Gene-expression correlates of the oscillatory signatures supporting human episodic memory encoding

Stefano Berto1, Miles R. Fontenot1, Sarah Seger2, Fatma Ayhan1, Emre Caglayan3, Ashwinikumar Kulkarni1, Connor Douglas1, Carol A. Tamminga3, Bradley C. Lega2✉ and Genevieve Konopka1✉

In humans, brain oscillations support critical features of memory formation. However, understanding the molecular mechanisms underlying this activity remains a major challenge. Here, we measured memory-sensitive oscillations using intracranial electroencephalography recordings from the temporal cortex of patients performing an episodic memory task. When these patients subsequently underwent resection, we employed transcriptomics on the temporal cortex to link gene expression with brain oscillations and identified genes correlated with oscillatory signatures of memory formation across six frequency bands. A co-expression analysis isolated oscillatory signature-specific modules associated with neuropsychiatric disorders and ion channel activity, with highly correlated genes exhibiting strong connectivity within these modules. Using single-nucleus transcriptomics, we further revealed that these modules are enriched for specific classes of both excitatory and inhibitory neurons, and immunohistochemistry confirmed expression of highly correlated genes. This unprecedented dataset of patient-specific brain oscillations coupled to genomics unlocks new insights into the genetic mechanisms that support memory encoding.

Genome-wide association studies (GWAS) and gene expression profiling of the human brain have unlocked the ability to investigate the genetic basis of complex brain phenomena. These datasets have principally been applied to noninvasive imaging studies, especially correlations with structural magnetic resonance imaging (MRI) or resting-state functional MRI1–4. Existing methods have relied on published datasets of gene expression from postmortem brains, which means that neurophysiological and behavioral data are not from the same individuals who contributed gene expression data5–7. This limits the potential impact of such approaches to determine how genes support key cognitive processes such as episodic memory and highlights the need to develop new datasets in which individuals contribute both neurophysiological and gene expression data. Another issue affecting previous studies is that neurophysiological measurements such as resting-state functional MRI are not directly linked to cognitive phenomenon. Thus, we previously attempted to correlate gene expression levels with oscillatory signatures of successful memory encoding8, as the fundamental role of these oscillations in supporting memory behavior has been well established in rodents and humans9,10. These oscillatory signatures are measures of the degree to which memory encoding success modulates oscillatory power in a given frequency band. They were quantified using intracranial electrodes implanted for seizure mapping purposes, with recordings made as participants performed an episodic memory task. We used a large database of intracranial electroencephalography (iEEG) recordings obtained over 10 years to piece together a distribution of these oscillatory signatures across brain regions. We identified genes correlated with these oscillatory signatures, including those previously linked to memory formation in rodent investigations, genes linked to neuropsychiatric disorders such as autism spectrum disorder (ASD) and novel genes that are prime targets for further investigation. However, as with other studies, this dataset did not have the benefit of both neurophysiological and gene expression information from the same individuals.

With the goal of explicating links between gene expression and brain oscillations and identifying propitious targets for neuromodulation to treat memory disorders, here, we compiled an unprecedented dataset from 16 human participants who first underwent iEEG during which we measured oscillatory signatures of episodic memory encoding using a well-refined signal-processing pipeline. These participants then underwent a temporal lobectomy, during which an en bloc resection of the lateral temporal lobe permitted the acquisition of high-quality tissue specimens that were processed immediately after removal from a common brain region (Brodmann area 38 (BA38)) from which in vivo recordings had been previously obtained. This approach allowed us to identify genes linked with mnemonic oscillatory signatures by correlating gene expression information with iEEG data obtained from the same individuals. Prioritization was performed using the following different steps: multivariate analyses (MVAs) followed by decomposition by brain oscillation using correlations; gene regulatory network connectivity and cell-type-specific expression and/or epigenomic state; and immunofluorescence staining confirmation. This robust analytical approach to combine human electrophysiological data by iEEG and genomic data from the same participants highlighted genes that might be relevant for mechanisms of episodic memory.

We made the a priori decision to focus on BA38 in this analysis for the following reasons: (1) the region has been shown to exhibit strong memory-related oscillatory signatures in multiple investiga-

1Department of Neuroscience, UT Southwestern Medical Center, Dallas, TX, USA. 2Department of Neurosurgery, UT Southwestern Medical Center, Dallas, TX, USA. 3Department of Psychiatry, UT Southwestern Medical Center, Dallas, TX, USA. ✉e-mail: Bradley.lega@utsouthwestern.edu; Genevieve.Konopka@utsouthwestern.edu
An inevitable feature of our dataset is that the participants suffered from intractable epilepsy, which presents an important caveat to the interpretations of the results. However, recent experiments have shown that blood-oxygenation-level-dependent patterns elicited during successful encoding in patients with epilepsy participating in cognitive studies do not show significant differences compared to healthy controls\(^{15}\). Moreover, since we examined gene–oscillatory signature correlations across these individuals rather than in comparison to an alternative cohort of data, we could institute standard methodologies to partially account for this concern. These included strict artifact-rejection routines and the exclusion of data from regions of seizure onset, as well as using matched post-mortem gene expression samples from both unaffected individuals and patients with epilepsy to adjust gene expression levels.

Results

Generation of within-individual memory oscillatory signatures and a gene expression dataset. To determine the relationship between memory-related brain oscillations and gene expression, we analyzed iEEG recorded as participants encoded episodic memories along with gene expression data from the same 16 individuals (Supplementary Table 1). Oscillatory signatures of successful memory encoding (subsequent memory effects (SMEs)) were calculated from recorded iEEG signals by comparing oscillatory patterns during successful versus unsuccessful memory encoding. We use the term “oscillations” to describe oscillatory power extracted in predefined frequency bands, but address issues related to the use of this term in the Discussion. We used the free-recall task, a standard test of episodic memory for which oscillatory patterns have been well described\(^{14}\), and calculated oscillatory signatures utilizing our well-established signal-processing pipeline\(^{15,16}\) (Fig. 1 and Methods). On average, participants remembered 24.3% of memory items, with a rate of list intrusion (erroneous recollection) of 5.4%. These characteristics are consistent with previous publications of the performance of participants undergoing iEEG during this task\(^{11}\). Further behavioral characteristics, including response probability curves by serial position and conditional response probability curves, are shown in Extended Data Fig. 1a,b. These revealed expected patterns for free recall, including primacy and recency effects and temporal contiguity for immediate lags.

SMEs were extracted from electrodes located in the temporal pole by first normalizing the iEEG signal following wavelet decomposition and statistically comparing oscillatory values between successful versus unsuccessful encoding events across 56 log-spaced frequencies from 2 to 120 Hz. This was done using a permutation procedure, whereby trial labels are shuffled 1,000 times within each electrode recording. We made the a priori decision to average oscillatory data for each individual across all electrodes localized to the anterior temporal pole (BA38) by expert neuroradiology review, as this seemed the most generalizable approach. Extended Data Fig. 1c shows that the variance of SME values across participants for all bands is greater than the variance within participants, which supports the validity of this approach. Data were averaged over a mean of 3.6 electrodes per participant. The resulting oscillatory signatures were averaged into six predefined frequency bands before entering these data into our model to estimate gene correlation values (Fig. 1d,f). The proportion of electrodes exhibiting significant differences in oscillatory power between successful and unsuccessful encoding demonstrated that significant memory-related oscillatory patterns were present (Extended Data Fig. 1d). Significant effects at the individual level are also shown (Fig. 1d), and these results were consistent with previous work\(^{17}\) related to memory patterns in the anterior temporal lobe. In addition, the correlation between observed SME values revealed an expected relationship between low- and high-frequency SMEs (Extended Data Fig. 1e). We note that observed differences may be due to functional changes in narrowband oscillations or broadband power shifts (or a mix of the two). Extended Data Fig. 1f,g shows the results of an oscillation detection analysis, which indicated that narrowband oscillations were present in our data, and we comment on this issue in the Discussion. These 16 study participants then underwent a temporal lobectomy operation. This surgery was performed by a single surgeon (B.C.L.) using a technique that was standardized across these participants for obtaining tissue from BA38 (Fig. 1e). None of the individuals included in this study had gross or radiographic lesions, such as temporal sclerosis or cortical dysplasia. Participants with seizure onset in the temporal pole were not included in our data.

We generated whole-transcriptome RNA-sequencing (RNA-seq) data from the 16 BA38 samples. In addition to the 16 individuals with matched oscillatory signature measurements and gene expression data, we generated BA38 RNA-seq data from an additional 11 temporal lobectomies from individuals for whom we did not obtain oscillation measurements, and postmortem tissue from 12 healthy individuals and 8 patients with epilepsy to validate our predictions using permutations/bootstrap (Fig. 1f and Methods). Principal component analysis revealed that gene expression was uniform across samples, with no outliers (Extended Data Fig. 1h–m). Variance explained by technical, biological and sequencing covariates was analyzed and removed before further analyses (Extended Data Fig. 1n). These adjusted gene expression values were used to calculate gene–oscillatory signature correlations across individuals for each frequency band and co-expression networks.

Memory oscillatory signatures are correlated with gene expression. To determine the relationship between memory oscillatory signatures and gene expression, we performed a MVA followed by decomposition by brain oscillation using a Spearman’s rank correlation that included the aforementioned permutations/bootstraps (Methods). Correlations between gene expression and brain oscillations were performed across participants, with each participant contributing a single gene-expression value and a single SME value per frequency band. The MVA detected a total of 753 genes with false-discovery rate (FDR)-corrected P values of <0.05 (Fig. 2a) for SME–gene expression correlations. The F-statistics for the significant genes we identified were robust and greater than for nonsignificant genes (Extended Data Fig. 2a). We next decomposed the MVA by a correlative analysis to identify genes whose expression correlated with memory-related oscillatory signatures in each of the six frequency bands (“SME genes”; multivariate, FDR <0.05; Spearman’s rank correlation ρ and permutations P <0.05). Of the 753 genes detected by MVA, 300 genes were linked with memory effects in specific frequency bands, with a high proportion associated with 2–4 Hz delta band oscillations (Fig. 2a and Supplementary Table 2). The majority of the identified genes were specific to one frequency band, with primarily only a small number of genes shared by delta and one other frequency band (Fig. 2b). Spearman’s ρ values were robust and greater than for random expectation (Extended Data Fig. 2b,c). These results further confirmed the significance of the identified genes.

Data from these 16 individuals also included a control behavioral paradigm in which individuals performed simple mathematical problems, which allowed us to observe oscillatory signatures linked to this separate cognitive domain (Methods). We performed the same analysis as above to test whether gene–oscillatory signature associations were specific for mnemonic processing. Our
dataset also included cortical thickness estimates for BA38 for each individual, which were extracted from our FreeSurfer processing routine, allowing us to perform an additional control analysis looking for genes correlated with this measurement. We did not observe an overlap with these alternative data, and all the genes highlighted below using co-expression network analysis were memory-specific (that is, gene–oscillation correlations were specific for memory-related oscillatory effects). Finally, we looked for gene correlations with memory performance (that is, behavioral data without regard to any oscillatory signature observations). Only one gene associated with oscillatory signatures overlapped with those identified in these control analyses, thereby reinforcing the unique memory-relevant information obtained by examining gene–oscillatory signature correlations (Fig. 2c).

Networks refine molecular pathways associated with memory. We sought to understand the functional properties of the genes identified as correlated with oscillatory signatures of successful memory encoding. We performed consensus weighted gene co-expression network analysis (WGCNA; Methods, Extended Data Fig. 3a and Supplementary Table 3) using gene expression from resected tissue together with the postmortem gene expression datasets. We placed the memory genes into a systems-level context to identify co-expression networks (for example, modules of highly correlated genes) linked with brain oscillations to further prioritize genes. We required that identified modules were robust across these multiple expression datasets (Methods), and this identified a total of 26 modules. Of these, six were significantly associated with oscillatory-signature-correlated genes (Fig. 3a and Extended Data Fig. 3b).

Two modules were significantly associated with delta oscillatory signatures, one module with both delta and low-gamma oscillations, and three modules were significantly associated with beta oscillatory signatures (Fig. 3a). Notably, we did not detect module

---

**Fig. 1 | Within-individual study design and quality control.** a, Representation of the position of each electrode in BA38 for the 16 participants. b, Schematic of iEEG memory testing. Intracranial electrodes were used to record oscillations as participants performed an episodic memory task. SMEs were calculated by contrasting brain activity recorded as individuals either remembered (green) or forgot (red) each item. c, Example SME recorded from BA38 (full time frequency representation, color axis represents the z-transformed P value for successful/unsuccessful contrast). d, Individual-level SME values in our data. A solidus indicates a significant SME (P < 0.05; two-sided Student’s t-test with permutation procedure) at the individual level. Warm colors indicate power increases during successful encoding. The gray bar plot indicates the total number of electrodes localized to BA38 for each participant. The red bar plot indicates the recall fraction for each individual, and the green bar plot indicates the measured cortical thickness in mm as determined using the FreeSurfer volume-extraction routine. e, Postoperative MRI and computed tomography overlay image after implantation of intracranial electrodes; image was used for localization. f, Human BA38 RNA-seq data from resected tissue were integrated with brain oscillation data derived from SME analysis to identify protein-coding genes whose gene expression support SMEs (SME genes). Permutations/bootstraps with additional human BA38 samples from independent sources were performed. SME genes were prioritized using co-expression networks and specified at the cell-type level using snRNA-seq and snATAC-seq data from BA38.
**Fig. 2** | Genes associated with SMEs are distinct. **a**, Heatmap showing the F-statistics for the initial SME genes (753 genes; FDR < 0.05). We next used correlation analysis to decompose the genes by brain oscillations (Spearman’s rank correlation, ρ < 0.05, permutations/bootstraps P < 0.05). We identified a total of 300 genes using this iterative filtering process that represents brain oscillation–gene expression correlations by wavelength. **b**, Upset plot indicating shared and specific SME genes between brain oscillations. Most genes are uniquely correlated to a specific wavelength. **c**, Venn diagram showing shared and specific genes between genes associated with memory effect (SME), genes associated with the math task (Math), genes associated with cortical thickness (Thickness) and genes associated with recalled words (Memory performance). Only one gene overlaps between SME and memory performance.

associations for genes correlated with cortical thickness or recall fraction (behavioral measurement), whereas genes correlated with oscillations during the mathematical tasks were associated with two independent modules, which provides further confirmation that genes associated with memory encoding and their networks are distinct (Fig. 3b). In addition, in three of these modules (WM4, WM12 and WM21), we identified significant enrichment for the SME genes (Extended Data Fig. 3c). Moreover, SME genes within these three modules showed higher connectivity compared with other genes, which suggests that oscillatory-signature-associated genes have a central role in the transcriptome of BA38 (Extended Data Fig. 3d). We also observed convergence of genes and modules associated with oscillatory-signature-correlated genes from our previous work4, which examined gene-oscillatory signature associations across cortical regions (Fig. 3c). The convergence of these findings using different patient populations and methods gives confidence to our inferences regarding the link between these genes and mnemonic processes. The two modules positively associated with delta oscillatory signatures (WM4 and WM12) were enriched for genes implicated in ion channel activity (Fig. 3d). Notably, WM4 contained previously identified genes correlated with memory oscillatory signatures (Fisher’s exact test, FDR-corrected P = 0.003, odds ratio (OR) = 4.4), whereas WM12 is enriched for a previously identified synaptic-related module correlated with memory oscillatory signatures (Fisher’s exact test, FDR-corrected P = 1.0 × 10−40, OR = 4.1) (Fig. 3c).

Because ‘hub genes’, genes with high intramodular connectivity, have been previously shown to drive module and network structure13, we examined the correlated modules for any hub genes that might have previously been linked to memory or cognition. SHANK2, one of the WM12 hub genes, encodes a synaptic scaffolding protein. Mutations in SHANK2 have been linked with ASD, intellectual disability and schizophrenia14,15. Moreover, SHANK2 has been associated with learning and memory deficits15, thereby further confirming the pivotal role of this WM12 hub gene in memory encoding. Importantly, modules associated with different oscillatory frequency bands exhibited different functional properties. In contrast to the delta-associated modules, modules linked with beta oscillatory signatures (WM11 and WM22) were significantly associated with alternative splicing and chromatin remodeling (Fig. 3d). In accordance with previous results4, we observed that both modules were enriched for genes in SME15, a module linked to beta oscillatory signatures containing genes implicated in splicing (Fisher’s exact test, FDR-corrected P = 2.2 × 10−40, OR = 3.9 (WM11) and P = 2.1 × 10−40, OR = 3.8 (WM22)) (Fig. 3c). These data may support alternative splicing regulation as a mechanism for variation in oscillatory signatures observed across individuals.

**Modules of memory oscillatory signatures are linked with neuropsychiatric disorders.** We next investigated the association of SME modules with genomic data from brain disorders. Using comprehensive transcriptomic and genetic data from multiple disorders (Methods), we assessed enrichment for genes dysregulated in neuropsychiatric disorders and GWAS enrichment using linkage disequilibrium (LD) score regression. The WM4 module associated with delta oscillatory signatures was significantly enriched for downregulated genes in ASD (Fisher’s exact test, FDR-corrected P = 4.3 × 10−4, OR = 2.95) and variants associated with ASD (Fisher’s exact test, FDR-corrected P = 0.001) (Fig. 4a,b and Supplementary Table 4). WM12 showed enrichment for GWAS associated with attention-deficit/hyperactivity disorder (ADHD; FDR = 0.001), bipolar disorder (BD; FDR = 0.003), major depressive disorder (MDD; FDR = 0.006), schizophrenia (SCZ_2018; FDR = 5.8 × 10−4) and variants associated with educational attainment (FDR = 0.03) and intelligence (FDR = 0.002) (Fig. 4b and Supplementary Table 4). Most importantly, we did not detect any significant enrichment for epilepsy-associated loci in the memory-related modules (Fig. 4b), and the enrichment for variants associated with non-brain-related...
traits and disorders was minimal (Extended Data Fig. 4a). We also found an enrichment for WM4 (FDR = 8.9 × 10^{-6}, OR = 3.3) and WM12 (ASD: FDR = 3.9 × 10^{-6}, OR = 3.4; ASD (scored 1–3): FDR = 3.2 × 10^{-4}, OR = 4.5) in ASD-associated genes from the SFARI Gene database (Fig. 4c).

We next compared the correlated modules with those found in a meta-analysis of transcriptomic data across neuropsychiatric disorders^22. Both WM4 and WM12 are enriched for a module severely affected in ASD, with RBFOX1 as a predominant hub (geneM1; FDR = 1.5 × 10^{-9}, OR = 7.9 (WM4) and FDR = 2.0 × 10^{-9}, OR = 3.92 (WM12)) (Extended Data Fig. 4b). Interestingly, RBFOX1 is also a hub in WM12 (Fig. 3d), which provides further support for the role of this gene in neuropsychiatric disorders and memory. The beta module WM21 was enriched for schizophrenia variants (SCZ_2018; FDR = 0.03) (Fig. 4a,b and Supplementary Table 4), whereas the beta module WM22 was enriched for a splicing module affected in schizophrenia (geneM19; FDR = 2.6 × 10^{-9}, OR = 6.6) (Extended Data Fig. 4b). Overall, the association of delta and beta oscillatory-signature-correlated modules with neuropsychiatric disorders for which memory is impaired provide further support for the role of these genes and pathways in episodic memory.

**Modules of memory oscillatory signatures are associated with specific cell types.** To develop cell-type-specific associations for the identified correlated genes, we performed single-nucleus RNA-seq (snRNA-seq) analysis on tissue from six participants, four of whom contributed oscillatory data (Supplementary Table 1). We sequenced the transcriptomes of 17,632 nuclei (Extended Data Fig. 5a), detecting an overall median of 11,498 unique molecular identifiers (UMIs) and 4,069 genes (Extended Data Fig. 5b,c). We accounted...
for technical and biological covariates before dimensionality reduction (Methods). We initially identified 24 clusters. We next used a publicly available snRNA-seq dataset from middle temporal gyri to further define our initial clusters by both cell-type and layer specificity (Methods and Supplementary Table 5). After the comparison based on marker enrichment (Methods), we focused on a robust set of 20 transcriptionally defined clusters (Fig. 5a). The proportion of cells were similarly distributed by participant in all clusters (Extended Data Fig. 5d,e). In total, we defined nine inhibitory neurons, eight excitatory neuron and three major non-neuronal clusters (Extended Data Fig. 5f,g). These clusters showed high expression of known major markers for their respective cell types (Fig. 5b and Supplementary Table 5).

We found that the delta-correlated modules WM4 and WM12 were strongly enriched for excitatory and inhibitory neurons (Fig. 5c). Specifically, WM4 and WM12 were highly enriched for combinations of ROBR THEMIS FEZF2 deep-layer excitatory neurons. These deep-layer neurons have been associated with memory encoding circuitry receiving GABAergic inputs from the hippocampus. In addition, delta rhythmicity might arise from deeper layer intrinsic bursting neurons that project to other subcortical regions. Therefore, these results further underscore the importance of these deep-layer excitatory neurons in episodic memory encoding. Moreover, both modules showed enrichments for combinations of SST VIP PVALB inhibitory neurons. Interestingly, fast-spiking parvalbumin (PVALB)-containing basket cells decisively control excitatory output, and they are required for memory consolidation regulating neocortical-hippocampal circuitry. Meanwhile, somatostatin (SST)-expressing neurons target distal dendrites of pyramidal cells, and they play a role in memory circuitry and cortical oscillatory synchronization. While SST PVALB interneurons specifically inhibit pyramidal neurons, VIP neurons both inhibit and disinhibit pyramidal neurons and might be implicated in working memory circuitry.

In addition, the module negatively associated with delta oscillatory signatures, WM21, was enriched for glia cells, with a predominance of oligodendrocyte-related genes (Fig. 5c), which provides support for a possible role for oligodendrocytes in memory circuits and neuronal synchrony as previously reported elsewhere. Moreover, using snRNA-seq data from brain tissue of patients with ASD or Alzheimer disease (Methods), we found that WM4 is significantly enriched for genes dysregulated in layer 2–4 excitatory neurons and SST inhibitory neurons in ASD, whereas WM21 is significantly enriched for oligodendrocyte markers affected in Alzheimer disease (Extended Data Fig. 5i). These results confirm the role of the modules associated with delta oscillatory signatures as linked to cognitive disorders at the cell-type level.

WM4 and WM12 are both enriched for delta-oscillatory-sigature-correlated genes, cognitive-disease-related variants and multiple neuronal types. To validate our approach for the purpose of identifying targets for the future development of neuromodulation strategies specific to brain disorders and cell types, we selected one hub gene from one of the delta modules. ILIRAPL2, which encodes an interleukin-1 (IL-1) receptor accessory protein, is a
hub gene in the WM4 module. Intriguingly, along with its paralog IL1RAPL1, IL1RAPL2 promotes functional excitatory synapse and dendritic spine formation\(^1\) and is associated with ASD\(^2\). Our snRNA-seq data showed that IL1RAPL2 has the greatest expression in RORB\(^+\) deep-layer excitatory neurons, but it is also expressed in SST\(^+\)LAMPS\(^+\) upper layer inhibitory neurons (Fig. 5d). Fluorescence immunohistochemistry (IHC) analysis of independently obtained tissue sections showed that IL1RAPL2 has the greatest overlapping expression with a marker of excitatory neurons (CAMKII\(^+\)), some overlap with a marker of inhibitory neurons (GAD67\(^+\)) and no overlap with a marker of astrocytes (GFAP\(^+\)) or a marker of oligodendrocytes (OLIG2\(^+\)) (Fig. 5e,f). Along with its role in excitatory synapse formation, the snRNA-seq and memory oscillatory signature association indicated that IL1RAPL2 might play an essential role in regulation of memory encoding in humans. Together, these results underscore the importance of further studies focused on the role of IL1RAPL2 in memory and excitatory–inhibitory synaptic etiologies.

**Discussion**

We set out to understand the genomic underpinnings of oscillatory patterns that support episodic memory encoding in humans, with the goal of identifying genes that are proximate targets for neuromodulation strategies to treat memory disorders. Using an unparalleled dataset from 16 human participants that included measurements of brain oscillations linked to successful episodic memory encoding and transcriptomic data from the temporal pole in the same individuals, we identified modules of genes that link specific cell types and cellular functions with memory-related oscillatory signatures.

Our analysis is fundamentally different from previous attempts to correlate gene expression with behavioral measurements such as memory performance\(^3\)–\(^5\). Oscillatory correlates of successful memory encoding represent an ‘intermediate step’ between gene regulation and memory behavior. Oscillations are localized to the brain region in which they are recorded using intracranial-depth electrodes and are dissociable into frequency bands with distinct properties. Linking neurophysiological measurements (such as these oscillatory signatures) with gene expression data will establish specific testable hypotheses in subsequent investigations for these identified genes. The hub genes described in Fig. 5d may represent the most proximate targets for subsequent testing using animal models or other approaches.

Our work sheds light on the molecular mechanisms that give rise to oscillatory correlates of successful memory encoding\(^6\). Our observation that delta oscillatory signatures are linked to ion channel genes and that these genes tend to be expressed in oligodendrocytes leads to the fascinating implication that the generation of low-frequency oscillatory patterns linked to mnemonic processing in humans is at least partially dependent on glial modulation of oscillations. This is based on our observations across all participants and on the single-nucleus expression analysis. This conclusion is supported by the role of oligodendrocytes in learning and memory acting on depolarization of membrane potential\(^7\)–\(^9\), which accelerates axonal conduction and ion channel activity as reflected by the delta-associated modules with positive association (WM4 and WM12).

---

**Fig. 5 | SME-specific modules are enriched for excitatory and inhibitory neurons. a, UMAP representation of the 20 classes of cell types using the BA38 snRNA-seq data. Each dot represents a nucleus. Excitatory neurons (Exc) are highlighted in a dark blue gradient, the inhibitory neurons (Inh) in a red gradient and the non-neuronal cells (Astro, Olig and OPC) in a light blue gradient. Cell types were annotated using a publicly available single-cell dataset. A Fisher’s exact enrichment test between cell markers of the two datasets was performed. Major cell types tend to cluster near one another. b, Violin plots representing gene markers for the major cell types detected. The y axis represents the log-normalized expression (log(norm exp)) of each marker gene in each cluster. The markers for excitatory neurons (for example, CUX and RORB) are highlighted in blue. The markers for inhibitory neurons (for example, GAD1 and RELN) are highlighted in red. The markers for non-neuronal cells (for example, FGFR3, MOBP and VCAN) are highlighted in green. c, Bubble chart showing the enrichment of the SME modules for cell-type markers defined using Seurat. The color gradient represents the log (FDR) and the bubble size represents the OR from a Fisher’s exact enrichment test of genes in modules from this study with genes expressed in specific cell types defined by our snRNA-seq data. d, Violin plot representing the log-normalized expression level of IL1RAPL2. The adjacent dot plot represents the average expression (gradient) and percentage of cells (size) expressing IL1RAPL2. The order of cell types follows the six modules significantly associated with memory-related brain oscillations. d, Violin plot representing the log-normalized expression level of IL1RAPL2. The adjacent dot plot represents the average expression (gradient) and percentage of cells (size) expressing IL1RAPL2. The order of cell types follows the labels of c. e, f, IHC of independent human temporal lobe specimens demonstrates the specific expression of IL1RAPL2 in excitatory (CAMKII\(^+\)) and inhibitory neurons (GAD67\(^+\)) in BA38 (e), but not in oligodendrocytes (OLIG2\(^+\)) or astrocytes (GFAP\(^+\)) (f).
Moreover, genes expressed in these positively associated modules were overrepresented in deep layers of excitatory neurons implicated in memory-encoding circuitry and delta-rhythmity formation\(^{41-43}\) and in \(\text{SST}^\dagger\text{VIP}^\dagger\text{PVALB}^\dagger\)-expressing interneurons important for mediating cortical–hippocampal communication during memory encoding\(^44\). These results further support the role of the identified genes in memory encoding and specifically highlight cell types that might be implicated in episodic memory.

We observed interesting properties for genes correlated with delta oscillations, but not theta oscillations, which runs contrary to rodent data that universally implicate theta frequency activity in successful memory formation. However, in the human temporal lobe, oscillations outside the 4–9 Hz range routinely exhibit memory-relevant properties, including cross-frequency coupling; thus, our findings are in line with previous observations using oscillatory signatures of successful memory encoding in humans\(^45\). In humans, these low-frequency oscillations represent a consistent feature of oscillatory signatures of memory formation, including influence on the timing of single unit activity\(^45-47\). The significant representation of genes correlated with delta oscillatory signatures...
Articles

expression values. Finally, several of the key genes we identified (for example, IL1RAPL2 and SMAD3) have been independently shown to be linked to memory processing in data from non-epileptic individuals and genetically modified rodent models\(^1\). Even though these correlative analyses do not imply causality, these genes have been highlighted by stringent correlative statistics, by high connectivity in the modules associated with memory oscillations and by cell-type expression specificity. Using this analytical approach, we defined IL1RAPL2 and SMAD3 as genomic markers for episodic memory for further investigation at the molecular level in model systems.

An important issue one must consider when using participants who underwent neurosurgery to obtain both oscillation and gene expression data relates to timing. Specifically, the use of human participants simply does not allow collection of tissue specimens immediately after behavior-related oscillations are observed. Brain oscillations are dynamic, occurring during specific behavior, but gene expression snapshots are taken later in time, when the participants underwent temporal lobectomy. In practical terms, this means that genes we identify as being linked to oscillatory signatures of successful memory formation necessarily must persist in their...
expression at least over a period of weeks, and that our study can- not identify genes whose expression is differentially induced (across participants) due to mnemonic stimuli over shorter time scales. We also note that while we use the term “oscillations” to describe power extracted in six predefined frequency bands, we acknowl- edge that the measurement of SMEs may reflect power differences that arise due to differences in both narrowband oscillations and broadband power shifts. We include examples of narrowband oscil- lations detected in our data using the multiple oscillation detection algorithm (MODAL; Methods). Future investigations may establish whether gene-expression correlation patterns are additionally cor- related with such broadband power shifts during encoding11, incor- porating slope shifts or a quantification of episodes in which bursts of oscillations occur. Broadly stated, this area remains an active area of investigation in human electrophysiology.

Collectively, this translational work establishes an experimental and analytical approach for deconstructing human behavioral and cognitive traits, such as memory, using integrative physiological and multi-omics techniques. Integration of single-nucleus tran- scriptomic and epigenomic data allowed us to identify the cell-type specificity of the memory-related gene co-expression modules and potential regulators of these modules. This molecular characteriza- tion of human memory highlights key genes that can be further studied in model systems. We anticipate that this within-individual approach can be used in future studies to highlight molecular pathways of other human complex traits with the goal of identi- fying therapeutic targets and linking clinical and genomic data at the individual level. Importantly, investigations using animal and in vitro models will be necessary to definitively characterize the memory-related properties of the genes identified in our analysis.

Online content
Any methods, additional references, Nature Research report- ing summaries, source data, extended data, supplementary infor- mation, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/ s41593-021-00803-x.

Received: 25 November 2019; Accepted: 19 January 2021; Published online: 8 March 2021

References
1. Morgan, S. E. et al. Cortical patterning of abnormal morphometric similarity in psychosis is associated with brain expression of schizophrenia-related genes. Proc. Natl Acad. Sci. USA 116, 9061–9069 (2019).
2. Lombardo, M. V. et al. Large-scale associations between the leukocyte transcriptome and BOLD responses to speech differ in autistic early language outcome subtypes. Nat. Neurosci. 21, 1680–1688 (2018).
3. Romero-García, R., Warrier, V., Fullmore, E. T., Baron-Cohen, S. & Bethlehem, R. A. I. Non-specific and transcriptionally downregulated genes are associated with cortical thickness differences in autism. Mol. Psychiatry 24, 1053–1064 (2019).
4. Wang, G. Z. et al. Correspondence between resting-state activity and brain gene expression. Neuron 88, 659–666 (2015).
5. Patania, A. et al. Topological gene expression networks recapitulate brain anatomy and function. Natw. Neurosci. 3, 744–762 (2019).
6. Le, B. D. & Stein, I. L. Mapping causal pathways from genetics to memory-related properties of the genes identified in our analysis.
7. Nakamura, K. & Kubota, K. The primate temporal pole: its putative role in object recognition and memory. Behav. Brain Res. 77, 53–77 (1996).
8. Hill, P. F., King, D. R., Lega, B. C. & Rugg, M. D. Comparison of fMRI correlates of successful episodic memory encoding in temporal lobe epilepsy patients and healthy controls. NeuroImage 207, 116397 (2020).
9. Sederberg, P. B., Howard, M. W. & Kahana, M. J. A context-based theory of recency and contiguity in free recall. Psychol. Rev. 115, 893–912 (2008).
10. Arora, A. et al. Comparison of logistic regression, support vector machines, and deep learning classifiers for predicting memory encoding success using human intracranial EEG recordings. J. Neural Eng. 15, 066028 (2018).
11. Lin, J. J. et al. Theta band power increases in the posterior hippocampus predict successful episodic memory encoding in humans. Hippocampus 27, 1040–1053 (2017).
12. Sederberg, P. B., Kahana, M. J., Howard, M. W., Donner, E. J. & Madsen, J. R. Theta and gamma oscillations during encoding predict subsequent recall. J. Neurosci. 23, 10809–10814 (2003).
13. Langfelder, P. & Horvath, S. WGCNA: An R package for weighted correlation network analysis. BMC Bioinformatics 9, 559 (2008).
14. Berkel, S. et al. Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. Nat. Genet. 42, 489–491 (2010).
15. Poykov, S. et al. Rare SHANK2 variants in schizophrenia. Mol. Psychiatry 20, 1487–1488 (2015).
16.Won, H. et al. Autistic-like social behaviour in Shank2 mutant mice improved by restoring NMDA receptor function. Nature 486, 261–265 (2012).
17. Zhang, Y. et al. Transcriptome-wide isoform-level dysregulation in ASD, schizophrenia, and bipolar disorder. Science 362, eaat1278 (2017).
18. Dembrow, N. C., Zemelman, B. V. & Johnston, D. Temporal dynamics of L5 dendrites in medial prefrontal cortex regulate integration versus coincidence detection of afferent inputs. J. Neurosci. 35, 4501–4514 (2015).
19. Silva, L. R., Armitai, Y. & Connors, B. W. Intracellular oscillations of neocortex generated by layer 5 pyramidal neurons. Science 251, 432–435 (1991).
20. Kim, E. J., Juvasvitt, A. L., Kyubwa, E. M., Jacobs, M. W. & Callaway, E. M. Three types of cortical layer 5 neurons that differ in brain-wide connectivity and function. Neuron 88, 1253–1267 (2015).
21. Xia, F. et al. Parvalbumin-positive interneurons mediate neocortical-hippocampal interactions that are necessary for memory consolidation. eLife https://doi.org/10.7554/eLife.27865 (2017).
22. Naka, A. et al. Complementary networks of cortical somatostatin interneurons enhance layer specific control. eLife https://doi.org/10.7554/eLife.43696 (2019).
23. Veit, J., Hakim, R., Jadi, M. P., Sejnowski, T. J. & Adesnik, H. Cortical gamma band synchronization through somatostatin interneurons. Nat. Neurosci. 20, 951–959 (2017).
24. Pi, H. J. et al. Cortical interneurons that specialize in disinhibitory control. Nature 503, 521–524 (2013).
25. Lee, S., Kruglikov, I., Huang, Z. J., Fishell, G. & Rudy, B. A disinhibitory circuit mediates motor integration in the somatosensory cortex. Nat. Neurosci. 16, 1662–1670 (2013).
26. Kamigaki, T. & Dan, Y. Delay activity of specific prefrontal interneuron subtypes modulates memory-guided behavior. Nat. Neurosci. 20, 854–863 (2017).
27. Pepper, R. E., Pitman, K. A., Cullen, C. L. & Young, K. M. How do cells of the oligodendrocyte lineage affect neuronal circuits to influence motor function, memory and mood? Front. Cell. Neurosci. 12, 399 (2018).
28. Um, J. W. & Ko, J. LAR-RPTPs: synaptic adhesion molecules that shape synapse development. Trends Cell Biol. 23, 465–475 (2013).
29. Kantojarvi, K. et al. Fine mapping of Xg11.1-q21.33 and mutation screening of RP96AK6, ZNF711, ACSLA, DLG3, and ILIRAPL2 for autism spectrum disorders (ASD). Autism Res. 4, 228–233 (2011).
30. Tasic, B. et al. Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. Nat. Neurosci. 19, 335–346 (2016).
31. Azevedo, F. A. et al. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled up primate brain. J. Comp. Neurol. 513, 532–541 (2009).
32. Zhang, Y. et al. Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. Proc. Natl Acad. Sci. USA 104, 20552–20557 (2007).
33. Papasavvopoulos, A. et al. Common Kir6.1 alleles are associated with human memory performance. Science 314, 475–478 (2006).
34. Jacobs, J. & Kahana, M. J. Direct brain recordings fuel advances in cognitive electrophysiology. Trends Cogn. Sci. 14, 162–171 (2010).
35. Yamazaki, Y. et al. Oligodendroglycocytes: facilitating axonal conduction by more myelination. Neuroscientist 16, 11–18 (2010).
36. Baker, A. et al. Specialized subpopulations of deep-layer pyramidal neurons in the neocortex: bridging cellular properties to functional outcomes. J. Neurosci. 33, 5441–5455 (2018).
37. Carracedo, L. M. et al. A neocortical delta rhythm facilitates reciprocal interlaminar interactions via nested theta rhythms. J. Neurosci. 33, 10750–10761 (2013).
43. Jinno, S. et al. Neuronal diversity in GABAergic long-range projections from the hippocampus. *J. Neurosci.* 27, 8790–8804 (2007).
44. Kim, D. et al. Distinct roles of parvalbumin- and somatostatin-expressing interneurons in working memory. *Neuron* 92, 902–915 (2016).
45. Jacobs, J. Hippocampal theta oscillations are slower in humans than in rodents: implications for models of spatial navigation and memory. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 369, 20130304 (2014).
46. Yaffe, R. B. et al. Reinstatement of distributed cortical oscillations occurs with precise spatiotemporal dynamics during successful memory retrieval. *Proc. Natl Acad. Sci. USA* 111, 18727–18732 (2014).
47. Rutishauser, U., Ross, I. B., Mamelak, A. N. & Schuman, E. M. Human memory strength is predicted by theta-frequency phase-locking of single neurons. *Nature* 464, 903–907 (2010).
48. Duzel, E., Penny, W. D. & Burgess, N. Brain oscillations and memory. *Curr. Opin. Neurobiol.* 20, 143–149 (2010).
49. Kahana, M. J. The cognitive correlates of human brain oscillations. *J. Neurosci.* 26, 1669–1672 (2006).
50. Vaz, A. P., Inati, S. K., Brunel, N. & Zaghoul, K. A. Coupled ripple oscillations between the medial temporal lobe and neocortex retrieve human memory. *Science* 363, 975–978 (2019).
51. Lega, B., Burke, J., Jacobs, J. & Kahana, M. J. Slow-theta-to-gamma phase-amplitude coupling in human hippocampus supports the formation of new episodic memories. *Cereb. Cortex* 26, 268–278 (2016).
52. Muñoz, M. D., Antolín-Vallespin, M., Tapia-González, S. & Sánchez-Capelo, A. Smad3 deficiency inhibits dentate gyrus LTP by enhancing GABA neurotransmission. *J. Neurochem.* 137, 190–199 (2016).
53. Donoghue, T. et al. Parameterizing neural power spectra. *Nat. Neurosci.* 23, 1655–1665 (2020).
54. Herweg, N. A., Solomon, E. A. & Kahana, M. J. Theta oscillations in human memory. *Trends Cogn. Sci.* 24, 208–227 (2020).

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature America, Inc. 2021
Methods

Experimental model and participant details. Participants and memory task. The research protocol was approved by the Institutional Review Board at UT Southwestern, and informed consent was obtained from each participant. Participants contributing gene expression data were retracted from a data set of individuals with epilepsy. All cases of temporal lobe epilepsy were identified from the aseg.stats files (in millimeters) for each participant (one value per participant).

Resected brain samples. All surgical samples included in this study were BA38 resections from patients with temporal lobe epilepsy. The brain specimen was dropped into ice-cold 1X PBS in a 50-ml conical tube immediately after removal from the patient. After four to five inversions, the tissue sample was transferred to a PBS tub with ice-cold 1X PBS for a second wash. The specimen was then moved to a Petri dish and grossly dissected by scalpel into ~12 subsamples and immediately frozen in individual Eppendorf tubes in liquid nitrogen as the tubes were filled. Care was taken to avoid major blood vessels. Gray matter was prioritized over tracts of white matter in an attempt to increase homogeneity and consistency of results across all samples. Time from removal of brain to flash freezing ranged from roughly 2 min for the first piece to about 7 min for the last subsample. Three to four of the subsamples were extracted for RNA, and the subsample with the highest RIN value was selected for RNA-seq. See Supplementary Table 1 for detailed demographic information.

Tissue preparation for sequencing. Postmortem brain samples. Twelve samples of BA38 were obtained from the Dallas Brain Collection. These tissue samples were donated from individuals without a history of neurological or psychiatric disorders, as previously published. Eight samples of BA38 were obtained from the University of Maryland Brain and Tissue Bank. These samples were donated from individuals with epilepsy. See Supplementary Table 1 for detailed demographic information.

RNA-seq. Total RNA was purified using an miNeasy kit (217004, Qiagen) following the manufacturer’s recommendations. RNA-seq libraries from mRNA were prepared in house as previously described. Sequencing was performed on randomly pooled samples by the McDermott Sequencing Core at UT Southwestern on an Illumina NextSeq 500 sequencer. Single-end, 75-base-pair (bp) reads were generated. Data collection and analysis were not performed blind to the conditions of the experiments. No statistical methods were used to determine sample sizes because of the limitation of availability of human brain surgical tissues.

Isolation of nuclei from resected brain tissues (snRNA-seq). Nuclei were isolated as previously described using Chromium Single Cell 3’ v3 (120337, 10X Genomics) according to the manufacturer’s protocol. Libraries were sequenced using an Illumina NovaSeq 6000 at the North Texas Genome Center (UT Arlington).

Isolation of nuclei from resected brain tissue (snATAC-seq). For snATAC-seq, a subsample of 21+ min (95% confidence interval).

FreeSurfer segmentation. FreeSurfer extraction form T1 mprage volume acquisition was used to quantify the cortical thickness in the temporal pole. Volume data for the temporal pole were identified from the aseg.stats files (in millimeters) for each participant (one value per participant).
Finally, nuclei were resuspended in 1× Nuclei Buffer at a concentration of 4,000 nuclei per μl for snATAC-seq. Droplet-based snATAC-seq libraries were prepared using Chromium Single Cell ATAC kit solution v.1.0 (10x Genomics) and followed the Chromium Single Cell ATAC reagent kit user guide: CG00016 Rev B. The library was sequenced using an Illumina NextSeq 500 at the McDermott Sequencing Core at UT Southwestern. 

**Immunofluorescence staining of human tissue.** Fresh surgically resected tissue was fixed in 4% paraformaldehyde in 1× PBS for 24–48 h at 4°C and then cryoprotected in a 30% sucrose solution. The tissue was sectioned at 7 μm using a cryostat (Leica). Sections underwent heat-induced antigen retrieval in citrate buffer (pH6.0) for 10 min at 95°C. Sections were blocked with 2% fetal bovine serum (FBS) in 0.1 M Tris (pH7.6) for 1 h at room temperature. After blocking, the sections were incubated with primary antibodies in 0.1 M Tris pH7.6/2% FBS overnight at 4°C and subsequently incubated with secondary antibodies in 0.1 M Tris pH7.6/2% FBS for 1 h at room temperature. Sections were immersed in 0.25% Sudan Black solution to quench lipofuscin autofluorescence and counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Sections were mounted and cover slippes using ProLong Diamond Antifade mountant (P36970, Thermo Fisher Scientific). The following antibodies and dilutions were used: goat anti-IL1RAPL2 (PA47039, Thermo Fisher Scientific 1:20); rat anti-SMA3D (MAB4308, R&D Systems, 1:100); rabbit anti-CamKII (PA514315, Thermo Fisher Scientific, 1:50); chicken anti-GFAP (ab4674, Abcam, 1:400); mouse anti-GAD67 (MAB5406, Millipore, 1:200); mouse anti-Olig2 (MAB5305, Millipore, 1:200); species-specific secondary antibodies were purchased from Abcam and were used at a 1:800 dilution. Alexa Fluor 488, Alexa Fluor 555, or Alexa Fluor 647 (Thermo Fisher Scientific, 1:800). Images were acquired using a x63 oil objective on a Zeiss LSM 880 confocal microscope. Experiments using secondary antibody only were conducted for each antibody to ensure specificity. The anti-IL1RAPL2 antibody was validated by Thermo Fisher Scientific using flow cytometry of human HepG2 cells. The anti-SMA3D antibody was validated by R&D Systems using flow cytometry in human PC-3 cells and IHC in human pancreatic cancer tissue and MDA-MB-231 cells. The anti-CamKII antibody was validated by Thermo Fisher Scientific using IHC in human brain and western blotting in human 293 cells. The anti-GFAP antibody was validated by Abcam across many species, including human. Protocol validations include IHC and immunofluorescence. A total of 211 references are provided for this antibody at https://www.abcam.com/igf1-antibody-ab67474.html. The anti-GAD67 antibody was validated by Millipore in human brain via IHC. Over 75 references are provided at http://www.emdmillipore.com/US/en/product/Anti-GAD67-Antibody-clone-1G10_2_MM_NF-MAB5406/Anchor_BRO. The anti-Olig2 antibody has been validated by Millipore in human via IHC, and 15 references are provided at http://www.emdmillipore.com/US/en/product/Anti-Olig2-Antibody-clone-211F1_LMM_NF-MAB5405/documentation. Immunofluorescence staining was performed in four different surgically resected tissues (data not shown), and a representative optimized image is shown in Figs. 5e,f, and 6e,f.

**Computational methods.** **Bulk RNA-seq mapping, quality control and expression quantification.** Quality control was performed using FastQC (v.0.11.9). Reads were aligned to the human hg38 reference genome using STAR (v.2.5.2b)

- For each sample, a BAM file including mapped and unmapped reads that spanned splice junctions was produced. Secondary alignment and multi-mapped reads were further removed using in-house scripts. Only uniquely mapped reads were retained for further analyses. Quality control metrics were performed using RsQC (v.2.6.4)

- with the hg38 gene model provided. These steps included the number of reads after multiple-step filtering, ribosomal RNA reads depletion and defining reads mapped to exons, untranslated regions and intronic regions. Picard tool was implemented to refine the quality control metrics (http://broadinstitute.github.io/picard/).

- To calculate sequencing statistics, Gencode annotation for hg38 (v.24) was used for reference alignment annotation and downstream quantification. Gene expression was measured using UMI tools. Residuals were extracted and average gene expression added as follows:

  - Gene expression = residuals + average gene expression

  - Adjusted gene expression = residuals + average gene expression

We applied two residualizations: (1) resected tissues and (2) resected tissues plus frozen tissues. 

The adjusted CPM from the 16 participants were used for SME correlation and quantile regression. The adjusted CPM from resected tissue and frozen tissue were used for the consensus WGCNA analysis and permutations/bootstrap analysis.

MVA. We performed a MVA based on the following model:

Gene expression ~ SME: band + EpDur + RIN + batch + (1/participants)

Due to the limited sample size and because we did not want to over-parameterize the model, we utilized the three fixed covariates that explained the highest variance in the data: EpDur, RIN and batch. Contrasts were used to compare SME association between waves. Genes with FDR < 0.05 were considered to be differentially associated with SME. The analysis was performed using edgeR (v.3.30.2) 35. These results were integrated with the correlative analysis to define the final 300 SME genes. The code used for this analysis is available at GitHub (https://github.com/konopkalab/Within_Subject).

Correlation analysis and permutation analysis. Spearman’s rank correlation was performed between each of the six memory brain oscillations and gene expression. We also utilized this method for six math brain oscillations, thickness and behavioral performances.

For this analysis, we used within-individual bulk RNA-seq from BA38 resected tissue from the 16 participants with calculated SMEs (WrS).

We next performed permutations/bootstrap analysis using the following three datasets:

1. Additional participants: bulk RNA-seq from BA38 resected tissue without SMEs from an additional 11 participants (ArS).
2. Independent data healthy: bulk RNA-seq from BA38 frozen tissue from 12 participants (HfS).
3. Independent data epilepsy: bulk RNA-seq from BA38 frozen tissue from 8 participants with epilepsy (EFS).

Bootstrap was applied by randomly subsampling 16 participants (as WrS) from the composite data and recalculating the correlation 100 times. We then calculated a Monte Carlo P value comparing the observed effect with the simulated effects for each gene as follows:

\[
\sum(\text{abs(simulated } P) > \text{abs(observed } P)) / 100
\]

We calculated two Monte Carlo P values: (1) BootP based on WrS + ArS (only resected tissues) and (2) BootP All, based on WrS + ArS + HfS + EFS (resected tissues and frozen tissues).

We additionally applied a permutation approach, shuffling the gene expression of WrS and recalculating 100 times the correlation between oscillations and gene expression. We then calculated a Monte Carlo P value (PermP). Nominal P value < 0.05, PermP < 0.05, BootP < 0.05 and BootP All < 0.05 were used to filter for significant correlations, as reported in Supplementary Table 2.

**Co-expression network analysis.** To identify modules of co-expressed genes in the RNA-seq data, we carried out WGCNA (v.1.69) 18. We applied a consensus analysis based on WrS + ArS + HfS + EFS data, defining modules highly preserved across multiple datasets. This method was applied to mitigate the potential noise across different types of data. A soft-threshold power was automatically calculated to achieve approximate scale-free topology (\(R^2 = 0.85\)). Networks were constructed with the blockwiseConsensusModules function with biweight midcorrelation (bicor). The modules were then determined using the dynamic tree-cutting algorithm. To ensure the robustness of the observed network, we used a permutation approach, recalculating the networks 200 times and comparing the observed connectivity per gene with the randomized one. None of the randomized networks showed similar connectivity, thereby providing robustness to the network inference. Module sizes were chosen to detect small modules driven by potential noise on the filtered data. A cut-off was used to more aggressively split the data and create more specific modules. Spearman’s rank correlation was used to compute module eigengene–memory oscillatory signature associations.

**snRNA-seq analysis.** snRNA-seq data from BA38 were processed using the miklasq command from 10x Genomics Cell Ranger (v.3.0.1). Extracted paired-end fastq files (26/28 bp (v2, v3) long R1: cell barcode and UMI sequence information; 124-bp long R2: transcript sequence information) were checked for read quality using FastQC (v.0.11.9). Gene counts were obtained by aligning reads to the hg38 genome using an in-house pipeline. UMI tools (v.1.0.0) 36 were used to generate a whitelist of barcodes and to extract reads to match the detected barcodes. Reads were aligned to the human hg38 reference genome using STAR (v.2.5.2b)

- Genecode annotation for hg38 (v.24) was used as reference alignment annotation. Gene level expression was calculated using featureCounts (v.1.6.0) 70 by gene.
removed. Downstream analysis was performed using Seurat (v.3.9.9)27. Briefly, we normalized the expression data and integrated the two different batches of sequencing by SC-transform, retaining 3,000 variable genes. We constructed a k-nearest neighbor graph based on the Euclidian distance in 30 principal component space and identified distinct clusters of cells using the Leiden algorithm (resolution of 0.8). Visualization of clusters was performed by applying the function RunUMAP() based on uniform manifold approximation and projection (UMAP) in two dimensions. Cell-type markers were identified by Wilcoxson’s rank-sum test (two-sided; Benjamini–Hochberg-adjusted; FDR <0.05, log(fold change) >0.3, p<1x10−5). Clusters were annotated based on marker enrichment, with markers defined by middle temporal gyrus data 7. Briefly, data were downloaded from the Allen portal (https://portal.brain-map.org/atlas-and-data/rnaseq). Seurat was used to define the markers for each cluster by Wilcoxson’s exact test (one-sided with the alternative greater; Benjamini–Hochberg-adjusted). Labels for BAA8 cell types were selected by using the highest significant enrichment defined by an OR with FDR <0.05. These labels were used for all downstream analysis and snATAC-seq integration. The code used for this analysis is available at GitHub (https://github.com/konopkalab/Within_Subject).

snATAC-seq analysis. snATAC-seq data from BAA8 of three participants were processed using the Cell Ranger ATAC pipeline. Seurat extension Signac (v.1.1.0) was used to perform single-cell clustering, visualization and annotation. Each cell was clustered using total fragments in peaks <1,500 or >15% of the total fragments were not considered for further analysis. Clustering and creating a gene activity matrix were done using the default parameters. Only the cells with >0.5 confidence in annotation were considered for downstream analysis. The gene activity matrix was produced by counting fragments in the gene body with cell-type enrichment (WM4 and WM12: excitatory–inhibitory clusters; WM21: oligodendrocyte–oligodendrocyte progenitor cell clusters). Motif enrichment testing was applied to the upstream regions of the genes in each module. Enrichment analysis was performed only for the modules with cell-type enrichment (WM4 and WM12: excitatory–inhibitory clusters; WM21: oligodendrocyte–oligodendrocyte progenitor cell clusters). Fragments for excitatory–inhibitory clusters and oligodendrocyte–oligodendrocyte progenitor cell (OPC) clusters were extracted separately from the Cell Ranger's fragments.tsv file. For each cell type, the fragments.tsv file was adjusted to contain 200 bp around the cut site, and peaks were called using MACS2 (v2.1.1) 61. The CIS-BP database for human was used (http://cisbp.cbbr.utoronto.ca/index.php). Only TFs with directly determined motifs were kept. TFs were filtered for presence in >30% of cells in the cluster that was being tested for enrichment. A motif matrix (peaks in rows, motifs in columns) was created using CreateMotifMatrix from Signac. Using the FindMotif function from Signac, the enrichment of each TF was tested for the upstream peaks of module genes versus upstream peaks of all genes using Wilcoxson’s exact test (two-sided; Benjamini–Hochberg adjustment). Peak visualization was done using FGV (v2.8.13)62. The code used for this analysis is available at GitHub (https://github.com/konopkalab/Within_Subject).

Functional enrichment. Functional annotation of the genes within the modules was performed using GE2 (Genes2functions)63 and confirmed by TopGene Ontology and Kyoto Encyclopedia of Genes and Genomes databases. Expressed genes (15,192) were used as background. A one-sided hypergeometric test was performed to test overrepresentation of functional categories. A Benjamini–Hochberg-adjusted P value was applied as a multiple comparisons adjustment. Neuropsychiatric genes. ASD-associated genes used for Fig. 4c were downloaded from SFARI Gene database 64. ASD (1–3) are ASD genes with a score between 1 and 3. ASD-associated genes used for GWAS were selected by using the highest significant enrichment defined by an OR with FDR <0.05. These genes were used for all downstream analysis and enrichment. Gene set enrichment. Gene set enrichment was applied to correlated genes and SME genes from our previous study as shown in Fig. 3c, SME genes from the current study as shown in Extended Data Fig. 3c, neuropsychiatric differentially expressed genes as shown in Fig. 4a and Extended Data Fig. 4b. ASD genes as shown in Fig. 4c, and cell-type markers as shown in Fig. 4c and Extended Data Fig. 5h,l. We used a Fisher’s exact test in R with the following parameters: alternative = “greater”, conf. level =0.05. We reported OR and Benjamini–Hochberg-adjusted P values (FDR).

Statistical analysis and reproducibility. No statistical methods were used to predetermine sample sizes because of the limited availability of human brain surgical tissues. Participants were not randomly selected for inclusion in the study based on the availability of human tissue/oscillation data. However, tissue pieces were randomized for processing for RNA-seq. Data collection and analysis of human participants were not performed blind to the conditions of the experiments. However, separate individuals carried out wet-bench and dry-bench analyses. Thus, the researcher was blinded to patient/oscillation characterization while processing tissue for RNA and data analysis. The final analysis required including participant covariate information, so the researcher was unblinded to participant characteristics and oscillation data at that point. For SME values, bulk RNA-seq transcriptomic data, snRNA-seq transcriptomic data and scATAC-seq epigenomic data distributions were assumed to be normal, but this was not formally tested. Nonparametric tests were used to avoid uncertainty when possible. Data collection and analysis were not performed blind to the conditions of the experiments.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The RNA-seq dataset used for memory oscillatory signature analysis in this study are available at GEO with accession number GSE139914.

Code availability

Custom R codes for the quality control, MVA, correlative analysis, permutation/bootstrap, WGCNA, snRNA-seq analysis, snATAC-seq analysis, visualizations, functional enrichments, GWAS enrichment and gene set enrichments are available at https://github.com/konopkalab/Within_Subject.

References

55. Natu, V. S. et al. Stimulation of the posterior cingulate cortex impairs episodic memory encoding. J. Neurosci. 39, 7173–7182 (2019).
56. Goyal, A. et al. Functionally distinct high and low theta oscillations in the human hippocampus. Nat. Commun. 11, 2469 (2020).
57. Watrous, A. J., Miller, J., Qasim, S. E., Fried, I. & Jacobs, J. Phase-tuned neuronal firing encodes human contextual representations for navigational goals. elife https://doi.org/10.7554/elife.32554 (2018).
58. Fischl, B. et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33, 341–355 (2002).
59. Ghose, S., Gleason, K. A., Potts, B. W., Lewis-Ameczua, K. & Tammenga, C. A. Differential expression of metabolic glutamate receptors 2 and 3 in schizophrenia: a mechanism for antipsychotic drug action? Am. J. Psychiatry 166, 812–820 (2009).
60. Takahashi, J. S. et al. ChIP-seq and RNA-seq methods to study circadian control of transcription in mammals. Methods Enzymol. 551, 285–321 (2015).
61. Habib, N. et al. Massively parallel single-nucleus RNA-seq with DroNc-seq. Nat. Methods 14, 955–958 (2017).
62. Zheng, G. X. et al. Massively parallel digital transcriptional profiling of single cells. Nat. Commun. 8, 14094 (2017).
63. Yeh, Y. H. et al. Transforming growth factor-beta and oxidative stress mediate tachycardia-induced cellular remodelling in cultured atrial-derived myocytes. Cardiovasc. Res. 91, 62–70 (2011).
64. Del Cid-Pellitero, E., Plavski, A., Mainville, L. & Jones, B. E. Homeostatic changes in GABA and glutamate receptors on excitatory cortical neurons during sleep deprivation and recovery. Front. Syst. Neurosci. 11, 17 (2017).
65. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 55–64 (2013).
66. Wang, L., Wang, S. & Li, W. RSeQC: quality control of RNA-seq experiments. Bioinformatics 28, 2184–2185 (2012).
67. Anders, S., Pyl, P. T. & Huber, W. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166–169 (2015).
68. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140 (2010).
69. Smith, T. Heger, A. & Sudbery, I. UMI-tools: modeling sequencing errors in unique molecular identifiers to improve quantification accuracy. Genome Res. 27, 491–499 (2017).
70. Liao, Y., Smyth, G. K. & Shi, W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**, 923–930 (2014).
71. Stuart, T. et al. Comprehensive integration of single-cell data. *Cell* **177**, 1888–1902.e21 (2019).
72. Becht, E. et al. Dimensionality reduction for visualizing single-cell data using UMAP. *Nat. Biotechnol.* **37**, 38–44 (2019).
73. Hodge, R. D. et al. Conserved cell types with divergent features in human versus mouse cortex. *Nature* **573**, 61–68 (2019).
74. Zhang, Y. et al. Model-based analysis of ChIP-Seq (MACS). *Genome Biol.* **9**, R137 (2008).
75. Weirauch, M. T. et al. Determination and inference of eukaryotic transcription factor sequence specificity. *Cell* **158**, 1431–1443 (2014).
76. Robinson, J. T. et al. Integrative genomics viewer. *Nat. Biotechnol.* **29**, 24–26 (2011).
77. Falcon, S. & Gentleman, R. Using GOstats to test gene lists for GO term association. *Bioinformatics* **23**, 257–258 (2007).
78. Chen, J., Bardes, E. E., Aronow, B. J. & Jegga, A. G. ToppGene suite for gene list enrichment analysis and candidate gene prioritization. *Nucleic Acids Res.* **37**, W505–W511 (2009).
79. Banerjee-Basu, S. & Packer, A. SFARI Gene: an evolving database for the autism research community. *Dis. Models Mech.* **3**, 133–135 (2010).
80. Velmeshev, D. et al. Single-cell genomics identifies cell type-specific molecular changes in autism. *Science* **364**, 685–689 (2019).
81. Mathys, H. et al. Single-cell transcriptomic analysis of Alzheimer’s disease. *Nature* **570**, 332–337 (2019).
82. de Leeuw, C. A., Mooij, J. M., Heskes, T. & Posthuma, D. MAGMA: generalized gene-set analysis and candidate gene prioritization. *PLoS Comput. Biol.* **11**, e1004219 (2015).
83. Grove, J. et al. Identification of common genetic risk variants for autism spectrum disorder. *Nat. Genet.* **51**, 404–413 (2019).
84. Jansen, I. E. et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer’s disease risk. *Nat. Genet.* **51**, 912–919 (2018).
85. International League Against Epilepsy Consortium on Complex Epilepsies. Genome-wide mega-analysis identifies 16 loci and highlights diverse biological mechanisms in the common epilepsies. *Nat. Commun.* **9**, 5269 (2018).
86. Lee, J. J. et al. Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. *Nat. Genet.* **50**, 1112–1121 (2018).
87. Savage, J. E. et al. Genome-wide association meta-analysis in 269,867 individuals identifies new genetic and functional links to intelligence. *Nat. Genet.* **50**, 912–919 (2018).
88. Bipolar Disorder and Schizophrenia Working Group of the Psychiatric Genomics Consortium.Genomic dissection of bipolar disorder and schizophrenia, including 28 subphenotypes. *Cell* **173**, 1705–1715.e16 (2018).
89. Davies, G. et al. Study of 300,486 individuals identifies 148 independent genetic loci influencing general cognitive function. *Nat. Commun.* **9**, 2098 (2018).
90. Wray, N. R. et al. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nat. Genet.* **50**, 668–681 (2018).
91. Pardinas, A. F. et al. Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection. *Nat. Genet.* **50**, 381–389 (2018).
92. Martin, J. et al. A genetic investigation of sex bias in the prevalence of attention-deficit/hyperactivity disorder. *Biol. Psychiatry* **83**, 1044–1053 (2018).
93. Sohail, M. et al. Polygenic adaptation on height is overestimated due to uncorrected stratification in genome-wide association studies. *elife* https://doi.org/10.7554/eLife.39702 (2019).
94. Hoffmann, T. J. et al. A large multiethnic genome-wide association study of adult body mass index identifies novel loci. *Genetics* **210**, 499–515 (2018).
95. Morris, A. P. et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat. Genet.* **44**, 981–990 (2012).
96. Estrada, K. et al. Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. *Nat. Genet.* **44**, 491–501 (2012).
97. Schukert, H. et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat. Genet.* **43**, 335–338 (2011).

Acknowledgements

We thank the patients for participating in the study and the donors and their families for the additional tissue samples. We also thank K. Gleason for assistance with postmortem samples. G.K. is supported by an Jon Heighton Scholarship in Autism Research at UT Southwestern. This work was supported by NIMH (F30MH105158) to M.R.F.; NIDA (ST32DA007290-05) and NHBIL (17322KL139438-GA1) to F.A.; NINDS (NS106647), a UT BRAIN Initiative Seed Grant (366582), the Chilton Foundation, and the National Center for Advancing Translational Sciences of the NIH under the Center for Translational Medicine's award number UL1TR001105 to B.C.L. and G.K.; NINDS (NS107357) to B.C.L.; and NIMH (MH103517), The Chan Zuckerberg Initiative, an advised fund of Silicon Valley Community Foundation (HCA-A-1704-01747), and the James S. McDonnell Foundation 21st Century Science Initiative in Understanding Human Cognition—Scholar Award (220020467) to G.K. Postmortem human tissue samples were obtained from the NIH NeuroBioBank (The Harvard Brain Tissue Resource Center, funded through HHSN-271-2013-537 00030C, the Human Brain and Spinal Fluid Resource Center, VA West Los Angeles Healthcare Center; and the University of Miami Brain Endowment Bank) and the UT Neuropsychiatry Research Program (Dallas Brain Collection). We also thank the UT Southwestern Neuroscience Microscopy Facility for providing imaging resources.

Author contributions

S.B., B.C.L. and G.K. analyzed the data and wrote the paper. M.R.F. and C.D. collected surgical samples, processed RNA and generated bulk RNA-seq libraries. M.R.F. contributed to the design of the project. E.A. generated the snRNA-seq and snATAC-seq data and performed IHC. A.K. preprocessed the snRNA-seq data. E.C. preprocessed the snATAC-seq data. C.A.T. provided postmortem human brain tissue. S.S. analyzed the oscillation data. B.C.L. conducted all surgical procedures and memory testing. B.C.L. and G.K. designed and supervised the study and provided intellectual guidance. All authors contributed to the design of the project. F.A. generated the snRNA-seq and snATAC-seq data and performed IHC. A.K. preprocessed the snRNA-seq data. E.C. preprocessed the snATAC-seq data. C.A.T. provided postmortem human brain tissue. S.S. analyzed the oscillation data. B.C.L. conducted all surgical procedures and memory testing. B.C.L. and G.K. designed and supervised the study and provided intellectual guidance. All authors discussed the results and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41593-021-00803-x.

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1038/s41593-021-00803-x.

Correspondence and requests for materials should be addressed to B.C.L. or G.K.

Peer review information

*Nature Neuroscience* thanks Andrew Jaffe, Ueli Rutishauser, Ziv Williams, and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Reprints and permissions information is available at www.nature.com/reprints.
Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Data quality control. 

a, Box plots depicting the probability of recall for items presented at each serial position. Primacy and recency effects are visible, consistent with expectations for performance in the free recall episodic memory paradigm. Whiskers on box plots represent maximum and minimum values. Boxes extend from the 25th to the 75th percentiles, the center lines represent the median. Loess regression with confidence intervals is superimposed to depict the overall distribution. Smooth curves are shown with 95% confidence bands. 

b, Lag conditional response probability curves in our data (lag CRP), indicating expected temporal clustering behavior. Loess regression with confidence intervals depicts the overall distribution. Smooth curves are shown with 95% confidence bands. 

c, Boxplot showing the comparison of within-subject variance (across all measured electrodes at each band, blue box plot,) with the variance across subjects (at each band, yellow box plot). Across subjects variance is significantly greater than within-subject variance. Reported p-value from Wilcoxon rank sum test (one-sided with alternative greater). Boxplots extend from the 25th to the 75th percentiles, the center lines represent the median. 

d, Scatter plot showing the fraction of all BA38 electrodes exhibiting a significant subsequent memory effect at each frequency. We observed significant differences predicting recall success across the frequency spectrum, including the delta and gamma bands. Loess regression with confidence intervals depicts the overall distribution. Smooth curves are shown with 95% confidence bands. 

e, Distribution of SME values for each brain oscillation and cross-correlation based on Spearman’s rank correlation. 

f, Barplots showing the fraction of electrodes at which oscillations were detected in each frequency band in the recalled and non-recalled conditions. 85% of electrodes exhibited an oscillation in at least one of the delta, theta, or alpha frequency bands. 

g, Scatter plot showing individual electrode examples of power curves used for oscillation detection via the MODAL algorithm, both before and after subtraction of the best fit line. 

h, Principal component analysis of the subjects used for the within-subject analysis. Variance explained by each principal component is highlighted in the axis. 

i, Barplot showing the variance explained by each covariate adjusted across 10 principal components (wVE) for the within-subject data. Technical, biological and sequencing covariates calculated by PICArD (see Methods) are included. 

j, Principal component analysis of all the subjects used in this study. PMep = post-mortem epileptic subjects, UT = within-subjects, PMctl = post-mortem healthy subjects. 

k, Variance explained by each covariate adjusted across 10 principal components (wVE). Type corresponds to the three different types of data included in the analysis (PMep, UT, PMctl). Technical, biological and sequencing covariates calculated by PICARD (see Methods) are included. 

l, Association between the first two components and covariates based on adjusted gene expression. X-axis corresponds to the -log10(P-value) from linear regression modeling between PCs and covariates.
Extended Data Fig. 2 | SME gene robustness and overlap with other tasks. a, Boxplot showing the difference between F-statistics of the SME genes (Multivariate analysis) compared with the other genes. Stars correspond to the Wilcoxon’s rank sum test (N, Sign = 753, NotSign = 14439; one-sided with alternative greater; p < 0.0001 = ****; Benjamini-Hochberg adjusted: Delta, FDR = 2.3x10−249, Theta, FDR = 3.2x10−205, Alpha, FDR = 4.1x10−140, Beta, FDR = 2.1x10−159, Low Gamma, FDR = 7.2x10−207, High Gamma, FDR = 1.3x10−63). Boxes extend from the 25th to the 75th percentiles and the center lines represent the median. b, Violin plots showing the rho^2 of the genes significantly associated with each brain oscillation. Standard errors are calculated based on the rho^2 distribution of the significantly correlated genes. Dots represent the median rho^2 for the specific brain oscillation. 100 random permutations were applied to calculate the Perm values (see Methods). Stars correspond to the Wilcoxon’s rank sum test (unadjusted, one-sided with alternative greater; p < 0.0001 = ****).
Extended Data Fig. 3 | WGCNA highlights modules associated with memory oscillations. a, Representative network dendrogram for the consensus WGCNA. Heatmap shows the correlation between memory oscillatory signatures and genes. Red = positively correlated, Blue = negatively correlated. b, Heatmap showing the module association between memory oscillatory signatures and module eigengenes (Spearman’s rank correlation). Warm colors represent positive correlations and cool colors represent negative correlations. P-values for each correlation together with exact correlation values are contained within each box. c, Bubble-chart showing the enrichment for 300 SME genes decomposed by brain oscillation. Gradient color represents the -log10(FDR) and bubble size represents the odds ratio (Or) from a Fisher’s exact enrichment test of each module with disease-relevant gene lists. Y-axis shows the brain oscillations labels. X-axis indicates the modules of the present study. d, Boxplots showing the differential connectivity (for example, number of edges) between SME genes and non-SME genes in the modules associated with memory oscillatory signatures with SME genes enriched. Stars correspond to the results of a Wilcoxon’s rank sum test (one-side test with alternative greater;\( p < 0.001 = ****\), \( p < 0.01 = **\), \( p < 0.05 = *\); Benjamini-Hochberg adjusted: WM4, \( FDR = 0.016\), WM12, \( FDR = 0.048\), WM21, \( FDR = 4.5\times10^{-4}\)). Boxes extend from the 25th to the 75th percentiles and the center lines represent the median.
Extended Data Fig. 4 | Memory-related modules are enriched for gene co-expression modules associated with neuropsychiatric disorders. a. Bubble-chart showing the enrichment for loci associated with human traits used as negative controls. Gradient color represents the -log_{10}(FDR) from linkage disequilibrium gene set analysis performed by MAGMA. Y-axis shows the acronyms for the GWAS data utilized for this analysis (see Methods). b. Bubble-chart showing the enrichment for modules of co-expressed genes dysregulated in ASD, SCZ or BD. Gradient color represents the -log_{10}(FDR) and bubble size represents the odds ratio (OR) from a Fisher’s exact enrichment test. Y-axis shows the acronyms for the modules associated with neuropsychiatric disorders utilized for this analysis (see Methods). X-axis shows the modules of the present study. Modules significantly correlated with memory-related oscillations are highlighted in bold text.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | snRNA-seq quality control metrics and module enrichment for cell-types dysregulated in cognitive disorders. 

**a**, Barplot showing the total number of nuclei identified per subject. Colors correspond to the two different batches. 

**b**, Quality control boxplots for snRNA-seq with number of genes detected, number of UMIs and percentage of mitochondrial genes. Colors correspond to the two different batches. Boxes extend from the 25th to the 75th percentiles and the center lines represent the median. Dots represent outliers. 

**c**, Scatter plot showing the relationship between number of UMIs (X-axis) and detected genes (Y-axis). Each sample is indicated in a different color. 

**d**, UMAP plots showing the distribution of nuclei in each subject. Colors correspond to the two different batches. 

**e**, Proportion of nuclei representing the identified clusters. Colors correspond to the six different subjects analyzed. 

**f**, UMAP plots showing the distribution of the three major cell-classes: GABAergic (blue), Glutamatergic (red), and non-neuronal (green). 

**g**, Pie chart showing the proportion of the three major cell-type classes (GABAergic, Glutamatergic, and non-neuronal cells). 

**h**, Bubble-chart showing the enrichment of the SME modules for cell-type markers dysregulated in ASD. Color gradient represents the -log10(FDR) and bubble size represent the odds ratio (OR) from a Fisher’s exact enrichment test. Y-axis shows the acronyms for the cell-types defined in the ASD study. 

**i**, Bubble-chart showing the enrichment of the SME modules for cell-type markers dysregulated in Alzheimer disease (AD). Color gradient represents the -log10(FDR) and bubble size represent the odds ratio (OR) from a Fisher’s exact enrichment test. Y-axis shows the acronyms for the cell-types defined in the AD study.
Extended Data Fig. 6 | snATAC-seq quality control metrics. **a**, Barplot showing the total number of nuclei identified per subject. **b**, Quality control boxplots for each snATAC-seq sample demonstrating the total number of peaks, the number of reads in the peaks and the percentage of reads in peaks. Boxes extend from the 25th to the 75th percentiles and the center lines represent the median. Dots represent outliers. **c**, Scatter plot showing the relationship between total number of reads (X-axis) and percentage of reads in the peaks (Y-axis). Each sample is indicated in a different color. **d**, Heatmap of the pairwise similarity between cluster identities. Y-axis shows the snrNA-seq clusters. X-axis shows the snATAC-seq clusters. Gradient corresponds to the percentage of cells for the corresponding prediction label. **e**, UMAP plots showing the distribution of nuclei in each subject. **f**, UMAP plots showing the distribution of the three major cell-classes: GABAergic (blue), Glutamatergic (red), and non-neuronal (green). **g**, Pie chart showing the proportion of the three major cell-type classes.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  - Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
  - Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

| Data collection | R v4.0.3, FreeSurfer v6.0, Matlab R2018a |
|-----------------|----------------------------------------|
| Data analysis   | Bulk RNA-seq: Reads were aligned to the human hg38 reference genome using STAR (2.5.2b) and only uniquely mapped reads were retained for further analyses. Gencode annotation for hg38 (version 24) was used as reference alignment annotation and downstream quantification. Gene level expression was calculated using HTseq (v0.9.1) using intersection-strict mode by gene. Counts were calculated based on protein-coding genes from the annotation file. Counts were normalized using counts per million reads (CPM) with edgeR (v3.32.0) package in R. Gene expression was balanced for technical and biological covariates.

snRNA-seq: Single-nuclei RNA-seq data from BA38 was processed using mkfastq command from 10X Genomics CellRanger (v3.0.1). Gene counts were obtained by aligning reads to the hg38 genome using an in-house pipeline. UMI-tools (v1.0.0) were used to match barcode and reads. Reads were aligned to the human hg38 reference genome using STAR (2.5.2b). Gencode annotation for hg38 (version 24) was used as reference alignment annotation. Gene level expression was calculated using featureCounts (v1.6.0) by gene. UMIs were further calculated using UMI-tools (v1.0.0). Batches were integrated by Seurat (v3.9.9) integration pipeline using SCtransform. Standard pipeline was used for downstream analysis and cell-markers definition.

snATAC-seq: Single-nuclei ATAC-seq data from BA38 was processed using Cell Ranger ATAC pipeline. Seurat extension Signac (v1.1.0) was used for batch integration, filtering, clustering. Identified clusters were cross-referenced to the snRNA-seq data using Seurat integration workflow. Peaks were identified by MACS2 (v2.1.1). The CIS-BP database for human was used for transcription factor motif enrichment. Peak visualization was done using IGV (v2.8.13).

Other R libraries used:
ggplot2 (v3.3.2), ggpubr (v0.4.0), tidyverse (v1.3.0), WGCNA (v1.69), GOstats (v2.56.0), ComplexHeatmap (v2.7.1), openxlsx (v4.2.3)
Custom R codes used for this analysis are deposited and available at:
https://github.com/konopkalab/Within_Subject

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data Availability

The RNA-seq dataset used for memory oscillatory signature analysis in this study are available at GEO with accession number: GSE139914.

Code Availability

Custom R codes for the quality control, multivariate analysis, correlative analysis, permutation/bootstraps, WGCNA analysis, snRNA-seq analysis, snATAC-seq analysis, visualizations, functional enrichments, GWAS enrichment, and gene set enrichments are available at https://github.com/konopkalab/Within_Subject

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [x] Life sciences
- [ ] Behavioural & social sciences
- [ ] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical methods were used to pre-determine sample sizes because of the limited availability of human brain surgical tissues. |
|-------------|-----------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | For Bulk RNA sequencing analysis: no data was excluded.  
For Single Nuclei RNA Sequencing analysis: cells with more than 10,000 UMI and 5% of mitochondrial gene expressed were removed. The thresholding was determined by determining outliers from the distribution of the data.  
For Single Nuclei ATAC Sequencing analysis: Cells with total fragments in peaks less than 1500 or less than 15% of the total fragments were not considered for further analysis. The thresholding was determined by determining outliers from the distribution of the data. |
| Replication | Findings were not replicated due to the nature and limited availability of human brain surgical tissues. Nevertheless, we used additional data from independent healthy post-mortem brain tissue to increase the confidence of our findings. |
| Randomization | Subjects were not randomly selected for inclusion in the study based on the availability of human tissue/oscillation data. However, tissue pieces were randomized for processing for RNA-seq. |
| Blinding | Data collection and analysis of human subjects were not performed blind to the conditions of the experiments. However, separate individuals carried out wet-bench and dry-bench analyses. Thus, the researcher was blinded to patient/oscillation characterization while processing tissue for RNA and data analysis. The final analysis required including subject covariate information, so the researcher was unblinded to subject characteristics and oscillation data at that point. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.
### Antibodies

**Antibodies used**

- goat α-IL1RAPL2 (#PA5-47039, Thermo Fisher Scientific 1:20), rat α-SMAD3 (#MAB4038, R&D Systems, 1:100), rabbit α-CaMKII alpha (#PA514315, Thermo Fisher Scientific, 1:50), chicken α-GFAP (#ab4674, Abcam, 1:400), mouse α-GAD67 (#MAB5406, Millipore, 1:200), mouse α-OLIG2 (#MABN50,Millipore, 1:200), species-specific secondary antibodies produced in donkey and conjugated to Alexa Fluor 488, Alexa Fluor 555, or Alexa Fluor 647 (Thermo Fisher Scientific, 1:800).

**Validation**

Secondary antibody only experiments were conducted for each antibody to ensure specificity. IL1RAPL2 antibody was validated by Thermo Fisher Scientific using flow cytometry of human HepG2 cells. SMAD3 antibody was validated by R&D Systems using flow cytometry in human PC-3 cells and IHC in human pancreatic cancer tissue and human MDA-MB-231 cells. PMID 21289011 was provided as a citation. CamKII antibody was validated by Thermo Fisher Scientific using IHC in human brain and western blotting in human 293 cells. PMID 28408870 was provided as a citation for immunofluorescence in mouse neurons. GFAP antibody was validated by Abcam across many species including human. Protocol validations include IHC and immunofluorescence. 211 references are provided for this antibody at https://www.abcam.com/gfap-antibody-ab4674.html. GAD67 antibody was validated by Millipore in human brain via immunohistochemistry. Over 75 references are provided at http://www.emdmillipore.com/US/en/product/Anti-GAD67-Antibody-clone-1G10.2,MM_NF-MAB5406#anchor_BRO. OLIG2 antibody has been validated by Millipore in human via IHC. 15 references are provided at http://www.emdmillipore.com/US/en/product/Anti-Olig2-Antibody-clone-211F1.1,MM_NF-MABN50#documentation.

### Human research participants

**Population characteristics**

All participants were adults with temporal lobe epilepsy. Demographics including age, sex, race, ethnicity, and duration of epilepsy are including in Supplementary Table 1.

**Recruitment**

All participants were recruited from the UTSW surgical epilepsy program, undergoing intracranial EEG to map seizure onset location. All participants were approached for participation who completed a full session of the free recall task with minimum performance (recall fraction > 10%) who were to undergo temporal lobectomy operation. As such all participants suffered from temporal lobe epilepsy. Participants were recruited during preoperative visit prior to temporal lobectomy.

**Ethics oversight**

The research protocol was approved by the Institutional Review Board at UT Southwestern and informed consent was obtained from each participant.

Note that full information on the approval of the study protocol must also be provided in the manuscript.