL-10, IL-2 and IL-4 in the MCI-AD and MCI-LB groups than the PD-MCI group. Due to the differences in age, co-morbidity and sampling methods between these groups, this finding is exploratory only and is not therefore recorded in table 2.

Correlations

We also sought to investigate whether there were any correlations between PD motor severity using the UPDRS III score and cytokine level. Control participants were excluded. Serum and plasma samples were both included in the correlation analysis. We found negative correlations between MDS-UPDRS III

and IL-2 (r=-0.234, p=0.017) and IL-4 (r=-0.301, p=0.002). (Figure 1). There was no correlation between MMSE and inflammatory markers in any of the MCI groups.

PD-MCI vs PD

There were no significant differences in any of the cytokines between PD-MCI and PD without cognitive impairment, except IL-8 which was significantly higher in PD without MCI (p = 0.026). The PD group without cognitive impairment had higher levels of IL-6 and TNF-alpha compared to controls.

Discussion

In a previous study we found that peripheral inflammatory markers were high in patients with MCI-AD and MCI-LB and normal in patients with DLB and AD dementia, implying that inflammation is highest early in disease [16]. In the current study we aimed to investigate this further in patients with PD-MCI. We found that whilst patients with MCI-AD and MCI-LB had significantly higher levels of inflammation compared to controls, the PD-MCI group had similar levels of inflammatory markers to controls, with only slight, non-significant increases in some cytokines. However, inflammatory markers were elevated in the PD non-cognitively impaired group. This suggests that inflammation may play a role in the onset of cognitive decline leading to AD dementia and DLB, but in PD, inflammation may be most prominent prior to the onset of MCI.

The aetiology of PD is debated; however, inflammation is associated with PD and may play a role in the onset of disease. It has been suggested that the pathological hallmarks of PD may be triggered by infection of the GI tract, contributing to the pre-motor symptoms of PD before also invading the CNS and leading to the more characteristic motor symptoms [24]. It is postulated that these infections communicate to the brain via the vagus nerve [25] which may then trigger microglial activation, contributing towards dopaminergic neuronal damage [26]. Studies have suggested that infections such as Helicobacter Pylori may be a particularly important source of enteric inflammation in PD [27]. These findings suggest that infection and therefore inflammation are involved in the early, pre-CNS stages of disease. We previously reported that there is evidence for increased inflammation in patients with PD, consistent with this, and that increased levels of inflammation predict faster disease progression and are associated with cognitive impairment over 3 years

of follow up [17]. This suggests not only that early inflammation may be involved in the onset of neurodegeneration, but also that a higher level of inflammation predicts a faster rate of decline.

There are few studies investigating inflammation in PDD, and those have found mixed results. Several studies have found no significant difference in the level of cytokines in patients with PDD compared to PD and controls and one study found that whilst some cytokines are raised in patients with PDD, some have lower levels compared to PD and controls [29]. Our findings add that there does not appear to be an overall increase in inflammation in PD-MCI. However previous analysis of this ICICLE-PD cohort has shown that in patients with PD higher levels of pro-inflammatory cytokines are associated with having a lower MMSE over 54 months [17] of longitudinal follow-up. This analysis looked at an older subset of ICICLE-PD patients (those over 60) to match the criterion in the MCI-AD and MCI-LB groups. This difference may therefore be age related, or may relate to the criteria used to define PD-MCI which may exclusively capture those with more advanced cognitive impairment. Furthermore, the current study included only cross sectional rather than longitudinal data. Further research is needed, especially prospective studies with serial assessments of inflammatory markers, to more fully explore the role that inflammation plays over time in PD.

In the current study we also found a weak but significant association between immune markers (IL-2 and IL-4) with motor severity, providing preliminary evidence that the immune activation may decrease as motor severity increases, although longitudinal studies are needed to test this finding. Furthermore, as IL-2 is generally considered to be pro-inflammatory and IL-4 anti-inflammatory, there may also be a complex disruption to the balance of pro and anti-inflammatory cytokines, particularly in the early stages of disease. There is increasing evidence to suggest that inflammation occurs early in neurodegenerative disease progression. In AD, studies have shown that inflammation occurs at the pre-dementia stages [12], and the level of inflammation seems to decrease with disease progression [11]. This finding has been replicated in patients with DLB [16]. A longitudinal study found that increased levels of IL-6 increases the risk of PD, suggesting that inflammation occurs at the pre-dementia stage [30]. In keeping with the data that we report here indicating that inflammatory markers are raised in PD cases without cognitive impairment, previous findings from the ICICLE-PD study have shown that inflammatory markers are raised in PD compared to controls [17]. One explanation is that inflammation may be highest in the earliest stages of PD and predictive of disease progression, but it is possible that this may wane once MCI manifests.

Clinically, this study provides useful information into when anti-inflammatory medications may be of benefit. It has been shown in previous studies that inflammation occurs early in MCI-AD and MCI-DLB, and

inflammation decreases as disease progresses to dementia[16], suggesting that anti-inflammatory medications may be of use in early stages of disease in these patients. However, this study has shown that there does not appear an increase in the level of inflammatory markers in patients with PD-MCI to controls, perhaps because inflammation occurs in the early stages of PD, prior to the onset of MCI. In these patients therefore, it seems unlikely that there would be any benefit of using anti-inflammatory medications at the onset of MCI.

Our analysis has some important limitations. As we included participants from two separate studies there were some differences in demographics and methodology. As outlined in the methodology, the way in which participants were recruited differed between participants from the LewyPro study and those from the ICICLE-PD study (from memory services and movement disorder services respectively), leading to the possibility of selection bias between groups. Whilst plasma was used to measure cytokines in the MCI-AD and MCI-LB patients, serum was used for the PD-MCI patients. Although the MSD manufacturer states that there is little difference between using plasma and serum for the cytokines measured [23], we felt that we were unable to confidently carry out analysis between groups. An additional limitation was the small sample size of each MCI group, with fewer than 50 participants in each group, affecting the power of analysis. Our findings should therefore be regarded as preliminary and in need of replication using consistent methodology across all groups and larger numbers of participants in each group. The PD-MCI participants were younger than other MCI groups, and there were differences in the gender distribution across groups. Whilst we controlled for this in analysis, future studies should match for these variables between MCI groups. Finally, as this was a cross sectional study correlation findings between disease severity and inflammatory markers were preliminary only, and longitudinal studies are needed in order to further investigate the association.

Conclusion

We found that whilst PD patients without MCI appear to have higher levels of inflammation than controls, those with PD-MCI seem to have similar levels of inflammation to controls. Those with MCI-AD and MCI-LB had increased inflammatory markers compared to control subjects. Taken with our earlier report in patients with PD who had higher levels of inflammation than controls, this suggests that immune activation might alter during the course of these diseases, perhaps being highest nearer to disease onset at clinical presentation, lower once MCI develops. Future studies should focus on investigating the temporal profile of inflammatory markers in prospective studies of patients with PD with serial blood samples to ascertain whether, as in dementia, there is early inflammation which

decreases as disease progresses, and therefore whether and when during the course of disease anti-inflammatory medications may be beneficial.

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Tables

Table 1: Demographics

	Control LewyPro (n = 20)	MCI-AD (n = 21)	MCI-LB (n = 38)	Control ICICLE-PD (n = 64)	PD-MCI (n = 44)	PD-normal cognition (n = 112)
Age	75.9 +/- 1.6	78.5 +/- 1.4	75.6 +/- 1.2	69.5 +/- 0.8	71.1 +/- 0.1	69.5 +/- 6.7
Gender (M:F)	16:4[b]	7:14[a,c]	25:13[b]	34:30[e]	33:11[d,f]	44:68[e]
MMSE	29.1 +/- 0.2[b,c]	26.5 +/- 0.6[a]	26.4 +/- 0.4[a]	29.3 +/- 0.1[e,f]	27.8 +/- 0.3[d]	28.9 +/- 1.1[d]
MDS-UPDRS III	N/A	15.7 +/- 1.4	27.2 +/- 2.6	N/A	30.4 +/- 1.1	26.3 +/- 10.5
CIRS	6.7 +/- 0.1	9.5 +/- 3.9	9.5 +/-4.3	2.1 +/- 0.2	3.0 +/- 0.3	2.5 +/- 1.5
LEDD (mg/day)	0	0	263.1 +/- 160.6	0	212.7 +/- 128.3	174.1 +/- 156.4
Anti-dementia med use (n, %)	N/A	5 (23.8)	17 (44.7)	N/A	0	0
Anti-inflammatory med use (n, %)	3 (15%)	1 (4.7%)	0	8 (12.5%)	6 (13.6%)	41 (36.6%)

Table 1: Results are presented as the mean +/- standard deviation. MCI-AD = Mild cognitive impairment due to Alzheimer's disease, MCI-LB = Mild cognitive impairment with Lewy bodies, PD-MCI = Parkinson's disease with Mild Cognitive Impairment, PD-Normal cognition = PD with no cognitive impairment. M:F = Male:Female. MMSE = Mini Mental State Examination. MDS-UPDRS III = Movement Disorders Society Unified Parkinson's Disease Rating Scale Part III. CIRS = Cumulative Illness Rating Scale. LEDD = levodopa equivalent dose per day. a = significant difference from controls (LewyPro), b = significant difference from MCI-AD, c = significant difference from MCI-LB, d = significant difference from controls (ICICLE), e = significant difference from PD-MCI, whereby the control LewyPro, MCI-AD and MCI-LB groups are being compared against each other, and the control ICICLE-PD and PD-MCI groups are being compared against each other. A significant difference is when p ≤ 0.05 after Bonferroni correction

Table 2: Inflammatory markers

	Control LewyPro	MCI-AD	MCI-LB	Control ICICLE-PD	PD-MCI	PD-normal cognition
CRP, mg/l	3.6 +/- 5.7	3.4 +/- 3.3	4.1 +/- 6.4	3.5 +/- 6.9	4.2 +/- 10.0	3.4 +/- 8.4
IFN-gamma, pg/ml	7.6 +/- 6.1	8.8 +/- 12.1	6.5 +/- 4.3	10.9 +/- 21.5	15.3 +/- 54.4	8.8 +/- 12.0
IL-10, pg/ml	$0.5 +/- 0.6^{b,c}$	$0.9 +/- 0.4^{a}$	$1.0 +/- 0.5^{a}$	0.2 +/- 0.2	0.2 +/- 0.3	0.4 +/- 0.8
IL-2, pg/ml	$0.4 +/- 0.4^{b,c}$	$3.6 +/- 0.9^{a}$	$3.7 +/- 0.9^{a}$	0.08 +/- 0.1	0.3 +/- 0.7	0.1 +/- 0.4
IL-4, pg/ml	$0.0 +/- 0.1^{b,c}$	$0.8 +/- 0.2^{a}$	$0.8 +/- 0.2^{a}$	0.0 +/- 0.1	0.0 +/- 0.0	0.0 +/- 0.0
IL-6, pg/ml	1.7 +/- 1.0	1.1 +/- 3.3	2.9 +/- 10.4	$0.7 +/- 7.6^{†}$	1.0 +/- 0.2	$1.0 +/- 1.8^{d}$
IL-8, pg/ml	4.8 +/- 1.5	4.3 +/- 2.9	6.8 +/- 17.5	8.6 +/- 3.8	$8.1 +/- 7.5^{†}$	$10.7 +/- 7.2^{e}$
TNF-alpha, pg/ml	$4.3 +/- 1.3^{b,c}$	$3.1 +/- 3.5^{a}$	$2.2 +/- 2.0^{a}$	$1.9 +/- 1.0^{†}$	2.6 +/- 1.7	$2.8 +/- 1.3^{d}$

Table 2: Results are presented as mean +/- standard deviation. MCI-AD = Mild Cognitive Impairment due to Alzheimer's disease, MCI-LB = Mild Cognitive Impairment with Lewy Bodies, PD-MCI = Parkinson's disease with Mild Cognitive Impairment. PD-normal cognition = Parkinson's disease with no cognitive impairment. CRP = C – reactive protein, IFN-gamma = interferon gamma, IL = interleukin, TNF-alpha – tumour necrosis factor alpha. a = significant difference from controls (LewyPro), b = significantly different from MCI-AD, c = significantly different from MCI-LB, d = significantly different from controls (ICICLE-PD), e = significantly different from PD-MCI group, whereby the control (LewyPro), MCI-AD and MCI-LB groups are compared, and the control (ICICLE-PD) and PD-MCI groups are compared. A difference is significant when p ≤ 0.05 after Bonferroni adjustments.

Declaration of Interest

J O'Brien reports grants from Avid/Lily during the conduct of this study. He also reports personal fees from GE Healthcare, personal fees from TauRx, grants and personal fees from Avid/Lily and personal fees from Axon outside of the submitted work.

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All other authors have no further competing interests to declare.