Title

LPS distinctively alters iPSC-microglia transcriptomes to resemble Alzheimer's disease genetic mouse model microglia

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Key words

iPSC microglia, Alzheimer's disease, ATPγS, IFN-γ, LPS, PGE_2

Summary statement

Using scRNA-seq to measure the iPSC-microglia response we find convergence between ATPγS and LPS+IFN-γ, however comparison to genetic mouse models indicates LPS as a better model for AD.

Abbreviations

ATPγS	Adenosine 5'-O-(3-thio) triphosphate
IFN-γ	Interferon gamma
iPSC	Induced pluripotent stem cells
LPS	Lipopolysaccharide
PGE_2	Prostaglandin E2
DEGs	Differentially Expressed Genes

Abstract

Alzheimer's disease (AD) is the most common form of dementia and risk-influencing genetics implicates microglia and neuroimmunity in the pathogenesis of AD. iPSC-microglia are increasingly used as a model of AD but the relevance of historical immune stimuli to model AD is unclear. We performed a detailed cross-comparison over time on the effects of combinatory stimulation of iPSC-microglia, and in particular their relevance to AD. We used single cell RNA-seq to measure the transcriptional response of iPSC-microglia after 24 and 48h of stimulation with PGE_2 or LPS+IFN-γ either alone or in combination with ATPγS. We observed a shared core transcriptional response of iPSC-microglia to ATPγS and to LPS+IFN-γ, suggestive of a convergent mechanism of action. Across all conditions we observed a significant overlap ~~and functional links~~although directional inconsistency to genes that change their expression levels in human microglia from AD patients. Using a data-led approach, we identify a common axis of transcriptomic change across AD genetic mouse models of microglia and show that only LPS provokes a transcriptional response along this axis in mouse microglia and LPS+IFN-γ in human iPSC-microglia.

Introduction

Microglia have well established roles in inflammation, phagocytosis and brain homeostasis, appear to promote neuronal survival during early development (Ueno et al.), participate in synaptic pruning (Paolicelli et al.) and regulate neuronal excitability (Badimon et al.). Microglia constantly survey and react to changes in their environment. The normal functioning of microglia is key to brain homeostasis, whilst their functional disruption, prolonged activation or ageing may contribute to pathological conditions (Luo et al.). Age-related morphological changes in human microglia include the loss of fine branches and cytoplasmic fragmentation (Streit et al., 2004), and transcriptomic changes such as the upregulation of the amyloid beta formation pathway and the downregulation of TGFβ pathway (Olah et al.). Genes associated with a higher risk of developing Alzheimer's disease (AD) are significantly associated with microglia-specific expression patterns (Agarwal et al.), while gene expression analyses also highlight key roles for microglia in AD (Zhang et al., Mukherjee et al.) and other neurodegenerative diseases.

As neuroimmune cells, microglia respond to a large variety of stimuli (Cho et al.), including lipopolysaccharide (LPS), Interferon gamma (IFN-γ), Prostaglandin E2 (PGE_2) and ATP studied here. The bacterial endotoxin, LPS, is a potent pro-inflammatory stimulus for microglia and activator of innate immunity. IFN-γ is a soluble cytokine predominantly released from T cells and natural killer cells (Mosser and Edwards). It is known to regulate leukocyte migration (Reyes-Vazquez et al.) and has an elevated expression in models of injury and pathology of the nervous system (Roselli et al.). IFN-γ primes microglia, resulting in changes in morphology and the release of proinflammatory cytokines, to thereby heighten microglial responses to other stimuli including LPS. For example, the combination of LPS+IFN-γ potentiates the response of murine macrophages by increasing nitric oxide production (Lowenstein et al., Held et al.). PGE_2 is an endogenous lipid immune modulator that elicits diverse functions through binding to different types of EP receptors (EP1, increasing Ca^{2+}, EP2 and EP4 increasing cAMP, and EP3 reducing cAMP) (Kawahara et al.). The activation of the PGE_2/EP2 pathway may promote inflammation in diverse models of neurodegeneration (Liang et al., Shie et al., Jin et al.), and targeting EP2 with agonists aims to reduce inflammation, restore healthy microglia function (Amaradhi et al.) and even improve age-related cognitive decline (Minhas et al., 2021). However, the activation of the PGE_2/EP4 pathway has shown anti-inflammatory effects in Aβ models of AD (Woodling et al.), leading to a dual PGE_2 function that can be context dependent (Andreasson, Caggiano and Kraig). PGE_2 is also known to exert its effect in other cell types, for example by promoting astrocyte proliferation (Zhang et al.). ATP is released as a transmitter by both neurons (Pankratov et al., Bodin and Burnstock) and astrocytes (Guthrie et al., Anderson et al., Lalo et al.),

but also acts to signal damage when released from injured cells (Rodrigues et al., 2015) and in response to hypoxia (Melani et al.). Extracellular ATP induces microglial chemotaxis both *in vitro* and *in vivo* (Davalos et al., Ohsawa et al.). The microglial response to external ATP is proposed to be mediated through P2 purinergic receptors (Walz et al.), while the ATP-dependent release of ATP in both microglia and astrocytes is suggested as a mechanism to mediate the long range migration of microglia toward sites of injury (Dou et al., 2012).

Whilst the effects of inflammatory stimuli on their own have been investigated, changes in response over time, the consequences of combined inflammatory activation in human models and, importantly, their utility for the study of AD are less well explored. To model inflammatory effects, we used human induced Pluripotent Stem Cell (iPSC)-derived microglia, following a highly efficient protocol that broadly recapitulates microglia ontogeny from primitive embryonic macrophages from the yolk sac (Haenseler et al., Buchrieser et al.). We took advantage of cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) (Stoeckius et al.) to simultaneously measure the transcriptional response of iPSC-microglia at a single cell resolution to diverse stimuli (LPS+IFN-γ, PGE_2 and ATPγS) after different exposure times. We confirmed the relevance of challenged iPSC-microglia as models for AD, by finding both a higher than expected overlap with genes that change their expression in microglia from AD patients and an unusually high number of protein interactions with the products of genes within AD GWAS loci. We also performed a meta-analysis on microglia from mouse models of AD, identifying a disease axis along which microglia from WT and transgenic AD mouse models are consistently separated. We observed segregation between homeostatic and activated response microglia along the disease axis, as well as a minor shift from microglia of post-mortem AD patients. This framework singles out LPS as the only insult we tested that shifts the transcriptional profile of microglia towards a disease state in both mouse and in human iPSC-microglia.

Results

We set out to study the response of induced Pluripotent Stem Cell (iPSC)-derived microglia to a series of individual and combined stimuli. More importantly, we investigated whether the iPSC-microgia *in vitro* response is relevant for Alzheimer's disease by focusing on both human and mouse models of the disease.

Individual homogenous populations of iPSC-microglia show consistent responses to stimuli across biological replicates

We exposed iPSC derived microglia to either ATPγS [1mM], lipopolysaccharide (LPS) with interferon gamma (IFN-γ) [10 ng/ml], or to prostaglandin E2 (PGE_2) [500 nM] and measured the transcriptional response after 24 and 48 hours. Additionally, iPSC microglia were exposed to either PGE_2 or LPS+IFN-γ, with ATPγS added after 24h and the combined response measured after a further 24h (**Fig. S1A**). Prior calcium imaging expeirments in iPSC microglia demonstrated that 24 hours pre-treatment with either PGE_2 or LPS+IFN-γ for 24 hours led to an increased response to ATPγS (**Fig. S2**). We therefore sought to investigate how treatment with these inflammatory stimuli may alter microglia molecular networks. Across a total of 8 conditions, and across 4 biological replicates, the transcriptional response was measured at the single cell level using CITE-seq for multiplexing (Stoeckius et al.). All comparisons were made to 0h controls (untreated).

After de-multiplexing we obtained the transcriptome of 20,231 single cells and performed unbiased clustering analysis to identify cells with similar transcriptional profiles (see **Methods**). We detected eight cell clusters (**Fig. S1B**) that segregated cells by experimental condition and by donor-to-donor differences (**Fig. S1C**), with the exception a small cluster of 469 cells (cluster 6) which did not express microglial markers but appeared to be fibroblast-like cell population (**Fig. S3B, S4A**). We further detected a small population of proliferating microglia (cluster 7, n = 302 cells) (**Fig. S3C, Fig. S4B**). We excluded both fibroblast-like cells and proliferating microglia from further analysis. In the remaining microglia-like populations, we observed a consistent transcriptional response across biological replicates upon exposure to the same stimuli (**Fig. 1A, B**). iPSC-microglia treated with LPS+IFN-γ could be further segregated by time of exposure (24 and 48h), while iPSC-microglia treated with ATPγS (either alone or in combination with other stimuli) clustered separately, indicating global similarity within treatments that converge across biological replicates. However, the expression profiles of cells treated with PGE_2 were more similar to untreated control cells, suggesting a milder response.

Functional convergence of DEGs after 24 hours stimulation ATPγS treatment and LPS+IFN-γ treatment

Principal component analysis showed separation of iPSC-microglia treated with LPS+IFN-γ along the first component (7.45% of the variance) and of iPSC-microglia treated with ATPγS along the second component (6.03% of the variance, **Fig. 1C**). Given the observed clustering per donor even within control iPSC-microglia (**Fig. S5**), we integrated our gene expression data across donors (**Fig. S6**) and performed differential expression analysis grouping by donor (**see Methods**). The largest number of differentially-expressed genes was found after 24 hour exposure to LPS+IFN-γ (n = 904, combined p value < 0.05) closely followed by the 24 hour stimulation with ATPγS (n = 802, combined p value < 0.05). Fewer gene expression changes were found in response to PGE_2 after 24 hour exposure (n = 152, combined p value < 0.05, **Fig. 1D**). Despite the wide range of differentially expressed genes (DEGs) detected in response to the different stimuli (LPS+IFN-γ, PGE_2 and ATPγS) and the distinct principal components, we found a set of 73 overlapping DEGs at 24h across all treatments (**Fig. 2A**, hypergeometric test pairwise comparisons, LPS+IFN-γ and ATPγS n = 514, p ~ 0; LPS+IFN-γ and PGE_2 n = 89, p = 8.23×10^{-62}; ATPγS and PGE_2 n = 112, p = 4.51258×10^{-101}). In particular, the strongest correlation between the gene expression fold changes at 24h was observed between the exposure to LPS+IFN-γ and to ATPγS (r = 0.625, p < 2.2×10^{-16}, **Fig. S7**), suggesting a convergent mechanism between these two different stimuli.

Using Gene Ontology annotations and controlling for the microglia-like gene background, we found strikingly similar sets of enriched biological processes across DEGs, that broadly segregated between up and down-regulated genes. However, enriched GO terms from down-regulated genes with PGE_2 tend to cluster with GO terms from up regulated genes in response to the other stimuli. In particular, we found high similarity between the ATPγS treatment and the LPS+IFN-γ treatment at 24 hours when compared to control (**Fig. 2B**). Among down-regulated genes in response to both ATPγS and LPS+IFN-γ at 24h we found an enrichment of genes associated with reduced gene expression including translational initiation, nuclear transcribed mRNA catabolic process nonsense mediated decay, as well as SRP-dependent co-translational targeting to membrane, oxidative phosphorylation, mitochondrial ATP synthesis, and genes involved in plasma lipoprotein particle clearance. Genes involved in the immune response were enriched among both up and down-regulated genes in response to both ATPγS and LPS+IFN-γ, but only among down-regulated genes in response to PGE_2. Notably, an enrichment of genes involved in the cellular response to LPS as well as IFN-γ-mediated signalling pathway was found among up-regulated genes with ATPγS, again, pointing towards a common mechanism in the iPSC-microglia response to ATPγS and to LPS+IFN-γ. In contrast, among

the up-regulated DEGs in response to PGE_2 at 24h there was no enrichment of genes already implicated in the response to LPS alone.

Distinct temporal gene expression patterns in response to LPS+IFN-γ versus PGE_2

The DEGs in response to LPS+IFN-γ at both 24h and 48h following exposure were more similar to each other than those DEGs in response to PGE_2 across the same time points. Specifically, when comparing the sets of DEG in response to LPS+IFN-γ at both 24 and 48 hours we observed a higher overlap (n = 605, Jaccard index = 0.609, Hypergeometric test p value ~ 0, **Fig. 2C**) than in response to PGE_2 (n = 66, Jaccard index = 0.303, Hypergeometric test p value $1.484x10^{-95}$, **Fig. 2D**). While similar biological processes are enriched at both 24h and 48h in response to LPS+IFN-γ, direct comparison between the two time points reveals that a fraction of differentially regulated at 24h are returning to baseline at 48h (**Fig. S8C, E**) and thus DEGs show opposite directions from 0-24h and from 24-48h. In contrast, when we compared the response to PGE_2 at 24 and 48h, we found biological processes uniquely enriched at each time point. For example, in contrast to ATPγS and LPS+IFN-γ, inflammatory response genes were down-regulated 24h after PGE_2 treatment but after 48h after exposure pathways shared with ATPγS and LPS+IFN-γ were also enriched among PGE_2-DEGs, including upregulated regulation of interferon gamma production pathway and inflammatory response and down-regulation of genes involved in nuclear-transcribed mRNA catabolic process (**Fig. S8E**). Our results show that while LPS+IFN-γ provokes a broad, intense and transient response, PGE_2 by contrast has a reduced but more complex and in some aspects delayed response, consistent with its dual pro and anti-inflammatory role (**Fig. S8D, E**).

Lack of wide-spread synergistic effects on the combined treatments with ATPγS

Although ATPγS treatment alone provoked a strong cellular response, little additional effect was observed when this treatment was combined with the prolonged exposure of either LPS+IFN-γ or PGE_2 (**Fig. S9**). Specifically, only 20 DEGs were uniquely identified in the combined treatment of LPS+IFN-γ 48h and ATPγS at 24 hours as compared to ATPγS alone (**Fig. S9B**) while only 2 unique DEGs were found in the combined effect of PGE_2 48h and ATPγS 24h as compared to ATPγS alone (**Fig. S9D**). Additionally, in the combined treatments with ATPγS we found almost the same set of biological pathways once we controlled for the effects of the individual treatments (**Fig. S9I**). We observed similar fold changes in response to LPS+IFN-γ at 48h with and without the addition of ATPγS at 24h, while only strong changes are observed in the combined treatment of PGE_2 at 48h with the addition of ATPγS (**Fig. S9E, F**). Gene expression changes in the combined PGE_2 and ATPγS

are quite similar to those observed in ATPγS alone (**Fig. S9H**). Taken together, these findings suggest the lack of widespread synergistic effects on LPS+IFN-γ or PGE_2 treatments when either are combined with ATPγS.

Combined protein-protein interaction network highlights a core similar response to LPS+IFN-γ and to ATPγS

Using an integrated protein-protein interaction network (see **Methods**), we found more interactions than expected by chance among the protein products of the DEGs in iPSC-microglia in response to each of the different stimuli once we control for degree and gene length (estimated p value < 0.0001 based on randomisations, see **Methods**, **Fig. S10A**). These results further support the functional convergence within each set of identified DEG. By focusing on a subset of the protein- protein interaction network containing the genes with the most marked changes at 24 hours (absolute log FC >= 1.5, combined p value < 0.05), we observed a high level of similarity in the direction and strength of the gene expression changes upon ATPγS and LPS+IFN-γ, in addition to functional clustering among up and down regulated genes (**Fig. S7, Fig. 3**).

DEGs in iPSC-microglia across all treatments significantly overlap with genes that change in microglia of AD patients

In Alzheimer's disease (AD), a large fraction of risk genes are highly expressed in microglia as compared to other cell types and efforts to characterize cell-type specific transcriptional changes from post-mortem tissue of patients with AD have been recently reported (Mathys et al., Grubman et al.). Grubman et al. characterized cell specific gene expression changes from the entorhinal cortex of six patients with AD and six controls, while Mathys et al. focused on cell specific changes in the prefrontal cortex of 24 individuals with AD and 24 controls. Both reported microglial-specific changes in AD patients as compared to controls (62 DEGs in the entorhinal cortex and 122 DEGs in prefrontal cortex, **Fig. 4A**). While there is heterogeneity between AD datasets, the overlap of 12 genes between datasets is higher than expected by chance (Hypergeometric test, p value = 4.525×10^{-12}). We compared the transcriptional changes in our challenged iPSC-microglia and the microglia specific changes observed both post-mortem AD studies (**see Methods**). We observed a small but higher than expected overlap between the genes differentially expressed in iPSC-microglia following all challenges and those DE in microglia from AD patients from both studies, including genes that change in the early state of the pathology (**Fig. 4B, C**). However, differences were observed in the direction of effect. In the iPSC-derived stimulated microglia, most of the gene

expression changes occurred in the same direction (such as the up regulation of Serglycin, SRGN). Only a few genes showed divergent expression patterns, like Secreted Phosphoprotein 1 (SPP1) up regulation with LPS+IFN-γ and down regulation in PGE_2 treatments. Another discordant example was the Chemokine (C-C motif) ligand 3 (CCL3), upregulated in response to ATPγS and LPS+IFN-γ and downregulated in PGE_2 at 24 hours (**Fig. 4D**). By contrast, we observed more changes in gene expression going into opposite directions when comparing the challenged iPSC-microglia to the post-mortem microglia of AD patients. For example, mitochondrial and ribosomal genes were down-regulated in iPSC-microglia and up-regulated in the post-mortem AD microglia. Thus, we perturb a small but significant subset of genes altered in post-mortem AD microglia when challenging iPSC-microglia with different stimuli. However, while few differences in directionality are observed between iPSC-microglia challenged with these different stimuli, larger differences in directionality exist between these challenged iPSC-microglia and post-mortem AD microglia.

DEGs in iPSC-microglia are linked through protein-protein interactions to genes that change in microglia of AD patients and to AD GWAS risk genes

Using the combined protein-interaction network, we found more protein-protein interactions (PPIs) than expected by chance between DEGs in post-mortem AD microglia and DEGs in our stimulated iPSC-microglia suggesting functional convergence into shared pathways (PPIs controlled for cell type specific effects, CDS length and node degree, **see Methods**, **Fig. S11A**). We also found more PPIs between genes lying within AD GWAS risk loci and each of every set of DEGs in challenged iPSC-microglia (**Fig. S11B**). The functional links between the *in vitro* perturbations in iPSC-microglia and the genetic risk of developing AD, as well as the post-diagnosis gene expression changes observed in post-mortem AD, suggest all these challenged iPSC-microglia could be relevant models for AD study.

Meta-analysis of mouse microglia allows the identification of a disease axis that segregates WT microglia from transgenic AD model microglia

As a final comparison for our challenged human iPSC-microglia, we compared them to *in vivo* purified microglia across a range of published AD mouse models. While a small fraction of AD-relevant risk genes lack a 1:1 human: mouse orthologue (Mancuso et al.), genetic mouse models are useful as they allow the study of behaviour, cognitive decline and recapitulate some physiopathological features of the disease. We performed a gene expression meta-analysis of purified mouse microglia across a series of transgenic models of AD including genetic mutations in amyloid precursor protein (APP), presenilin (PS1), Microtubule Associated Protein Tau (MAPT) and

the Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) (Wang et al., Song et al., Orre et al., Srinivasan et al., Friedman et al.). After data re-processing and accounting for batch effects (**see Methods**), the first principal component (accounting for 14.43% of the variance) segregated WT from transgenic models carrying genetic mutations associated to AD (**Fig. 5A, S11A, S11B, S11C**). We refer herein to the first principal component as the *disease model axis*. Along this data-driven disease axis, the most severe model (5xFAD) showed the most segregation while microglia with a TREM2 knock out clustered with WT microglia.

Next, we asked if the orthologues of genes lying within AD GWAS risk loci were enriched among the genes driving the gene expression differences along the disease axis (**see Methods**). From 116 AD GWAS loci genes, we identify 55 with one-to-one ortholog correspondence from human to mice expressed across the microglia gene datasets used in the meta-analysis. However, when we focused on the top 500 genes with the lowest loadings along the disease model axis (corresponding to a reduced expression in AD models), we found more AD GWAS loci genes than expected by chance (Hypergeometric test; p value = 0.00197) including: HBEGF, CASS4, OARD1, CNN2, IL6R, BZW2, BIN1, FRMD4A and ADAM10 (**Fig. S12D**). We confirmed the overlap in different sized windows, from top 50 to 1000 genes with 50 gene increments. A significant overlap with AD GWAS loci genes held true when testing from 200-300, 400-750 and 900-1000 top genes with lowest loadings (adjusted p value < 0.05). We also found more protein-protein interactions to AD GWAS loci genes than expected by chance in both the top genes with the highest and lowest loadings along the disease model axis (**Fig. S12E**). Genes with the highest PC1 loadings showed enrichment of genes involved in the innate immune response (including response to bacterium), the regulation of cytokine production, as well as genes involved in beta-amyloid clearance. On the other hand, genes with the lowest loadings along PC1 showed enrichment of genes in involved in the positive regulation of defence response, negative regulation of cell proliferation, and blood vessel morphogenesis (**Fig. S13**). In summary, the meta-analysis of mouse microglia revealed a disease model axis of microglia gene expression variation that aligns with the disease severity observed in the genetic mouse models of AD, where genes driving the differences along this axis are both enriched in AD GWAS loci genes, have more protein-protein interactions to AD GWAS loci genes than expected by chance and are enriched in genes in pathways relevant to the disease models of AD.

Disease axis from genetic mouse models of AD segregates homeostatic from activated response microglia

We next asked if the disease model axis could also segregate the recently reported ARM (activated response microglia) subtypes/states that localise with Aβ accumulation in the transgenic mouse APP

knock-in model (App^{NL-G-F}) (Sala Frigerio et al.). We re-normalized and aggregated gene expression data of the App^{NL-G-F} mouse model by either microglia subtype, genotype, sex, age, tissue and projected the transcriptional profiles into the disease axis created from the meta-analysis of mouse models of AD (**See Methods, Fig. 5B**). We observed that the largest segregation along the disease axis occurred when we compared homeostatic microglia, which localised as microglia from WT in other studies, and ARM, which localised similarly to AD model microglia. To a lesser degree, we also observed segregation along the disease axis by genotype, age and sex, in agreement with previous observations in which microglia from female mice progress more rapidly to an ARM state (Sala Frigerio et al.). Differences between homeostatic and ARM microglia along the disease axis were further confirmed when projecting analogous gene expression data from the APP/PS1 mice model reported in the same study (**Fig. 5C**). In this second dataset we also observed that APOE KO moved microglia along the disease axis towards a transcriptional profile more similar to the WT, consistent with previous observations where its deletion prevents the main inflammatory response to Aβ plaques (Sala Frigerio et al.).

LPS treatment shifts the transcriptional profile of microglia towards a disease state

Following the data-led establishment of a framework that segregates at the transcriptional level WT microglia from mouse genetic AD model microglia and that captures differences between homeostatic and ARM subtypes/states, we then asked which different inflammatory stimuli, if any, drive microglia along this disease model axis towards a transcriptional state similar to that observed in the disease models of AD. To this end, we reanalysed the transcriptional profiles recently reported (Cho et al.) that systematically assess the microglia response to an array of stimuli across 96 different conditions. Once we accounted for batch effects, we projected each treated microglia transcriptional profiles onto the disease model axis (**see Methods**). We observed that after 4h treatment with high doses of LPS microglia transcriptional profiles showed the largest shift along the disease axis (**Fig. 6A**). Similarly, we created a pseudo-bulk from our human iPSC-microglia averaging expression per donor and per treatment, based only on those genes with *one-to-one* ortholog correspondence between species, accounting for batch effects and projected the transcriptional profiles into the disease axis (**see Methods**). Again, only iPSC-microglia treated with LPS+IFN-γ shifted along the disease axis (**Fig. 6B**). We further performed a randomization analysis in which we ranked all samples along PC1 and tested if the transcriptional profiles of microglia stimulated with LPS ranked higher along PC1 than expected by chance. Both in mouse primary microglia and in human iPSC we observed a higher ranking along PC1 in microglia treated with LPS (estimated p value, $p_{Mouse} < 1x10^{-5}$, $p_{Human} = 0.00208$). Finally, encounter a large overlapping set of functional

pathways shared among the up regulated genes in response to LPS+IFN-γ and those with the highest loadings along the disease axis (**Fig. S13B**). Taken together, despite the core similarities observed in the response to ATPγS and to LPS+IFN-γ, it is the response to LPS by both mouse microglia and human iPSC-microglia that best promotes a transcriptional shift towards a state more similar to that of the activated response microglia from the mouse AD models.

Minor shift along disease axis of human post-mortem microglia from AD patients

Finally, we projected the gene expression profiles of human post-mortem microglia from individuals with AD and healthy controls (Mathys et al., Grubman et al.) onto the disease model axis created (**Fig. S14**). We observed a small but consistent shift along the disease axis, where transcriptional profiles of microglia from individuals with AD segregate along the disease axis closer to the transgenic models of AD, and those from controls towards the profiles of microglia from WT mouse.

Discussion

In this study, we compared the transcriptomic response of iPSC-microglia to a range and combination of different stimuli at different exposure times and then asked whether any of these challenges provoked a cellular response that that could be useful when modelling AD. Our single cell approach allowed us to remove contaminating fibroblast-like cells, proliferating microglia and focus on the large fraction of iPSC-microglia **(Fig. S3)**. We showed a consistent response to the different stimuli across four biological replicates (**Fig. S6**), where the main sources of variation correspond to the exposure type (**Fig. 1C**), with LPS+IFN-γ and ATPγS provoking the largest number of transiently differentially expressed genes (**Fig. 1D**) with the strongest functional convergence in terms of shared enriched biological pathways, as compared to a milder but more complex response to PGE_2 (**Fig. 2, 3, S8**). Few additional effects were observed when combining treatments which supports both functional convergence and dominance of individual effects (**Fig. 1D, S9**).

In comparison to microglia nuclei obtained from human post-mortem AD, while there is a significant overlap in the DEGs (**Fig. 4B, C**), the direction of change is largely not concordant (**Fig. 4D**). This lack of agreement on direction could reflect the temporal nature of immune-stimulation (**Fig. 2, S8**) and that the post-mortem microglia are likely to be far more neuropathologically heterogeneous than the comparatively controlled and homogeneous iPSC-microglia challenges. In terms of convergent biological processes, across all iPSC-microglia treatments we found significantly more protein-protein interactors than expected by chance to either DEG in microglia from AD patients or to genes lying in AD GWAS loci (while controlling for the microglial background), indicating that by stimulating iPSC-microglia we are perturbing gene networks functionally associated to AD.

To further pursue the question of relevance of iPSC microglia models to AD, we employed an unbiased approach to reveal a shared axis of gene expression variation that distinguished purified wild types microglia from AD model microglia across a wide range of AD mouse models (**Fig. 5**). The discovered axis reflects large and small shifts in gene expression across a great many genes, rather than a smaller number of independently statistically significant changes in a subset of genes. Human post-mortem microglia showed a consistent but small change along the disease model axis separating AD cases from controls. The lack of a stronger segregation of human post-mortem microglia along the microglia disease axis from AD mouse models might reflect distinct biology or differences in comparative timing and heterogeneity in the transcriptional profiles of AD post-mortem microglia. Placing the gene expression profiles from all human *in vitro* iPSC-microglia challenges and all *in vitro* mouse microglia challenges considered in this study onto this disease

model axis singled out the expression changes invoked by LPS in mouse and LPS+IFN-γ in human iPSC-microglia as the challenge that distinctively produces a gene expression reaction similar to that shared among AD genetic mouse models (**Fig. 6**). Nevertheless, given the lack of stimulation of LPS alone in iPSC-microglia we were unable to confirm the result using LPS alone in human.

While overall, we observed a great similarity between the response to ATPγS and to LPS+IFN-γ suggesting shared mechanisms of action, we speculate that key differences could be operating upstream of these shared mechanisms that may shift the transcriptional profile towards a state resembling that of the mouse disease models of AD. As observed in mice, LPS alone is able to shift the transcriptional profile of microglia towards a more AD disease model state while IFN-γ alone does not show this shift (at least in the observed time/doses) (**Fig. 6A**). While it would be of interest to test the effect of other stimuli, for example Aβ fibrils, our current results propose that from the stimuli we tested LPS as provoking the most AD-relevant microglia stimulus given its similarity to the genetic mouse models. LPS also has advantages in terms of assay reproducibility, availability and scalability. While LPS is not known to, nor likely to, cause AD, the Toll-like receptor 4 that mediates the LPS response is thought to have a role in Alzheimer's disease (Park and Lee, Calvo-Rodriguez et al.).

Our data-led approach to identifying an AD disease model transcriptional axis for microglia can be revisited with new model data and further investigated for disease insight. While there is a strong agreement between the response to LPS and the genetic mouse models of AD, still for most of overlapping DEGs the directionality of change is not consistent with human post-mortem microglia. Noticeably, an exception, *SPP1* was among the top genes driving the shift along the disease axis from the genetic mouse models of AD, was exclusively upregulated in the iPSC-microglia treated with LPS+IFN-γ at both 24 and 28h, has an increased expression in two different studies of post-mortem human AD microglia (Grubman et al., Mathys et al.), and it is characteristic of the ARM subtype (Sala Frigerio et al.). A microglia population expressing Spp1 has been described in the axon tracts of the pre-myelinated brain during early post-natal development in mouse (Hammond et al.) and has also been associated with a specific microglia population from a model of toxic demyelination and in human microglia of Multiple Sclerosis patients (Masuda et al.). The role of *SPP1* both during normal conditions and development and in disease warrants further study.

Methods

Cell culture, differentiation and processing

Two male (SFC841-03-01 (Dafinca et al.), SFC854-03-02 (Haenseler et al.)) and two female iPSC lines (SFC180-01-01 (Haenseler et al.), SFC856-03-04 (Haenseler et al.)) were used for the study. They were originally reprogrammed from healthy donors recruited through StemBANCC/Oxford Parkinson's Disease Centre (participants were recruited to this study having given signed informed consent, which included derivation of hiPSC lines from skin biopsies, Ethics Committee: National Health Service, Health Research Authority, NRES Committee South Central, Berkshire, UK [REC 10/H0505/71]), and are all listed in hPSCreg and available from EBiSC. They were differentiated to primitive macrophage precursors and subsequently skewed to microglia-like cells in monoculture according to (Haenseler et al.). Primitive macrophage precursors were plated in IBIDI dishes (IBIDI µ-Dish 35 mm, low, cat no 80136) at a starting density of 500.000 cells per IBIDI dish (approx. 125.000 cells/cm^2). Cells were treated with LPS 10ng/ml and IFN-γ 10ng/ml or with PGE_2 500nM for 24h or 48h in a final volume of 500ul of media per IBIDI dish. ATPγS 1mM was added for the second 24h where relevant (**Fig. S1**). Note that our experimental design compares all 24 or 48h treatments to 0h controls where relevant, and thus is unable to distinguish in vitro changes due only to culturing cells without treatment for 24 or 48 hours.

Cells were lifted by incubating them with 200ul accutase (ThermoFisher) 3min at 37° C. Cells were then collected 2x 500ul PBS and pelleted by spinning at 600g for 5min at 4° C. Next, cells were resuspended in 100ul staining buffer (2% BSA, 0.02% PBS-Tween20) and incubated with 7ul Fc blocking reagent (Biolegend) for 10min. Then 1ug of cell hashing antibodies was added to each of the samples. Each cell line had 8 IBIDI dishes corresponding to the 8 different treatment conditions and 8 hashing antibodies. After 30 min incubation at 4° C, cells were washed 2 times: first wash by spinning them at 600g for 5min and resuspending them in 500ml staining buffer spinning, second wash by spinning the cells at 600g 5min and resuspending them in 200ul staining buffer. Finally, cells were resuspended in 150ul PBS, filtered through a 40um cell strainer and counted. Note that cultures were staggered and RNA extracted at the same time to avoid batch effects. All the treatments from a cell line were pooled together and were loaded on a 10X Chromium. For SFC841-03-01, SFC856-03-04 and SFC180-01-01 cell line 10.000 cells per pool were loaded in one 10X Chromium lane, for SFC854-03-02 cell line 5000 cells per pool were loaded on two 10X Chromium lanes.

Calcium imaging

For ratiometric Ca^{2+} imaging, microglia from male line (SFC841-03-01) were incubated in aCSF (in mM 130 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$ and 10 glucose, pH 7.4, Osm 290–310) containing 5µM Fura-2 AM and 80µM pluronic acid (Thermo Fisher Scientific) for 1hr at 37°C after their 24hr pre-treatment incubation. After incubation with Fura-2 AM, the cells were washed with aCSF to get rid of extracellular dye and left to sit for 30min at room temperature before imaging. During imaging recording, the first few minutes were recorded with only aCSF. Vehicle or ATPγS (50µM) was washed on and off the cells in a time dependent manner. The fluorescence of Fura-2 was excited alternatively at wavelengths of 340 and 380nm by means of high-speed wavelength-switching device on Zeiss microscope. Zeiss image analysis software allowed selection of several 'region of interest' within the field of view. Ratiometric 340/380 calculation was performed with a background subtraction. The 340/380 ratio were then analysed by measuring the average value in a user-defined time window using custom scripts in MATLAB. The data were smoothed using robust local regression MATLAB function at 20%.

Data processing

We used Cell Ranger pipeline (v 2.1.0) to process the sequencing data including alignment with STAR and single cell 3' gene counting. CITE-seq Count python tool was used to demultiplex samples by antibody hashtag. Then, we used the *HTODemux* function from Seurat to identify doublets and keep singlets (n = 20231). We kept only protein coding genes detected in at least 100 cells. Thus we obtained gene expression data for 12335 genes across 20231 cells. In the filtered dataset we observed a median of 2309 genes, 10293 UMIs and 3.25% of mitochondrial reads. Gene expression was normalized against the total number of counts detected per cell. Gene expression data was scaled to a factor of $1x10^4$ before the transformation to logarithmic scale.

Dimensionality reduction and clustering

We performed Principal Component Analysis (PCA) on the scaled gene expression of the top 1000 most variable features (across 20231 cells). For visualization, we used Uniform Manifold Approximation and Projection (UMAP) based on the first 30 principal components (PCs). To identify communities of similar cells, we used the shared nearest neighbour (SNN) modularity optimization based clustering algorithm (*FindClusters* function in Seurat R package). To identify unbiased clusters we included the first 20 PCs and a granularity resolution of 0.1. Most cell clusters show expression of

microglial marker C1QB (**Fig. S3A**), except cluster 6, consisting of 469 cells which instead show increased expression levels of COL1A1 (**Fig. S3B**) and other common fibroblast markers (Muhl et al.) For a more comprehensive characterization, we considered the expression levels of a core set of 249 human microglial markers identified by Patir et al. from a meta-analysis of transcriptomic data (Patir et al.). Many human microglial makers were expressed across all cell clusters, except in cluster 6, the fibroblast cell population (**Fig. S3C, S4A**). We also detected a small population (cluster 7) of proliferating iPSC-microglia characterized by the expression of KIAA0101, UBE2C, TOP2A and CDK1 (**Fig. S4B**). We excluded from further analysis both cluster 6 (fibroblast-like) and cluster 7 (proliferating cells) and perform PCA again. Initially only on untreated control iPSC-microglia (n = 1751), where we used the first 30 principal components for UMAP and clustering (**Fig. S5**). Then, PCA were recalculated for iPSC-microglia across all experimental groups (n = 19460) and again, the first 30 PCs used for UMAP.

Data Integration

We performed an step across our biological replicates (donors). Gene expression data was divided into smaller datasets per donor, normalized and the top 1000 most variable features were identified. A total of 1620 features were repeatedly variable and were used to find anchors. Canonical correlation analysis were performed across each pair of datasets. Integrated data was scaled for principal component analysis. For visualization we used UMAP based on the first 30 PCs.

Differential expression

Differential expression analysis was performed in the integrated dataset, were we used the *FindConservedMarkers* function in Seurat R package. Each experimental condition is compared to the untreated control cell per donor independently using a Wilcoxon rank sum test. Therefore, each gene was tested four times, one per donor. The metap R package was used to combine p-values using the minimump function that implements the Tippett's method, for the meta-analysis of p values. Genes with a combined p value < 0.05 were considered differentially expressed.

Gene Ontology (GO) enrichment

For Gene Ontology enrichment analyses we used clusterProfiler R package. Gene Ontology annotations were accessed through Bioconductor (org.Hs.eg.db). We used as background population the set of genes expressed in our dataset (n = 12335). We used FDR to account for multiple testing

and considered enriched only those terms with an adjusted p value < 0.05. To reduce redundancy among enriched GO terms we used rrvgo R package using *Rel* similarity with a threshold of 0.85. Similarly, for GO mouse annotations (org.Mm.eg.db) was used and background population of genes used in the mouse microglia meta-analysis.

Combined protein-protein interaction network

We constructed a protein –protein interaction network based on the data available across a range of resources: BioGRID (Stark et al.)[accessed: 30-03-2020], HitPredict (Lopez et al.) [accessed: 30-03-2020], IntAct (Orchard et al.) [accessed: 30-03-2020], STRING (Szklarczyk et al.) [accessed: 30-03-2020, only links with experimental evidence score > 0], CORUM (Giurgiu et al.) [accessed: 30-03-2020], Reactome (Fabregat et al.) [accessed: 30-03-2020], BioPlex HCT116.v.1.0 [accessed: 30-03-2020], BioPlex 3.0 (Huttlin et al.) [accessed: 30-03-2020], MINT (Licata et al.) [accessed: 30-03-2020], InBioMap (Li et al.) [accessed: 30-03-2020]. All protein-protein interactions (PPIs) were either kept or mapped to Ensembl gene IDs. When we tested if the number of PPIs was higher among a set of genes than expected by chance we performed 10,000 randomizations. In each randomization we select an equally sized sample of genes matched for degree and CDS length and counted the number of PPIs among them. An estimated p value was derived from the number of randomizations where we detected more PPIs than the observed among the protein products of each set of DEGs.

Test for gene overlap

We used a hypergeometric test for the overlap between each pair of sets of differentially expressed genes. We adjusted for multiple testing using the Benjamini-Hochberg method. We used as background population of 12335 genes to estimate the expected proportions. When compared *Homo sapiens* and *Mus musculus*, only genes with *one to one* ortholog correspondence were taken into account.

Microglia response to diverse stimuli in mice

We re-processed the gene expression data from mouse microglia exposed to 96 different conditions *in vitro* available in GEO (GSE109329, (Cho et al.)). Quantify transcript abundances using Kallisto version kallisto_linux-v0.46.0 (Bray et al., 2016). The reference index was built based on coding (cdna) and non-coding RNA (ncrna) sequences with annotations from Ensembl release 98 available through the ftp website (Mus_musculus.GRCm38.cdna.all.fa.gz,

Mus_musculus.GRCm38.ncrna.fa.gz). We filtered out sequences in scaffold chromosomes. We filtered genes with no expression across all samples. For comparison between species only gene with one to one ortholog from *Homo sapiens* to *Mus musculus* were considered.

Differentially expressed genes in human AD patients

We used data from two independent studies that have reported microglia specific gene expression changes in AD patients compared to controls (Grubman et al., Mathys et al.). Genes reported by Mathys et al. (Supplementary Table 2 in their publication, FDR-adjusted p-value < 0.05, two-sided 2-sided Wilcoxon-rank-sum test), and those reported by Grubman on the accompanying website to their publication http://adsn.ddnetbio.com/ (AD vs Ctrl based on subclusters, n = 62 genes, FDR < 0.05, n = 62 genes, empirical Bayes quasi-likelihood F-test).

Meta-analysis of microglia from genetic mouse models of AD

Gene expression datasets from mouse microglia were obtained from GEO through a search of genetic models of Alzheimer's disease (search in GEO for "microglia mouse AD" in 2018). Microarray datasets included: FACS purified microglia from 8.5 month old WT, Trem2-/-, 5XFAD, and Trem2-/-5XFAD (GSE65067, (Wang et al.)), CD45+ and CD11b+ microglia from 8.5 month old mice expressing the common variant, R47H or no human TREM2 on a background of murine TREM2 deficiency and the 5XFAD mouse model of AD (GSE108595(Song et al.)), cortical microglia from 15-18 month old APPswe/PS1dE9 mice compared to wildtype littermates (GSE74615, (Orre et al.)). RNA-seq datasets included: FACs sorted microglia from 7 or 13 month old PS2APP or non-transgenic mice (GSE75431, (Srinivasan et al.)). Microglia (Cx3cr1::GFP+ sorted) from the cortex of 14-15 month old PS2APP or WT mice (GSE89482, (Friedman et al.)) Sorted Cd11b+ myeloid cells from 11-12 month old Tau-P301L and non-transgenic littermates (GSE93179, (Friedman et al.)). Sorted Cd11b+ myeloid cells from 6 month old Tau-P301S transgenic mice or non-transgenic littermates (GSE93180, (Friedman et al.)). For RNAseq datasets, fastq files were downloaded from GEO, transcript quantification performed with Salmon (version 0.9.1) for protein coding genes with Ensembl (release v91). Transcript counts for all studies were imported and summarized to gene levels counts with *tximport* R library and genes with less than 20 counts across all samples were filtered out. The filtered count matrix was normalized using *Rlog* transformation implemented in DESeq2 R library (Love et al.). For microarray datasets, CEL files were downloaded from GEO repository. We performed background subtraction, quantile normalization and summarization using the RMA algorithm implemented in oligo R library (Carvalho and Irizarry). Then, we used surrogate variable analysis to correct for batch

effects between the 7 studies through the *ComBat* function available in the sva R library (Chakraborty et al., 2012). Finally, performed principal component analysis using the *prcomp* function in R.

Projection into PC1 of mouse genetic AD models meta-analysis

We projected samples from a few datasets into the same dimensional space (PC1) from the meta-analysis created from the genetic mouse models of AD, one dataset to be projected at a time. For each dataset we projected into PC1, we corrected for batch effects using *ComBat* along with the rest of the datasets from the meta-analysis. Then, we centred the batch corrected data from the dataset to be projected and multiply it by the gene loadings of PC1 (contained in the rotation slot from the corresponding *prcomp* object in R). For the single cell datasets we averaged the gene expression by either experimental group, microglia subtype, genotype, age or sex before correcting for batch effects.

To test if LPS stimulated microglia tend to rank higher along PC1, we first ranked all the projected samples along PC1 (separately for mouse microglia, and for human iPSC microglia). We obtained the average rank for all the samples that included LPS (mouse microglia) or LPS+IFN-γ (iPSC-microglia) and compared it to the average rank of 100000 equally sized random samples. We obtained an estimated p value by counting the number of times that the random samples had an average higher rank along PC1.

Mouse microglia subtypes from single cell gene expression data

Counts were downloaded directly from GEO (GSE127892, GSE127884), meta-data extracted from loom files available at scope.bdslab.org (Sala Frigerio et al.). Counts from each dataset (APP/PS1 and APP-NF-G-L) were normalized and scaled using the *logNormalize* method with a scale factor of 10000 implemented in the *NormalizeData* and *ScaleData* functions from Seurat R package (Stuart et al.). Gene expression was averaged either by microglia subtype clusters, genotype, age or sex.

Protein-protein interactions to AD GWAS risk genes, and to DEG in microglia of AD patients

From the GWAS catalogue (Buniello et al.) we obtained all mapped genes to SNP's associated to Alzheimer's disease trait (EFO_0000249; accessed 2020-11-16, P value ≤ $1x10^{-8}$). From the set of 116 AD GWAS risk genes, we found that 72 had expression in our iPSC-microglia and were included in the

combined protein-protein interaction network described above. Then, we tested if the number of PPI between each set of DEGs and AD GWAS risk genes was higher than expected by chance. To this end, we contrasted the number of PPIs among the gene products of each set of DEGS in iPSC-microglia to those of 10,000 equally sized random samples from our background population (genes expressed in iPSC-microglia), while controlling for the CDS length and degree of the random sets in the PPI network. An estimated p value was drawn from the 10,000 randomizations. Same approach was used to test if the number of PPIs between each set of DEGs (iPSC-microglia) and DEG in microglia of AD patients was higher than expected by chance.

We also tested if the genes with the top 500 highest and lowest loading along the disease axis (PC1 of the meta-analysis) had more protein-protein interactions than expected by chance to AD GWAS genes. In this case the background population was reduced to genes detected in the meta-analysis that had a one to one ortholog relationship from mouse to human.

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Competing interests

ZC is co-founder and director of Oxford StemTech Ltd. and HumanCentric DD Ltd. CW is co-founder and director of HumanCentric DD Ltd.

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Data availability

The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO Series accession number GSE186301 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE186301). Underlying code will be also shared in Github upon publication at https://github.com/jmonzon87/PokeMicro.

Author contributions

E.B, A.E.H, J.D, S.A.C, Z.C, C.W conceptualization, J.D, S.A.C, Z.C, C.W resources, supervision, project administration and funding acquisition, J.M.S, D.A, software, formal analysis and data curation, J.M.S, D.A, E.B, L.W investigation, E.B planned and performed the wet lab experiments, L.W calcium imaging, J.M.S visualization, J.M.S writing original draft preparation; J.M.S, D.A, E.B, A.E.H, L.W, J.D, S.A.C, Z.C, C.W writing review and editing.

Figures

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Figure 1. iPSC-derived microglia show a similar response to treatments across biological replicates with the largest response to LPS+IFN-γ. A UMAP based on the first ten principal components of the top 1000 most variable genes across iPSC-microglia shows segregation of groups exposed to either LPS+IFN-γ or ATPγS, while those treated only with PGE_2 tend to cluster near controls. **B** UMAP shows similar segregation pattern across biological replicates (colours indicate the donors from which iPSC-microglia were derived from). **C** Principal component analysis based the top 1000 most variable genes of in iPSC-microglial cells (n = 19460). First two principal components are coloured by experimental group, density plots on side help to distinguish groups treated with LPS+IFN-γ along the first component and ATPγS along treated groups on the second component. **D** Number of differentially expressed genes (DEGs) detected between each treatment and control cells (Integrated data, combined p value < 0.05).

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Figure 2. Consistent increased expression of chemotaxis related genes and decreased expression of genes involved in translation and SRP-dependent co-translational protein targeting to membrane. A Venn diagram show the overlap between differentially expressed genes (combined p value < 0.05) at 24 hours after exposure to LPS+IFN-γ, PGE_2 and ATPγS. **B** Enriched biological processes found among differentially expressed genes detected after 24 and 48 hours in response to ATPγS, LPS+IFN-γ and PGE_2. Gene Ontology enrichment analysis was performed separately for up and down regulated genes. Heatmap shows the -log10 transformed adjusted p value for each enriched biological process in shades of pink (if adjusted p value < 0.05, otherwise grey). Showing only non-redundant terms based on their semantic similarity (**see Methods**). **C** Venn diagram shows a larger set of genes uniquely differentially expressed at 24 hours for LPS+IFN-γ compared to 48 hours. **D** Similarly, a larger set of unique DEGs in response to PGE_2 was found at 24 hours.

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Figure 3. Functional convergence among ATPγS and LPS+IFN-γ treatments at 24h through a combined protein-protein interaction network. Nodes indicate genes, and edges known protein-protein interactions between their gene products (**see Methods**). Protein-protein interaction network among the protein products of the differentially expressed genes with the largest fold changes in any of the treatments (absolute logFC >= 1.5, combined p value < 0.05). **A** Genes are coloured by the logFC after 24 hours in response to ATPγS. **B** Genes are coloured by the logFC after 24h in response to LPS+IFN-γ.

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Figure 4. Overlapping DEGs in stimulated iPSC-microglia and DEGs in microglia from Alzheimer's disease patients. A Venn diagram shows the overlap between DEGs in microglia from AD patients identified by Mathys et al in the prefrontal cortex and by Grubman et al. in the entorhinal cortex (Hypergeometric test, n = 12, p value $4.525x10^{-12}$). **B** Heatmap shows the number of overlapping iPSC-microglia DEGs and those DEGs in microglia from AD patients. It includes the subsets of DEGs that change in expression early in the pathology of AD (contrasting individuals that showed amyloid burden but few neurofibrillary tangles and modest cognitive impairment) and the subset of genes that change late in the pathology of AD (higher amyloid burden, presence of neurofibrillary tangles and cognitive impairment compared to the early pathology group). **C** We test if the overlap between DEGs was higher than expected by chance, the heatmap indicates the adjusted p value of the corresponding hypergeometric tests (Adjusted p value < 0.05 shown in pink shades, otherwise shown in grey). **D** Heatmap shows the direction and magnitude of the change (log transformed Fold Change) of the DEGs in iPSC-microglia and those DEGs in microglia from AD patients, grey squares indicate no significant change in expression (adjusted p value > 0.05).

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Figure 5. Disease axis from meta-analysis of microglia from genetic mouse models of AD segregates homeostatic and activated response microglia. A After accounting for batch effects first principal component segregates mouse microglia from WT and that of transgenic mouse models of AD across datasets. **B** Single cell gene expression of microglia from the knock in mice $APP^{NL\text{-}G\text{-}F}$ and WT was aggregated by either microglia type/cluster (ARM: Activated Response Microglia, CRM: Cycling/Proliferating Microglia, H1M: Homeostatic Microglia 1, H2M: Homeostatic Microglia 2, IRM: Interferon Response Microglia, TRM: Transit Response Microglia), genotype (K: $APP^{NL\text{-}G\text{-}F}$, W: WT), age (3, 6, 12 and 21 months) or sex (F: female, M: male) and projected into the first principal component or disease axis. Each dot represents the projected PC1 for the aggregated transcriptional profile of microglia across 10187 shared genes. **C** Single cell gene expression of microglia from male WT and APP/PS1 mice was aggregated by either microglia type/cluster (ARM: Activated Response Microglia, H1/2M: Homeostatic Microglia 1/2, IRM: Interferon Response Microglia, TRM: Transit Response Microglia), genotype (C: C57BL/6, D: APP/PS1-ApoeKO, E: C57BL/6-ApoeKO, P: APP/PS1) or age (17 and 18 months) and projected into the disease axis from the meta-analysis of mouse AD models.

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Figure 6. LPS shifts mouse primary microglia and human iPSC-microglia towards a more similar profile of that of mouse models of AD. A Gene expression data of *in vitro* mouse microglia stimulated with a large array of different stimuli was projected into the disease axis (or first principal component based on the meta-analysis of gene expression of microglia from genetic mouse models of AD). Each dot represents the projected PC1 based on the transcriptional profile of 10844 shared genes. Largest shifts along PC1 occur in microglia treated with high doses of LPS at 4 hours. **B** Gene expression data from our human iPSC-microglia was aggregated by experimental group and donor and projected into the disease axis (PC1) from the meta-analysis of microglia from genetic models of AD. Each dot represents the projected PC1 based on the transcriptional profile of 8156 common genes. iPSC-microglia treated with LPS+IFN-γ showed the largest shift along the PC1 projection. We used randomization analysis (**See Methods**) to test if the average rank of treatments that included LPS along PC1 was higher than expected by chance. Both in mouse primary microglia and in human iPSC-microglia, samples treated with LPS ranked higher along PC1 (estimated p value, $p_{Mouse} < 1x10^{-5}$, $p_{Human} = 0.00208$)

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