Cell-type specialization is encoded by specific chromatin topologies

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The three-dimensional (3D) structure of chromatin is intrinsically associated with gene regulation and cell function1–3. Methods based on chromatin conformation capture have mapped chromatin structures in neuronal systems such as in vitro differentiated neurons, neurons isolated through fluorescence-activated cell sorting from cortical tissues pooled from different animals and from dissociated whole hippocampi4–6. However, changes in chromatin organization captured by imaging, such as the relocation of Bdnf away from the nuclear periphery after activation7, are invisible with such approaches8. Here we developed immunoGAM, an extension of genome architecture mapping (GAM)2,9, to map 3D chromatin topology genome-wide in specific brain cell types, without tissue disruption, from single animals. GAM is a ligation-free technology that maps genome topology by sequencing the DNA content from thin (about 220 nm) nuclear cryosections. Chromatin interactions are identified from the increased probability of co-segregation of contacting loci across a collection of nuclear slices. ImmunoGAM expands the scope of GAM to enable the selection of specific cell types using low cell numbers (approximately 1,000 cells) within a complex tissue and avoids tissue dissociation2,10. We report cell-type specialized 3D chromatin structures at multiple genomic scales that relate to patterns of gene expression. We discover extensive ‘melting’ of long genes when they are highly expressed and/or have high chromatin accessibility. The contacts most specific of neuron subtypes contain genes associated with specialized processes, such as addiction and synaptic plasticity, which harbour putative binding sites for neuronal transcription factors within accessible chromatin regions. Moreover, sensory receptor genes are preferentially found in heterochromatic compartments in brain cells, which establish strong contacts across tens of megabases. Our results demonstrate that highly specific chromatin conformations in brain cells are tightly related to gene regulation mechanisms and specialized functions.

To explore how genome folding is related to cell specialization, we applied immunoGAM to mouse brain tissue slices and analysed three cell types with diverse functions (Fig. 1a): oligodendroglia (oligodendrocytes and their precursors (OLGs)) from the somatosensory cortex; pyramidal glutamatergic neurons (PGNs) from the cornu ammonis 1 (CA1) of the dorsal hippocampus; and dopaminergic neurons (DNs) from the ventral tegmental area (VTA) of the midbrain. OLGs are important for neuronal myelination and circuit formation11, whereas PGNs are important for temporal and spatial memory formation and consolidation12, and DNs are activated during cue-guided reward-based learning13. Publicly available GAM data from mouse embryonic stem (mES) cells1 were used for comparison (Supplementary Table 1).

We selected cell types from brain tissue slices by immunofluorescence with cell marker antibodies before genomic extraction (Supplementary Table 1). A detailed flowchart of ImmunoGAM quality control (QC) measures and normalization is shown in Extended Data Fig. 1a–d and Supplementary Table 2. GAM contact matrices, each from about 850 cells, had low biases in GC content and mappability (Extended Data Fig. 2a–c). We

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calculated local contact densities and topological domains using the insulation square method, and calculated compartments associated with open chromatin (compartment A) and closed chromatin (compartment B) using principal component analysis (PCA) (Supplementary Tables 3–5).

As an example of cell-type-specific organization, we considered the Pcdh locus, which contains three clusters of cell adhesion genes (Pcdha, Pcdhb and Pcdhg) and occupies two topologically associating domains (TADs) in mES cells, as previously described (Fig. 1c, see Extended Data Fig. 3a for replicates). Mapping contact densities using 100–1,000 kb insulation squares showed that the locus is generally open above 500 kb. Higher expression of Pcdha and Pcdhb coincides with increased long-range contacts between the three clusters in neurons and OLGs and with additional long-range contacts with the highly expressed Fgf1 gene in OLGs. We also discovered contacts spanning tens of megabases in brain cells. For example, strong contacts connected two regions approximately 3- and 5-Mb wide, separated by 35 Mb, which contained clusters of vomeronasal (Vmn) and olfactory (Olfr) receptor genes (Fig. 1d, see Extended Data Fig. 3b for replicates). Thus, the application of immunoGAM in specific brain cell types reveals large rearrangements in 3D chromatin architecture at short-range and long-range genomic lengths.

To further investigate how cell-type-specific 3D genome topologies relate to gene expression and chromatin accessibility, we produced or collected published single-cell RNA sequencing (scRNA-seq) data and single-cell assay for transposable-accessible chromatin with high-throughput sequencing (ATAC-seq) data from mES cells, the cortex, the hippocampus and the midbrain (Methods, Extended Data Fig. 4, Supplementary Table 6). After selecting cell populations equivalent to those captured by immunoGAM, we compiled cell-type-specific pseudobulk RNA-seq and ATAC-seq datasets.

**TADs extensively rearrange between cell types**

Complex and extensive cell-type-specific changes in TAD-level contacts were frequent, for example, at a 4-Mb region that contains Scn genes that encode sodium voltage-gated channel subunits (Fig. 2a, see Extended Data Fig. 5a for replicates). We obtained a total of approximately 2,300 TADs across cell types, with a median length of about 1 Mb, which is in line with previous reports (Extended Data Fig. 5b). Although pairwise comparisons of TAD border positions confirmed previous levels of conservation (78–89%; Extended Data Fig. 5c), multway comparisons showed high cell-type specificity (Fig. 2b, see Extended Data Fig. 5d for sparser combinations). One-third of the borders were unique and significantly more insulated in other cell types (Extended Data Fig. 5e), with some variability noted between biological replicates (59–65%) (Extended Data Fig. 5f). By contrast, only 8% of the total set of borders was shared by brain cells and 14% by all cell types. Shared borders showed significantly stronger insulation in brain cells than in mES cells (Extended Data Fig. 5g), which suggests that there is structural stabilization after terminal differentiation. Unique boundaries often contained expressed genes (52–55% in brain cells, 38% in mES cells) (Extended Data Fig. 5h) and genes with enriched Gene Ontology (GO) terms relevant to the specialized cell type (Fig. 2c, Supplementary Table 7), such as ‘membrane depolarization’ and ‘cognition’ in PGNs or genes important for dopaminergic differentiation and dopamine synthesis in DNs.

**Long neuronal genes melt in brain cells**

Many neuronal genes involved in specialized functions are long (>300 kb) and produce many isoforms owing to complex RNA processing. Chromatin reorganization was most apparent at long genes.
in both PGNs and DNś (Fig. 2d, e). For example, Grik2 loses contact density in PGNś compared to mES cells, especially around the transcription start site (TSS) and transcription end site (TES) (Fig. 2d). By contrast, Dscam decondenses across its entire gene body in DNs (Fig. 2e). To assess whether decondensation relates to the expression of long genes, we compared the insulation of the most and least expressed long genes (Extended Data Fig. 3i). Highly expressed genes were significantly less insulated at TSSś and TESś and throughout gene bodies in both DNs and PGNś, but not in OLGś or mES cells. The general contact loss at highly expressed long neuronal genes is reminiscent of the decondensation, or ‘melting’, observed by microscopy at polytene chromosome puffs19 or tandem gene arrays20.

To detect melting genome-wide in an unbiased manner, we devised a pipeline at long genes that undergo melting events. To assess whether decondensation relates to the expression of long genes, we compared the insulation of the most and least expressed long genes (Extended Data Fig. 3i). Highly expressed genes were significantly less insulated at TSSś and TESś and throughout gene bodies in both DNs and PGNś, but not in OLGś or mES cells. The general contact loss at highly expressed long neuronal genes is reminiscent of the decondensation, or ‘melting’, observed by microscopy at polytene chromosome puffs19 or tandem gene arrays20.

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at open chromatin in brain cells, although such events can occur (for example, Magi2 in OLGs or Dscam in DNs) (Supplementary Table 8).

We next examined in more detail melting in neurxin 3 (Nrxn3) and RNA binding Fox 1 homologue 1 (Rbfox1) genes, both of which are highly sensitive to topoisomerase I inhibition. Rbfox1 encodes a membrane protein involved in synaptic connections and plasticity. In mES cells, Nrxn3 spans two TADs with high contact density, localizes in compartment B and associates with the nuclear lamina and the nucleolus. In DNs, Nrxn3 extensively melts (replicate scores of 48 and 49), is highly transcribed and accessible and belongs to compartment A (Fig. 3a, see Extended Data Fig. 7a for all cell types and replicates). Rbfox1 encodes a RNA-binding protein that regulates alternative splicing. In mES cells, Rbfox1 lies within a dense contact domain in compartment A, has very low expression and low chromatin accessibility. It also has nucleolar-associated domain and partial lamina-associated domain memberships. Rbfox1 extensively melts in PGNs (scores of 65 and 39), which coincides with its highest expression and high accessibility in these cells (Fig. 3b, Extended Data Fig. 7b).

To further understand the melting process in the Nrxn3 region, we used a polymer-physics-based approach to generate ensembles of 3D models in mES cells and DNs from GAM matrices (Fig. 3c, Supplementary Tables 9 and 10). 3D models were validated by reconstructing in silico GAM matrices (Extended Data Fig. 7c). mES cell models showed intermingled globular domains, including the green and red domains that contain Nrxn3 (Supplementary Video 1, see Extended Data Fig. 7d for additional examples). In DNs, the melted green domain becomes highly extended and has high gyration radii (Fig. 3c, d, Supplementary Video 2), while the upstream (grey) and downstream (blue) domains condense (Fig. 3a, Extended Data Fig. 7e).

Next, we applied fluorescence in situ hybridization on cryosections (cryo-FISH) to visualize Rbfox1 in mES cells and PGNs (Fig. 3e, Supplementary Table 11). In mES cells, a fluorescence-labelled probe across Rbfox1 revealed circular loci (average area of 0.44 ± 0.17 μm², mean ± s.d.) often localized at the nuclear surface (59%) or the nuclear periphery (27%; Fig. 3f, g). The TSS and TES regions of Rbfox1 are significantly more separated in PGNs than in mES cells (two-sided Mann–Whitney test; P < 0.01; from left to right, P = 0.003, P = 0.179, P = 0.331; NS, not significant). J. Schematic summarizing the melting of long genes in neurons, which is accompanied by locus relocalization away from repressive nuclear landmarks.

Fig. 3 | Extensive decondensation and relocalization of highly expressed long neuronal genes. a, b, Examples of two melting genes. Nrxn3 occupies two dense TADs in mES cells but melts in DNs where it is most highly expressed and accessible (a; chromosome 12: 87.6–92.4 Mb). Rbfox1 is highly condensed in mES cells and melts in PGNs where it is highly expressed and accessible (b; chromosome 16: 4.8–9.8 Mb). Compartment tracks are shown for each cell type, and published lamina-associated domains (LADs) and nucleolus-associated domains (NADs) for mES cells. c, Polymer models show extensive Nrxn3 melting in DNs compared to mES cells. Colour bars show DN domain positions. d, Gyration radii of green melting domains are significantly higher in DNs than in mES cells (***P = 1.1 × 10⁻⁵; two-sided Mann–Whitney test, n = 450). Arrows indicate positions of exemplar models. e, Genomic regions covered by cryo-FISH probes across the entire Rbfox1 gene, or targeting the gene TSS, middle of the coding region (Mid) or TES (Supplementary Table 11 contains the probe list). f, Rbfox1 (pseudocoloured green) occupies small, rounded foci in mES cells, often at the nucleolus periphery (immunostained for nucleophosmin 1, ref. 48; pseudocoloured purple). In PGNs, Rbfox1 occupies larger, decondensed foci away from nucleoli. Arrows indicate Rbfox1 foci in mES cells (orange) and PGNs (blue). Scale bars, 3 μm. g, Rbfox1 is occupied significantly larger areas in PGNs than in mES cells (***P = 0.008; two-sided Mann–Whitney test; two experimental replicates (Rep. 1 and Rep. 2) with n = 13, 39 and 38, 25 respectively). Most Rbfox1 foci localize at the nucleolar periphery in mES cells, but away from the nucleolus in PGNs. h, Cryo-FISH experiments that target the TSS, Mid and TES regions of Rbfox1 (pseudocoloured cyan, green, purple) show extensive separation in PGNs compared with mES cells. Arrows indicate Rbfox1 foci in mES cells (orange) and PGNs (blue). Scale bars, 3 μm. i. The TSS and TES regions of Rbfox1 are significantly more separated in PGNs than in mES cells (two-sided Mann–Whitney test; **P < 0.01; from left to right, P = 0.003, P = 0.179, P = 0.331; NS, not significant). J. Schematic summarizing the melting of long genes in neurons, which is accompanied by locus relocalization away from repressive nuclear landmarks.
Fig. 4 | Neuron-specific genes establish specific contacts rich in putative TF-binding sites. a. GAM contacts from PGNs and DNs (mouse replicate 1) were normalized (Z-score) and subtracted to produce differential contacts matrices. The top 5% most differential contacts (top 5% diff.) ranged from 0.05 to 5 Mb. Contacts containing TF motifs within accessible chromatin in both contacting windows were selected in the most (top five) enriched in PGNs or DNs or with the highest discriminatory power (information gain; Extended Data Fig. 9f). b. Multiple TF pairs coincide in the same PGN (left) or DN (right) differential contacts. The most abundant groups of contacts are shown for each cell type. c. Differential contacts with the most enriched combination TF feature pairs contain expressed genes in both windows. d. Differential contacts with the most abundant TF feature pairs in PGNs contain differentially expressed genes (top), with PGN-specific roles (middle; one-sided Fisher’s exact permuted P < 0.01). The top enriched GO terms show that differential contacts between DN upregulated genes (bottom) contain genes upregulated in DNs (green) and other expressed genes. e. Differential contacts with the most abundant TF feature pairs in DNs contain differentially expressed genes (top) with DN-specific functions (middle; one-sided Fisher’s exact permuted P < 0.01). The top enriched GO terms show that differential contacts between DN upregulated genes (bottom) contain genes upregulated in DNs (green) and other expressed genes. f. Left, Egr1 is highly expressed (chromosome 18: 33.7–36.0 Mb) and contacts with its downstream domain in PGNs compared with DNs. Right, the differential contact matrix shows increased PGN-specific contacts in the entire region surrounding Egr1 (right). The Egr1-containing TAD (inset; chromosome 18: 34.65–35.85 Mb) has multiple putative TF-binding sites found within PGN-accessible regions, most notably surrounding the Egr1 gene (grey dashed box), not found in DNs. g. Schematics summarizing the presence of genes related to synaptic plasticity in PGN-specific contacts and drug addiction in DN-specific contacts, with accessible chromatin harbouring binding sites for differentially expressed TFs.

Together, polymer modeling from GAM data and single-cell imaging highlight that domain melting is a previously unappreciated topological feature of very long genes. Domain melting occurs when genes are highly expressed, or highly accessible, in brain cell types, and the process is robustly captured by immuneGAM (Fig. 3). The decondensation of long genes in brain cells relative to mES cells often coincides with extensive reorganization of their chromosomal contacts, preferentially alongside increased intrachromosomal contacts.

Differential hubs of expressed genes
To explore how extensive chromatin rearrangements relate to changes in cis-regulatory elements and expressed genes, we extracted the top (5%) most differential contacts between PGNs and DNs within 5 Mb (ref. 18) (Fig. 4a, a detailed pipeline is provided in Extended Data Fig. 9a). We searched for binding motifs in accessible regions, which typically cover about 1.3 kb of the 50-kb contacting windows (Extended Data Fig. 9b), from differentially expressed transcription factors (TFs) that covered >5% of differential contacts (16 DN-specific and 32 PGN-specific TFs; Extended Data Fig. 9c, d, Supplementary Table 12). Out of 1,275 possible combinations of TF motif pairs, we prioritized 19 pairs (combinations of 14 TF motifs) that were most enriched in a given cell type or with a high ability to distinguish cell types (information gain; a full pipeline and criteria are provided in Extended Data Fig. 9e, f, and see Supplementary Table 13 for all TF pairs). We searched for differential contacts containing the most common TF-pair combinations (Fig. 4b, a full list is shown in Extended...
and to compartment B in brain cells. Most genes are
P (blue box). All enriched GO terms had one-sided Fisher's exact permuted
brain cells relative to mES cells and move from compartment B in mES cells to
compartment A in brain cells (pink box), and for genes that decrease
expression in brain cells and move to compartment B compared to mES cells (blue box). All enriched GO terms had one-sided Fisher's exact permuted
P = 0.

Fig. 5 Sensory receptor gene clusters preferentially belong to B compartments in brain cells and form megabase-range interactions. a. Selected top enriched GO terms for genes that increase expression in all brain cells relative to mES cells and move from compartment B in mES cells to compartment A in brain cells (pink box), and for genes that decrease expression in brain cells and move to compartment B compared to mES cells (blue box). All enriched GO terms had one-sided Fisher's exact permuted
P = 0. b. Top enriched GO terms for genes silent in all cell types that gain membership to compartment B in brain cells. Most genes are Olfr and Vmn sensory receptor genes. All enriched GO terms had one-sided Fisher's exact permuted
P = 0. c. GAM contact matrices containing Vmn and orphan receptor genes (chromosome 7: 35–55 Mb) show large clusters of strong interactions between B compartments in OLGs, PGNs and DNs, but not mES cells. Dashed boxes indicate interacting regions.

Data Fig. 9g). In PGNs, homodimers and heterodimers for Neurod1 and/or Neurod2 putative binding sites characterized the most abundant contacts, together with Egr1, Eto5, Lhx2, Maf, Nr3c1, Pou3f2 and Ubp1 (Neurod group; 5,572 contacts). In DNs, contacts containing Neurod1 and Neurod2 appeared as heterodimers (660 contacts). The most frequent TF-motif pair in DNs, and the second most in PGNs, is a Ctcf homodimer (892 and 781 contacts, respectively). The next most abundant DN-specific contacts contained Foxa1 combined with Ctcf, Nr2f1 or Nr4a1 (Foxal–TF group; 1,612 contacts). All groups spanned 0.05–5 Mb and captured strong contacts (Extended Data Fig. 10a, b). The selected differential contacts rarely coincided with two TAD borders (Extended Data Fig. 10c) and often involved compartment A windows (Extended Data Fig. 10d). Networks of differential contacts, built on the basis of motif co-occurrence using all 50 differentially expressed TFs, confirmed connectivity between multiple TF motifs in PGNs, and between Foxa1 or Neurod and specific TFs in DNs (Extended Data Fig. 10e, f, Supplementary Table I4).

Many contacts in each TF-motif group contained expressed genes in both contacting windows (30–45% in DNs, 40–50% in PGNs) that were significantly above the genome-wide or top 5% contact frequencies (10–16%; Fig. 4c, Extended Data Fig. 10g). Many of these genes were differentially expressed between PGNs and DNs (1,490 and 975, respectively, out of 3,357 differentially expressed genes; Extended Data Fig. 10h). In PGN-specific contacts, both the Neurod and Ctcf–Ctcf groups contained PGN upregulated genes with GO terms related to synaptic plasticity (Fig. 4d). Two PGN upregulated genes, Dlg4 (which

is important for long-term potentiation24) and Shisa6 (which prevents desensitization of AMPA receptors during plasticity23) were present within a hub of Neurod contacts that contained other activity-related genes, including Map2k4 and Dnah9 (see Extended Data Fig. 10i for the differential contact matrix). DN upregulated genes found with the Foxal–TF (139 out of 1,844), the Neurod–TF (87) and the Ctcf–Ctcf (80) pair are involved in synaptic organization and addiction pathways (Fig. 4e). For example, Dnm3 has altered protein expression in an alcohol-dependence paradigm28 and makes contacts containing the Foxal–TF pair with Mrp14 (downregulated after nicotine exposure26), Cacynp (upregulated following alcohol exposure28) and Pou2f1 (a co-factor associated with alcohol dependence28) (see Extended Data Fig. 10) for the differential contact matrix). Of note, Egr1, an immediate early gene upregulated in activated neurons28, establishes PGN-specific contacts containing accessible regions covered by Egr1 and Neurod motifs (Fig. 4f, see Extended Data Fig. 10k for replicate data). Egr1 was highly upregulated in PGNs (log2(fold-change) = 3, PGNs compared to DNs) and gained contacts with its adjacent TAD. It also contained accessible chromatin peaks rich in TF motifs belonging to the Neurod group that are not seen in DNs. Binding of EGR1 protein to its own promoter is confirmed in published chromatin immunoprecipitation with sequencing (ChIP-seq) data from the cortex23.

Together, our strategy identifies hubs of chromatin contacts specific for different neuron types that contain putative binding sites for differentially expressed TFs (Fig. 4g). These interconnected hubs bring together distal genes with specialized neuronal functions, such as synaptic plasticity in PGNs or drug addiction in DNs.

**Extensive A/B compartment reorganization**

Last, we found broad changes in A/B compartmentalization between all cell types (Extended Data Fig. 11a, b), with lowest Pearson's correlations of compartment eigenvector values between brain cells and mES cells and highest correlations between neuronal replicates (Extended Data Fig. 11c). Only 12% of genomic windows changed from compartment B in mES cells to compartment A in brain cells or between compartment A in mES cells to compartment B in brain cells (7%; see Extended Data Fig. 11d, e for per-chromosome transitions). Similar mean and total genomic lengths occupied contiguously by A or B compartments characterized all cell types (Extended Data Fig. 11f). A-to-B transitions from mES cells to brain cells contained 335 genes more strongly expressed in brain cells than in mES cells (Extended Data Fig. 12a). Their enriched GO terms included 'behaviour' and 'gated ion channel activity' (Fig. 5a). A-to-B transitions in mES cells to brain cells contained mostly silent genes in all cell types (572 out of 715 genes), except 50 transcriptional regulation genes highly expressed in mES cells (Fig. 5a, Extended Data Fig. 12b).

We found that A-to-B transitions were enriched for sensory receptor genes such as Vmn (149 genes out of 572 silent genes in the group) and Olfr (179 genes), and these were often found in clusters21,23 (Fig. 5b). Although silent, only 35% of Vmn and 66% of Olfr genes belonged to compartment B in mES cells compared with 82–96% and 72–85%, respectively, in brain cells (Extended Data Fig. 12c). Vmn and Olfr genes were often involved in strong clusters of contacts in brain cells that spanned up to 50 Mb (Fig. 5c, additional examples in Fig. 1d, Extended Data Fig. 12d, e). Long-range contacts in brain cells were significantly stronger when B compartments contained Vmn or, to a lesser extent, Olfr genes (at distances >3 Mb) (Extended Data Fig. 12f). This result suggests that sensory genes are not only more likely to belong to heterochromatic B compartments but also to more strongly contact other B compartments in brain cells.

**Discussion**

Here we introduced immunoGAM to capture genome-wide chromatin conformation states of specialized cell populations in the mouse brain.
We discovered extensive reorganization of chromatin topology across genomic scales, including cell-type-specific TAD reorganization that involves genes relevant to brain cell specialization (Extended Data Fig. 12g).

We reported melting of long genes (>300 kb) with highest expression levels and/or accessible chromatin in brain cells. Single-cell imaging of Rbfox1 in PGNs showed that the most prominent decondensation occurred between TSSs and TESs. Many long genes have specialized regulation in brain cells, for example, by topoisomerase activity or DNA methylation, by long stretches of H3K27ac or H3K4me3 acting as enhancer-like domains or by large transcription loops. Their regulation is further complicated by intricate RNA processing dynamics, which are required for adaptive responses based on activation state. Many of the highlighted genes, including Nrnx3, Rbfox1, Grik2 and Dscam, have genetic variants associated with or directly causal of neuronal diseases.

Thus, understanding how gene melting relates to regulation will become important to understanding the mechanisms of neurological disease.

Cell-type-specific networks of contacts were enriched for putative binding sites of differentially expressed TFs and connected hubs of differentially expressed genes with specialized functions, which is reminiscent of transcription factories. DN-specific loops contained genes related to drug-exposure response and addiction paradigms. Midbrain VTA DNs are the first brain cells that respond to addictive substances, including amphetamines, nicotine and cocaine. Future studies can explore the relationship between DN-specific chromatin landscapes and the regulation of these critical genes, with potential implications for the onset of addiction. PGN-specific contacts connected hubs of synaptic plasticity genes. Of note, PGN-specific contacts at the Egr1 gene, which is involved in the activation of long-term potentiation, contained Egr1 binding motifs, which suggests that there may be self-activation mechanisms. Together with reports that de novo chromatin looping can accompany transcriptional activation, our work suggests that coordinated TF binding at distant locations in the linear genome, but in close contact due to the 3D chromatin landscape, may be critical for the induction of long-term potentiation.

Our results also highlighted the specialization of repressive long-range contacts in brain cells. Repressed Olfr genes form a large interchromosomal hub in mature olfactory sensory neurons to regulate specificity of single Olfr gene activation. We showed that sensory genes also form strong cis-contacts in brain cells not directly involved in sensory processes, a result confirmed in adult cortical neurons. Tight 3D compartmentalization of Vmm and Olfr genes may be important for their repression in brain cells, as Olfr genes can be stochastically activated and mis-expressed in neurodegenerative diseases.

Finally, we showed that immunoGAM requires low cell numbers (approximately 1,000 cells) from single individuals while retaining the spatial organization of cells within brain tissues. This highlights its potential to provide insights into the aetiology and progression of neurological disease. Collectively, our work showed that cell specialization in the brain and chromatin structure are intimately linked at multiple genomic scales.

Online content
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**Methods**

**Randomization, blinding, and sample size**

Randomization and blinding were not relevant for the current study. The experiments and the subsequent analyses were performed on wild-type animals or cell lines, for which no clinical trial, treatment or disease comparison was performed. Samples were processed in different laboratories by different people, and there was no selection criteria for the wild-type mice used in the study. The appropriate number of samples for a GAM dataset varies and depends on multiple parameters such as nuclear volume, level of chromatin compaction, quality of DNA extraction, and so on. Because most of these parameters can be assessed only after the data have been collected and processed, we recommend that the optimal resolution is defined during the collection of each GAM dataset, rather than trying to estimate optimal sample size before data collection. GAM data can be collected in multiple batches from the same starting material, therefore the sample size can be increased until the desired resolution is achieved. For scRNA-seq experiments in mES cells, no statistical method was used to predetermine sample size. Libraries were generated twice, from mES cells from different biological replicates, to account for experimental variability. For scATAC-seq experiments, no statistical method was used to predetermine sample size.

**Animal maintenance**

Collection of GAM data from DNAs was performed using one C57Bl/6NCrl (RRID: IMSR_CR:027; WT) mouse, which was purchased from Charles River, and from one tyrosine hydroxylase–green fluorescent protein (TH–GFP; B6.Cg-Tg(TH-GFP)21-31/C57B6) mouse, obtained as previously described. All procedures involving WT and TH–GFP animals were approved by the Imperial College London’s Animal Welfare and Ethics Review Body. Adult male mice aged 2–3 months were used. All mice had access to food and water ad libitum and were kept on a 12-h light/12-h dark cycle at 22–23 °C and 55 ± 10% humidity.

**Tissue fixation and preparation**

WT, TH–GFP and Satb2<sup>loxP/loxP</sup> mice were anesthetized under isoflurane (4%), given a lethal intraperitoneal injection of pentobarbital (0.08 μl, 100 mg ml<sup>-1</sup> Euthatal) and transcardially perfused with 50 ml ice-cold PBS followed by 50–100 ml 4% depolymerized paraformaldehyde (PFA; electron microscopy grade, methanol-free) in 250 mM HEPES–NaOH (pH 7.4–7.6). Sox10::cre:RCE::loxP-EGFP animals were killed using an intraperitoneal injection of ketaminexylazine followed by transcardial perfusion with 20 ml PBS and 20 ml 4% PFA in 250 mM HEPES (pH 7.4–7.6). Brains from WT or TH–GFP mice were removed, and the tissue containing the VTA was dissected from each hemisphere at room temperature and rapidly transferred to fixative. For Satb2<sup>loxP/loxP</sup> mice, the CA1 field hippocampus was dissected from each hemisphere at room temperature. For Sox10<sup>cre/cre</sup> mice, brain tissue containing the somatosensory cortex was dissected at room temperature. Following dissection, tissue blocks were placed in 4% PFA in 250 mM HEPES–NaOH (pH 7.4–7.6) for post-fixation at 4 °C for 1 h. Brains were then placed in 8% PFA in 250 mM HEPES and incubated at 4 °C for 2–3 h. Tissue blocks were then placed in 1% PFA in 250 mM HEPES and kept at 4 °C until tissue was prepared for cryopreservation (up to 5 days, with daily solution changes).

**Cryoblock preparation and cryosectioning**

Fixed tissue samples from different brain regions were further dissected to produce about 1.5 × 3 mm tissue samples suitable for Tokuyasu cryosectioning, with an approximate 220–230 nm thickness. Cryosections were washed (3 times, 30 min in total) in PBS, followed by 50–100 ml 4% depolymerized paraformaldehyde (PFA; electron microscopy grade, methanol-free) in 250 mM HEPES–NaOH (pH 7.4–7.6) for 1 h. The fixed tissue was transferred to 2.1 M sucrose in PBS and embedded for 16–24 h at 4 °C, before being positioned at the top of copper stub holders suitable for ultracryomicrotomy and frozen in liquid nitrogen. Cryopreserved tissue samples are kept indefinitely immersed under liquid nitrogen.

**Immunofluorescence detection of GAM samples for confocal microscopy**

For confocal imaging, cryosections were incubated in sheep anti-TH (L150; Pel Freez Arkansas, P60101-0), mouse anti-pan-histone H1 (1:500; Merck, MAB3422) or chicken anti-GFP (1:500; Abcam, ab13970) followed by donkey anti-sheep or goat anti-chicken IgG conjugated with Alexa Fluor-555 or Alexa Fluor-488 (for pan-histone; Invitrogen). Alexa Fluor-488 (for TH and GFP; Abcam) or donkey anti-mouse IgG conjugated with Alexa Fluor-555 or Alexa Fluor-488 (for pan-histone; Invitrogen).

For PGNs, cryosections were washed (3 times, 30 min in total) in PBS, permeabilized (5 min) in 0.3% Triton X-100 in PBS (v/v) and incubated (2 h, room temperature) in blocking solution (1% BSA (w/v), 5% fetal bovine serum (FBS)).
were mounted in Mowiol 4-88.

8C opaque, Carl Zeiss, 415190-9161-000). We used multiplex-GAM9, for the PEN membrane and collected into PCR adhesive caps (AdhesiveStrip (nuclear profiles (NPs)), individual NPs were laser microdissected from Microsystems, LMD7000) using a ×3 dry objective. Following detection was visualized using a Leica laser microdissection microscope (Leica Microsystems) using a ×63 oil-immersion objective.

Secondary antibodies were used at the same concentration. Cell stainings as follows: anti-TH (1:50), anti-pan-histone (1:50) or anti-GFP (1:50).

For laser microdissection, cryosections on PEN membranes were microwaved directly in the PCR adhesive caps for 4 h (or 24 h for 160 out of 585 WGA was performed using an in-house protocol. In brief, NPs were included for each dataset collection to keep track of contamination and a adaptor sequence. The pre-amplification step was done using 2× DeepVentr mix (2× Thermo polynuclease buffer (10×), 400 μm dNTPs, 4 mM MgSO4, in ultrapure water), 0.5 μM GAT 7N primers (5′-GTG GAT GGT TGA GGT AGT GTG GAG NNN NNN N) and 2 units 1 DeepVentr (exo-) DNA polymerase (New England Biolabs, M0259L) in the programmable thermal cycler for 11 cycles. Primers that annealed to the general adaptor sequence were then used in a second exponential amplification reaction to increase the amount of product. The exponential amplification was done using 2× DeepVentr mix, 10 mM dNTPs, 100 μM GAM-COM primers (5′-GTG AGT G_ATG GTG ATG GAG NN NNN N) and 2 units 1 DeepVentr (exo-) DNA polymerase in the programmable thermal cycler for 26 cycles. For a small number of NPs from DNAs (Supplementary Table 2), WGA was performed using a WGA4 kit (Sigma-Aldrich) using the manufacturer’s instructions; the recent formulation of this kit is no longer suitable for GAM data production from subcellular nuclear slices.

GAM library preparation and high-throughput sequencing

Following WGA, the samples were purified using SPIR beads (0.7:25 or 1.7 ratio of beads per sample volume). The DNA concentration of each purified sample was measured using a Quant-IT Pico Green dsDNA assay kit (Invitrogen, P7389) according to the manufacturer’s instructions.

GAM libraries were prepared using an Illumina Nextera XT library preparation kit (Illumina, FC-131-1096) following the manufacturer’s instructions with an 80% reduced volume of reagents. Following library preparation, the DNA was purified using SPIR beads (1.7 ratio of beads per sample volume) and the concentration for each sample was measured using a Quant-IT Pico Green dsDNA assay. An equal amount of DNA from each sample was pooled together (up to 196 samples), and the final pool was additionally purified three times using the SPIR beads (1.7 ratio of beads per sample volume). The final pool of libraries was analysed using DNA High Sensitivity on-chip electrophoresis on an Agilent 2100 Bioanalyzer to confirm the removal of primer dimers and to estimate the average size and DNA fragment size distribution in the pool.

NGS libraries were sequenced on an Illumina NextSeq 500 machine according to the manufacturer’s instructions using single-end 75 bp reads. The number of sequenced reads for each sample can be found in Supplementary Table 2.

Tn5-based libraries are preferred for GAM data sequencing to increase fragment sequence variation, which helps avoid the need for dark cycles in the current Illumina machines. This choice greatly reduces the cost of sequencing and decreases the frequency of noise reads from absent windows seen with the previous protocol.

GAM data sequencing alignment

Sequenced reads from each GAM library were mapped to the mouse genome assembly GRCh38 (December 2011, mm10) with Bowtie2 (v.2.3.4.3) using default settings. All non-uniquely mapped reads, reads with mapping quality <20 and PCR duplicates were excluded from further analyses.

GAM data window calling and sample QC

Positive genomic windows present within ultrathin nuclear slices were identified for each GAM library. In brief, the genome was split into equal-sized windows (50 kb), and the number of nucleotides sequenced in each bin was calculated for each GAM sample with bedtools. Next, we determined the percentage of orphan windows (that is, positive windows that were flanked by two adjacent negative windows) for every percentile of the nucleotide coverage distribution and we identified the percentile with the lowest percentage of orphan windows for each GAM sample in the dataset. The number of nucleotides that corresponds to the percentile
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with the lowest percentage of orphan windows in each sample was used as an optimal coverage threshold for window identification in each sample. Windows were called positive if the number of nucleotides sequenced in each bin was greater than the determined optimal threshold.

Each dataset was assessed for QC by determining the percentage of orphan windows in each sample, the number of uniquely mapped reads to the mouse genome and the correlations from cross-well contamination for every sample (Supplementary Table 2). Most GAM libraries passed the QC analyses (86–96% in each dataset; Extended Data Fig. 1b, c). To assess the quality of sampling in each GAM dataset, we measured the frequency with which all possible intrachromosomal pairs of genomic windows are found in the same GAM sample; we found that 98.8–99.9% of all mappable pairs of windows were sampled at least once at resolution 50 kb at all genomic distances. Each sample was considered to be of good quality if they had <70% orphan windows, >50,000 uniquely mapped reads and a cross-well contamination score determined per collection plate of <0.4 (Jaccard index). The number of samples in each cell type that passed QC is summarized in Extended Data Table 2a. Following QC analysis, we noted that the 160 (out of 385) DN replicates samples incubated with lysis buffer for 24 h had decreases in orphan windows (median = 26% and 36% for 24 h and 4 h, respectively) and increases in total genome coverage (median = 9% and 6% for 24 h and 4 h, respectively). Although these differences were minor, we recommend 24 h lysis for future work.

Publicly available GAM datasets from mES cells

For mES cells, GAM datasets were downloaded from the 4D Nucleome portal (https://data.4dnucleome.org/). We used 249 × 3 NP GAM datasets from mES cells (clone 46C), which were grown at 37°C in a 5% CO₂ incubator in Glasgow modified Eagle's medium (MEM), supplemented with 10% FBS, 2 ng ml⁻¹ leukemia inhibitory factor (LIF) and 1 mM 2-mercaptoethanol, on 0.1% gelatin-coated dishes. Cells were passaged every other day. After the last passage, 24 h before collection, mES cells were re-plated in serum-free ESGRO Complete Clonal Grade medium (Merck, SF001- B). The list of 4DN sample identity numbers is provided in Supplementary Table I.

Identification of compartments A and B

For compartment analysis, matrices of co-segregation frequency were determined using the ratio of independent occurrence of a single positive window in each sample over the pairwise co-occurrence of pairs of positive windows in a given pair of genomic windows². GAM co-segregation matrices at 250-kb resolution were assigned to either A or B compartments, as previously described. In brief, each chromosome was represented as a matrix of observed interactions O(i,j) between locus i and locus j (co-segregation) and separately for E(i,j), whereby each pair of genomic window is the mean number of contacts with the same distance between i and j. A matrix of observed over expected values O/E(i,j) was produced by dividing O by E. A correlation matrix C(i,j) was produced between column i and column j of the O/E matrix. PCA was performed for the first three components on matrix C before extracting the component with the best correlation to GC content. Loci with PCA eigenvector values with the same sign that correlate best with GC content were called A compartments, whereas regions with the opposite sign were B compartments. For visualizations and Pearson’s correlations between datasets, eigenvector values on the same chromosome in compartment A were normalized from 0 to 1, whereas values on the same chromosome in compartment B were normalized from −1 to 0. Compartments were considered common if they had the same compartment definition within the same genomic bin. Compartment changes between cell types were computed after considering compartments that were common between biological replicates unless otherwise indicated.

To identify and visualize gene expression differences among genes in changing compartments, k-means clustering was performed on triplicate pseudo-replicates of each cell type using a custom Python script (Extended Data Fig. 12a, b). The number of clusters were determined using the elbow method, with k-means = 6 for genes in compartment B in mES cells and compartment A in brain cells, and k-means = 5 for compartment A in mES cells and compartment B in brain cells.

mES cell culture for scRNA-seq and scATAC-seq

mES cells from the 46C clone, derived from E14tg2a and expressing GFP under the Sox1 promoter, were a gift from D. Henrique (Instituto de Medicina Molecular, Faculdade de Medicina, Lisboa, Lisbon, Portugal). mES cells were cultured as previously described. In brief, cells were

Insulation score and topological domain boundary calling

TAD calling was performed by calculating insulation scores in NPMI GAM contact matrices at 50-kb resolution, as previously described. The insulation square method was chosen as it was previously shown that the domain borders detected in GAM data are also found in Hi-C, for which they are the most robust (most insulated). The insulation score was computed individually for each cell type and biological replicate, with insulation square sizes ranging from 100 to 1,000 kb. TAD boundaries were called using a 500-kb insulation square size and based on local minima of the insulation score. This approach does not detect meta-TADs or sub-TADs, and results in numbers and lengths of domains were similar to previous reports. Future work with higher resolution GAM datasets will enable further analyses of the reorganization of domains at finer genomic scales to investigate changes in sub-TADs, which have been previously shown to occur following cell commitment to neuronal lineages.

Within each dataset, boundaries that were touching or overlapping by at least one nucleotide were merged. Boundaries were further refined to consider only the minimum insulation score within the boundary and one window on each side, to produce a 3-bin ‘minimum insulation score’ boundary. In comparisons of boundaries between different datasets, 150-kb boundaries were considered different when separated by at least one 50-kb genomic bin, that is, if the centre of the boundaries are separated by at least 200 kb (note chromosome Y was excluded from the analysis). In Fig. 2b, we considered the boundary coordinate as the genomic window within a boundary with the lowest insulation value. TAD border coordinates for all cell types can be found in Supplementary Table 3, and the full range of insulation scores (100–1,000 kb) for all cell types can be found in Supplementary Table 4. UpSet plots for TAD border overlaps, compartments and TF motif analyses were generated using either custom Python or R scripts or using the UpSetR package (v.1.4.0).
routinely grown at 37°C, 5% (v/v) CO₂, on gelatine-coated (0.1% v/v) Nunc T25 flasks in Gibco Glasgow’s MEM (Invitrogen, 21700-082), supplemented with 10%(v/v)fetal calf serum (Bioscience LifeSciences, 701, batch number 110006) for scRNA-seq or Gibco FBS (Invitrogen, 10270-106, batch number 41FLS126K) for ATAC-seq, 2,000 units ml⁻¹ LIF (Milipore, ESG107), 0.1 mMβ-mercaptoethanol (Invitrogen, 31350-010), 2 mM L-glutamine (Invitrogen, 25030-024), 1 mM sodium pyruvate (Invitrogen, 11360070), 1% penicillin–streptomycin (Invitrogen, 15140122) and 1% MEM non-essential amino acids (Invitrogen, 11140035). Medium was changed every day and cells were split every other day. mES cell batches tested negative for Mycoplasma infection, which was performed according to the manufacturer’s instructions (AppliChem, A3744-0020). Before collecting material for scRNA-seq or ATAC-seq, cells were grown for 48 h in serum-free ESGRO Complete Clonal Grade medium (Merck, SF001-B), supplemented with 1,000 units ml⁻¹ LIF, on gelatine-coated (Sigma, G1393-100 ml, 0.1% v/v) Nunc 10-cm dishes, with a change in medium in 24 h.

46C E14tg2 mES cells are not listed in the ICLAC Register of Misidentified Cell Lines. The 46C E14tg2 mES cell line was generated by insertion of an eGFP cassette under the control of the Sox1 promoter in E14tg2 cells. Cells aligned with the GTF sequence were identified in the GAM sequencing data from mES cells. In addition, genome sequencing data from GAM mES cell samples was mined for single nucleotide polymorphisms (SNPs). Although GAM sequencing reads are sparsely distributed across the genome, there was a 64% overlap of GAM mES cell SNPs with SNPs identified from the parental E14tg2 genome sequencing data (https://www.ncbi.nlm.nih.gov/sra?term=SRX389523; data not shown).

Single-cell mRNA library preparation

Two batches (denoted batch A and B) of single-cell mRNA-seq libraries were prepared according to the Fluidigm manual “Using the CI Single-Cell Auto Prep System to Generate mRNA from Single Cells and Libraries for Sequencing”, Cell suspension was loaded on 10–17 μm C1 Single-Cell Auto Prep IFCs (Fluidigm, 100-5760, kit 100-6201). After loading, the chip was observed under the microscope to score cells as singlets, doublets, multiplets, debris or other. The chip was then loaded again on Fluidigm C1 IFCs, and cDNA was synthesized and pre-amplified in the chip using a Clontech SMARTer kit (Takara Clontech, 634833). In batch B, we included Spike-In Mix 1 (1,000; Life Technologies, 445674) as per the Fluidigm manual. Illumina sequencing libraries were prepared using a Nextera XT kit (Illumina, FC-131-1096) and a Nextera Index kit (Illumina, FC-131-1002), as previously described. Libraries from each microfluidic chip (96 cells) were pooled and sequenced on 4 lanes on Illumina HiSeq 2000, 2×100 bp paired-end (batch A) or 1 lane on Illumina HiSeq 2000, 2×125 bp paired-end (batch B) at the Wellcome Trust Sanger Institute Sequencing Facility (Supplementary Table 15).

scRNA-seq data processing, mapping and expression estimates

To calculate expression estimates, mRNA-seq reads were mapped with STAR (spliced transcripts alignment to a reference, v.2.4.2a) and processed with RSEM using the ‘single-cell-prior’ option (RNA-seq by expectation-maximization, v.1.2.25). The references provided to STAR and RSEM were the GTF annotation from UCSC Known Genes (mm10, v.6) and the associated isoform–gene relationship information from the Known Isosforms table (UCSC), adding information for ERCC sequences in samples from batch B. Tables were downloaded from the UCSC Table browser (http://genome.ucsc.edu/cgi-bin/hgTables) and for ERCCs, from the Thermofisher website (http://www.thermofisher.com/order/catalog/product/4456739). Gene-level expression estimates in ‘Expected Counts’ from RSEM were used for the analysis.

Differential gene expression analysis

For differential expression analysis for all cell types, pseudobulk replicate samples were obtained by randomly partitioning the total number of single cells per dataset into three groups and pooling all unique molecular identifiers (UMIs) per gene of cells belonging to the same replicate. To determine differentially expressed genes, all six possible pairwise comparisons between samples were performed using DEseq2 (v.1.24.0) with default parameters. In addition, shrunken log fold-changes were added with the LfcShrink function, using default parameters. Genes classified as differentially expressed in at least one comparison were considered for further analysis (adjusted P value < 0.05; Benjamini–Hochberg multiple testing correction method). A summary table for the differential expression analysis of all cell types can be found in Supplementary Table 12. For the TF
motif analysis, only the differentially expressed genes obtained from the comparison between DNs and PGNs were considered for further analysis (Extended Data Fig. 9c, d).

**Tn5 purification**

The pTXB1 plasmid carrying the Tn5-intein-CBD fusion construct with the hyperactive Tn5 protein containing the E54K and L372P mutations was obtained from Addgene (plasmid 60240). Tn5 expression and purification was performed as previously described, except that the final storage buffer was 50 mM HEPES-KOH pH 7.2, 0.8 M NaCl, 0.1 mM EDTA, 1 mM dithiothreitol and 55% glycerol.

**Tn5 adapter mix preparation**

To generate 100 μM adapter mix, 200 μM Tn5MRev (5’-[phos]CTGTCGCTTATACACATC) was mixed with 200 μM Tn5M-A (5’TGGTGCGCCAGGCTCACATGTATAAGAGACG; Adapter_mixA, 1:1 ratio). Separately, 200 μM Tn5MRev was mixed with 1 volume of 200 μM Tn5M-B (5’-GTCCTCGTGGGCAGTGGGTGTAAGAGACG; Adapter_mixB, 1:1 ratio). The two mixtures were incubated for 5 min at 95 °C and gradually cooled to 25 °C at a ramp rate of 0.1 °C s⁻¹. Finally, the Adapter_mixA was mixed with Adapter_mixB at a 1:1 ratio for a final 100 μM adapter mix.

**mES cell ATAC-seq library preparation**

ATAC-seq libraries were generated from approximately 75,000 mES cell nuclei following the Omni ATAC protocol with a modified transposition reaction: TAPS-DMF buffer (50 mM TAPS-NaOH, pH 8.5, 23 mM MgCl₂, 50% DMF), 0.1% Tween-20, 0.1% digitonin, in 0.2x PBS. A total of 3 μl of the Tn5 mix (5.6 μg Tn5 and 0.143 volume of 100 μM adapter mix) was added to the transposition reaction mix. Libraries were prepared as described in the Omni ATAC protocol. The final library was sequenced with an Illumina NextSeq 500 machine according to manufacturer's instructions, using paired-end 75 bp reads (150 cycles).

**Isolation of the VTA for snATAC-seq**

Male C57Bl/6N (RRID: IMSR_CR:027; WT) mice, aged 7 and 9 weeks, were killed by cervical dislocation. Brains were removed and the tissue containing the midbrain VTA was dissected from each hemisphere at room temperature and rapidly frozen on dry ice. Frozen midbrain samples were kept at −80 °C until further processing.

**DN snATAC-seq library preparation**

Two 10X Genomics scATAC-seq libraries from the midbrain VTA, VTA-1 and VTA-2 (from mice aged 7 or 9 weeks, respectively), were generated from midbrain VTA samples according to the 10X Genomics manual “Nuclei Isolation from Mouse Brain Tissue for Single Cell ATAC Sequencing” Rev B for flash-frozen tissue with minor adjustments. In brief, 500 μl 0.1× lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.1% BSA, 0.01% Tween-20, 0.001% Nonidet P40 substitute, 0.001% digitonin, and 1× complete Mini, EDTA-free protease inhibitor cocktail, Millipore-Sigma, 11836170001) was added to the frozen samples and immediately homogenized using a pestle pestle (15 times), followed by 5 min incubation on ice. The lysate was pipetted mixed 10 times, then incubated 10 min on ice. Finally, 500 μl of chilled wash buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 2 mM MgCl₂, 1% BSA, 0.1% Tween-20) was added to the lysed cells, and the suspension was passed through a 30-μm CellTrics strainers (Th Geyer, 7648779). The final approximately 500 μl nuclei suspension was stained with DAPI (final concentration 0.03 μg ml⁻¹) for about 5 min.

Around 200,000 DAPI-positive events were sorted using a BD FACSAria III flow cytometer with 70-μm nozzle configuration with sample and sort collection device cooling set to 4 °C into 300 μl Diluted Nuclei buffer (commercial buffer from 10X Genomics) in a 1.5-ml Eppendorf tube. A first gate excluded debris in a forward scatter/side scatter plot (see examples in Extended Data Fig. 4h, i). A consecutive, second gate in a DAPI-A/DAPI-H plot was used to exclude doublets and nuclei with incomplete DNA content (BD FACSDiva software, v.8.0.2). The collected nuclei were centrifuged at 500g for 5 min at 4 °C and resuspended in 20 μl Diluted Nuclei buffer. The nucleus concentration was determined using a Countess II FL Automated Cell Counter in DAPI fluorescence mode.

snATAC-seq libraries were prepared per the Chromium Next Gem Single Cell ATAC Reagent kits v.1.1 User Guide. In brief, nuclei were loaded on a microfluidics chip together with transposition reagents, transposase enzyme, beads with oligo-DT tags and oil to create an emulsion. Afterwards, the transposase reaction takes place inside the droplets. The barcoded cDNA is recovered from the emulsion, amplified and cleaned using a bead purification process. The cDNA is then used for library construction, including enzymatic fragmentation, adapter ligation and sample index PCR. Libraries were sequenced with either an Illumina NextSeq 500 machine using paired-end 75 bp reads (for VTA-1, 150 cycles) or a NovaSeq 6000 using paired-end 75 bp reads (for VTA-2, 100 cycles).

**ATAC-seq data processing, mapping, processing and QC**

For bulk mES cell ATAC-seq, paired-end reads were mapped to the mouse genome (mm10) using Bowtie with the following parameters:--minins 25--maxins 2000--no-discard--dovetail--soft-clipped-unmapped-tlen. Low-quality mapped reads (MQ < 30) and mitochondrial reads were removed. Duplicated reads were removed with Sambamba (v.0.6.8). Reads passing quality checks were converted to BAM format for further analyses.

For VTA snATAC-seq, paired-end reads were demultiplexed and mapped to the mouse genome (mm10) using the 10X Genomics Cellranger software (version cellranger-atac-1.2.0). The two VTA snATAC-seq libraries were analysed using ArchR software (v.0.9.1). Doubles were removed following default parameters in ArchR. Next, low-quality cells (identified as TSS enrichment score <4 and <2,500 unique fragments per cell) were removed for further analyses.

Next, dimensionality reduction was performed using the Latent semantic indexing (LSI) dimensionality reduction method from ArchR, with default parameters (except iterations = 10, resolution = 0.2, var-features = 60,000). The ArchR addHarmony function was used to run the Harmony algorithm for batch correction with default parameters, followed by clusters calling. Gene scores were determined as specified by ArchR. DNs were identified as the cluster with higher gene scores for Th, a well-known DN marker, and confirmed by additional DN marker expression (for example, Lmx1b, Foxa2, Foxa1 and Slc6a3). The DN cluster is composed of 216 cells in total (113 from VTA-1 and 103 from the VTA-2). UMI duplicates were collapsed to one fragment. To visualize an approximation for gene expression, gene scores were calculated using the createArrowFiles (addGeneScoreMat = TRUE) function in ArchR.

**Processing of published OLG and PGN scATAC-seq**

scATAC-seq BAM files for OLGs were downloaded from the sciATAC-seq in vivo atlas of the mouse brain. Next, reads were extracted from the BAM file that corresponded to cells from the cluster identified as oligodendrocytes from the prefrontal cortex (458 cells), to produce a pseudobulk ATAC BAM file. The original data, mapped to the mm9 genome, were converted to mm10 using the liftOver tool from UCSC utilities (https://genome.ucsc.edu/cgi-bin/hg/liftOver).

scATAC-seq datasets were obtained from hippocampal PGNs. A BAM file containing all cell types was supplied by A. Adey (Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR, USA). Reads were extracted from the BAM file that corresponded to the NRI PGN population (270 cells) to produce a pseudobulk ATAC BAM file.

**Generation of normalized ATAC-seq bigwig tracks**

A size factor normalization was applied to generate ATAC-seq bigwig tracks comparable between mES cells, OLGs, PGNs and DNs. First, a count matrix was generated for all TSS regions (±250 bp), which contained reads from at least two of the four cell types.
The TSS list was extracted from the genes.gtf file included in the cell ranger reference data (red-data-cellranger-atac-mm10-1.2.0i; https://support.10xgenomics.com/single-cell-atac/software/pipelines/latest/advanced/references). To calculate size factors, the TSS count matrix was processed through DESeqDataSetFromMatrix and estimateSizeFactors from the DESeq2 package. For all cell types, the scale factor (SF) = (cell type size factor) × −1.

Each pseudobulk ATAC-seq BAM file from mES cells, PGNs and OLGs was converted to the bedGraph format using the genomeCoverageBed function from bedtools with the following parameters: -pc -bg -scale SF. For DNs, ATAC-seq fragment files were converted to the bedGraph format using the genomeCoverageBed function from bedtools with the following parameters: -g chrom.sizes -bg -scale SF. The mm10 chrom.sizes file was downloaded from UCSC using fetchChromSize from UCSC utilities (http://hgdownload.soe.ucsc.edu/admin/exe/). The bedGraph files were then converted to bigwig using the bedGraphToBigWig function from UCSC utilities.

**DN and PGN ATAC-seq peak calling**

ATAC-seq peaks were called in DNs following the iterative overlap peak merging procedure described in the ArchR package. First, two pseudobulk replicates were generated by running the addGroupCoverage function from bedtools with the following parameters: -pc -bg -scale SF. For DNs, ATAC-seq fragment files were converted to the bedGraph format using the genomeCoverageBed function from bedtools with the following parameters: -g chrom.sizes -bg -scale SF. The mm10 chrom.sizes file was downloaded from UCSC using fetchChromSize from UCSC utilities (http://hgdownload.soe.ucsc.edu/admin/exe/).

**RNA and ATAC-seq length-scaled ATAC reads per million**

To calculate length-scaled RNA reads per million (lsRRPM) for 479 long genes (>300 kb), the mES cell BAM file (paired-end) was read using the readAlignmentPairs function from the GenomicAlignments function from the GenomicAlignments package in R (v.1.20.1; https://bioconductor.org/packages/release/bioc/html/GenomicAlignments.html). For published single-cell datasets (OLGs, PGNs, DNs; single-end libraries), BAM files were loaded using the readAlignments function from the GenomicAlignment package. Owing to the very long length of some reads, all BAM fragments were resized to the 5′ end base pair to avoid overlapping with multiple features. Next, the following formula was used to compute lsRRPM values for each cell type and per gene:

\[ \text{lsRRPM} = \frac{\text{number of overlaps between ATAC fragments and long gene body gene length (10}^{-6}) \times \text{total number of RNA fragments (10}^{-6})}{\text{total number of ATAC fragments (10}^{-6})} \]

To calculate length-scaled ATAC reads per million (lsARPM) for 479 long genes (>300 kb), concordant paired-end fragments were extracted for all cell types using the readAlignmentPairs function from the GenomicAlignments package in R with the following total number of fragments: 37,261,746 (mES cells), 2,121,258 (OLGs), 4,594,229 (PGNs) and 8,939,526 (DNs). Next, the following formula was used to compute lsARPM values for each cell type and per gene:

\[ \text{lsARPM} = \frac{\text{number of overlaps between ATAC fragments and long gene body gene length (10}^{-6}) \times \text{total number of ATAC fragments (10}^{-6})}{\text{total number of ATAC fragments (10}^{-6})} \]

**GO analysis**

GO term enrichment analysis was performed using GOElite (v.1.2.4). In Extended Data Fig. 4n, DN snATAC-seq marker genes were extracted with the getMarkerFeatures function from ArchR with default parameters. Marker genes were selected as genes with log fold change values of >1 and false discovery rate of <0.01 in the DN cluster compared with all clusters from the VTA (total of 973 genes). All unique genes were used as the background GO dataset. In Fig. 2c, all genes expressed in at least one cell type, annotated to mm10, were used as the background dataset. In Fig. 4d, e, all genes expressed in PGNs or DNs were used as the background dataset, and in Fig. 5a, b, all unique genes were used. Default parameters were used for the GO enrichment: GO terms that were enriched above the background (significant permuted P values of <0.05, 2,000 permutations) were pruned to select the terms with the largest Z-score (>1.96) relative to all corresponding child or parent paths in a network of related terms (genes changed >2). GO terms which had a permuted P value of ≤0.01, contained fewer than 6 genes per GO term or from the ‘cellular_component’ ontology, were not reported in the main figures. A full list of unfiltered GO terms can be found in Supplementary Table 7.

**MELTRON pipeline**

To assess gene insulation differences, insulation square values at 10 length scales (100–1,000 kb) were calculated for genes >300 kb in length (n = 479; calculated for a minimum 8 × 50-kb bins, that is, 400 kb minimum length). Cumulative probability distributions of insulation square values were calculated for each dataset, and the brain cells were compared to mES cell probability distributions for each gene by computing the maximum distance between the distributions and applying a Kolmogorov–Smirnov test. P values were corrected for multiple testing using the Bonferroni method, and log_{10} P transformed to obtain a domain melting score. Domain melting scores for each gene in each comparison can be found in Supplementary Table 8. For visualization, empirical cumulative probabilities and insulation score values were smoothed using a Gaussian kernel density estimate (adjust = 0.3).

**Calculation of the trans–cis contact ratio**

To determine the interaction strength of contacts to all (trans) somatic chromosomes relative to interaction strength to their own (cis) chromosome, cis and trans NPMI-normalized matrices were calculated at 250-kb resolution. Bins detected in less than 3%, or more than 75%, of 3 NP samples were removed from the analysis. To be sensitive to outliers, NPMI values of both cis (NPMIc) and trans (NPMIt) contacts for every bin were summarized with the arithmetic mean. The trans–cis contact ratio was then obtained using the following formula:

\[ \text{trans–cis contact ratio} = \frac{\sum \text{NPMIc} + \text{genomic bins}(n_1)}{\sum \text{NPMIt} + \text{genomic bins}(n_2)} \]

Trans–cis values of bins spanning long genes were summarized with the median.

**Modelling and in silico GAM**

To reconstruct 3D conformations of the *Nrxn3* locus, we employed the Strings & Binders Switch (SBS) polymer model of chromatin. In the SBS model, a chromatin region is modelled as a self-avoiding chain of beads, including different binding sites for diffusing, cognate, molecular binders. Binding sites of the same type can be bridged by their cognate binders, which then drives polymer folding. The optimal SBS polymers for the *Nrxn3* locus in mES cells and DNs were inferred using PRISMR, a machine-learning-based procedure that finds the minimal arrangement of the polymer binding sites that best describe input pairwise contact data, such as Hi-C or GAM. Here, PRISMR was applied to the GAM experimental data by considering the NPMI-normalized pairwise contact data, such as Hi-C or GAM. To determine the interaction strength of contacts to all (trans) somatic chromosomes relative to interaction strength to their own (cis) chromosome, cis and trans NPMI-normalized matrices were calculated at 250-kb resolution. Bins detected in less than 3%, or more than 75%, of 3 NP samples were removed from the analysis. To be sensitive to outliers, NPMI values of both cis (NPMIc) and trans (NPMIt) contacts for every bin were summarized with the arithmetic mean. The trans–cis contact ratio was then obtained using the following formula:

\[ \text{trans–cis contact ratio} = \frac{\sum \text{NPMIc} + \text{genomic bins}(n_1)}{\sum \text{NPMIt} + \text{genomic bins}(n_2)} \]

Trans–cis values of bins spanning long genes were summarized with the median.
Next, to generate thermodynamic ensembles of 3D conformations of the locus, molecular dynamics simulations were run of the optimal polymers, using the freely available LAMMPS software (v. sjune2019). In these simulations, the system evolves according to the Langevin equation, with dynamics parameters derived from classical polymer physics studies. Polymers are first initialized in self-avoiding conformations and then left to evolve to reach their equilibrium globular phase. Beads and binders have the same diameter \( \sigma = 1 \), expressed in dimensionless units, and experience a hard-core repulsion by use of a truncated Lennard–Jones potential. Analogously, attractive interactions are modelled with short-ranged Lennard–Jones potentials. A range of affinities between beads and cognate binders were sampled in the weak biochemical range, from \( 3.0 \sigma \) to \( 8.0 \sigma \) (where \( \kappa_\text{T} \) is the Boltzmann constant and \( \tau \) the system temperature). In addition, binders interact nonspecifically with the polymer with a lower affinity, sampled from \( 0 \kappa_\text{T} \) to \( 2.7 \kappa_\text{T} \). For the sake of simplicity, the same affinity strengths were used for all different binding site types. The total binder concentration was taken above the polymer coil–globule transition threshold. For each of the considered cases, ensembles of up to 450 distinct equilibrium configurations were derived. Full details about the model and simulations are discussed in Barbieri et al. and Chiariello et al.

In silico GAM NPMI matrices were obtained from the ensemble of 3D structures by applying the in silico GAM algorithm, here generalized to simulate the GAM protocol with 3 NPs per GAM sample and to perform NPMI normalization. In silico GAM NPMI matrices can be obtained using previously published algorithms, by aggregating the content of three in silico slices into one tube, and then applying the NPMI normalization formula (see the section ‘Visualization of pairwise chromatin contact matrices’, therein). Specifically, the same number of slices were used as in the GAM experiments, 249 \( \times \) 3 NPs for mES cells and 585 \( \times \) 3 NPs for DNs. Pearson’s correlation coefficients were used to compare the in silico and experimental NPMI GAM matrices.

Example of single 3D conformations were rendered by a third-order spline of the polymer bead positions, with regions of interest highlighted in different colours. To quantify the size and variability of the 3D structures in mES cells and DNs, the average gyration radius \( (R_g) \) was measured from the selected domains encompassing and surrounding the gene, expressed in dimensionless units \( \sigma \) in 3d. Extended Data Fig. 7e. Analyses and plots were produced with the Anaconda Data package v.4.7.12, and 3D structure visualizations were produced with POV Ray, v.3.7 (http://www.povray.org/download/).

Cryosections for FISH experiments
Cryosections were fixed and cryopreserved hippocampal CAI tissue and mES cells were cryosectioned as previously described (see ‘Cryoblock preparation and cryosectioning’ above) with an approximate thickness of 400 nm. Distances between the objects were measured using Fiji software (v.2.0.0-rc-69/1.52p). All images were collected with a ×4 zoom at 1,024 \( \times \) 1,024 pixel resolution (pixel size of 0.0451 \( \mu m \), resolution of 22.1760 pixels \( \mu m^{-1} \)).

In situ GAM NPMI matrices were obtained from the ensemble of 3D structures by applying the in situ GAM algorithm, here generalized to simulate the GAM protocol with 3 NPs per GAM sample and to perform NPMI normalization. In situ GAM NPMI matrices can be obtained using previously published algorithms, by aggregating the content of three in situ slices into one tube, and then applying the NPMI normalization formula (see the section ‘Visualization of pairwise chromatin contact matrices’, therein). Specifically, the same number of slices were used as in the GAM experiments, 249 \( \times \) 3 NPs for mES cells and 585 \( \times \) 3 NPs for DNs. Pearson’s correlation coefficients were used to compare the in situ and experimental NPMI GAM matrices.

Example of single 3D conformations were rendered by a third-order spline of the polymer bead positions, with regions of interest highlighted in different colours. To quantify the size and variability of the 3D structures in mES cells and DNs, the average gyration radius \( (R_g) \) was measured from the selected domains encompassing and surrounding the gene, expressed in dimensionless units \( \sigma \) in 3d. Extended Data Fig. 7e. Analyses and plots were produced with the Anaconda Data package v.4.7.12, and 3D structure visualizations were produced with POV Ray, v.3.7 (http://www.povray.org/download/).

Cryo-FISH microscopy
Cryo-FISH images were collected sequentially with a Leica TCS SP8 STED confocal microscope (Leica Microsystems DMi6000B-CS) using Leica Application Suite X v.3.5.5.19976 and a HCPL APO CS2 \( \times 63 \) (1.40 oil objective (numerical aperture of 1.4, Plan Apochromat) (see ‘Immunofluorescence detection for confocal microscopy’) using the following settings: 405-nm excitation and 420–500-nm emission (for DAPI), 488-nm excitation and 510–535-nm emission (for probes labelled with ChromaTide Alexa Fluor-488 and for nucleophosmin), 568-nm excitation and 586–620-nm emission (for probes labelled with ChromaTide Alexa Fluor-568, 647-nm excitation and 657–700-nm emission (for probes labelled with Alexa Fluor-647), and 555-nm excitation and 586–640-nm emission (for immunofluorescence labelling of nucleophosmin with Alexa Fluor-555). All images were collected with a \( \times 4 \) zoom at 1,024 \( \times \) 1,024 pixel resolution (pixel size of 0.0431 \( \mu m \), resolution of 22.1760 pixels \( \mu m^{-1} \)).

Cryo-FISH image analysis
Images were analysed using Fiji software (v.2.0.0-rc-69/1.52p). All images were pre-processed as previously described. Genomic foci were visually identified, and areas of the manually defined objects were measured using the Fiji-Area tool. For the cryo-FISH experiment combined with immunofluorescence, the location of genomic loci in relation to the nuclear lamina or nucleolus was assessed on the basis of the overlap of foci with the nucleolus (identified by nucleophosmin) or the nuclear lamina (as defined by the periphery of the DAPI staining) by at least three pixels. To determine the distance between the TSS, middle and TES genic foci, we took the centre of mass of the selected objects, as defined by Fiji-Center of mass function (the brightness-weighted average of the x and y coordinates of all pixels within the selected areas). Distances between the objects were measured using the Fiji-Line tool between the centres of mass defined for
Determination of differential contacts between GAM datasets
Significant differences in pairwise contacts between a pair of GAM datasets were determined as previously described with modifications. In brief, genomic windows with low detection, defined as less than 2% of the distribution of all detected genomic windows for each chromosome, were removed from both datasets to be compared. Contacts were filtered to be within 0.5–5 Mb distance and above 0.15 NPMI, and NPMI contact frequencies at each genomic distance of each chromosome were normalized by computing a Z-score transformation, and a differential matrix (D) was derived by subtracting the two Z-score normalized matrices.

TF-binding site analysis
To find TF-binding motifs present within specific contacts, significant differential contacts were determined for DNs and PGNs. Accessible regions within the differential contacts were determined using scATAC-seq for PGNs and DNs. To account for methodological differences, including lower sequencing depth in PGN scATAC-seq data (Extended Data Fig. 4l), we considered only the peaks that occurred in >10% of cells (>10 cells in DNs; >13 in PGNs). Motif finding within accessible regions in significant contacts was performed using the Regulatory Genomics Toolbox (v.0.12.3; https://www.regulatory-genomics.org/motif-analysis/introduction/) with TF motifs (from the HOCOMOCO database, v.11) obtained for TFs expressed in either DNs or PGNs (R-log ≥ 2.5) to determine the percentage of windows containing each TF motif. Next, TF motifs were filtered based on (1) the percentage of windows containing the motif (>5%) and (2) the differential expression in either PGNs or DNs (log2(adjusted Pvalue) > 3, see Differential gene expression analysis above), which resulted in 50 TF motifs for feature pair analysis (33 TF motifs from PGNs and 17 from DNs; Extended Data Fig. 9c, d).

Feature pairs associated with specific contacts were determined as previously described and testing the 1,275 combinations of motif pairs (1,225 heterotypic motif pairs and 50 homotypic motif pairs). The number of contacts containing each pair of selected TF motifs (PGN

\[ \frac{\text{PGN} - \text{DN}}{\text{PGN} + \text{DN}} \]

and DNTF), together with the percentage of total significant differential contacts in PGNs and DNs (PGN and DN), were used to determine the enrichment score for all TF feature pair interactions (that is, the ratio between frequencies of contacts in PGNs or DNs, (PGN

\[ \frac{\text{PGN}}{\text{PGN}} \]

and DN)/ (DN)/DN)). The effectiveness of a TF pair for discriminating between contacts from PGNs and DNs was assessed by using the information gain measure90. Enrichment and information gain for all TF feature pair interactions, as well as differential expression values for TFs (DNs compared to PGNs), can be found in Supplementary Table 13. The top feature pairs were extracted on basis of the highest information gain (ten feature pairs), PGN enrichment (five feature pairs) and DN enrichment (five feature pairs) scores. Contact overlaps for top feature pairs were visualized using UpSet plots.

Network and community detection analysis of TF-binding sites in significant differential contacts
To determine the interconnectivity between different TF motifs found in accessible regions of significant differential contacts, the number of contacts for each pair of TF motifs (1,275 pairs) was determined. After filtering pairs of TF motifs involved in less than 20% of the total contacts (15,833 and 5,400 contacts minimum in PGNs and DNs, respectively), a network was built for each cell type with TF motifs as nodes and number of contacts as weighted edges. The Leiden algorithm was used to detect communities of strongly interconnected nodes, using the leiden package in R, with a resolution of 1.01 for both PGNs and DNs (Extended Data Fig. 10f, Supplementary Table 14).

GAM aggregated contact plots
To visualize the average contact intensity for a set of genomic contacts, NPMI contact frequencies at each genomic distance of each chromosome were first normalized by computing a Z-score transformation. The resulting Z-score values were determined for each contact and for each chromosome in a 4-bin radius (50-kb bins). For each chromosome, Z-score values for each set of contacts and for the surrounding bins were summarized by the arithmetic mean. Mean values computed for each chromosome were added together and divided by the number of chromosomes.

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

Data availability
Raw fastq sequencing files for all samples from DN, PGN and OLG GAM datasets, together with non-normalized co-segregation matrices, normalized pair-wise chromatin contacts maps and raw GAM segregation tables are available from the GEO repository under accession number GSE148792. Raw fastq sequencing files for mES cell GAM datasets are available from 4DN data portal (https://data.4dnucleome.org/). The 4DN sample IDs for all samples used in the study are available in Supplementary Table 1. All polymer model 3D structures produced for the analyses of this work are available in Supplementary Tables 9 and 10. Raw confocal and laser microdissection images, as well as images and ROIs for cryo-FISH experiments are available at: https://github.com/pombo-lab/WinickNg_Kukalev_Harabula_Nature_2021/tree/main/microscopy_images/.

Raw single cell mES cell transcriptome data are available from ENA data portal (https://www.ebi.ac.uk/ena/browser/home). The ENA sample IDs for all samples used in the study are available in Supplementary Table 15. Position sorted BAM files for ATAC-seq data from mES cells and DNs are available from the GEO repository under accession number GSE174024, together with processed bigwig files. A public UCSC session with all data produced, as well as all published data utilized in this study is available at http://genome-euro.ucsc.edu/s/Kjmorris/Winick_Ng_2021_GAMbrainpublicsession. Source data are provided with this paper.

Code availability
Processing and plotting scripts for MELTRON and insulation scores are available at: https://github.com/pombo-lab/Meltron/. Processing and plotting scripts for the trans–cis contact ratios are available at https://github.com/pombo-lab/GAM_trans_cis_ratio/. Custom python and R scripts for GAM window calling, GAM quality control, GAM genome sampling quality and resolution, production of NPMI matrices, aggregated maps, k-means clustering, calculation of insulation scores and compartment calling were deposited in https://github.com/pombo-lab/WinickNg_Kukalev_Harabula_Nature_2021/tree/main/code/.

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Extended Data Fig. 1 | ImmunoGAM experimental pipeline and GAM data quality control. a, ImmunoGAM experimental pipeline. VTA and CA1 dissections and cryoblock preparations are shown as examples. After fixation, brain tissue is dissected and cryopreserved in sucrose/PBS solution, before sectioning on an ultracryomicrotome (~220nm thick tissue slices; −100 °C). For confocal imaging, DAPI staining labels nuclear slices and helps to morphologically identify the CA1 PGN layer in the hippocampus, or was combined with TH immunolabelling to identify DNs in the midbrain, or with GFP immunolabelling to identify OLG lineage cells in the cortex (scale bars = 10 μm for OLGs and DNs, 100 μm for PGNs). For laser microdissection, nuclei were identified by indirect immunofluorescence using anti-pan-histone antibodies to morphologically select PGNs of the pyramidal neuron layer, or were combined with immunofluorescence detection of TH for DNs or GFP for OLGs. Laser microdissection images are shown as examples (scale bars = 30 μm for DNs, 200 μm for PGNs). Three nuclear slices were selected and laser microdissected from the tissue to fall into the same PCR lid, as described for multiplex GAM9 (scale bars = 30 μm for panels a and b, 400 μm for panels c–e). Genomic DNA content was extracted from each sample and amplified using whole-genome amplification, followed by Illumina Nextseq sequencing. b, Quality control parameters (uniquely mapped reads, genome coverage of positive windows, and percentage of orphan windows; see Methods) for all combined GAM samples collected from brain cell types. Each data point represents a GAM sample. Samples passing QC are shown in green, samples not passing QC in red. c, Percentages of uniquely mapped reads and orphan windows per GAM sample, shown separately for each dataset produced in this study. Samples not passing QC are shown in red, water control samples (laser-microdissected material not containing a nuclear profile) are shown in black. d, Normalized point-wise mutual information (NPMI) normalization corrects for differences in the co-segregation matrix caused by changes in the window detection frequency (WDF; see Methods). Example shown for PGNs replicate 1 (R1; chr7:60,000,000-80,000,000).
a Summary of GAM datasets used in this study

| Dataset    | GAM samples collected (3NPs per sample) | Samples that did not pass quality control | Water controls | GAM samples passing quality control | Number of cells per dataset | % locus pairs detected at least once within 5Mb (50-kb resolution) |
|------------|----------------------------------------|------------------------------------------|----------------|------------------------------------|-----------------------------|---------------------------------------------------------------|
| DNs R1     | 656                                    | 58                                       | 11             | 11                                 | 13                          | 585                                                          | 1755 99.8                                                |
| DNs R2     | 316                                    | 19                                       | 6              | 6                                  | 6                           | 281                                                          | 873 99.9                                                 |
| PGNs R1    | 218                                    | 7                                        | 2              | 2                                  | 2                           | 209                                                          | 627 99.9                                                 |
| PGNs R2    | 288                                    | 7                                        | 1              | 6                                  | 6                           | 275                                                          | 825 99.8                                                 |
| OLGs       | 335                                    | 46                                       | 4              | 4                                  | 0                           | 290                                                          | 870 99.7                                                 |
| mESCs      |                                        |                                           |                |                                    | 249                        | 747                                                          | 99.9                                                      |

b Normalization of biases in GAM PGNs R1 dataset

Extended Data Fig. 2 | Normalization of immunoGAM data. a, Summary of GAM datasets used in this study. VTADNs were collected from two animals, an 8-week old wild-type mouse and a 10-week old mouse carrying a TH-GFP reporter. PGNs were collected from two 8-week old wild-type littermate mice. Cortical OLGs were collected based on detection of GFP expression from a 3-week old Sox10-cre-LoxP-GFP mouse. GAM data from mES cell (clone 46C) was previously published and available from the 4DNucleome portal after quality control (https://data.4dnucleome.org; Supplementary Table 1).

50-kb windows for PGNs R1 were divided into equally sized groups depending on their GC content, mappability, window detection frequency (WDF) or DpnII restriction density. Heatmaps of mean observed/expected bias (represented as a fold change) are shown for co-segregation, D-prime (used for previous GAM normalizations), PMI and NPMI normalizations. NPMI normalization results in the lowest absolute bias percentage for all tested categories (box plots on right). Box plot definitions were as follows: 25 percentile lower limit, 75 percentile upper limit, and center line as the median; interquartile range (IQR) was 25 to 75 percentile; upper whisker was (75 percentile + (IQR*1.5)), lower whisker was (25 percentile – (IQR*1.5)) or zero if negative; outliers outside the whiskers were indicated with open circles. n = 100 for each bias tested, representing all combinations of deciles in PGNs R1. c, Absolute bias analysis for remaining immunoGAM datasets. Box plot definitions were as in panel b.
**Extended Data Fig. 3** ImmunoGAM contact matrices from replicate mice. 

**a.** GAM contact matrices centered on the Pcdh gene cluster for mESC, CA1 PGN replicate 2, and VTA DN replicate 2 (Chr18: 36,000,000-39,000,000; 50-kb resolution). ChIP-seq peaks for CTCF15 are shown below the mES cell matrix, showing extensive binding at the Pcdh locus. Dashed lines illustrate differences in contacts between Pcdh-α, -β and -γ genes for different cell types. Scale bars are adjusted to a range between the 0 value and the 99th percentile of NPMI values for each cell type.

**b.** Example matrices for replicate 2 of CA1 PGN and VTA DN, for Chr17: 0-60,000,000 at 50-kb resolution. Dashed lines illustrate vomeronasal (Vmn) and olfactory (Olfr) receptor gene clusters within B compartments, separated by ~35 Mb, are observed in brain cells but not in mES cells. Compartments A/B were classified using normalized PCA eigenvectors.
Extended Data Fig. 4 | Curation of scRNA-seq and snATAC-seq data from published datasets and datasets produced for the present study.

a, Schematic representation of scRNA-seq datasets used in this study. We collected published scRNA-seq datasets from cortex and hippocampus, and produced scRNA-seq from midbrain. From each of the brain tissues, we select the specific cell types that were matched with those collected for the presented GAM data. The selected datasets from each cell type were combined and visualized through UMAP embedding, coloured by expression of each marker gene: Sox10 for OLGs, Camk2a for PGNs and Th for DNs. Cluster contours are drawn to highlight separation between cell types. All marker genes were found highly expressed in their respective cell types. b, scRNA-seq datasets were also generated from mES cells. UMAP clustering is coloured by b-genes were found highly expressed in their respective cell types. c, Pearson’s correlation plot of gene expression in mES cells (clone 46C) between published bulk versus single-cell RNA-seq. Average single-cell expression is highly correlated with bulk RNA-seq (two-sided Pearson’s R product-moment correlation; \( R = 0.93 \), \( p < 2.2 \times 10^{-16} \)). Only genes common to both datasets are represented (total genes in bulk dataset = 22822, total genes in single cell dataset = 23208, common to both = 22045). d, Single cell expression of Rbfox3, a pan-neuronal marker, overlaid on the UMAP of single cell transcriptomes. e, Additional examples of UMAPs for single cell transcriptomes of cell-type markers. Pou5f1 and Sox2 were used as markers for mES cells, Olig2 and Pdgfra for OLGs, Wfs1 and Satb2 for PGNs, and Slc6a3 and Calb1 for DNs. All markers show higher expression in their respective cell types. f, Distribution of regularized log (R-log) values for pseudobulk scRNA-seq datasets. For each cell type, cells were randomly partitioned into 3 pseudobulk replicates before pooling and normalizing reads. The distribution of R-log values is bi-modal for all cell types and pseudobulk replicates. To consider expressed genes for downstream analysis, a 2.5 R-log threshold (dashed red lines) was applied in all datasets. Genes with R-log ≥ 2.5 in all three pseudobulk replicates are considered expressed for that cell type. g, Example scRNA seq pseudobulk tracks of sequenced reads for marker genes in each cell type. Tracks were RPKM normalized to allow for cell-type comparisons. Markers were: Esrrb for mES cells, Pdgfra for OLGs, Wfs1 for PGNs and Slc6a3 for DNs. All markers are specifically expressed in their respective cell types. h, Exemplar plots of fluorescence-activated cell sorting (FACS) and gating strategy in midbrain VTA samples. Two biological replicate samples from independent mice, VTA-1 (top) and VTA-2 (bottom) were sorted to determine percentage of intact nuclei. Debris was excluded with a first gate (left; SSC/FSC plots, \( n = 10000 \) for VTA-1 and VTA-2, a total of \( n = 200000 \) DAPI positive events were sorted) and damaged nuclei with a second gate using DAPI (right; DAPI H/ DAPI-A plots, \( n = 8687 \) and \( 8748 \) for VTA-1 and VTA-2, respectively). The frequencies of parent populations are indicated by circles within the plots, and the target intact nuclei are indicated by the boxed area. i, Table indicating the total number of recorded events for VTA-1 and VTA-2 exemplar FACS gating as shown in Extended Data Fig. 4h, as well as the number and percentage of intact nuclei. j, Distribution of fragment sizes for (sc)ATAC-seq data used in this study. Bulk ATAC-seq data was generated from mES cells. snATAC-seq was generated from midbrain VTA, from which 216 nuclei were classified as DNs (see Methods). OLG and PGN scATAC-seq was collected from published data (see Methods, Supplementary Table 6). k, Aggregated sequencing reads at 2kb genomic regions centered on transcription start sites (TSSs). Nucleosome-free regions (NFRs; < 147 bp) were extracted from the ATAC alignment BAM files in each cell type (i.e. fragments). NFRs are enriched at the TSS for all ATAC-seq datasets. l, Number of fragments per cell/nucleus for sc/snATAC-seq datasets. The number of unique fragments per nucleus was highest for DNs. m, Single-cell accessibility maps for DNs generated in the present study were visualized together by UMAP embedding, and coloured by expression of DN marker genes or marker genes for OLGs and PGNs. Per-cell gene scores were calculated for each DNs marker gene (see Methods). DNs expressed DN-specific markers (973 genes; over-representation as measured by Z-Score; see Methods for marker selection), containing terms relevant for dopamine metabolism, synaptic transmission and behaviour. All enriched GOs were highly significantly enriched (one-sided Fisher’s exact permuted p-values = 0).
Extended Data Fig. 5 | Identification of contact density changes, TAD borders, and differences in contacts between cell types. a, GAM contact matrices for replicates 2 obtained from PGNs and DNs, within a 2-Mb region (50-kb resolution; Chr2:64,800,000-66,800,000). Contact density maps, TAD borders, pseudobulk scRNA-seq, and pseudobulk scATAC-seq tracks are indicated for each cell type below matrices. b, Distributions of TAD lengths in each GAM dataset. TAD length was calculated as the distance between two boundary points (defined as lowest insulation score point within a boundary). c, Pairwise comparisons of TAD boundary overlap between cell types. TAD boundaries were determined using insulation square method, using square size of 500kb, and the minimum score considered +1 bin on either side, giving a constant total of 150-kb TAD boundaries. The matrix of percentages of common TAD boundaries is not symmetrical as the percentage of overlap between boundaries varies with the direction of the comparison. The first dataset in the comparison is specified on the y axis, and the second on the x-axis. d, Four-way comparison of TAD boundary overlap between all cell types is shown as an UpSet plot. TAD boundaries were defined as in 5c. e, Average insulation score profiles centered on cell-type specific TAD borders show low average insulation scores in the cell type where the borders are detected, with highly significant differences at central border window with all other cell types (two-sided Mann-Whitney test for central TAD border window in unique cell-type border and compared to all other cell types: "****"p < 0.0001; p = 1.1x10^{-20}, 1.2x10^{-15}, and 1.0x10^{-14} for mES cells compared to OLGs, PGNs and DNs, respectively; p = 6.0x10^{-19}, 2.4x10^{-12}, and 4.1x10^{-11} for OLGs compared to mES cells, PGNs and DNs, respectively; p = 1.0x10^{-15}, 3.8x10^{-10}, and 8.5x10^{-08} for DNs compared to mES cells, OLGs and PGNs, respectively). f, Venn plots show overlap between TAD boundaries in PGN or DN replicates 1 and 2. Overlaps were performed by comparing replicate 1 (R1) to replicate 2 (R2), and conversely R2 to R1. g, Average insulation score profiles of common TAD borders (first UpSet plot group) centered on the lowest insulation point within each TAD border are shown for each cell type (two-sided Mann-Whitney test for central TAD border window in mES cell border and compared to each brain cell-type: "****"p < 0.0001; p = 8.6x10^{-10}, 1.5x10^{-18}, and 1.0x10^{-18} for mES cells compared to OLGs, PGNs and DNs, respectively). h, Percentage of TAD borders containing expressed genes (R-log ≥ 2.5) in each cell type for the groups shown in d. Higher percentage of borders contain expressed genes in groups with shared borders in two or more cell types. In all cell types, brain cells have a higher percentage of borders with expressed genes compared to mES cells. i, Average insulation score profiles of common TAD borders (first UpSet plot group) centered on cell-type specific TAD borders show low average insulation scores in the cell type where the borders are detected, with highly significant differences at central border window with all other cell types (two-sided Mann-Whitney test for central TAD border window in unique cell-type border and compared to all other cell types: "****"p < 0.0001; p = 1.1x10^{-20}, 1.2x10^{-15}, and 1.0x10^{-14} for mES cells compared to OLGs, PGNs and DNs, respectively; p = 6.0x10^{-19}, 2.4x10^{-12}, and 4.1x10^{-11} for OLGs compared to mES cells, PGNs and DNs, respectively; p = 1.0x10^{-15}, 3.8x10^{-10}, and 8.5x10^{-08} for DNs compared to mES cells, OLGs and PGNs, respectively; p = 6.7x10^{-10}, 1.8x10^{-12}, and 8.5x10^{-08} for DNs compared to mES cells, OLGs and PGNs, respectively).
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Identification of domain melting in long expressed genes. a, Cumulative probability of insulation square scores ranging from 100 – 1000 kb for Grik2 in all cell types and replicates (left). Comparison between PGNs replicates 1 and 2 and mES cells, with maximum distance (d) and TAD melting scores (right). Cumulative probability distributions of insulation scores and domain melting scores for Grik2 in PGNs, Dscam in PGNs, and Magi2 in OLGs (right). All genes were compared to mES cells, with maximum distance (d) indicated for each comparison. b, Example of domain melting for Magi2 in OLGs. c, Correlation of replicate domain melting scores for replicates 1 and 2 in PGNs and DNs (two-sided Pearson's R product-moment correlation was calculated for all 479 long genes; ****p < 2.2x10^{-16} for both PGNs and DNs). d, Domain melting scores for each gene (n = 479) in PGNs R2 and DNs R2, compared to mES cells. Genes with melting scores > 5 are coloured in each cell type. Density estimates of length-scaled RNA reads per million (lsRRPM) transcription levels are shown for genes with melting scores > 5 (coloured by cell type) compared to non-melting genes (grey; two-sided Wilcoxon rank-sum test; ****p = 5.4x10^{-9} and 6.5x10^{-11} in PGNs and DNs, respectively). e, Melting genes have higher density of open chromatin regions throughout their gene bodies (length-scaled ATAC-seq RPM values; lsARPM), especially in PGNs and DNs, and to a minor extent in OLGs (two-sided Wilcoxon rank-sum test; p = 0.05, ****p = 0.0001) compared to non-melting genes (grey; two-sided Wilcoxon rank-sum test; ****p = 2.6x10^{-6} and 2.2x10^{-16} in PGNs and DNs, respectively). f, Domain melting scores compared to length-scaled ATAC-seq reads per million (lsARPM) transcription levels for each gene (n = 479) in PGNs R2 and DNs R2. Density estimates of lsARPM open chromatin levels are shown for genes with melting scores > 5 (coloured by cell type) compared to non-melting genes (grey; two-sided Wilcoxon rank-sum test; ****p = 2.6x10^{-6} and 2.2x10^{-16} in PGNs and DNs, respectively). g, Long genes within the top 3% melting scores in any cell-type (24 of 44 genes) have a higher likelihood of sensitivity to topoisomerase inhibition compared to genes with intermediate melting scores (42 of 261) and genes with no domain melting (27 of 174; two-sided χ² test; ****p-value = 5.0e-9). h, Heatmaps of genes with domain melting in OLGs, and with domain melting in at least 1 replicate for PGNs and DNs, clustered by change in transcription level (length-scaled RNA RPM; lsRRPM) from mES cells to brain cell type. ATAC-seq (length-scaled ATAC RPM; lsARPM), compartments in each cell-type, and percentage of mES cell lamina- and nucleolus-associated domain (LAD° and NAD°, respectively) in mES cells are shown for comparison. The density of the change in lsRRPM, lsARPM, and melting scores are shown for each cluster (violin plots on right). Compartment changes are shown as bar plots (lower right). i, mES cell LAD association (defined as > 50% of gene body with feature) for genes with or without melting domains in brain cell types and replicates. For DNs and OLGs, genes with domain melting were less likely to be LAD associated in mES cells, compared to non-melting genes (Two-sided Fisher's exact test; **p < 0.01, ***p < 0.001; p-values from left to right, p = 0.001, 0.272, 0.209, 0.003, 0.0001). j, mES cell NAD association (defined as > 50% of gene body with feature) for genes with or without melting domains in brain cell-types and replicates. For DNs and OLGs, genes with domain melting were less likely to be NAD associated in mES cells, compared to non-melting genes (Two-sided Fisher's exact test; *p < 0.05, **p < 0.01; p-values from left to right, p = 0.003, 0.272, 0.209, 0.055, 0.008).
Extended Data Fig. 7 | Characteristics and mechanisms of domain melting in long expressed genes. a, Contact density maps for each cell type and replicate, at the Nrxn3 locus, calculated using insulation square sizes ranging from 100 − 1000 kb. Contact density is reduced in PGNs and DNs replicate 2 (R2), similar to R1 but occurring in slightly differing regions of the gene. 

b, Contact density maps for each cell type and replicate, at the Rbfox1 locus. Contact density is reduced in OLGs and PGNs R2, in the same region as R1.

c, Ensembles of polymer models were produced for the Nrxn3 locus in mES cells and in DNs from experimental GAM data using PRISMR modelling (n= 450). The quality of the models was verified by applying in-silico GAM to the ensemble of polymers and comparison between NPMI-normalized contact matrices from in-silico and experimental immunoGAM (Pearson r = 0.72 and 0.79 for mES cells and DNs, respectively). Colour bars below in-silico matrices highlight the position of domains in DNs and are used to colour the polymer examples shown in Fig. 3c and Extended Data Fig. 7d.

d, Additional examples of polymer models for the Nrxn3 locus in mES cells and DNs. The Nrxn3 melted TAD is represented by the green coloured region and is more decondensed in DNs than mES cells. See Fig. 3c for location and colouring of the domains.

e, Distribution of gyration radii of all domains in polymer models for mES cells and DNs (see Fig. 3c for location and colouring of the domains; n= 450, two-sided Mann-Whitney test between mES cells and DNs; dashed lines indicate quartiles; ****p<0.0001; domains from left to right: p= 3.0e-151, 0.0005, 1.1e-92, 2.0e-147, 7.3e-40, 2.5e-67).

f, Exemplar images of whole gene cryo-FISH for Rbfox1 (green) in mES cells and PGNs, using probes that label the whole gene. Nucleoli (purple) were detected by an anti-nucleophosmin 1 antibody. Yellow inset of the ~400 nm section shows a single nucleus. Inset on nuclear section (yellow box) with Rbfox1 FISH signal and each imaging channel. Yellow outline indicates region of Rbfox1 signal used for area measurement and localization to nuclear landmarks.

g, Exemplar images of tri-colour cryo-FISH for Rbfox1 TSS (teal), Mid (green) and TES (purple) in mES cells and PGNs (see Fig. 3e for schematic). Yellow inset of the 400 nm section shows a single nucleus. Inset on nuclear section (yellow box) with Rbfox1 FISH signal and each imaging channel. Yellow outline indicates region of Rbfox1 signal used for center of mass distance measurements.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Melting genes often show increased contacts with their own chromosome.  

**a.** Melting genes are more likely to gain intra-chromosomal contacts in PGNs and DNs R1, but not OLGs, compared to mES cells (two-sided Wilcoxon rank-sum test; **p<0.01, ***p<0.001; p-values from left to right, p = 0.003, 0.0003, 0.329). Median trans-cis contact ratios were calculated for each gene with domain melting in DNs, PGNs, or OLGs, and compared to mES cells. **b.** Median trans-cis contact ratios were calculated for each gene with domain melting in PGNs R2 or DNs R2. Median trans-cis ratios were significantly lower for PGNs and DNs R2 melting genes when compared to mES cells (two-sided Wilcoxon rank-sum test; *p<0.05, ***p<0.0001; p-values were p = 0.037 and 0.0003 for PGNs and DNs, respectively). **c.** Correlation of median trans-cis ratios for all long genes (> 300kb) in R1 and R2 for PGNs or DNs. In PGNs, median trans-cis ratios were significantly correlated between replicates, with a high correlation value (Two-sided Pearson's R product-moment correlation; R = 0.9, ***p<0.0005). **d.** Median trans-cis contact ratios were calculated for each gene without domain melting. Non-melting genes show no preference for changes in trans-cis contact ratios between brain cells and mES cells (two-sided Wilcoxon rank-sum test). **e.** The Rbfox1 locus gains contacts with other chromosomes in PGNs, compared to mES cells. Trans-cis contact ratios were determined by the mean ratio between trans NPMI scores and cis NPMI scores (250kb genomic bins), and normalizing each ratio as a percentile for each chromosome. Inset (grey shaded region) shows a 7Mb region (Chr16: 3,000,000-10,000,000) containing the Rbfox1 gene (blue shaded region). **f.** Trans-cis contact ratios are shown for chromosome 12 in mES cells and DNs. Inset (grey shaded region) shows a 7Mb region (Chr12: 85,000,000-92,000,000) containing the Nrxn3 gene (green shaded region). **g.** Median trans-cis ratios for genes with melting domains, separated by association with NAD association (defined as > 50% of gene body with feature). For DNs, median trans-cis ratios were significantly decreased when compared to mES cells, regardless of association with NADs (two-sided Wilcoxon rank-sum test; *p<0.05, **p<0.01; p-values from left to right, p = 0.927, 0.233, 0.100, 0.010, 0.044, 0.003). For PGNs, median trans-cis ratios were significantly decreased for non-NAD associated genes (**p<0.01), and trending toward significance for NAD-associated genes, when compared to mES cells (p=0.1). OLGs had no significant differences in median trans-cis values for both NAD associated and non-associated genes, when compared to mES cells. **h.** Median trans-cis ratios for genes without melting domains, separated by association with NAD association (defined as > 50% of gene body with feature). NAD-associated genes had significantly lower trans-cis values in all brain cell types when compared to mES cells (two-sided Wilcoxon rank-sum test; **p<0.01; p-values from left to right, p = 0.002, 0.205, 0.013, 0.147, 0.002, 0.911). For all brain cell types, non-melting genes that were not associated with NADs had no significant differences in median trans-cis values when compared to mES cells.
Significant differential contacts < 5Mb distance (window pairs):

- **PGNs R1**: 173,904
- **DNs R1**: 167,232

Accessible chromatin peaks:

- **PGNs**: 53,256
- **DNs**: 55,362

Map accessible regions to unique windows:

- **PGNs**: 23,793
- **DNs**: 17,636

Select motifs for in-depth analysis based on:

1. Accessible motifs within unique windows > 5%
2. Differential TF expression -log10(p. adj.) > 3

- **PGNs**: 33 TFs
- **DNs**: 17 TFs

Heterotypic TF pairs: 50*49/2 = 1225

Homotypic TF pairs: 50

Total TF pairs: 1275

Keep window pairs with at least 1 feature pair

- **PGNs**: 45,483 (26% of differential contacts)
- **DNs**: 19,330 (12% of differential contacts)

Extract unique windows:

- **PGNs**: 45,699
- **DNs**: 43,902

HOCOMOCO v11 Motif database: 529 motifs

Filter motifs by TF expression (rlog > 2.5): 218 TFs

Motif finding based on presence within accessible peaks

Top 5% most differential contacts

- **PGNs R1**: 1,739,044
- **DNs R1**: 1,672,323

Differential TF pairs table for each window pair:

Case 1: Feature A in window 1 and Feature B in window 2
Case 2: Feature A in window 2 and Feature B in window 1

Heterotypic TF pairs: 50*49/2 = 1225

Homotypic TF pairs: 50

Total TF pairs: 1225

Keep window pairs with at least 1 feature pair

- **PGNs**: 45,483 (26% of differential contacts)
- **DNs**: 19,330 (12% of differential contacts)

Calculate Information gain and enrichment of TF pairs between PGNs and DNs

Select feature pairs of interest:

1. Top 10 feature pairs by Info Gain Score
2. Top 5 feature pairs by PGNs enrichment
3. Top 5 feature pairs by DNs enrichment
Extended Data Fig. 9 | Analysis of transcription factor binding sites and differentially expressed genes in GAM differential contacts between DNs and PGNs.

a, GAM contacts from PGNs and DNs (mouse replicate 1) were normalized (Z-Score) and subtracted to produce differential contacts matrices. Top 5% differential contacts ranged 0.05-5 Mb. Contacts containing TF motifs within accessible chromatin on each contacting window were selected in most (top 5) enriched in PGNs or DNs or with highest discriminatory power (information gain).

b, Distribution of the number of ATAC-seq peaks per 50kb GAM window in DNs and PGNs (upper panel; mean(μ) = 2.6 and 2.0 in DNs and PGNs, respectively). Number of base pairs covered by ATAC-seq peaks per 50kb GAM window in DNs and PGNs (lower panel; μ = 1270 and 1326 in DNs and PGNs, respectively).

c, Correlation plot of cell type and replicates for differential gene expression analysis. Pseudobulk replicates correlate most highly with one another, followed by brain cell types. Right, heatmap of differentially expressed (DE) genes between PGNs and DNs, clustered by cell type.

d, Selection of TF motifs based on percentage of TF motifs in accessible regions within unique windows (> 5%) and differential expression between PGNs (Benjamini-Hochberg corrected two-sided Wald test; log10(p. adj.) < 3) and DNs (log10(p. adj.) ⩾ 3). PGN-selected TFs (33) are shown in blue, DN-selected TFs (17) are shown in green. A list of selected TFs are shown below, with TF motifs continuing after the TF enrichment analysis in (f) coloured in blue (PGNs) or green (DNs).

e, Full pipeline to determine pairs of genomic windows in GAM differential contacts containing transcription factor binding sites.

GAM contacts from PGNs and DNs were normalized and compared to produce a differential Z-Score matrix with a 0.05-5 Mb distance range. The top 5% differential contacts with > 0.15 NPMI values for each dataset were extracted from the differential matrices. Accessible chromatin regions were mapped to the top differential contacts. Next, TF motifs were filtered based on expression in at least one cell type. Accessible regions in differential contacts were used to determine the percentage of TF motifs within unique windows. To find TFs with the potential to drive contact specificity between DNs and PGNs, we chose for further analyses the TF motifs that were found in DN or PGN accessible regions within differential contacts which (1) were present in at least 5% of contacts, and (2) the TFs were differentially expressed between DNs and PGNs (-log10(p.adj.) ⩾ 3). The 50 TFs which met the requirements were further investigated to determine the frequency of each motif pair (TF feature pair) in PGN and DN differential contacts. The top-20 TF feature pairs were selected for further analyses based: (a) on Information gain score (top 10 feature pairs selected), and (b) on enrichment in either PGNs (top 5 selected) or DNs (top 5 selected).

f, TF motif pairs selected by enrichment scores in DNs or PGNs, or by the highest Information gain scores.

g, Overlaps of top 20 TF feature pair contacts for PGN and DN significant differential contacts. The top 40 groups with overlapping TF features are shown for each cell type.
Extended Data Fig. 10 | Features of top differential contacts containing pairs of TF binding sites. 

a, Percentage of contacts at each genomic distance for top differential contacts found in TF feature pair groups. Contacts in all groups are enriched at distances > 2 Mb.

b, Aggregated maps of average Z-scores for TF-containing contact groups in PGNs and DNs. The Z-Score was determined for each contact and a 200kb (4 genomic bin) radius. For each group, chromosome- and distance-matched contacts were randomly sampled three times from the genome-wide distribution (one exemplar is shown for each group).

c, Percentage of contacts (< 2 Mb) that fall within a TAD border in both windows, one window or no windows. For both cell types, most contacts do not overlap with TAD borders, with a slight no differences detected for top differential contacts found in TF feature pair groups, except a modest increase for contacts that have both windows with a border for Ctcf-Ctcf containing contacts in both PGNs and DNs.

d, Overlap of TF-containing contact groups with compartment identity in each contacting window. For both cell types, TF-containing contact groups were more likely to be in A-compartment in both contacting windows, compared to the genome-wide average and all top differential contacts.

e, TF motif network and community analysis. After determining the number of contacts for each TF pair, only pairs involved in > 20% of total TF-containing contacts were considered. A network was built with each TF as a node and contacts as the edge weight. Community detection was performed using a Leiden algorithm, before visualizing the network.

f, Network analysis and community detection for TF motifs found within DN or PGN differential contacts.

g, Overlap of TF-pair containing contacts with 1000 random circular permutations of PGN and DN expressed gene regions shows that the observed enrichments of contacts with genes in both windows are significantly higher than the expected distribution (two-sided t-test; **empirical p = 0.001 for all observed values tested). The enrichments were also seen, to smaller degree than for the TF-pair containing contacts, for all contacts between A-compartment windows.

h, Number of PGN or DN differentially expressed (DE) genes found in differential contacts according to sets of TF feature pairs.

i, Differential Z-Score matrix showing PGN-upregulated genes that form contacts across a ~4.5-Mb linear genomic distance (pink box; Chr11: 65,400,000-70,400,000). Upper right inset shows PGN significant differential contacts containing the Neurod group (contacts are shown in pink). Genes highlighted in blue are upregulated in PGNs. 

j, Differential Z-Score matrix showing DN-upregulated genes that form contacts across a ~5-Mb linear genomic distance (pink boxes; Chr1: 160,000,000-166,000,000). Upper right inset shows DN significant differential contacts containing the Foxa1-TF group (contacts are shown in orange). Genes highlighted in green are upregulated in DNs.

k, GAM contact matrices showing a 2.3-Mb region surrounding the Egr1 gene for PGNs R1 and R2 (Chr18: 33,700,000-36,000,000).
Extended Data Fig. 11 | Identification of compartments and differences between cell types. a, Open and closed chromatin compartments (A and B, respectively) display different genomic distributions in mES cells, OLGs, PGNs and DNs. Mouse replicates 1 and 2 (R1 and R2, respectively) are shown. Purple, compartment A; orange, compartment B. b, Comparison of compartment A/B membership in GAM datasets from PGNs and DNs and their replicates. Compartment changes show good overlap between replicates. Purple, compartment A; orange, compartment B. c, Pearson’s correlation of eigenvectors shows the largest differences between mES cells and brain cell types. d, UpSet plot showing all combinations of compartments changes Most genomic windows share membership to compartments A, followed by B, in all cell types. The most frequent compartment changes occur from compartment B in mES cells to A in all brain cells (pink box), followed by changes from A in mES cells to B in all brain cells (blue box). e, Compartment changes for each cell type comparison in each chromosome. Only compartments common to both replicates were used in the comparison. Brain cell types have higher overlap with each other as compared to mES cells. PGNs and DNs had the most overlap for most chromosomes. f, Violin plots of the distribution of compartment lengths show similar lengths between cell types. Right, percentage of the genome covered by A or B compartments in each cell type shows similar distribution between cell types.
Extended Data Fig. 12 | See next page for caption.
Extended Data Fig. 12 Genomic regions involved in strong long-range contacts in brain cells regions contain sensory receptor clusters in B compartments. a, Heatmap of gene expression for genes that change compartments between compartment B in mES cells to compartment A in all brain cells. Clustering of genes by expression shows six distinct clusters where clusters 3 and 4 contain genes that increase their expression between mES cells and all brain cell types. Gene ontology (GO) in Fig. 5a was done on genes from clusters 3 and 4 combined (pink box). Expression is calculated as the R-log value for each cell type (see Methods). b, Heatmap of gene expression for genes that change from compartment A in mES cells to compartment B in brain cells. Clustering of genes by expression identifies five clusters. Genes in cluster 4 are expressed in mES cells and show lower expression in the brain cell types; they were used for GO analysis presented in Fig. 5a (light blue box). Genes in clusters 2 and 3 are not expressed in mES cells nor brain cells; they were combined and used for GO analyses presented in Fig. 5b (dark blue box). Expression is calculated as the R-log value for each cell type. c, A higher proportion of Olfr and Vmn genes are found in B compartments in brain cells, compared to mES cells. d, GAM contact matrices show interactions between an Olfr/Vmn gene cluster and a second Olfr cluster (dashed boxes) separated by 25 Mb (Chr7: 80,000,000-110,000,000). The contacts between the two receptor clusters are strongest in OLGs, where the B compartment is strongest. e, GAM contact matrices show strong interactions that span a 30Mb distance between compartment B regions in OLGs, PGNs and DNs (purple circle), but not mES cells (Chr7: 52,000,000-95,000,000). Dashed boxes indicate contacts containing Olfr and Vmn gene clusters. f, Distribution of the top 20% of Z-Score normalized contacts for each genomic window at distances > 3 Mb (Two-sided Mann-Whitney U test; exact p-values are indicated on the plot). g, Summary diagram. The 3D genome is extensively reorganized in brain cells to reflect its gene expression specialization. (i) Contacts are rearranged at multiple scales, where formation of new TAD borders can coincide with genes important for cell specialization in all cell types. (ii) Domain melting occurs at very long genes which are highly transcribed and with high chromatin accessibility in brain cells. (iii) The most specific contacts in neurons contain complex networks of binding sites of neuron-specific transcription factors. Contacts bridge genes expressed in the neurons where the contacts are observed, with specialized functions, such as in synaptic plasticity (PGNs) and addiction (DNs). (iv) Finally, B compartments contain clusters of sensory receptor genes silent in all cell types which form strong contacts across tens of megabases.
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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: Leica Laser Microdissection v8.2; Leica Application Suite X v3.5.3.15976; BD FACSDiva v6.0.2.

Data analysis: bedtools v2.29.2; UpSetR v1.4.0; DrawR v8.1.4; samtools v0.1.19; deaptools v1.1.3; DISeqC2 v1.34.0; bowtie2 v2.3.4.3; 10x Genomic Cell Ranger v1.2.0; ARCHER v0.9.1; GenoMCArgetments v1.2.0.1; Regulatory Genomics Toolbox v0.12.2; kallisto package v0.3.1; STAMP v2.4.2a; RSEM v1.2.25; FastQC v0.11.4; Picard tools v2.5.0; Samba v0.6.8; GOTools v1.3.24; IGV v2.4.10; ANACONDA package v1.7.13; povray v3.8; Fiji software v2.1.0.0; v3.6; UCSC utilities; MELTRON and trans-cis contact ratio pipelines were deposited in https://github.com/pombo-lab/Meltron; custom python and R scripts for GAM window calling, GAM quality control, GAM genome sampling quality and restriction, production of NPMI matrices, aggregated maps, k-means clustering, calculation of insulation score and compartment calling were deposited in https://github.com/pombo-lab/Winkler-Kokalev_Harbula_Nature_2021.

For manuscripts utilizing custom algorithms or software that are not yet described in published literature, software must be made available to editors and 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

Raw fastq sequencing files for all samples from ON, PGN and OLG GAM datasets, together with non-normalized co-segregation matrices, normalized pair-wise chromatin contact maps and raw GAM segregation tables are available from the GEO repository under accession number GSE54364. Raw fastq sequencing files for
mESC GAM datasets are available from 4DN data portal (https://data.4dn数据中心/). The 4DN sample IDs for all samples used in the study are available in Supplemental Table 1.

Raw confocal and laser microdissection images, as well as images and ROIs for CRYO-FISH experiments are available at: https://github.com/pombe-lab/Windmill_Kukalev_Harabula_Nature_2021/tree/main/MIcroscopy Images.

Raw single cell mESC transcriptomes data are available from ENA data portal (https://www.ebi.ac.uk/ena/browser/nome). The ENA sample IDs for all samples used in the study are available in Supplemental Table 13. Raw single cell and bulk ATAC-seq bam-files for DNAs and mESCs, respectively, are available from the GEO repository under accession number GSE174034, together with processed bigwig files. A public UCSC session with all data produced, as well as all published data utilised in this study is available at: http://genome-euro.ucsc.edu/rk/morris/Winck_Ng_2021_GEMsubstratpublication.

UCSC Table Browser: http://genome-euro.ucsc.edu/cgi-bin/hgTables; ECRG: https://www.thermofisher.com/order/catalog/product/A456735; Cell ranger nfdata-cell-ranger-atac-mm-101.2.01: https://support.10xgenomics.com/single-cell-atlas/software/biopolices/latest/advanced/references#overview; HOCOMOCO database v71: https://hocomoco11.lcc.unsw.edu.au/

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.

☐ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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

Life sciences study design

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

Sample size

The appropriate number of samples for a GAM dataset varies and depends on multiple parameters such as nuclear volume, level of chromatin compaction, quality of DNA extraction, etc. Since most of these parameters can be assessed only after the data has been collected and processed, we recommend that the optimal resolution is defined during the collection of each GAM dataset, rather than trying to estimate optimal sample size before data collection. GAM data can be collected in multiple batches from the same starting material, therefore the sample size can be increased until the desired resolution is achieved.

Resolution is determined by comparing the distribution of intra-chromosomal co-segregation frequencies for all possible pairs of loci at a given resolution, using the standard Poisson distribution. In case multiple datasets from different samples are analysed together, we recommend choosing the highest possible resolution appropriate for every dataset involved in the analysis. In the present study, we measured co-segregation frequencies for all GAM datasets, finding that 98.8 - 99.5% of all mapable pairs of windows were sampled at least once at 20 kb resolution considering all genomic distances. The script to test the quality of genome sampling at given resolution was uploaded to GitHub (https://github.com/pombe-lab/Windmill_Kukalev_Harabula_Nature_2021/blob/main/code/SAM.define.working.resolution.py).

For single cell ATAC-seq data, no statistical method was used to determine sample size, as in https://www.nature.com/articles/jad5930-018-00075-3-proof-t.pdf

For scRNA-seq (mESCs), no statistical method was used to determine sample size. Libraries were generated twice, from mESCs from different biological replicates, to account for experimental variability.

Data exclusions

The quality of individual GAM libraries was determined using a combination of several quality metrics: clustering of positive windows, sequencing depth and lack of sample contamination. Due to the nature of genome sampling by ultra-highly crosslinking, good quality positive windows are expected to cluster near to each other, while noise is expected to behave randomly and not cluster on the linear genome sequence. Positive windows in low-quality GAM samples (i.e. from the water controls, or sample not amplified during the whole-genome amplification reaction) often do not cluster with other positive windows, termed "orphan windows". In this study, an individual GAM sample was considered to be of good quality if it had > 70% orphan windows > 50,000 uniquely mapped reads and no signal of cross-well contamination, as determined by low A30 index score to the distribution of positive windows in all samples processed at the same time.

For single-cell ATACseq (midbrain VTA), single cells were considered of low quality (and removed from the analysis) if HTSS enrichment score was < 4 and there were ≥ 2500 unique fragments per cell. After processing of raw data and clustering, the DN population was identified (see Methods) and single-cell IDs were extracted. ATAC-seq fragments derived from DN single-cells were subset from the original VTA position sorted BAM file and grouped into a subset containing only DN fragments. The subset file was uploaded to GEO (GSE174034).

For sRNA-seq (mESCs), libraries were excluded from the analysis if they were derived from cells that appeared as debris or doublets/ multilets upon visual inspections of the 18 chip, or if the libraries appeared as outliers in number of sequencing reads or mapping statistics, as fully detailed in the Methods section.

Replication

For the two neuronal cell types, single animal replicates were produced and had similar results in all metrics tested. Variations in replicates are reported through the male and supplemental data.

Randomization

Randomization was not relevant to our study. The experiments and the subsequent analysis were performed on wild type animals or cells, where no treatment or disease comparison was performed. As described in the Methods section, our samples were processed in different labs by different people. There was no selection criteria for the wild type mice used in the study.

Blinding

Blinding was not relevant to our study. We did not perform clinical trials, nor compared disease models or different treatments. As described in the Methods section, our samples were processed in different labs by different people. There was no selection criteria for the wild type mice used in the study.
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.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ❌  | Antibodies            |
| ❌  | Eukaryotic cell lines |
| ❌  | Paleontology and archaeology |
| ❌  | Animals and other organisms |
| ❌  | Human research participants |
| ❌  | Clinical data |
| ❌  | Dual use research of concern |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ✅  | ChIP-seq               |
| ✅  | Flow cytometry         |
| ✅  | MRI-based neuroimaging |

### Antibodies

**Antibodies Used**

- **Pel-Freez Arkansas**, Catalog number P60101-0; Sheep anti-tyrosine hydroxylase, Lot number 4ac12175p.
- **Merck**, Catalog number MAB3422, Mouse anti-phospho-ERK1/2 (Thr202/Tyr204), Lot number 41438.
- **Abcam**, Catalog number ab106172; Chicken anti-2G2, DOT serum, Lot number 12473.
- **Invitrogen**, Donkey anti-sheep IgG AlexaFluor-488, Catalog number A-11051.
- mouse anti-ras in cell line 823 was a kind gift from Harris Busch.
- **Abcam**, Goat anti-Chicken Ig, AlexaFluor-488, Catalog number ab100260.
- **Invitrogen**, Donkey anti-mouse IgG , AlexaFluor-488, Catalog number A-10005.
- **Invitrogen**, Donkey anti-mouse IgG, AlexaFluor-555, Catalog number A-10078.

**Validation**

- **Pel-Freez Arkansas**, Catalog number P60101-0; Sheep anti-tyrosine hydroxylase, Lot number 4ac12175 - validated by Western blot in rat caudate lyate [here](https://www.pel-freez.com/wp-content/uploads/2011/11/2011-P60101-00.docx.pdf).
- **Merck**, Catalog number MAB3422, Mouse anti-phospho-ERK1/2 (Thr202/Tyr204), Lot number 244216G - validated by Western blot on Jurkat lysate [here](https://www.merck-kga.de/search/product/Anti-Phospho-ERK12-Clone-H11-4-MM-MF-MAB3422?referrer=1&https%3A%2F%2Fwww.google.com%2Fsafebrowsing%3Furl%3D&anchor%3DProductInformation).
- **Abcam**, Catalog number ab13970, Chiken anti-2G2, Lot number GR126981-21 - validated by Western blot in whole cell lysates of mouse astrocytes expressing a GFP plasmid [here](https://www.abcam.com/GFP-antibody-ab13970?utm_medium=sanp&campaign=mv0F740ZvB&source=rev_fedeadlee&gclid=CjwKCAjzemHbA9t0wea5FVDKnWjlJ2b0GhcE0k_h1m-bgUpjX0CjgC7mVNdmg6GBT0bCoeluQAOGhBwE).
- mouse anti-ras in cell line 823 was validated by Western blot in Hela nuclear extract [here](Valdez, B.C. et al. Identification of the Nuclear and Nucleolar Localization Signals of the Protein α20. J. Biol. Chem. 290, 23767-23775 (1994)).

### Eukaryotic cell lines

#### Policy Information about Cell lines

**Cell line source(s)**

- The mouse embryonic stem cells clone 46C derived from E14tg2 cells were provided by Dr. Domingos Henriques from Instituto de Medicina Molecular, Faculdade de Medicina Lisboa, Lisbon, Portugal.

**Authentication**

- 46C E14tg2 mESC are not listed in the ICLAC Register of Misidentified Cell Lines. The 46C E14tg2 mESC line was generated by insertion of an eGFP cassette under the control of the Sox1-Oct4 promoter in E14tg2 cells. Cells aligned with GFP sequence were identified in the G418 sequencing data from mESCs. Additionally, genome sequencing data from G418 mESC samples was mined for SNPs. Though G418 sequencing reads are sparsely distributed across the genome, there was a 66% overlap of G418 mESC SNPs with SNPs identified from the parental E14tg2 genome sequencing data [here](https://www.ncbi.nlm.nih.gov/geo/?term=SRX695228).

**Mycoplasma contamination**

- The cells were negative for Mycoplasma contamination. The Mycoplasma test was performed according to the manufacturer's instructions [here](https://www.chemcat.com/ATCC/402020).

**Commonly misidentified lines**

- No commonly misidentified cell lines were used in the study. (46C E14tg2 mESC are not listed in the ICLAC Register of misidentified cell lines [here](https://claco.org/databases/cross-contaminations/))

### Animals and other organisms

#### Policy Information about studies involving animals, ARRIVE guidelines recommended for reporting animal research

**Laboratory animals**

- All animals used in this study were from the species Mus musculus.
- The following mouse strains were used:
  - C57BL/6N (RRID: IMSR:CR:0077); W1 for mHATAC-eg experiments, mice, adult male, ages 7 and 9 weeks.
  - C57BL/6N mice were housed in a temperature controlled room at 22°C with humidity of 55±10% in individually ventilated cages with 12 hours light/12 hours dark cycle with free access to food and water ad libitum.
  - C57BL/6N (RRID: IMSR:CR:0077); W1 for G418 experiments, mice purchased from Charles River, adult male, 2-3 months old.
  - C57BL/6N (RRID: IMSR:CR:0077); W1 for G418 experiments, mice purchased from Charles River, adult male, 2-3 months old.
  - C57BL/6N and G418 mice had access to food and water ad libitum and were kept on a 12:12h light/dark cycle at 20-23°C at
Flow Cytometry

Plots

- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g., CD4-FITC).
  - The axis scales are clearly visible, include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
Male C57BL/6 (BD Transgenic Model) mice, ages 8 and 9 weeks, were sacrificed by cervical dislocation. Brains were removed and the tissue containing the telencephalic VTA was dissected from each hemisphere at room temperature and rapidly frozen on dry ice. Frozen tissue was homogenized in 500 µl cold 1X PBS buffer (130 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 1% SDS, 0.01% Tween-20, 0.01% Nonidet P-40 Substrate, 0.001% Dithiothreitol). Chilled wash buffer (500 µl, 100 mM Tris-HCl, pH 7.4, 30 mM NaCl, 2 mM MgCl₂, 1% SDS, 0.1% Tween-20) was added to the homogenate, and the suspension was passed through 30 micrometer GelTrica strainers (Th. Geyer, cat# 76485779). The final ~500 microliter nuclei suspension was stained with DAPI (final concentration 0.08 microg/ml) for 5 min.

Instrument
BD FACSAria III Flow Cytometer

Software
BD FACSDiva v 8.2.1

Cell population abundance
Target population (intract nuclei) abundance was between 1-5% (see Extended Data Figure 4h–i)

Gating strategy
A first gate excluded debris in a FSC/SSC plot, and a consecutive, second gate in a DAPI-A/DAPI-H plot was used to exclude doublets and nuclei with incomplete DNA content.

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

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