The gene expression signature of electrical stimulation in the human brain

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Abstract

Direct electrical stimulation has been used for decades as a gold standard clinical tool to map cognitive function in neurosurgery patients[1-8]. However, the molecular impact of electrical stimulation in the human brain is unknown. Here, using state-of-the-art transcriptomic and epigenomic sequencing techniques, we define the molecular changes in bulk tissue and at the single-cell level in the human cerebral cortex following direct electrical stimulation of the anterior temporal lobe in patients undergoing neurosurgery. Direct electrical stimulation surprisingly had a robust and consistent impact on the expression of genes related to microglia-specific cytokine activity, an effect that was replicated in mice. Using a newly developed deep learning computational tool, we further demonstrate cell type-specific molecular activation, which underscores the effects of electrical stimulation on gene expression in microglia. Taken together, this work challenges the notion that the immediate impact of electrical stimulation commonly used in the clinic has a primary effect on neuronal gene expression and reveals that microglia robustly respond to electrical stimulation, thus enabling these non-neuronal cells to sculpt and shape the activity of neuronal circuits in the human brain.

Main

Electrical stimulation of the human brain has become an indispensable clinical tool for diagnosis and therapy[1-8]. In neurosurgical patients, cortical stimulation is the gold standard diagnostic tool to identify the location of functionally relevant brain regions that are critical for speech, language, or motor function[9,10] to avoid potential damage to these regions during surgery. Transient changes in gene expression underlie the ability of the brain to adapt to changes in the environment or brain perturbation, such as electrical stimulation. Dynamic transcriptomic patterns are essential for cognition[11-13], affective processing[14], addiction[15], and the initiation of behaviors[16,17]. Thus, electrical stimulation likely exerts neural effects via alterations in gene expression. Although diagnostic and therapeutic brain stimulation is conducted daily in thousands of patients worldwide, the molecular impact of electrical stimulation in the human brain remains unknown. Recent advances in molecular sequencing techniques have revolutionized transcriptomics by enabling mapping of changes in transcription and chromatin accessibility in single cells[18]. However, these experiments have only been conducted in rodents[19,20] and neurons derived from human induced pluripotent stem cells[21]. A recent pioneering study utilized a single nuclei molecular approach to link human brain transcriptomics signatures with oscillatory signatures of memory consolidation in epileptic patients undergoing an episodic memory task, but these gene expression measures were obtained many days after assessment of intracranial recording data during the task[22,23]. Therefore, research is needed to evaluate the more immediate effects on single-cell early gene expression, such as tens of minutes after neural system perturbation with electrical stimulation. Studies investigating gene expression profiles after electrical stimulation are particularly difficult to perform in human brains due to the requirement that tissue is sampled at precise temporal windows before and after stimulation.

Here, in patients undergoing clinical neurosurgical resection of pathological epileptogenic sites in the mesial temporal lobe, samples from tissue in the anterior

temporal lobe (ATL) that required clinical resection to access the deeper epileptogenic site for treatment were obtained before and after electrical stimulation. By analyzing gene expression at baseline and minutes after stimulation from the same patient, along with our analysis of samples taken from patients that did not receive electrical stimulation, we are able to distinguish genes responsive to electrical stimulation from the genes altered nonspecifically by surgical or disease-based factors that would be stable across samples. Our parameters for electrical stimulation were based on those regularly used in clinical mapping[24,25]; moreover, we referenced the human results to a similar electrical stimulation paradigm in mice, revealing a similar transcriptional profile. Finally, using a single-nuclei multi-omics approach, we provide insight into the cell-type-specific transcriptomic and epigenomic responses to electrical stimulation in the human brain, highlighting important effects beyond neurons, including microglia. This study provides fundamental insights into changes in cell-type specific molecular signatures in the human cortex after electrical stimulation, laying the groundwork for a molecular understanding of the impact of this fundamental tool in clinical neurosurgery, diagnosis, and treatment.

Results

We recruited eight adult neurosurgical patients undergoing surgical resection of seizure foci following clinical monitoring to treat epilepsy. The patients provided informed consent to take part in this research and were informed that tissue samples would only be taken from tissue that would require clinically resection for treatment. The patient participants underwent an anterior temporal lobectomy for access to a medial temporal lobe epileptogenic site, during which samples were resected from the neocortex and processed immediately after removal. The participants were evenly distributed between two experimental paradigms. In the first group with the electrically stimulated paradigm (4 participants), a baseline tissue sample was resected from the ATL after the craniotomy and durotomy, exposing the temporal lobe. Then, an adjacent region of the cortex was stimulated using a stimulation protocol commonly used for bipolar electrical stimulation (50 Hz) for 2 minutes[24,25] And resected approximately thirty minutes later (32.5 ± 11.2 min), a sample was taken from the stimulated region **(Fig. 1a; Supplementary Fig. 1a, Supplementary data 1).** In the second group with the

unstimulated paradigm (4 participants), a sample was taken at baseline, and then a second sample was taken without stimulation about 30 minutes later (37.3 ± 12.5 mins, **Fig. 1b; Supplementary Fig. 1a)**. The pre-stimulation sample and the unstimulated paradigm groups serves as controls for changes in baseline gene expression and changes in gene expression occurring as a result of the surgical procedure. Among the 8 participants, five were under general anesthesia, whereas three participants were awake during the surgical procedure. All the resected tissue samples from both the stimulated and unstimulated paradigms were taken well outside the seizure focus (27.8 ± 7mm from the border of seizure focus**, Supplementary Fig. 1b**).

Tissue samples were first subjected to bulk whole-transcriptome RNA sequencing (RNA-seq) to identify differentially expressed genes following electrical stimulation. Bulk RNA-seq analysis from the stimulated and corresponding baseline samples revealed 124 differentially expressed genes following electrical stimulation, with 112 up-regulated and 12 down-regulated genes **(Fig. 1c**; **Supplementary data 2)**. Enrichment network analysis was used to identify the pathways most represented among the differentially expressed genes in the stimulated paradigm. The top significant pathways were enriched with genes involved in cytokine activity, DNA-binding transcription activator activity (RNA Pol II), cytokine receptor binding, DNA-binding transcription activator activity, nuclear glucocorticoid receptor binding, chemokine activity, CCR chemokine receptor binding, RNA pol II specific DNA binding, chemokine receptor binding, and protein phosphatase activity **(Fig. 1d).** Notably, these genes were not significantly enriched for previously identified genes induced in regions showing seizure activity in the human brain **(Supplementary Fig. 2)**[26].

Bulk RNA-seq analysis from the unstimulated paradigm comparing unstimulated samples with corresponding baseline samples identified differential expression of only 16 genes, with nine up-regulated and seven down-regulated genes **(Fig. 1e, Supplementary data 3).** Only one gene, *NR4A3*, was found to be differentially expressed in both the stimulated and unstimulated groups **(Fig. 1g).** The lack of overlap between the differentially expressed genes observed in our stimulated and unstimulated groups suggests that the changes in gene expression that we see following electrical

stimulation do not reflect disease state or surgical effects such as craniotomy, brain temperature, and anesthesia, but reflect changes due to electrical stimulation.

We used a similar electrical stimulation paradigm in the mouse non-primary auditory cortex to investigate whether these gene expression changes are unique to human samples and to determine whether they are related to disease state [26,27]. Bulk RNA-seq was performed from samples collected 30 minutes following electrical stimulation and unstimulated samples collected at the same time from the contralateral side (**Fig. 2a**). Bulk RNA-seq identified 44 upregulated and 107 downregulated genes (**Fig. 2b, Supplementary data 4**). Pathway analysis identified from the upregulated genes were enriched for cytokine activity, chemokine activity, cytokine receptor binding, and CCR chemokine receptor binding. (**Fig. 2c**). We also found upregulated pathways linked to transcription and post-transcription regulatory pathways, such as mRNA 3'-UTR binding, mRNA3'-UTR AU-rich region binding, and transcription co-repressor activity. Comparing the stimulation-responsive genes from human bulk RNA-seq and mouse bulk RNA-seq revealed a significant correlation (R^2=0.0415, p-value <0.00001), especially cytokine-related genes were commonly upregulated following electrical stimulation in both humans and mice (**Fig. 2d**). Some of the pathways commonly altered by electrical stimulation between humans and mice are cytokine activity, chemokine activity, chemokine receptor binding, cytokine receptor activity, and the CCR chemokine receptor binding. Some common genes enriched in these chemokine and cytokine-related pathways are *CCL3*, *CCL4*, *CXCL1*, *IL1A*, and *TNF*. We further validated the expression of the cytokine activity-related genes *Ccl3* and *Ccl4* using qPCR analysis from mouse brain following electrical stimulation (**Fig. 2e**). The differentially expressed genes following electrical stimulation in the human cortex correlated significantly with learning-induced genes in the mouse cortex (R^2=0.2027, p-value <0.00001). Immediate early genes such as *Arc*, *Fos*, *Egr1*, *Nr4a1*, and *FosB* were upregulated in both datasets **(Supplementary Fig. 3)**. However, learning did not induce expression of cytokine activity related genes in mouse cortex. Thus, our findings of gene expression signature in response to electrical stimulation in the human and mouse brain reveal a molecular signature that partially conserved across species.

Next, we investigated the cell types exhibiting differential gene expression following electrical stimulation in the human brain by utilizing single nuclei multiomics (RNA and ATAC) on samples from the stimulated paradigm from 3 participants (**Fig 3a, Supplementary Fig. 4**). Cell clustering analysis identified seven major cell types in our samples—excitatory neurons, microglia, VIP-Sncg-Lamp5 inhibitory neurons, PValb-Sst inhibitory neurons, oligodendrocytes, oligodendrocytes precursor cells (OPC), and astrocytes (**Fig. 3b**). To determine the differentially expressed genes in each cell type following electrical stimulation, we performed a pseudobulk RNA-seq analysis due to its superior performance for detecting differential expression in single-cell RNA-sequencing analyses [28] and the stringent nature of this analysis. The pseudobulk analysis revealed that the microglia displayed the highest differential gene expression of all cell types with 31 upregulated genes (**Fig. 3c, Supplementary data 5**). Genes related to cytokine activity (*CCL3*, *CCL4*, *CCL3L1*, and *IL1B*,) were upregulated exclusively within microglia. Five genes were upregulated in oligodendrocytes (*FOS*, *HSPA1A*, *JUNB*, *GADD45B*, and *FOSB*), and only two genes were differentially expressed (one upregulated: *LHFPL3*, and one downregulated: *ROBO2*) in astrocytes (**Supplementary data 5**). Surprisingly, no differentially expressed genes were detected within the neuronal cell types (excitatory and inhibitory neuronal cell types). Comparing the upregulated genes in microglia with human and mice bulk RNA seq revealed 5 genes (*Fos*, *Dusp1*, *Ccl3*, *Ccl4*, *Zfp36*) that are commonly upregulated following electrical stimulation (**Fig. 3d**). *Atf3*, *Cd83*, *Egr3*, *Nr4a1*, *Il1b*, *Mcl1*, *Nedd9*, *Spp1*, *Nfkbid*, *Rgs1*, and *Ccl3l1* were found to be human microglia-specific genes induced by electrical stimulation that do not change in the mice cortex (**Fig 3d**). Pathways enriched among the upregulated genes in microglia included cytokine-related pathways such as cytokine activity, chemokine activity, CCR chemokine receptor, and cytokine receptor binding (**Fig. 3e**). Microglia exhibited activation of transcription regulatory pathways such as DNA-binding transcription activator activity and RNA polymerase-specific DNA binding TF binding (**Fig. 3e**). These results reveal that electrical stimulation increases the expression of genes that are involved in cytokine activity and transcription regulation in microglia.

To investigate if electrical stimulation has an impact on the epigenome, we assessed chromatin accessibility using the single nuclear assay for transposase-accessible chromatin with sequencing (snATAC-seq)[29] on human samples from the stimulated paradigm. We focused on promoter accessibility (-2 to +2 kb from TSS) to analyze changes in chromatin accessibility in snATAC-seq data (**Supplementary data 6**). The differentially accessible regions that were either enriched or depleted following electrical stimulation in microglia significantly correlated (R^2=0.0262, p-value<0.002) with the microglia gene expression from snRNA-seq (**Fig. 3f**), suggesting that the genes that exhibit induced expression following electrical stimulation also exhibit increased chromatin accessibility. Genes related to transcription regulators, such as NR4A1 and FOS, showed a positive correlation between transcriptome and chromatin accessibility. We also found enriched promoter accessibility and increased gene expression for CCL4 in microglia following electrical stimulation (**Fig 3g**). A recent study demonstrated that ambient RNA from neurons may contaminate non-neuronal cells in single-nuclei transcriptomic data[30]. Our snRNA seq data revealed the upregulation of several IEGs, such as *FOS*, *EGR3*, *JUNB,* and *NR4A1* within microglia. These genes are often seen to be upregulated in neurons following neuronal activation. However, our snATAC-seq data from the same samples also showed increased chromatin accessibility in microglial populations within IEG promoters, thus suggesting that the microglia-specific IEG expression is not due to neuronal ambient RNA contamination. Lastly, we analyzed genome-wide transcription factor (TF) binding motifs from the upregulated peaks following electrical stimulation in microglia using chromatin accessibility data. Genomewide TF motif analysis revealed enrichment of binding motifs for ELK3, ELK1, YY2, NRF1, HINFP, and ELK4 (**Fig. 3h, Supplementary data 7**). Interestingly, ELK4, a transcription factor downstream of MAPK signaling, is predicted to positively regulate the expression of *Ccl3* and *Ccl4*[31]. Thus, our sn-ATAC-seq results reveal a signature of transcription factor motifs and chromatin accessibility underlying electrical stimulation-induced gene expression in human microglia.

Our experimental design included internal controls (baseline/unstimulated samples) from each participant, enabling us to investigate the impact of electrical stimulation across individual participants. Therefore, we next examined the cell type-specific

response to electrical stimulation from within participants using our newly developed deep-learning computational tool, NEUROeSTIMator[32], to map the active population of cells following electrical stimulation in each participant, comparing stimulated samples with corresponding baseline controls. We estimated cell activity from the single nuclei RNA data from individual populations of cells with either baseline or stimulated from each participants (**Fig. 4a**). We used paired linear regression with bootstrap sampling to assess differences in estimated activity between conditions for each cell type (**Fig. 4b**). Interestingly, we observed that the microglial cluster showed a significant increase in activity scores following stimulation, and this effect was consistent across all three participants [β = 0.100301, p-value < 2e-16] (**Fig. 4c**). This data supports our bulk RNA seq and snRNAseq data. However, we observed variability in electrical stimulation-mediated activation of excitatory neurons across participants. We detected significant differences in activity when using all three participants [β = -0.053920, p-value < 2e-16]. One pair of samples showed a significant increase in activity [β = 0.032528, p-value = 0.000807], while 2 out of 3 pairs showed a significant decrease in activity [β = -0.022086, -0.083254, p-values = 1.58e-8, 4e-9] (**Fig. 4c**). Variability in activity estimates was also observed in inhibitory neuronal sub-types across participants (**Fig. 4c**).

To further investigate the epigenomic signature of cellular activity following electrical stimulation, we estimated activity from snATAC-seq data using a similar approach (**Fig. 5a**). Activity was estimated for cells using gene-level counts in promoter regions and paired linear regression with bootstrap sampling was used to assess activity between baseline and electrical stimulation (**Fig. 5b**). Consistent with the transcriptomics data, microglia showed a significant increase in promoter-informed activity estimates, and this effect was consistent across all three participants [β = 0.060977, p-value < 2e-16] (**Fig. 5c**). Neuronal clusters showed variability in activation in activity when considering all three participants [$\beta_{Excitatory}$ = 0.016163, p-value$_{Excitatory}$ = 0.000247; $\beta_{Inhibitory}$ = 0.028302, p-value$_{Inhibitory}$ = 2.283e-7]. Only one participant showed a significant increase in the excitatory[β = 0.04712, p-val = 0.000314] and inhibitory neurons (Sst-Pvalb) [β = 0.08863, p-value = 0.00201]. These findings suggest that microglia exhibit robust transcriptional and epigenomic responses to electrical stimulation and that neuronal cell types exhibit greater variability between patient participants.

Discussion

Electrical stimulation is a model for studying the human cortex with protocols that are used clinically for mapping the function of specific brain regions. Here, we take a molecular approach, defining the transcriptomic and epigenomic signatures of electrical stimulation at the single cell level in the human cortex in neurosurgical patient participants. Researchers have correlated human brain transcriptomics with prior recorded oscillatory signatures of memory consolidation[22,33], but have not previously examined the changes in gene expression that are rapidly and directly driven by electrical stimulation. Most work thus far has emphasized the role of neurons in responding to external stimuli, but our work reveals the critical role of microglia in sculpting the activity and function of brain circuits. In microglia, genes related to cytokine signaling showed the greatest induction pattern, and this was conserved across species. As previously hypothesized, we observed the induction of activity-dependent genes in our bulk RNA sequencing results, but to our surprise, single nuclei multiomics experiments revealed pronounced microglia-specific transcriptomic activation following electrical stimulation that was supported by analysis using NEUROeSTIMator[32], a deep-learning computational model. Identifying a microglial transcriptomic response following electrical stimulation represents an important conceptual advance in our understanding of the impact of this common form of electrical stimulation used clinically.

Microglia are critical modulators of neuronal function, acting to suppress excessive activity by inhibiting surrounding neurons, including excitatory neurons[34]. Although classified as non-excitable cells, microglia exhibit electrophysiological stimulus-response features, and changes in their membrane potential affect crucial microglial functions including phagocytosis, chemotaxis, and cytokine release[35]. Our analyses identified critical molecular components within microglia that change with electrical stimulation, including chemokine-encoding genes such as *CCL3* and *CCL4*, which act to alter microglial motility, influence neuronal-microglial interactions, and shape neuronal connectivity[34,36-39]. These chemokines are ligands for C-C chemokine receptor type 5 (CCR5), which regulates neuronal excitability and memory allocation in the hippocampus[40]. Previous rodent studies have shown that neuronal activation using

chemogenetic approaches leads to distinct gene expression changes in microglia, including genes encoding chemokines[34]. Our work also revealed that genes encoding transcription factors such as FOS and NR4A1 are induced in microglia in response to electrical stimulation. Further, we observed increased promoter accessibility at these genes, underscoring the conclusion that these genes are transcriptionally upregulated in microglia after stimulation and broadening the potential functional impact of these immediate early genes beyond their more frequently studied role in neurons. Indeed, NR4A1 acts in neurons as a transcription factor essential for memory consolidation[41,42] and functions in microglia as a molecular rheostat, contributing to the maintenance of a threshold that prevents microglial activation[43]. Furthermore, our study of chromatin accessibility defines a cell-type-specific signature of transcriptional motifs driving these processes that includes motifs for ELK4 and NRF1, which have been identified in other studies as transcriptional motifs associated with the differential regulation of molecular pathways in specific subsets of microglia[44,45].

This study represents a rare opportunity to define the molecular impact of electrical stimulation in patients undergoing anterior temporal lobectomy due to therapy refractory seizures. Although presenting a unique opportunity, it is important to note the caveats that come from this research carried out in a setting focused on clinical efficacy in patients that have epilepsy. Thus, we can not control all of the parameters as we might in an experiment carried out in a model organism, although we have worked to address three potential limitations of our study. First, our experimental design includes a group that does not receive electrical stimulation, thus controlling for the impact of the surgical procedure, craniotomy, brain temperature changes, and levels of anesthesia when present. We also show that electrical stimulation in a mouse model, in which we can control many additional parameters, gives rise to similar molecular changes. Importantly, our mouse experiment included the use of an inactive electrode, thus controlling for mechanically induced changes in gene expression. Second, the stimulation pattern used is safe and efficacious for brain mapping in human patients but is not optimized to modulate activity in specific circuits and cell types in the brain. Indeed, the stimulation protocol used leads to the functional disruption of brain regions[1], and thus, we may expect to see reductions in activity-dependent gene expression in

excitatory neurons, as seen in two patients in our NEUROeSTIMator analysis. Third, it goes without saying that these patients have severe therapy refractory epilepsy. However, the resected samples were taken a considerable distance from the seizure foci (see Suppl. Fig. 1b), our gene induction signatures do not resemble those seen in actively spiking tissue[26], and our molecular changes are seen in a mouse model that does not exhibit seizures.

Despite these caveats, we were able to identify a reliable cell-specific signature of the impact of electrical stimulation in the living human brain. This study demonstrates for the first time a unique transcriptomic and epigenomic signature in microglia following direct electrical stimulation, representing a conceptual advance in our understanding of how the brain responds to an important clinical tool used to map brain function. These findings have the potential to inform the clinical practice of diagnostic and therapeutic brain stimulation. Clinical and translational research has focused mainly on the immediate effects of stimulation in neuronal populations, while microglia receive little attention, often being discussed with regard to neuroimmunological aspects[46]. Although, for clinical mapping, clinicians rely on the immediate neuronal effects on behavior (e.g., speech arrest), there is growing evidence that therapeutic effects of electrical stimulation can accumulate gradually over time[47]. We could not test a range of stimulation frequencies and intensities or examine a broad range of brain areas because of clinical limitations, but our results raise the intriguing hypothesis that microglia, not just neurons, shape plasticity after repetitive stimulation. Our work further highlights that cytokine and chemokine-based mechanisms enable microglia to respond to electrical stimulation and sculpt circuit function, making these potential targets to modify circuit activity with pharmacological approaches. Microglia exist in different subpopulations depending on transcriptional state and age[48,49], and our epigenetic work has revealed particular transcriptional motifs that mark microglial subtypes important for cortical plasticity. It will be especially interesting in future work to extend our studies to stimulation protocols used therapeutically as well as natural stimuli to probe the critical role of microglial signaling in mediating the response of the human brain to experience.

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Author contributions

S.C., M.A.H., and T.A. conceived the project, designed the experiments, and interpreted the data. S.C. and T.A. wrote the manuscript with assistance from C.I.P., L.L., and T.N.J., and inputs from other co-authors. H.K. performed human surgery and electrical stimulation and resected the tissue. A.E.R and C.K.K. processed with IRB and MRI imaging registration. H.O, M.A.H, T.N-J, and K.V.L provided advice on surgical procedures and electrical stimulation. M.M.H. provided a pathological assessment. L.C.L., K.V.N., and B.J.P. collected the tissue and images during resection. S.C., B.J.P., L.C.L., U.M., L.L. performed human or mouse tissue samples experiments. C.I.P. and Y.A.A. performed distance to the seizure site analysis. U.M. and L.L. performed mouse brain imaging and data analysis. Y.V., A.I.G., and B.B. performed the bioinformatics analysis with inputs from E.B. and J.J.M. S.A.H. performed the mouse surgery and electrical stimulation experiments. K.V.N, C.K.K, Z.K. Y.K, C.I.P, M.H, J.J.M, E.B, U.M, and T.N.J. provided input on data analysis and interpretation. All authors discussed the results and commented on the manuscript.

Competing interests

T.A. is a scientific advisor to Aditum Bio and Radius Health and serves on the scientific advisory board of Embark Neuro. The other authors declare no conflicting interests.

Figure legends

Figure 1. **Electrical stimulation of the human cortex induces changes in genes associated with cytokine activity and transcription regulation. a, b**. Schematics of stimulated (**a**) and unstimulated (**b**) paradigms in the human anterior temporal lobe. For both paradigms sample A was taken at T = 0 minutes then an adjacent sample B was taken at either 30 min after stimulation (**a**, stimulated paradigm, 4 participants) or 30 min after sample A was taken (**b**, unstimulated paradigm, 4 participants). **c.** Volcano plot showing gene expression changes in the stimulated samples. The most significant genes (FDR < 0.05) are labeled in red (upregulated) or blue (downregulated). **d.** Cnet plot showing pathway enrichment analysis of the genes significantly (FDR < 0.05) differentially expressed in the stimulated samples. **e**. Volcano plots showing gene expression changes in the unstimulated samples. The most significant genes (FDR < 0.05) are labeled in red (upregulated) or blue (downregulated).

Figure 2. Electrical stimulation of mouse cortex induces changes in genes associated with cytokine activity and transcription regulation. a. The mouse auditory cortex was stimulated, tissue was collected 30 min later, and the contralateral auditory cortex was obtained as baseline control. **b.** Volcano plot showing gene expression changes after stimulation. The most significant genes (FDR < 0.05) are labeled in red (upregulated) or blue (downregulated). **c.** Cnet plot showing pathway enrichment analysis of the genes significantly (FDR < 0.05) affected by electrical stimulation. **d.** Quadrant plot comparing genes induced by electrical stimulation in mice with genes induced by electrical stimulation in human brain. Genes upregulated in both mice and human brains after stimulation are labeled. The size, opacity, and color intensity of each data point denotes the minimum false discovery rate value for a gene between each transcriptomic datasets. **e.** qPCR analysis of the genes related to cytokine activity comparing stimulated versus baseline controls in mice cortex. n=7/group.

Figure 3. Single nuclei multiomics reveal cell type-specific transcriptomic and epigenomic changes following electrical stimulation. a. Single nucleus multiomic experimental approach. **b.** UMAP shows the specific cell types from each cluster. **c.** Volcano plot showing differentially expressed genes in microglia using pseudobulk analysis comparing stimulated vs baseline samples from human brain (FDR<0.1). **d.** Upset plot comparing DEGs in mouse, human bulk RNA seq, and human microglia snRNA-seq after electrical stimulation. **e.** Cnet plot showing pathway enrichment analysis of the genes significantly (FDR < 0.1) affected by electrical stimulation in human microglia. **f.** Quadrant plot comparing DEGs from snRNA-seq following electrical stimulation in human microglia and genes with open chromatin accessibility from snATAC-seq in microglia following electrical stimulation. Genes upregulated in both mice and human brains after stimulation are labeled. **g**. Quadrant plot comparing gene expression changes (log10(FDR)*log2fold-change) between snRNA-seq (x-axis) and snATAC-seq (y-axis) data in microglia after stimulation. Genes in red in the top-right corner of the plot are significantly upregulated at the gene expression level and exhibit increased chromatin accessibility. The dotted lines represent FDR thresholds of 0.01. The dashed line represents the linear regression applied to this quadrant plot. **g.** DNA motifs are overrepresented in the set of peaks differentially accessible in microglia after stimulation. Motifs are ranked based on significance from the most significant left to right. **h.** The coverage plot shows ATAC peaks at the CCL4 locus. Each track represents a normalized chromatin accessibility signal from the ATAC assay for each cell type and condition (baseline or stimulated).

Figure 4. NEUROeSTIMator for snRNA-seq. Cell-type specific differences in activity estimates. a. UMAP of cells representing the activity estimated from the RNA gene counts in the baseline and stimulated conditions. Darker blue points are cells with higher estimated activity. **b.** The bootstrap distribution of the difference between activity estimates (stimulated – baseline) by cell type. Horizontal bars indicate the 95% confidence interval of the mean difference in activity estimate. **c.** Activity estimates in selected cell types in baseline (green) and stimulated (orange) conditions.

Figure 5. NEUROeSTIMator for snATAC-seq. Cell-type specific differences in activity estimates. a. UMAP of cells representing the activity estimated from the ATAC

counts in the baseline and stimulated conditions. Darker blue points are cells with higher estimated activity. **b.** The bootstrap distribution of the difference between activity estimates (stimulated – baseline) by cell type. Horizontal bars indicate the 95% confidence interval of the mean difference in activity estimate. **c.** Activity estimates in selected cell types in baseline (green) and stimulated (orange) conditions.

Materials and Methods:

Patient participants: The study participants were 8 adult neurosurgical patients (6 female, 2 male, age 19-63 years old, median age 41 years old) with medically refractory epilepsy. The patients were undergoing surgical resection of seizure foci following non-invasive electroencephalography (EEG) or invasive iEEG monitoring. All patients were diagnosed with intractable epilepsy. All patients underwent ATL resection surgery for epilepsy of various etiologies **(Extended data fig 1).**

Two of the patient participants, L472 had a cavernoma and L475 had a dysembryoplastic neuroepithelial tumor. X out of Y participants were required to be awake during the surgical resection, and the rest were anesthetized. All patient participants except #6 were non-smokers. Participant's age, sex, surgery, and awake or sedative information were recorded (**Extended Data Table 1)**. All participants were native English speakers, 7 were right-handed, 1 was left-handed, and all had left language dominance as determined by Wada tests. All participants underwent audiometric evaluation before the study, and none were found to have hearing deficits or word recognition scores deemed sufficient to affect the findings presented in this study. The vision was self-reported as normal or corrected to normal with glasses or contact lenses. As determined by standard neuropsychological assessments, cognitive function was in the average range in all participants. Research protocols were approved by the University of Iowa Institutional Review Board (IRB 201910791, 201911084) and the National Institutes of Health. Written informed consent was obtained from all participants.

Procedure: Surgery was performed awake, under general anesthesia or monitored anesthetic care. Standard craniotomy was performed by the same senior epilepsy neurosurgeon in all patients to reach the epilepsy focus for resection, which involved the

anterior and medial temporal lobe in all patients except one who had a temporal encephalocele and surrounding anterolateral temporal cortical focus. Before neurosurgical ATL resection, cortical tissue from the anterior temporal lobe was sampled by the neurosurgeon and handed over to the research team for analysis. The experimental condition was defined by an electrical "stimulation paradigm" and a control "no-stimulation paradigm**"**. The location of the sampled tissue is plotted on anatomic brain reconstructions **(Extended data fig 1).** The 30 minutes in between the control and experimental samples was clinically required for the clinical EEG team to record inter-ictal activity with surface recording grids placed gently on the brain surface.

There were 4 participants (1 iEEG and 3 EEG patients) who underwent the "stimulation paradigm" and 4 (2 iEEG and 2 EEG patients) who underwent the "no-stimulation paradigm" **(Extended Data Table 5)**.

In the "stimulation paradigm", a baseline sample was obtained from the anterior temporal cortex that would be resected in the planned surgical resection. The area directly adjacent to where the baseline sample was collected was stimulated with direct bipolar electric stimulation (50 Hz frequency, 0.2 ms pulse duration, 2 min stimulation duration, and 10 V voltage). The stimulated area was then sampled after a period of 30 minutes to allow for gene expression[50,51]. In the "no stimulation paradigm", no direct electric stimulation was performed and the area directly adjacent to the baseline sample was collected 30 minutes after initial baseline sampling.

After sampling the tissue samples were immediately placed in a sterile container on dry ice. The average weight of the baseline sample was 88.0 ± 33.2 mg (mean, standard deviation) and the adjacent sample was 113.4 ± 62.7 mg. After the collection of all samples in this fashion, they were weighed and transferred to a freezer at -80° C for storage until further testing.

Sample Localization to MNI space: All samples were from the same cortical region. Intraoperative photos of the sample sites were obtained during the time of surgery. Using patient participant matched preoperative T1 sequence MRI, the sample sites were mapped onto their anatomic brain reconstructions **(Extended data fig. 1)**. They were also mapped onto MNI space coordinates.

Human cortical tissue RNA extraction, library preparation, and sequencing: Total RNA was extracted from sampled human brains using miRNeasy Mini Kit (Qiagen, CA, USA). The tissue samples were homogenized in QIAzol (Qiagen, CA. USA) stainless steel beads (Qiagen, CA, USA). Chloroform was then used for phase separation. RNA containing an aqueous layer was further purified using the RNeasy MinElute spin column. RNA was finally eluted in RNase-free water. RNA concentrations were estimated using a Nanodrop (Thermo Fisher Scientific, MA, USA) and Qubit (Thermo Fisher Scientific, MA, USA). RNA libraries were prepared at the Iowa Institute of Human Genetics (IIHG), Genomics Division, using the Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus (Illumina Inc., San Diego, CA). The KAPA Illumina Library Quantification Kit (KAPA Biosystems, Wilmington, MA) was used to measure library concentrations. Pooled libraries were sequenced on Illumina NovaSeq6000 sequencers with 150-bp paired-end chemistry (Illumina) at the Iowa Institute of Human Genetics (IIHG) core.

Bulk RNA sequencing analysis: RNA-seq data were processed with the bcbio-nextgen pipeline (https://github.com/bcbio/bcbio-nextgen, version 1.2.9). The pipeline uses STAR[52] to align reads to the hg38 or mm10 reference genome and quantifies expression at the gene level with feature Counts [53]. All further analyses were performed using R. For gene-level count data, the R package EDASeq was used to account for sequencing depth (upper quartile normalization) [54]. Latent sources of variation in expression levels were assessed and accounted for using RUVSeq (RUVs mode using all features) [55]. Appropriate choice of the RUVSeq parameter k was guided through inspection of principal components analysis (PCA) plots. Specifically, the smallest value k was chosen where PCA plots demonstrated replicate sample clustering in the first three principal components[56]. Differential expression analysis was conducted using the edgeR package[57]. Codes to reproduce the RNA-seq differential gene expression analysis are available at https://github.com/YannVRB/Human-brain-stimulation.git.

All the transcriptomics data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE224952.

Downstream pathway analysis:

The enrichment analysis of differentially expressed genes-associated pathways and molecular functions from the stimulated and unstimulated RNA-seq was performed with the Gene Ontology (GO–molecular function) databases using clusterProfiler package in R. Only the pathways with an adjusted p-value < 0.05 were considered as significant and displayed. Further, the enrichment data were visualized using 'cnetplot' function of clusterProfiler.

Single-nuclei multiomics (nuclei isolation, library preparation, sequencing: Nuclei were isolated from brain tissue using the Chromium Nuclei Isolation Kit (10X Genomics). Briefly, frozen tissue was dissociated with pestle in lysis buffer, passed through nuclei isolation column and spun at 16,000 rcf for 20 sec at 4^0C. Flowthrough was vortexed and spun at 500 rcf for 3 mins at 4^0C. Pellet was resuspended with debris removal buffer and centrifuged at 700 rcf for 10 mins at 4^0C, nuclei resuspended in wash buffer and centrifuged again at 500 rcf for 5 mins at 4^0C. Pellet was resuspended in resuspension buffer and nuclei were counted using a hemocytometer. Nuclei were directly processed for droplet capture for single cell multiome ATAC + gene expression using a chromium controller (10X Genomics). Chromium Next GEM Single Cell Multiome ATAC + Gene v1 chemistry was used to create single nuclei ATAC and RNA libraries from the same cell. Two baseline and two stimulated samples were used for independent replicates. Libraries were sequenced on an Illumina Novaseq 6000 with a 150 bp paired end read setup.

Single-nuclei multiomic data processing and analysis: To analyze the RNA part of the human brain stimulation multiomic data, gene counts were normalized and log transformed (LogNormalize), and the top 2,000 most variable features between each nuclei were identified using FindVariableFeatures (selection.method = 'vst'). Features that are repeatedly variable across nuclei and datasets were selected for integration (SelectIntegrationFeatures). We then identified anchors (FindIntegrationAnchors), which took the list of 4 individual Seurat objects for each sample as input and used these anchors to integrate the four datasets together (IntegrateData). The following analyses were performed on the integrated Seurat object. Linear dimensionality reduction was

performed by principal component analysis (runPCA, npcs = 25). A k-nearest-neighbors graph was constructed based on Euclidean distance in PCA space and refined (FindNeighbors, npcs = 30), then nuclei were clustered using the Louvain algorithm (FindClusters, resolution = 0.5). Clusters were visualized with UMAP (runUMAP, dims = 30). Cell types were annotated by label transfer cell labels from an existing human primary motor cortex reference dataset from the Allen Institute (doi: 10.1038/s41586-021-03465-8) (FindTransferAnchors and TransferData). Cell types identification was validated by expression of specific biomarkers. Prior of running differential gene expression analysis, as recommended by recent publications (https://doi.org/10.1038/nmeth.4612 and https://doi.org/10.1038/s41467-020-19894-4), we used an aggregation-based (pseudobulk) workflow. We aggregated all cells within the same cell type and sample using the AggregateExpression function. This returns a Seurat object where each 'cell' represents the pseudobulk profile of one cell type in one individual. After we aggregated cells, we performed celltype-specific differential expression between stimulated and baseline samples using DESeq2.

To analyze ATAC part of the human brain stimulation multiomic data, prior to integrating the four Seurat object, the default assay was switched to ATAC, and peak calling was performed. Since the set of peaks identified by Cellranger often merges distinct peaks that are close together, creating a problem for motif enrichment analysis and peak-to-gene linkage, we identified a more accurate set of peaks by calling peaks using MACS2 (CallPeaks) on all cells together. Peaks on nonstandard chromosomes and in genomic blacklist regions were removed (keepStandardChromosomes and subsetByOverlaps). Normalization was performed with a frequency-inverse document frequency normalization which normalizes across cells and peaks (RunTFIDF). Then, a feature selection was performed using all the peaks as input (FindTopFeatures). The dimensional reduction was performed on the TF-IDF normalized matrix with the selected peaks using a singular value decomposition (RunSVD). To mimic the open chromatin conformation of a gene, a gene activity matrix was calculated using a window of 1000bp before and after the transcription start site of each protein coding gene (GeneActivity). Differentially accessible transcription start sites in individual clusters between baseline and stimulated samples were calculated using a logistic regression

framework (FindMarkers, test.use = 'LR', latent.vars = 'nCount_peaks', Padj < 0.05). Motif and transcription factor enrichment analysis for Microglia cluster was performed using FindMotifs on genome-wide all peaks assay of the Seurat object. The top six enriched motifs in microglia cluster are shown. Genomic locations of typical genes like CCL4 were presented (CoveragePlot). It also includes co-accessibility between peaks and transcription start site of genes. Codes to reproduce the multiomic data analysis are available at https://github.com/YannVRB/Human-brain-stimulation.git.

NEUROeSTIMator analysis. Gene-level counts for each cell were used as input for the NEUROeSTIMator model [33] to estimate transcriptional signatures of cell activity. Activity was also estimated for cells using gene-level counts in promoter regions from the ATAC assay. Estimated activity was assessed for significant differences between the baseline and stimulated conditions across the donors and cell types using paired linear regression. P-values were adjusted for multiple testing with the Benjamini-Hochberg correction. To account for variable sample sizes across cell types and participants, bootstrap resampling was performed (n = 100 cells per donor, replicates = 1000) for each cell type in each condition to generate a distribution of the mean difference in estimated activity between conditions.

To account for variation in sample size across cell types and participants, bootstrap resampling was performed (n = 100 cells per participant, replicates = 1000) for each cell type of the mean difference in estimated activity scores between conditions (mean stimulated – mean baseline). The distributions were analyzed by mean and 95% confidence interval.

Animals: Adult male C57BL/6J mice were purchased from The Jackson Laboratory were 3 to 4 months of age during experiments. All mice had free access to food and water; lights were maintained on a 12-hour light/12-hour dark cycle.

Mouse electrical stimulation: Stimulation experiments were performed in anesthetized adult male C57BL6/J mice. Anesthesia was induced with 5% isoflurane by inhalation and maintained at 1.8-2% for the duration of the experiment. The mouse was placed in a stereotax (Kopf) and a midline incision was made and the skin retracted to expose the temporal muscle bilaterally. The dorsal insertion of both temporal muscles was

removed, and the muscles retracted. A 2-3 mm craniotomy was made over area AuV (centered at 2.9 mm posterior, 4.2 mm lateral, 2.8 mm ventral from Bregma based on Paxinos atlas) bilaterally to expose the cortical surface and a small square of gel foam soaked in ACSF was applied on top of the dura to prevent it from dehydrating. For each mouse the side of electrical stimulation and sham stimulation were alternated, with the sham side serving as the baseline control for gene expression profiling. Electrical stimulation was delivered through a bipolar ball electrode constructed from two silver wires in which the uninsulated tips were melted under a butane flame (1 mm tip size, 2 mm tip spacing). For both electrical and sham stimulations, the gel foam was removed and the electrode was gently lowered to make contact with the cortical surface. For electrical stimulation, a biphasic pulse train was then delivered for two minutes (8 mA, 50 Hz, 200 µs pulse width). For the sham stimulation no current was delivered but the electrode was left in place for 2 minutes. Following electrical or sham stimulation the electrode was slowly retracted, and the exposed dura was covered with gel foam. Selection for hemisphere was performed randomly, which resulted in the order of electrical and sham stimulation alternating from mouse to mouse. No more than 5 minutes elapsed between electrical or sham stimulation of both sides. After both sides were stimulated (electrical or sham) the mouse was left in the stereotax under anesthesia for 30 minutes before euthanasia and tissue collection. Tissue samples were immediately stored at -80^{0}C in RNA later solution (Ambion).

RNA extraction, cDNA preparation and qPCRs from mouse auditory cortex: Tissue samples were homogenized in Qiazol (Qiagen) using stainless steel beads (Qiagen). Chloroform was added and centrifuged at 12,000*g* at room temperature for 15 min to separate RNA in the aqueous phase. RNA was precipitated in ethanol and cleared using RNeasy kit (Qiagen). RNA eluted in nuclease-free water was then treated with DNase (Qiagen) at room temperature for 25 min to remove genomic DNA. RNA was further precipitated in ethanol, sodium acetate (pH 5.2) and glycogen overnight at −20°C. RNA was precipitated by centrifugation at 30,000g for 20 min, precipitate washed with 70% ethanol and the dried RNA pellet was resuspended in nuclease-free water. RNA concentration was measured using NanoDrop (Thermo Fisher Scientific). 1 µg of RNA was used for complementary DNA (cDNA) preparation using the SuperScript IV First-

Strand Synthesis System (Ambion). Real-time reverse transcription polymerase chain reactions (RT-PCRs) were performed on the QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems, Life Technologies). Data were normalized to housekeeping genes (*Tubulin*, *Pgk1*, and *Actin*), and $2^{(-\Delta\Delta Ct)}$ method was used for gene expression analysis.

Mouse cortex spatial transcriptomics analysis (Yann): Spatial transcriptomics data were obtained from the Visium Spatial Gene Expression platform, as described previously[42]. This dataset provides comprehensive gene expression profiles across various brain regions, including the mouse cortex. To focus on the mouse auditory cortex, the closest homolog to the human anterior temporal lobe, we manually selected spatial spots from the Visium data corresponding to this brain region. This selection was based on anatomical landmarks and known spatial coordinates for the auditory cortex. For each of the 14 mice in the dataset, we subsetted the data to include only these selected spots, thereby isolating the gene expression profiles specifically from the auditory cortex.

The dataset of 14 mice was divided into two experimental conditions: 7 mice underwent Spatial Object Recognition after 1 hour (SOR-1h), while the other 7 mice were kept in their homecage environment without specific tasks. Based on the Visium spots data overlaying the mouse auditory cortex, differential gene expression analysis was performed to identify genes that were significantly differentially expressed between the SOR-1h and homecage conditions.

Statistics: For the qPCR analysis, the Wilcoxon matched pairs signed rank test and one sample Wilcoxon test was performed.

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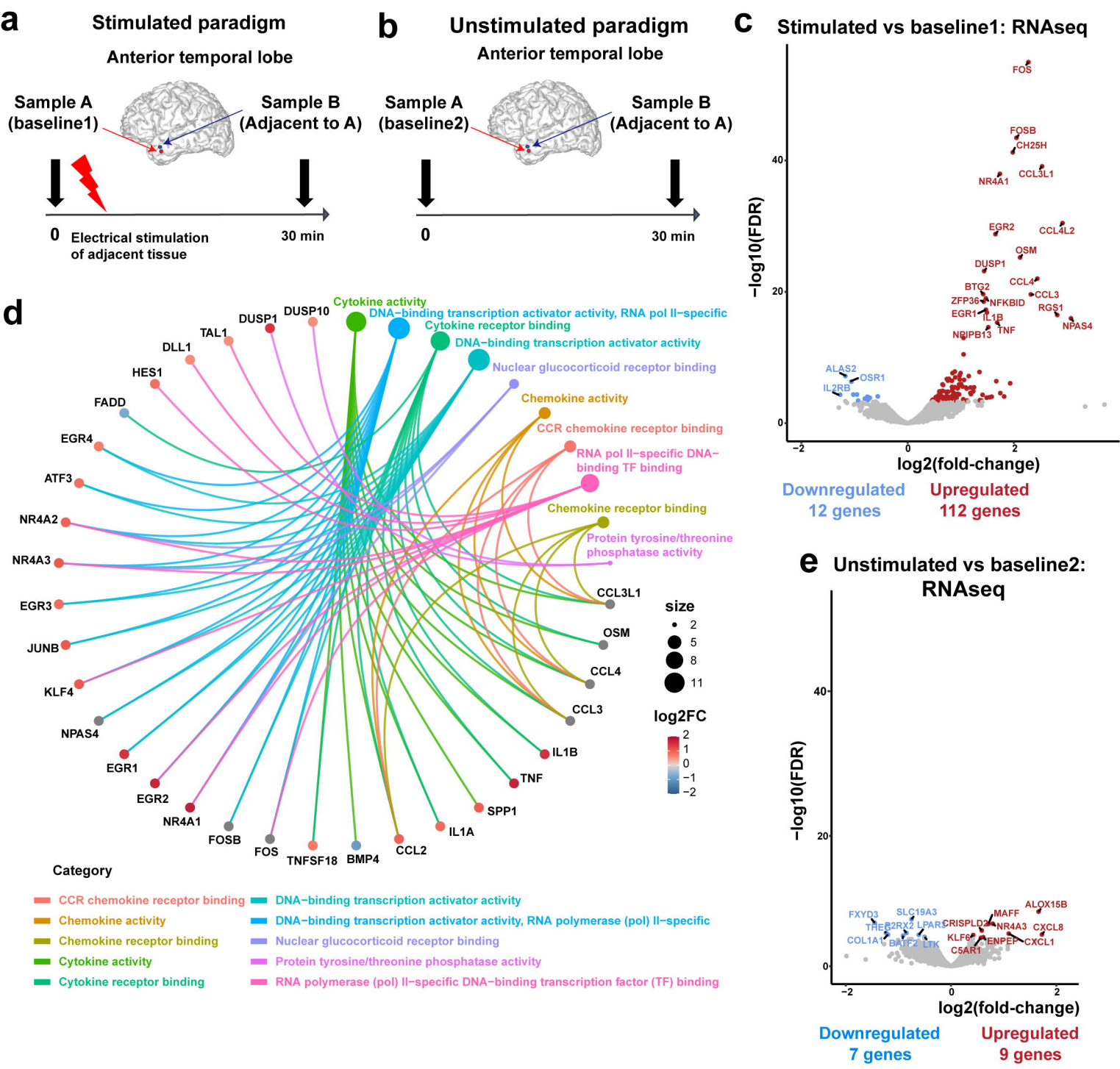
a
Stimulated paradigm
Anterior temporal lobe
Sample A (baseline1)
Sample B (Adjacent to A)
0
Electrical stimulation of adjacent tissue
30 min
b
Unstimulated paradigm
Anterior temporal lobe
Sample A (baseline2)
Sample B (Adjacent to A)
0
30 min
c
Stimulated vs baseline1: RNAseq
−log10(FDR)
log2(fold-change)
Downregulated 12 genes
Upregulated 112 genes
d
Cytokine activity
DNA−binding transcription activator activity, RNA pol II−specific
Cytokine receptor binding
DNA−binding transcription activator activity
Nuclear glucocorticoid receptor binding
Chemokine activity
CCR chemokine receptor binding
RNA pol II−specific DNA−binding TF binding
Chemokine receptor binding
Protein tyrosine/threonine phosphatase activity
size
log2FC
Category
CCR chemokine receptor binding
Chemokine activity
Chemokine receptor binding
Cytokine activity
Cytokine receptor binding
DNA−binding transcription activator activity
DNA−binding transcription activator activity, RNA polymerase (pol) II−specific
Nuclear glucocorticoid receptor binding
Protein tyrosine/threonine phosphatase activity
RNA polymerase (pol) II−specific DNA−binding transcription factor (TF) binding
e
Unstimulated vs baseline2: RNAseq
−log10(FDR)
log2(fold-change)
Downregulated 7 genes
Upregulated 9 genes

Figure 1

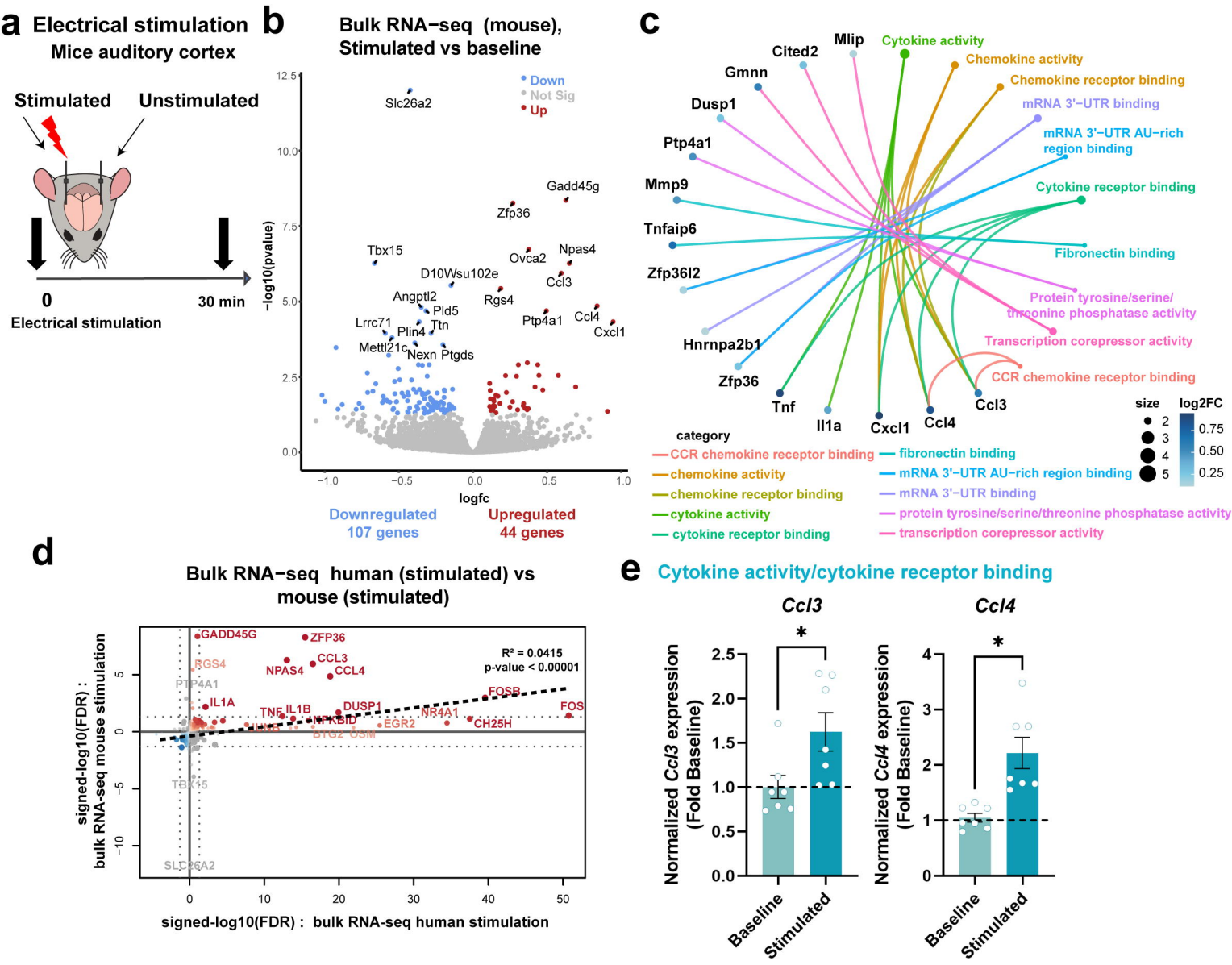
a
Electrical stimulation
Mice auditory cortex
Stimulated
Unstimulated
0
30 min
Electrical stimulation
b
Bulk RNA−seq (mouse), Stimulated vs baseline
Down
Not Sig
Up
−log10(pvalue)
logfc
Slc26a2
Gadd45g
Zfp36
Tbx15
Ovca2
Npas4
D10Wsu102e
Ccl3
Angptl2
Rgs4
Pld5
Lrrc71
Ttn
Ptp4a1
Ccl4
Cxcl1
Plin4
Mettl21c
Nexn
Ptgds
Downregulated
107 genes
Upregulated
44 genes
c
Cited2
Mlip
Cytokine activity
Chemokine activity
Gmnn
Chemokine receptor binding
Dusp1
mRNA 3'−UTR binding
Ptp4a1
mRNA 3'−UTR AU−rich region binding
Mmp9
Cytokine receptor binding
Tnfaip6
Fibronectin binding
Zfp36l2
Protein tyrosine/serine/threonine phosphatase activity
Transcription corepressor activity
Hnrnpa2b1
CCR chemokine receptor binding
Zfp36
Tnf
Il1a
Cxcl1
Ccl4
Ccl3
size
log2FC
category
CCR chemokine receptor binding
chemokine activity
chemokine receptor binding
cytokine activity
cytokine receptor binding
fibronectin binding
mRNA 3'−UTR AU−rich region binding
mRNA 3'−UTR binding
protein tyrosine/serine/threonine phosphatase activity
transcription corepressor activity
d
Bulk RNA−seq human (stimulated) vs mouse (stimulated)
R² = 0.0415
p-value < 0.00001
GADD45G
ZFP36
CCL3
CCL4
NPAS4
RGS4
PTP4A1
IL1A
FOSB
FOS
TNF
IL1B
DUSP1
NR4A1
EGR2
CH25H
TBX15
SLC26A2
signed-log10(FDR) : bulk RNA-seq mouse stimulation
signed-log10(FDR) : bulk RNA-seq human stimulation
e
Cytokine activity/cytokine receptor binding
Ccl3
Ccl4
Normalized Ccl3 expression (Fold Baseline)
Normalized Ccl4 expression (Fold Baseline)
Baseline
Stimulated

Figure 2

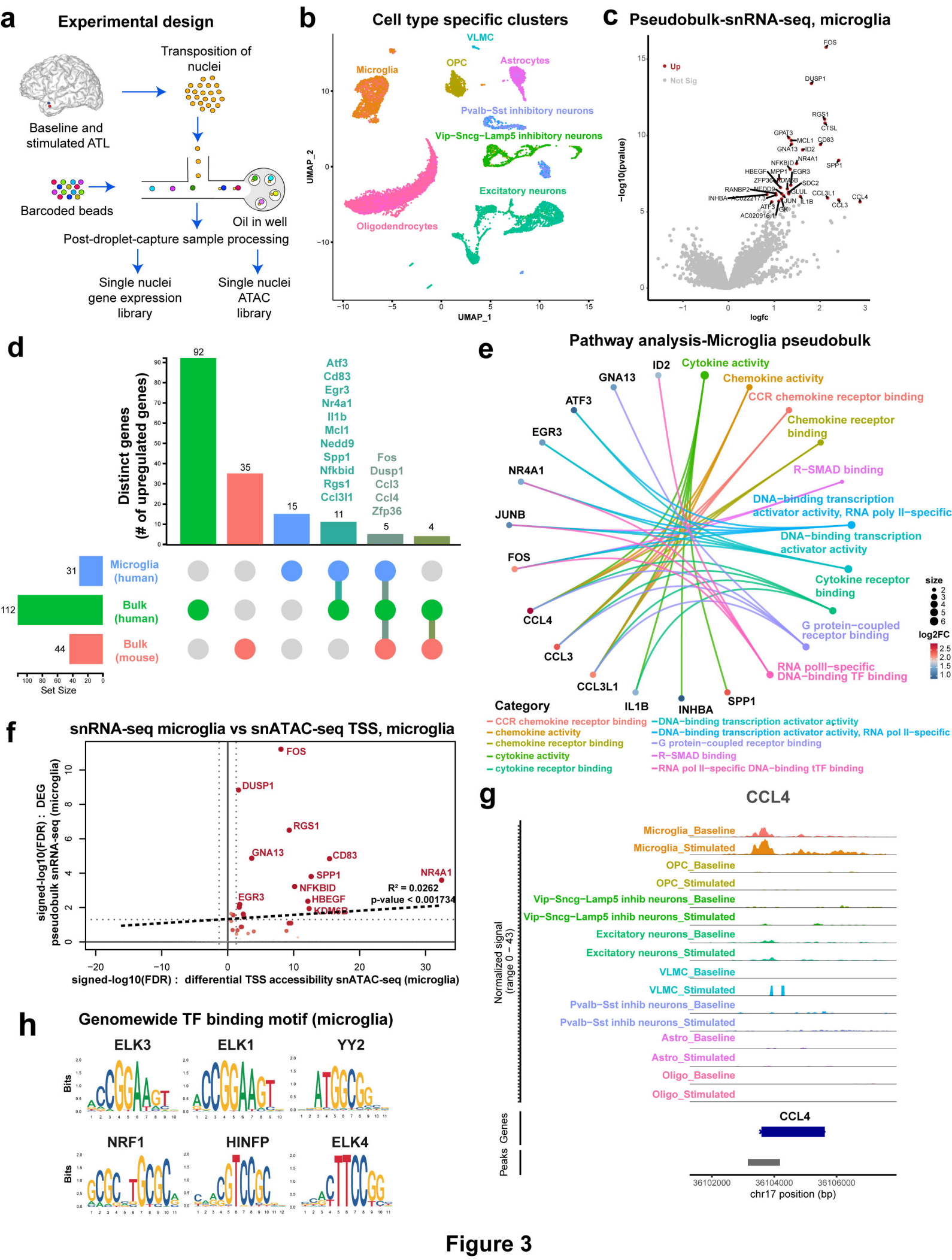
a
Experimental design
Transposition of nuclei
Baseline and stimulated ATL
Barcoded beads
Oil in well
Post-droplet-capture sample processing
Single nuclei gene expression library
Single nuclei ATAC library
b
Cell type specific clusters
VLMC
Microglia
OPC
Astrocytes
Pvalb−Sst inhibitory neurons
Vip−Sncg−Lamp5 inhibitory neurons
Excitatory neurons
Oligodendrocytes
UMAP_1
UMAP_2
c
Pseudobulk-snRNA-seq, microglia
Up
Not Sig
−log10(pvalue)
logfc
d
Distinct genes (# of upregulated genes)
92
35
15
11
5
4
Atf3 Cd83 Egr3 Nr4a1 Il1b Mcl1 Nedd9 Spp1 Nfkbid Rgs1 Ccl3l1
Fos Dusp1 Ccl3 Ccl4 Zfp36
31
112
44
Microglia (human)
Bulk (human)
Bulk (mouse)
Set Size
e
Pathway analysis-Microglia pseudobulk
Cytokine activity
Chemokine activity
CCR chemokine receptor binding
Chemokine receptor binding
R−SMAD binding
DNA−binding transcription activator activity, RNA poly II−specific
DNA−binding transcription activator activity
Cytokine receptor binding
G protein−coupled receptor binding
RNA polII−specific DNA−binding TF binding
ID2
GNA13
ATF3
EGR3
NR4A1
JUNB
FOS
CCL4
CCL3
CCL3L1
IL1B
INHBA
SPP1
size
log2FC
Category
CCR chemokine receptor binding
chemokine activity
chemokine receptor binding
cytokine activity
cytokine receptor binding
DNA−binding transcription activator activity
DNA−binding transcription activator activity, RNA pol II−specific
G protein−coupled receptor binding
R−SMAD binding
RNA pol II−specific DNA−binding tTF binding
f
snRNA-seq microglia vs snATAC-seq TSS, microglia
FOS
DUSP1
RGS1
GNA13
CD83
SPP1
NR4A1
NFKBID
EGR3
HBEGF
KDM6B
R² = 0.0262
p-value < 0.001734
signed-log10(FDR) : DEG pseudobulk snRNA-seq (microglia)
signed-log10(FDR) : differential TSS accessibility snATAC-seq (microglia)
g
CCL4
Microglia_Baseline
Microglia_Stimulated
OPC_Baseline
OPC_Stimulated
Vip−Sncg−Lamp5 inhib neurons_Baseline
Vip−Sncg−Lamp5 inhib neurons_Stimulated
Excitatory neurons_Baseline
Excitatory neurons_Stimulated
VLMC_Baseline
VLMC_Stimulated
Pvalb−Sst inhib neurons_Baseline
Pvalb−Sst inhib neurons_Stimulated
Astro_Baseline
Astro_Stimulated
Oligo_Baseline
Oligo_Stimulated
Normalized signal (range 0 − 43)
Genes
Peaks
36102000
36104000
36106000
chr17 position (bp)
h
Genomewide TF binding motif (microglia)
ELK3
ELK1
YY2
NRF1
HINFP
ELK4
Bits

Figure 3

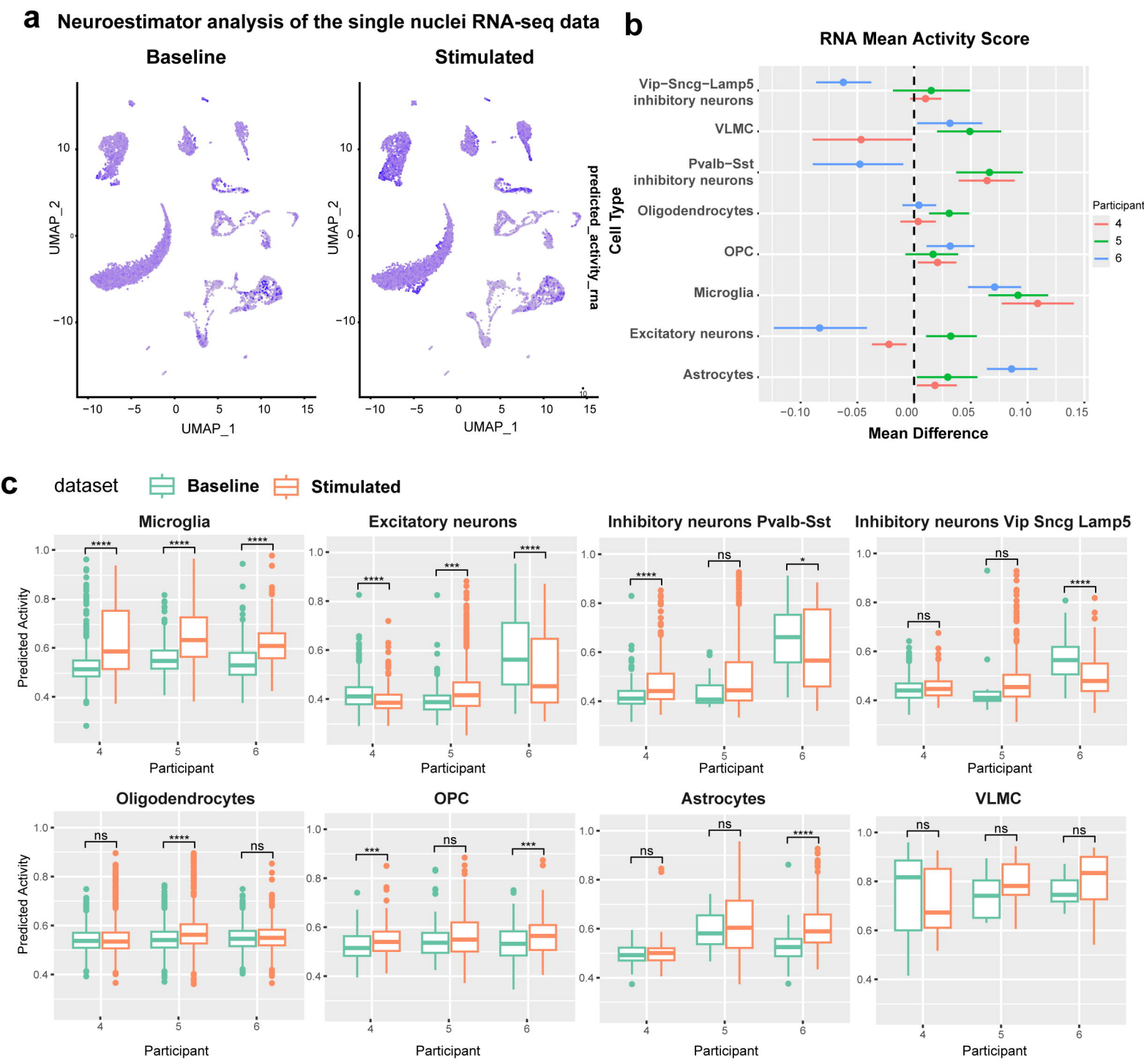
a
Neuroestimator analysis of the single nuclei RNA-seq data
Baseline
Stimulated
UMAP_2
UMAP_1
predicted_activity_rna
b
RNA Mean Activity Score
Cell Type
Vip−Sncg−Lamp5 inhibitory neurons
VLMC
Pvalb−Sst inhibitory neurons
Oligodendrocytes
OPC
Microglia
Excitatory neurons
Astrocytes
Mean Difference
Participant
4
5
6
c
dataset
Baseline
Stimulated
Microglia
Excitatory neurons
Inhibitory neurons Pvalb-Sst
Inhibitory neurons Vip Sncg Lamp5
Oligodendrocytes
OPC
Astrocytes
VLMC
Predicted Activity
Participant

Figure 4

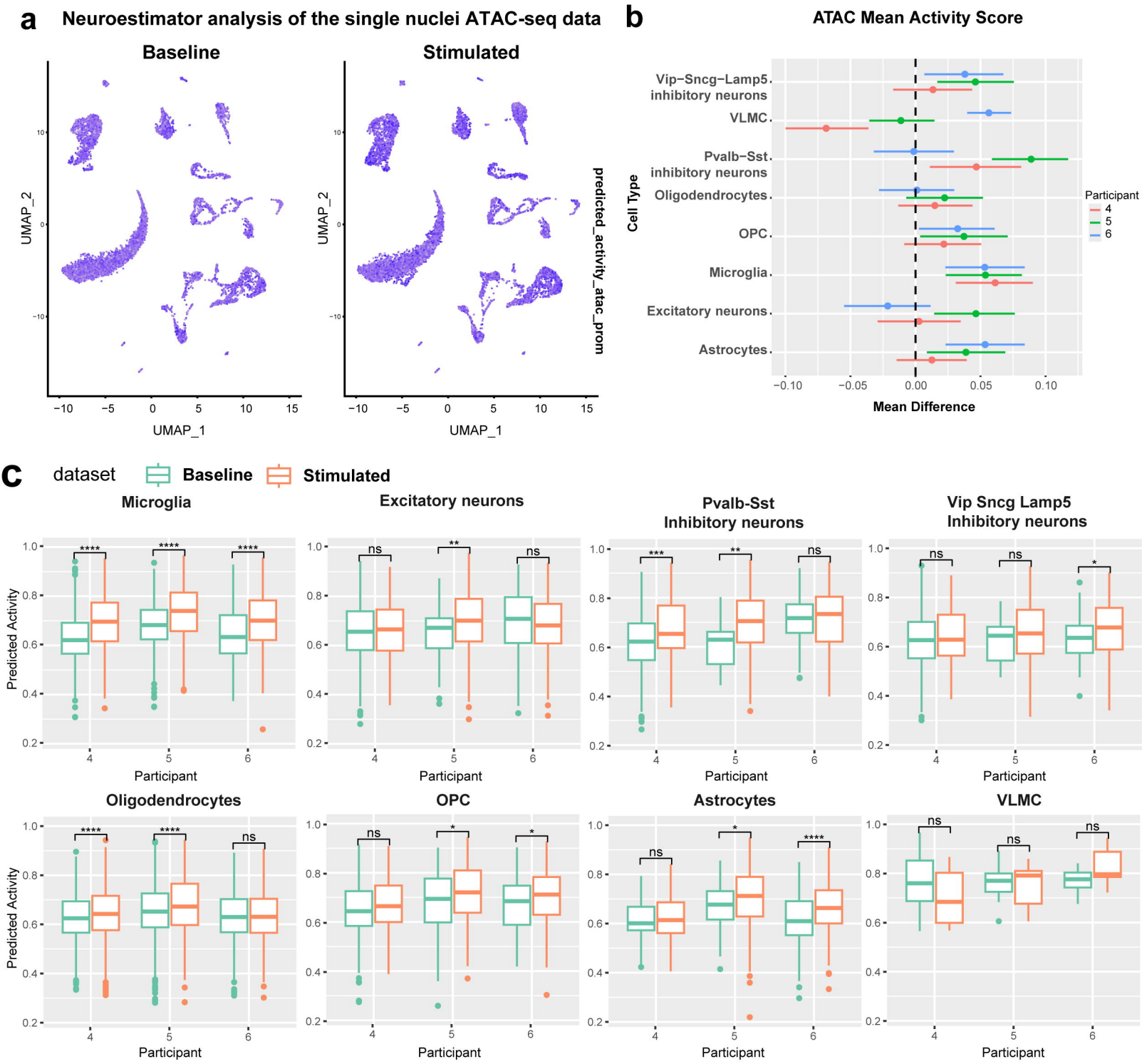
a
Neuroestimator analysis of the single nuclei ATAC-seq data
Baseline
Stimulated
UMAP_2
UMAP_1
predicted_activity_atac_prom
b
ATAC Mean Activity Score
Vip−Sncg−Lamp5 inhibitory neurons
VLMC
Pvalb−Sst inhibitory neurons
Oligodendrocytes
OPC
Microglia
Excitatory neurons
Astrocytes
Cell Type
Mean Difference
Participant
4
5
6
c
dataset
Baseline
Stimulated
Microglia
Excitatory neurons
Pvalb-Sst Inhibitory neurons
Vip Sncg Lamp5 Inhibitory neurons
Oligodendrocytes
OPC
Astrocytes
VLMC
Predicted Activity
Participant

Figure 5