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The dynamics of chromatin architecture in brain development and function

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Abstract

The brain is comprised of many different cell types with specialized functions which respond and adapt to the continuously changing environment, through tight spatiotemporal regulation of gene expression. The three-dimensional (3D) organisation of the genome is increasingly recognized as a major feature of gene regulation in brain cells, for the activation, repression and poising of gene expression, and in coupling transcription with RNA processing and transport. Here, we discuss the importance of dynamic chromatin organisation in the developmental patterning of the brain, and its role in fine tuning brain activity and plasticity. A better understanding of how disease-associated mutations interfere with chromatin organisation and long-range gene regulation will help reveal the molecular mechanisms underlying complex neurodevelopmental and neuropsychiatric disorders.

Introduction

The brain is a highly heterogeneous tissue that contains many interconnected neuronal and non-neuronal cell types with unique functions, which undergo coordinated development and maturation in space and time. Neurons are post-mitotic cells with an extremely long life span, which nevertheless rapidly adapt and respond to the changing environment whilst maintaining their homeostatic state and undergoing further functional specialization. Changes in chromatin states and in 3D genome architecture have been reported in connection with gene regulation during cellular plasticity in complex brain functions, and are increasingly important to understand the causative factors of disorders such as schizophrenia, depression, or Alzheimer's disease.

Regulation of chromatin states, through histone or DNA modifications and transcription factor binding, is linked with different brain functions and neurological disorders [1]. Gene expression is regulated locally through recruitment of the transcription machinery, through physical contacts between genes and their regulatory regions, and through physical associations of the genome with specialized nuclear landmarks, such as the nuclear lamina (**Figure 1)** [2–5]. Topologically associating domains (TADs) are ~1 megabase (Mb) domains of self-interacting regions, demarcated by boundaries which are often enriched for CCCTC-binding factor (CTCF) and cohesin, and whose disruption can lead to gene misexpression and disease [4–6]. At larger genomic scales, chromosomes segregate into compartments, classified as compartments A, enriched for active genes, and compartments B, containing mostly inactive regions [4,5]. In interphase, chromosomes occupy discrete nuclear territories. They establish inter-chromosomal contacts, which have been associated with autism-spectrum disorders [7,8]. Forming, maintaining and reorganizing chromatin architecture is a highly regulated process which implicates a wide variety of chromatin factors and changes in gene expression [9,10] through mechanisms that remain largely unexplored in brain cells.

The spatiotemporal expression of genes during development, which is increasingly influenced by environmental factors as the brain matures, is associated with extensive rewiring of 3D genome topology [11–13]. Dynamic chromatin changes are increasingly thought to help maintain plasticity of the post-mitotic cells, for example during neuronal activation [14,15], and are often associated with complex brain disorders [14], evolutionary traits [12] or cognitive processes, such as memory [16,17]. Here, we discuss how chromatin architecture relates with different aspects of the mammalian brain function, from development to neuronal activity, in health and in neurodevelopmental and psychiatric diseases.

Dynamics of chromatin architecture in brain development

The mammalian brain undergoes many changes throughout the individual's lifetime. During pre-natal development, progenitor cells differentiate into a wide variety of brain cells in a spatiotemporal coordinated manner. Adaptations of the brain cell networks occur in the first years of life and continue throughout adulthood as the brain adapts to organismal changes and the environment. Recent studies highlight how remodelling of chromatin architecture is highly cell-type specific and associated with the various transitions that brain cells undergo.

Rewiring of the enhancer-promoter interactome is a feature of lineage specification during early brain development and in specialized brain functions (**Figure 2a**) [11–13]. Expression of transcription factors and chromatin remodelers regulates transitions between active/inactive chromatin states, which lead to restructuring of the 3D chromatin architecture [13,18,19]. For example, the Polycomb repression network is disrupted as cells differentiate during cortical neurogenesis, whilst new chromatin loops form between neuronal transcription factor sites [18]. Widening of TADs and pruning of chromatin interactions occurs as human neurons differentiate and appears to reflect neuronal lineage specification. This process is specific for both human and murine neurons when compared to other cell types such as embryonic stem cells or glia cells. Interestingly, there is also an enrichment in neuronal-specific contacts that harbor schizophrenia-associated single nucleotide polymorphisms (SNPs) [13]. Mutations that reconstitute the disease-associated SNPs lead to misregulation of distal genes, consistent with previous observations in samples from schizophrenia patients [13]. In mouse dopaminergic neurons differentiated *in vitro*, TADs become increasingly inter-connected in hierarchical metaTAD structures that preferentially organize active regions (**Figure 2b**) [20]. The increased long-range contacts in metaTADs could be a consequence of the non-dividing nature of the neurons which may enable formation of longer range interacting domains, and may in turn result from or have roles in gene expression. Dynamic switches in A/B compartments also occur during the acquisition of the neuronal fate (**Figure 2c**) [12,18]. A study in mouse cortical neurons using Hi-C shows that the interactions between B compartments become stronger as opposed to those between A compartments [18]. Strengthening the interactions between inactive regions may contribute to cell fate commitment by partially restricting the possible states of 3D genome folding in the non-dividing neurons, whilst allowing for dynamic changes in the active regions during the regulation of plasticity. Large-scale dynamic shifts in chromatin condensation and topology are also observed in granule neurons using an electron microscopy-based approach [21].

Brain development and maturation continues after birth with refinement of cellular specialization and the synaptic networks, a challenging developmental time when many neuropsychiatric disorders arise. Changes in chromatin organization accompany the acquisition of unique functions by the different subclasses of neurons. Mouse cortical neurons

show large cell-type specific variations in gene transcription and enhancer repertoires at different post-natal stages [22]. Single-cell chromosome conformation capture during mouse cortical maturation shows rewiring of chromatin loops involving genes important for synaptogenesis, increased insulation in B compartments and large genomic rearrangements [23,24].

One aspect that remains poorly explored is the role of the environment in the dynamic changes in chromatin architecture during neuronal maturation. For the post-mitotic neurons, a dynamic chromatin architecture could be a key mechanisms to adapt to the changes in the environment and acquire more specific functions.The specialization of individual cells in response to the environment may be in part mediated through large-scale rewiring of chromatin organization, or itself lead to rewiring of 3D genome topology that may promote new gene expression states and responses (**Figure 2d**). Mature olfactory sensory neurons sense a wide variety of odors through expression of unique sets of olfactory genes. Their exquisite specificity and diversity of expression is achieved during neuronal maturation through the rewiring of inter-chromosomal contacts between clusters of enhancers and cell-specific sets of olfactory receptor genes [25,26]. The way in which the environment influences chromatin organization dynamics in the developing neurons might be specific for the brain region and the stimulus applied. Sensory deprivation in mice does not interfere with the dynamic changes in chromatin organization that occur during maturation of visual cortex neurons [23]. Future studies of how different environmental factors affect different brain regions will help understand how early life events shape cognition, towards the discovery of better diagnosis and treatments for neurological disorders.

The prolonged lifetime and continuous activity of neurons make them vulnerable to accumulating damage as the organism ages. Stochastic cell-to-cell variation in chromatin marks and transcription during aging can be linked to age-related cognitive impairments [27,28]. Global changes in chromatin architecture during aging have been reported in several cell types [29] but remain largely unexplored in the aging brain. It will be interesting to follow the repercussions of a prolonged lifespan on the neuronal plasticity of chromatin architecture and whether regulatory chromatin interactions, such as inter-chromosomal contacts, are stronger or/and more frequent as the neurons age. An increased understanding of these changes and the underlying mechanisms could provide valuable insights into the etiology of neurodegenerative disorders and age-related cognitive impairments.

Dynamics of chromatin architecture in neuronal activity

Neuronal activity induces changes in gene expression that are essential for complex cognitive processes. Several chromatin modifications regulate gene expression during neuronal activation in learning and memory [30]. Cell-type specific variation in chromatin

architecture are also increasingly reported to occur in neurons in different activation states suggesting that 3D genome organisation is modulated or helps fine tune activity-induced gene expression in different learning paradigms.

In the mouse cerebellum, motor learning induces cell-type specific rewiring of long-range enhancer-promoter interactions, which in turn regulate transcription of several gene modules and regional compartmentalization, potentially to co-regulate their transcription [17]. Deletion of cohesin, important for promoting chromatin interactions, disrupts chromatin architecture and impairs motor learning [17]. A similar activity-dependent rewiring of promoter-enhancer contacts involving activity-dependent genes occurs during activation of mouse cortical neurons [15]. In mouse hippocampal neurons, memory encoding is associated with increase chromatin accessibility, followed by transitions of large chromatin domains from inactive to active regions in memory consolidation, and by restructuring of enhancer-promoter regions during memory retrieval [16].

Dynamic changes in chromatin organization also emerge as a mechanism to fine-tune the timing and specificity of activity-induced gene expression (**Figure 3**). Rewiring of the promoter-enhancer contacts contributes to the order in which genes are activated *in vitro* in mouse cortical neurons. For example, immediate-early genes (IEGs) form shorter and faster interactions with their enhancers after the activation signal, whilst secondary response genes (SRGs) form longer-range interactions over a longer period of time [14]. Rewiring of chromatin interactions may also help regulate the type of response to different stimuli. In mouse cortical neurons, the IEG *cFos* interacts with different enhancers depending on the stimulus applied [31]. Prolonged neuronal activation in the mouse cortex in *status epilepticus* leads to repositioning of the *Bdnf* gene from the inactive lamina-associated regions to the nuclear interior coinciding with its expression [32]. Further insights into how chromatin organization modulates brain activity are expected from studying the consequences of mutations in chromatin remodelling factors [33]. Conditional knockouts of several chromatin remodelers in the adult mouse brain impair neuronal activity and cognitive processes, especially long-term memory [34,35]. For example, conditional knockouts of CTCF impair chromatin organization of the memory-related genes *Bdnf* and *Arc* in the mouse hippocampus [36].

The brain contains a wide variety of neurons with distinct molecular and functional properties that develop over time. Neurons respond to stimuli in a cell-type and stimulus-specific manner and can acquire further specialized functions through changes in morphology and firing properties. Adapting to new functions while maintaining homeostasis is a challenging process that requires cellular plasticity and coordination. Increased cellular plasticity may be imparted through dynamic changes in genome architecture which in turn may help maintain newly acquired functions, through for example regulation of proteins and factors required for the new synaptic connections. Since changes in genome architecture are stimulus-specific

and can lead to gene co-regulation, they may provide a mechanism for a rapid synchronization of networks of neurons that respond to a specific input, and for fine tuning their response to a variety of simultaneous inputs. It also remains to be investigated whether changes in chromatin architecture have a role beyond gene regulation. For example, if chromatin architectural changes impact the structure of the nucleus and/or the diffusion of the molecules throughout the cell body.

Altered chromatin architecture in complex brain disorders

Neurodevelopmental, neuropsychiatric and neurodegenerative disorders are amongst the most prevalent human diseases. Genome-wide association studies (GWAS) have found many variants associated with complex brain disorders with the vast majority located in non-coding regions of the genome. They are thought to affect candidate regulatory elements of distal genes via long-range chromatin interactions, in a cell-type, developmental-stage and disease-specific manner. Identification of causal variants, and the cells in which the affected elements are active, remains challenging due to the complexity of the brain and of the clinical phenotypes. Recent advances that integrate GWAS with chromatin architecture and cell state emerge as promising tools to find candidate regions for further functional analysis [37,38].

Several studies show that reorganization of chromatin architecture during brain development often involves regions containing disease-associated variants [12,13,38,39]. H-MAGMA is a recently developed computational tool that assigns noncoding variants to genes based on their interactions. By applying H-MAGMA on Hi-C datasets from human cortical cells in pre- and post-natal tissues, the variants associated with neuropsychiatric diseases were often found associated with the regulation of early developmental genes, whilst those associated with neurodegenerative diseases were more often encountered in genes expressed in the mature brain [37].

Disease-associated variants often form cell-type specific regulatory interactions. A study on different populations of brain cells derived from human induced pluripotent stem cells shows, for example, that variants associated with unipolar disorder occur in regions important for the excitatory and hippocampal neurons, whilst Parkinson's disease variants are enriched exclusively in astrocytes [40]. Likewise, analysis of human post-mortem cortical tissue shows that sporadic Alzheimer's disease variants are largely found in microglia-specific chromatin interactions, whilst variants associated with different neuropsychiatric disorders are confined to neuronal-specific enhancer-promoter networks [41].

Studying chromatin architecture in post-mortem samples could also help explain the comorbidities that often accompany brain disorders. Analysis of human post-mortem midbrain dopaminergic neurons identified many domains harboring risk variants for schizophrenia that

are involved in inter- and intra-chromosomal contacts with distal regions harbording risk variants for increased body mass [42]. Interestingly, these interactions are enriched for regulatory motifs specific for dopaminergic and adipogenesis pathways, as well as reward and addiction, which could in part explain the predisposition of some of schizophrenia patients to metabolic related co-morbidities [42]. However, a larger number of samples will be essential to gain further insights into individual variability in predisposition to disease and response to treatments.

Although most reports on the role of chromatin architecture in complex brain disorders remain to this date correlative, they provide a substantial resource for future mechanistic explorations. In contrast only a small number of studies of neuronal dysfunction in disease provide direct links for a causal role of aberrant chromatin architecture [43–45]. For example, disease-associated tandem repeats are enriched in a subset of TAD boundaries. Expansion of the repeats at the *FMR1* gene in patients with Fragile X syndrome disrupts the local TAD boundary and regulatory interactions, resulting in a potential silencing mechanism of the *FMR1* gene in the diseased neurons [45]. Future advancements in the molecular tools that permit large-scale parallel investigations of multiple regions will provide the means to dissect the complexity of the brain.

Future studies will also have to account for environmental inputs and the contribution of sex to advance our understanding of the complexity of the disorders and explain disease predisposition and severity. For example, sleep is emerging as a crucial regulator of brain activity which is often disrupted in autism spectrum disorders. Gene expression changes have been reported in the neurons of sleep-deprived mice, but how these relate to chromatin organization and the underlying genetic factors remains to be investigated [46]. Studies on sex-specific effects of stress on the brain also show promising results [47]. For example, the fluctuating hormonal levels during the female oestrus cycle modify chromatin interactions between neuronal-specific genes and might help explain increased predisposition to anxiety and depression [48].

Dissecting brain heterogeneity

The extent to which chromatin organization differs among different brain cell types is beginning to emerge. Approaches that capture orthogonal information can help assign cell identity to genome-wide chromatin maps. For example, parallel mapping of chromatin architecture and DNA methylation using single-nucleus methyl-3C has enabled the reconstruction of cell-type specific chromatin maps from 14 different cortical cell types [49]. Current genomics approaches to map chromatin architecture often require tissue dissociation, cell sorting, which can affect cell physiology [50]. They often need large cell numbers which

are frequently pooled from different individuals, loosing the power to study individual responses or disease processes, which are essential especially in neuropsychiatry and neurodegeneration. Microscopy and genomics approaches that rely on tissue sections are beginning to overcome these limitations. For example, we have recently adapted genome architecture mapping (GAM) to map chromatin contacts in low numbers of specific brain cells, while preserving the architecture of the tissue, detecting cell-type specific chromatin architecture in mature neurons and oligodendrocytes [24].

Cell-to-cell variability in 3D genome topology within the same population of cells is another aspect that remains unexplored, particularly in the brain. Super-resolution chromatin tracing imaging based on the oligo-based technology shows diverse chromatin configurations at the TAD level in individual human fibroblast or cancerous cells, with some regions more likely than others to have defined TAD boundaries in single cells [51]. Similar observations were made in mouse embryonic stem cells using a sequencing-based approach called single-cell SPRITE (scSPRITE), which also showed cellular heterogeneity at the level of promoter-enhancer interactions [52]. How heterogeneity in 3D genome folding translates to fine modulation of gene expression and cellular functions in single cells remains to be explored, and requires further developments to allow multi-modal mapping of chromatin contacts, cell states and gene expression in the same cell. These efforts promise to revolutionize our views over what defines a cell type, by providing a fine molecular phenotyping of cell populations. In the brain, this could be used for example to study how a group of neurons within a population selectively respond to environmental cues during cognitive processes and how these responses are affected in complex brain disorders (**Figure 4**).

Although less explored, studying how individual genetic variability modulates brain function and responses to environmental challenges will be an additional goal to unravel the aetiology of complex brain disorders. Integrating GWAS with gene expression and chromatin architecture shows promising results in the discovery of disease-causative variants [37,38]. Grasping this vast amount of complexity remains challenging, as we often miss the environmental factors that interact with individual genetic variability in large scale population studies. Future molecular and computational tools that allow faster, finer and more integrative mapping of chromatin features will pave the path towards our understanding of brain complexity and function, and further help in developing diagnosis and personalized treatments.

Conclusions and future perspectives

Dynamic changes in chromatin architecture are emerging as key modulators of brain development and function. Through the action of chromatin factors, chromatin architecture helps regulate the expression of the transcription factor repertoire required for the differentiation and specialization of the vast and diverse population of brain cells. Changes in

chromatin topology also enable and fine tune the activity of brain cells, potentially contributing to the high plasticity of the brain to adapt to the environment. How changes in genome architecture integrate with those at the synaptic level remains to be explored. Future studies that compare chromatin architecture features in neurons and other cell types are also poised to deepen our understanding of the mechanisms that enable post-mitotic neurons to survive for a long time whilst responding and adapting to the environment.

Molecular and computational tools that dissect different levels of heterogeneity from orthogonal data will help understand how the brain can accomplish its unique functions. Over the years, technological advancements have led to a deeper and more robust classification of brain cell types, but several aspects of chromatin architecture remain poorly explored. For example, complex (multi-way) chromatin interactions are a feature of genomic regions with highest transcriptional activity in mouse embryonic stem cells [53,54]. The longevity of neurons suggest that complex chromatin interactions may be even more prevalent in neurons, and be especially implicated in the non-coding disease-associated variants. The application of genome editing to dissect the molecular mechanisms associated with 3D genome folding is also especially challenging in the brain, requiring the use of suitable CRISPR-based tools that edit the genome or manipulate chromatin architecture in specific brain cell types and developmental times. Given the dynamic nature of the cellular processes in the brain, further advances in technologies and model systems are needed to investigate how the brain functions in complex processes such as learning and memory, and to open the paths towards personalized diagnosis and treatments for complex brain disorders.

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This study presents high resolution maps of non-coding elements involved in human cortical neurogenesis by comparing chromatin accessibility in the germinal zone and the cortical plate in the human cerebral cortex. Hi-C interaction maps link distal regulatory elements to specific genes, and show that there are human gained enhancers involved in the neurogenesis process enriched in the outer radial glia cells. They also find that these enhancers contain variants associated to educational attainment, risk for diseases and intracranial volume. The study highlights the importance of chromatin organization in early neuronal development.

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Rajarajan et al. perform Hi-C in human induced pluripotent stem cells differentiated into glia and neuronal cells. They observe cell-type specific changes in chromatin architecture at different scales that reflect lineage commitment and cell function. Neurons have more longer-range chromatin loops in contrast to neuronal precursors or glial cells. They observe that neuronal topologically associating domains (TADs) are slightly wider, potentially as a result of sub-TADs joining. These changes are dynamic and a large proportion of interactions involve schizophrenia-risk loci predominantly in neurons.

* 14. Beagan JA, Pastuzyn ED, Fernandez LR, Guo MH, Feng K, Titus KR, Chandrashekar H, Shepherd JD, Phillips-Cremins JE: **Three-dimensional genome restructuring across timescales of activity-induced neuronal gene expression**. *Nat Neurosci* 2020, **23**:707–717.

Neuronal activation leads to a cascade of events, starting with fast transcription of immediate early genes (IEGs) and a slower and longer-term activation of the secondary response genes (SRGs). Beagan et al. study chromatin architecture at the IEGs and SRGs in response to activation and inhibition in mouse cortical cultured neurons. Using 5C-seq, they show that neuronal activation leads to changes in chromatin topology at the IEGs and SRGs loci. They show that neuronal activation leads to dynamic changes in chromatin interactions and classify these interactions into: invariable, de novo and decommissioned. Additionally, IEGs (Arc and cFos) form few shorter-range loops within 20 min of activation, prior to peak mRNA levels. In contrast, SRGs (Bdnf) engage in both activity-induced and pre-existing loops over longer-distances and these loops take a longer time to form. The authours suggest this as a potential mechanism through which activity-dependent response of the neurons is regulated in a temporal manner. Additionally, they indicate that the formation of these loops might be disrupted in complex disorders such as autism spectrum disorders and schizophrenia.

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Fernandez-Albert et al. developed a nuclear tagging approach that allows isolation of neurons following activation to perform neuronal-cell type specific transcriptional and chromatin states for *in vivo* explorations. By applying the tagging approach in mouse forebrain neurons in the context of novel object exploration and in pathological conditions (status epilepticus), they

show that activation leads to changes in chromatin interactions, accessibility and other epigenetic modifications. There are overlaps in the mechanisms that induce changes in chromatin in response to context-learning and epilepsy, but they trigger different epigenetic signatures. They identify changes that occur fast in response to activation (e.g. rapid increase in chromatin accessibility at immediate early genes), but also some changes that persist over time. AP1-induced chromatin loops persist over a longer time after neuronal-activation, which suggests this as a potential mechanism for memory-associated paradigms.

** 16. Marco A, Meharena HS, Dileep V, Raju RM, Davila-Velderrain J, Zhang AL, Adaikkan C, Young JZ, Gao F, Kellis M, et al.: **Mapping the epigenomic and transcriptomic interplay during memory formation and recall in the hippocampal engram ensemble**. *Nat Neurosci* 2020, doi:10.1038/s41593-020-00717-0.

Marco et al. use the targeted recombination in active populations (TRAP) mouse system to map chromatin architecture and state during different stages of memory formation. The authors show that during memory encoding chromatin accessibility increases followed by transition of large chromatin domains from inactive to active regions during late stages of memory consolidation. Reactivation of the neurons during memory retrieval leads to rewiring of promoter-enhancer interactions and regulation of genes important for the synaptic compartments. The reorganization of chromatin in reactivated neurons involves a large subset of regions primed during memory encoding.

** 17. Yamada T, Yang Y, Valnegri P, Juric I, Abnousi A, Markwalter KH, Guthrie AN, Godec A, Oldenborg A, Hu M, et al.: **Sensory experience remodels genome architecture in neural circuit to drive motor learning**. *Nature* 2019, **569**:708–713.

Yamada et al. show that sensory experience in the mouse granule neurons leads to active remodeling of chromatin organization. Neuronal activation induces reorganization of long-distance enhancer-promoter chromatin interactions and restructuring of the transcriptionally active compartments. Conditional CRISPR knock out of cohesin in the anterior dorsal cerebellar vermis granule neurons disrupts chromatin interactions, impairs transcription and motor learning.

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Tan et al. use Dip-C in mouse olfactory sensory neurons. They identify single-cell specific chromatin organization with olfactory receptor (OR) genes and enhancers predominantly clustering in the interior of the nucleus in contrast to other cell types. Individual olfactory neurons harbor multiple aggregates of OR genes, in support for the theory that individual neurons are guided by individual-specific ORs.

* 37. Sey NYA, Hu B, Mah W, Fauni H, McAfee JC, Rajarajan P, Brennand KJ, Akbarian S, Won H: **A computational tool (H-MAGMA) for improved prediction of brain-disorder risk genes by incorporating brain chromatin interaction profiles**. *Nat Neurosci* 2020, **23**:583–593.

Sey et al. developed a computational method (H-MAGMA) that uses Hi-C data to assign sets of desired SNPs to cognate genes. They apply H-MAGMA to five psychiatric disorders and four degenerative disorders Hi-C datasets from human fetal and adult brain. They show that

neuropsychiatric disorders exhibit neurodevelopmental origins, whilst neurodegenerative disorders had adult origins. Fetal enrichment showed predominantly neurodevelopmental disorders (autism spectrum disorder, attention-deficit/hyperactivity disorder), while the adult enrichment was more robust in late onset disorders such as bipolar disorder, schizophrenia and major depressive disorder. Using Hi-C and expression data from iPSCs-derived neurons and astrocytes, they also show that psychiatric disorder genes tend to be expressed in excitatory neurons, whilst the neurodegenerative-disorders show an increase in transcription over time in more cell types.

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Nott et al. perform chromatin architecture and state analysis on different cell types isolated from the human cortex and identify cell-type specific enhancer-promoter interactions and transcription binding motifs. They perform linkage disequilibrium score regression analysis and identify a strong enrichment of disease-associated variants within neuronal enhancers and promoters for different neuropsychiatric and behavioral traits, whilst sporadic Alzheimer's disease variants were found mostly in microglia enhancers.

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Sun et al. analyze Hi-C data from human embryonic stem cells and human fetal cortical neurons and identify that a subset of disease-associated short tandem repeats (daSTRs) are more enriched significantly in TAD boundaries with a extremely high densitiy of CpG islands than normal-length repeats. The authors propose a model in which the combination of high-density CpG and TAD boundary increases the susceptibility to STR instability. They perform 5C in samples from patients with Fragile X syndrome and show that at the *FMR1* gene daSTRs disrupt the TAD and ablate CTCF occupancy. These architectural changes correlate with silencing of the *FMR1* gene, and result potentially from the disruption of regulatory interactions between the gene promoter and neighbouring enhancers.

Figures

Figure 1

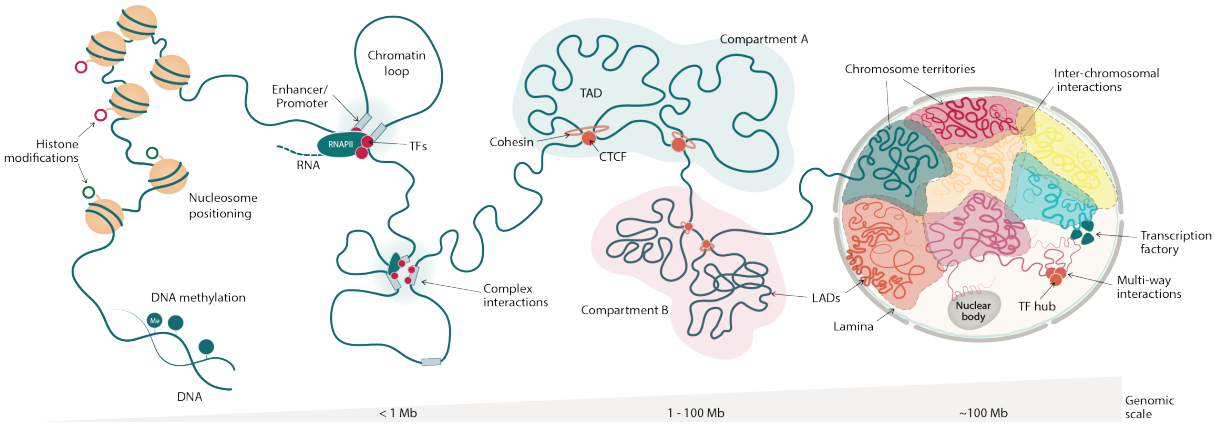

Figure 1. Levels of genome organization. The mammalian genome is tightly packed inside the nucleus at multiple levels. At the finest scale, DNA is wrapped around histone proteins into nucleosomes that form the chromatin fibre. Epigenetic modifications such as DNA methylation or histone post-translational modifications modulate chromatin compaction and availability to the transcriptional machinery. Chromatin interactions bring together distal regulatory elements with their target genes through chromatin looping to regulate gene expression. These interactions can be complex (e.g. multiple enhancers regulate one promoter). At the submegabase scale, chromatin is folded into domains of interacting chromatin known as topologically associating domains (TADs). TAD boundaries are often enriched in CTCF and cohesin. At larger genomic scales, open/active chromatin and condensed/inactive chromatin segregate spatially into compartments A and B, respectively. Repressed and highly condensed chromatin often associate with the nuclear lamina to form lamina-associated domains (LADs) or with the nucleolar periphery, a large nuclear compartment specialized in the synthesis and processing of the large 45S ribosomal RNAs (not represented). Chromatin is also organised in relation to other subnuclear compartments, such as transcription factories, transcription factor (TF) hubs or splicing speckles. Interphase chromosomes occupy discrete territories inside the nucleus, that form inter-chromosomal interactions.

Figure 2

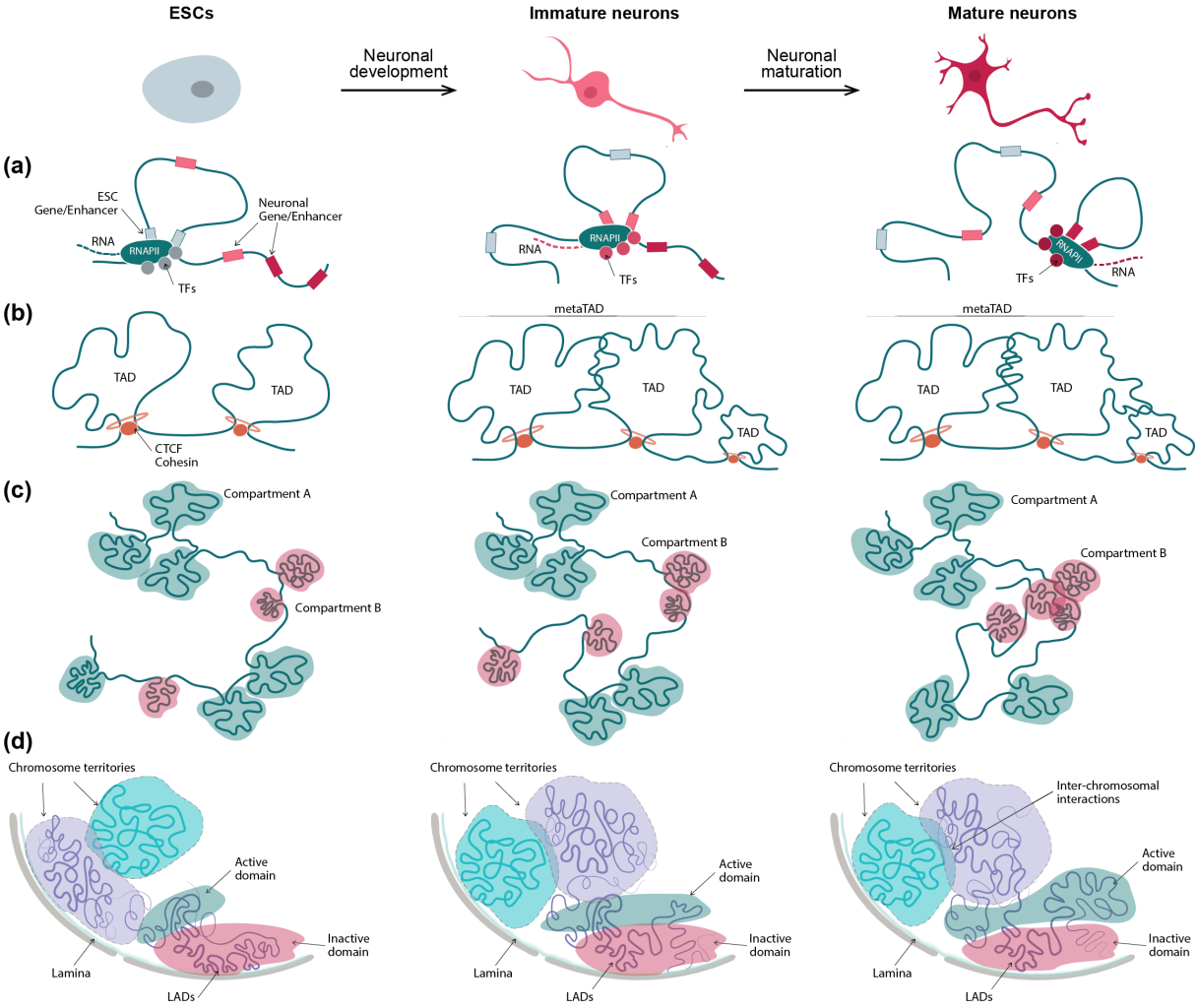

Figure 2. Dynamic changes in chromatin architecture contribute neuronal development and maturation.

Cell-type specific changes at different levels of chromatin architecture contribute to acquisition of cellular specialization and function during brain development and maturation.

(a) Global rewiring of promoter-enhancer interactions that control expression of genes important for neuronal development and maturation. Disease-associated variants are present in the dynamic interacting regions .

(b) Topologically associating domains (TADs) reorganise during ESC differentiation into neurons, including widening of TADs or formation of metaTADs [13,20].

(c) Changes in compartment identity during neuronal development and maturation are associated with gene activation and downregulation. Interactions between B compartments become stronger [18,23,39].

(d) Large chromatin domains change their location within the nucleus [21]. Chromosomes can reposition from a preferred association to the nuclear periphery to the nuclear interior [23]. Inter-chromosomal interactions regulate expression of genes important for cellular specialization during neuronal maturation [25,26]. Chromatin regions can transition from the silent environment at the lamina (lamina-associated domains; LADs) to a transcriptionally permissive nuclear interior where they can become transcriptionally active [55].

Figure 3

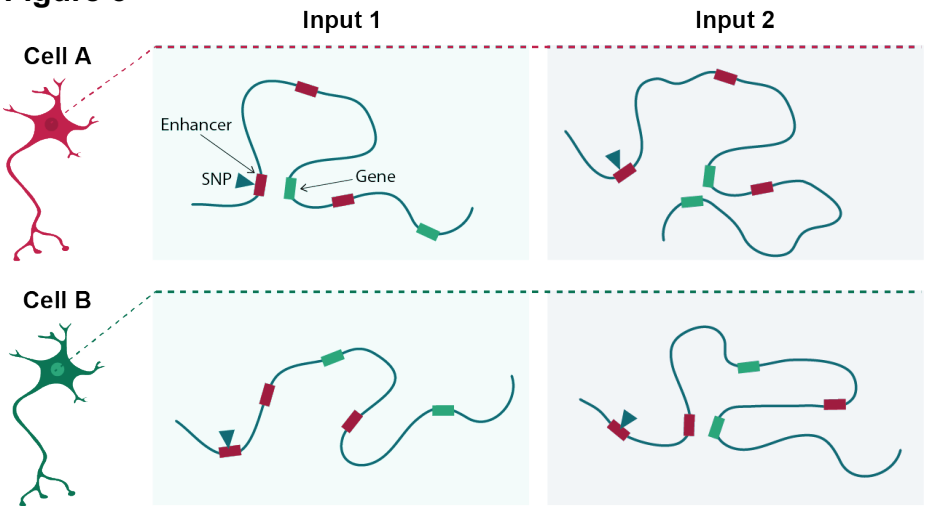

Figure 3. Cell-type specific changes in chromatin architecture modulate neuronal activity. Chromatin architecture changes dynamically during neuronal activation , in a cell-type and input (stimulus) specific [17,31]. Rewiring of promoter-enhancer interactions fine tunes the spatiotemporal expression of activity-induced genes [14,17]. Disease-associated single nucleotide polymorphisms (SNPs) often reside in non-coding regions and can cause misexpression of distal genes through altering chromatin interactions during neuronal activation [14], and these could have effects in specific cells or upon specific stimuli.

Figure 4

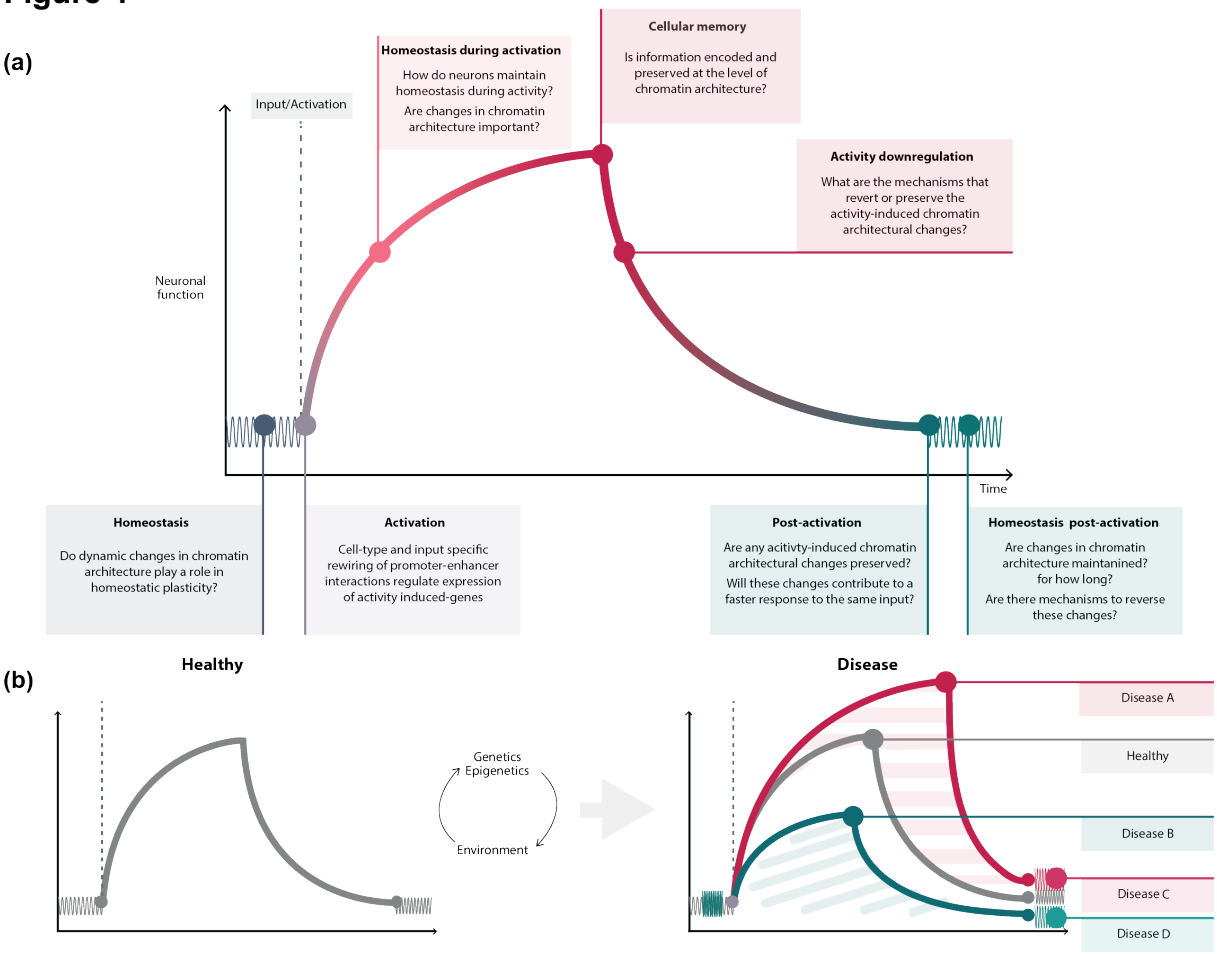

Figure 4. Key steps in neuronal function and specific aspects that may depend on dynamic chromatin architecture remodelling.
(a) Neurons respond and adapt fast to environmental cues through changes in synaptic plasticity. Homeostatic plasticity is a mechanism that preserves the structural and molecular integrity of neurons to avoid hyper- or hypo-excitability [56]. Regulation of homeostatic plasticity at the synaptic level has been extensively studied. Epigenetic modifications are also emerging as additional mechanisms [57] and chromatin-based mechanisms have been proposed [56]. Cell-type and input-specific changes in chromatin architecture occur during neuronal activation [14,15,17,31,32,32]. Synaptic changes that remain after activation are a form of storing information at the cellular level [57]. Future studies that map chromatin architecture pre- and post-activation will help reveal whether information is also stored through activity-induced chromatin architectural changes that are preserved over time [16]. The role of chromatin dynamics in how neurons maintain homeostasis during activity downregulation and post-activity also remain to be explored.
(b) Genetic, epigenetic and environmental factors regulate different levels of neuronal activity in the healthy brain. However, mutations and/or environmental stress can hamper neuronal activity and lead to complex brain disorders characterized by either hyper- (Disease A) or hypo-activity (Disease B), or by inefficient homeostatic control (Disease C, D). Disease-associated variants have been identified in cell-type specific interactions that regulate neuronal gene expression during development [13], maturation [22] or activity [14].