The interplay of seizures-induced axonal sprouting and transcription-dependent Bdnf repositioning in the model of temporal lobe epilepsy

Anna Skupien-Jaroszek, Agnieszka Walczak, Iwona Czaban, Karolina Pels, Andrzej Antoni Szczepankiewicz, Katarzyna Krawczyk, Błażej Ruszczycki, Grzegorz Marek Wilczynski, Joanna Dzwonek, Adriana Magalska

1 Laboratory of Molecular and Systemic Neuromorphology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland, 2 Laboratory of Molecular Basis of Cell Motility, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

☯ These authors contributed equally to this work.
† Deceased.
* a.magalska@nencki.edu.pl (AM); j.dzwonek@nencki.edu.pl (JD)

Abstract

The Brain-Derived Neurotrophic Factor is one of the most important trophic proteins in the brain. The role of this growth factor in neuronal plasticity, in health and disease, has been extensively studied. However, mechanisms of epigenetic regulation of Bdnf gene expression in epilepsy are still elusive. In our previous work, using a rat model of neuronal activation upon kainate-induced seizures, we observed a repositioning of Bdnf alleles from the nuclear periphery towards the nuclear center. This change of intranuclear position was associated with transcriptional gene activity. In the present study, using the same neuronal activation model, we analyzed the relation between the percentage of the Bdnf allele at the nuclear periphery and clinical and morphological traits of epilepsy. We observed that the decrease of the percentage of the Bdnf allele at the nuclear periphery correlates with stronger mossy fiber sprouting—an aberrant form of excitatory circuit formation. Moreover, using in vitro hippocampal cultures we showed that Bdnf repositioning is a consequence of transcriptional activity. Inhibition of RNA polymerase II activity in primary cultured neurons with Actinomycin D completely blocked Bdnf gene transcription and repositioning occurring after neuronal excitation. Interestingly, we observed that histone deacetylases inhibition with Trichostatin A induced a slight increase of Bdnf gene transcription and its repositioning even in the absence of neuronal excitation. Presented results provide novel insight into the role of BDNF in epileptogenesis. Moreover, they strengthen the statement that this particular gene is a good candidate to search for a new generation of antiepileptic therapies.
Introduction

The Brain-Derived Neurotrophic Factor (BDNF) is one of the most important neurotrophins in the brain. Acting via its synaptic receptor Tropomyosin-related kinase B (TrkB), BDNF is involved in neuronal differentiation, survival, and synaptic plasticity [1–3]. Thus, it plays an important role in the number of neurological and psychiatric disorders such as Parkinson’s disease [4], schizophrenia [5], depression [6], bipolar disease [7], and epilepsy [8–10].

Currently, it is known that BDNF is involved in the event of aberrant synaptic plasticity called mossy fiber sprouting, observed in temporal lobe epilepsy (TLE), which is one of the most common types of epilepsy in adults [11]. The level of both BDNF protein [12] and mRNA [13–15] were described to be elevated after seizures in the temporal lobe and hippocampi of epileptic patients. Experiments performed on animals and in vitro models showed that BDNF causes hypertrophy of granule neurons [16] and increased mossy fiber branching [17]. Moreover, intrahippocampal infusion of BDNF induced mild seizures with the development of mossy fiber sprouting [18]. Those findings support the involvement of BDNF in the aberrant synapse formation in TLE, however, underlying molecular mechanisms are still not clear.

The current trend in neuroscience is to look for mechanisms of neuronal functioning at a gene expression level. BDNF encoding gene is a so-called delayed, immediate-early gene induced in the later phase of neuronal activation [19]. It consists of 9 exons differentially expressed in humans, mice, and rats [20]. Regulation of BDNF expression after neuronal excitation has been quite well understood at the level of transcription factors and chromatin modifications. It is known that the 3’ end of the protein-coding exon is spliced to one of the eight 5’ exons, which are controlled by their unique promoters [20–22]. Moreover, Bdnf can be epigenetically down-regulated through DNA methylation and histone deacetylation. The aforementioned epigenetic changes result in the recruitment of REST/NRSF complex [23] and MeCP2 [24,25] and chromatin remodeling. Conversely, the up-regulation of the gene can be triggered by DNA demethylation and/or histone acetylation, which was already presented in both in vitro [20] and in vivo studies [26]. Importantly, the level of histone H3 acetylation at the Bdnf promoters IV and VI may underlie sustained up-regulation of transcription following chronic electroconvulsive shock [27]. Fukuchi et al. [28] showed that valproic acid, an inhibitor of histone deacetylases commonly used antiepileptic drug, increases expression of Bdnf under control of promoter I.

Studies of the last decade have shown that the genome is spatially organized within the nucleus [29]. Rearrangements of chromatin are involved in the regulation of gene expression, and the radial position of genes reflects their expression [30]. In differentiated cells, the nuclear periphery is a repressive environment, where heterochromatin is recruited to the nuclear lamina [31]. Artificial localization of gene at the nuclear periphery, by tethering to the inner nuclear membrane, is sufficient to induce silencing of its expression [32]. The role of chromatin structure in the regulation of gene expression in neurons remains still unexplored. Crepaldi and colleagues [33] showed that activity-dependent genes, including Bdnf, were repositioned to transcription factories after KCl induced depolarization in cultured cortical neurons. Moreover, in our previous studies [34] we showed that during neuronal excitation and epileptogenesis Bdnf alleles had been detached from the nuclear lamina and repositioned from the nuclear periphery toward the nuclear center. The observed phenomenon was associated with changes in Bdnf expression. However, it was not clear whether Bdnf repositioning had been a cause or a consequence of the gene transcriptional activity. Therefore, in the current study, we are addressing this interesting question.

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Materials and methods

Animals

The experiments were performed on young, adult male Wistar rats, weighing 170–250 g, obtained from Mossakowski Medical Research Centre, Polish Academy of Sciences. Animals were kept under a 12 h light/dark cycle, with unlimited food and water supplies. All procedures were performed with the consent of the 1st Local Ethical Committee in Warsaw (Permission number LKE 306/2017).

Induction of seizures

Seizures were evoked by two doses of kainate (5 mg/kg, Sigma-Aldrich) (0.5% solution in saline, pH 7), administered intraperitoneal in 1 h intervals, and scored as described by Helliier et al [35]. The animals were taken for further studies regardless of whether they fulfilled the criterion of the full status epilepticus, or not [35]. To reduce kainate-induced mortality (up to 10%), diazepam (25mg/kg) was administrated intraperitoneally 3–6 hours after seizure onset. To monitor animal health, the rats were constantly observed by experimenters, for 6 hours after kainate injection, and 4 hours/day in the subsequent 4 weeks. All animals were euthanized with an overdose of pentobarbital sodium (Nembutal 150 mg/kg, i.p.) and perfused with 4% paraformaldehyde in PBS at the end of the experiments, and the brain tissues were collected.

Estimation of clinical and morphological traits

Clinical and morphological traits such as the intensity of seizures, ruffling of the fur, forelimb clonus, body, and head tremor, losing posture, immobility and aggression have been estimated for 6 hours after kainate injection, and 4 hours/day in subsequent 4 weeks. The intensity of seizures upon kainate treatment was scored according to the 6-grade modified Racine’s scale (0-lack of seizures to 5- fully developed tonic-clonic seizures with a loss of posture) [35]. Ruffling of the fur was scored on a 3-grade scale (0-lack of ruffling to 2-extensively ruffled fur). Forelimb clonus was scored according to a 7-grade scale (0-lack of clonus to 6-very strong forelimb clonus). Body and head tremor was scored according to a 6-grade scale (0-lack of tremor to 5-very strong tremor). Loss of posture was scored according to the 4-grade scale (0-lack of loss of posture to 4 constant loss of posture). Immobility was scored according to the 10-grade scale (0-completely active to 9-lack of activity). Aggression was scored on a 4-grade scale (0-lack of aggression to 3-extreme aggression). To minimize the bias, two independent observers performed the scoring of clinical parameters. Mossy fiber sprouting was verified by immunofluorescent staining for synaptoporin and scored on a 5-grade scale (0-lack of sprouting to 4-very strong sprouting). The mean synaptoporin intensity was measured for each individual using ImageJ. Measurements were done on confocal images in a specific area of the molecular layer of the dentate gyrus depicted in Fig 2B. Additionally, two independent observers estimated sprouting visually in the same brain area directly using a microscope.

Primary neuronal hippocampal cultures and treatment

Primary neuronal cultures were prepared from the hippocampi of P0 rat brains as described previously [36]. Chemical long-term potentiation (cLTP) was initiated by stimulating the cells for 2 hours with 50 μM of picrotoxin, 50 μM forskolin, and 0.1 μM rolipram (all from Sigma-Aldrich). Transcription was inhibited by 2h exposure to 8mg/ml of Actinomycin D (Sigma-Aldrich). Histone deacetylases were inhibited by 12h treatment with 200 nM Trichotstatin A (Abcam) or 250 nM romidepsin (Cayman Chemical Company) for 2h.
Fluorescent in situ hybridization

Fluorescent in situ hybridization was performed according to the protocol of Cremer et al. (2008) [37] on 30 μm-thick brain cryosections of the 4% paraformaldehyde-perfused, 8 kainate-treated, and 4 control animals, as well as on 3 independent primary hippocampal neuronal cultures fixed with 4% paraformaldehyde at DIV 14 (day in vitro). As templates for Bdnf FISH probes, CH230-449H21 BAC obtained from Children’s Hospital Oakland Research Institute were used. Probes were verified on rat metaphase spreads. The probes were labeled using the standard nick-translation procedure. Biotinylated probes were detected using Alexa Fluor 488- conjugated avidin (Invitrogen), followed by FITC-conjugated rabbit anti-avidin antibody (Sigma-Aldrich).

Immunostaining

Immunostaining for synaptoporin was performed on 30 μm-thick brain cryosections of the 4% paraformaldehyde-perfused animal using standard immunofluorescent staining protocol [38]. 1 μg/ml of rabbit polyclonal anti synaptoporin (Synaptic Systems) antibody was used. The intensity of immunostaining was estimated using the 5-grade scale (0-no staining to 4-strong staining). The neuronal damage was examined by staining with Fluoro-Jade B (Millipore) according to the method of Schmued et al [39].

Image acquisition

Fluorescent specimens were examined under TCS SP8 confocal microscope (Leica) or Zeiss 800 confocal microscope (Zeiss), by sequential scanning of images, with a pixel size of 80 nm and axial spacing of 210 nm, using a PlanApo oil-immersion 63 (1.4 numerical aperture) objective.

Quantitative image analysis

The minimal distance from the nuclear periphery and Bdnf alleles in neuronal nuclei of animals was calculated using custom-written software, Segmentation Magick, described in Ruszczycki et al. [40]. For neuronal cultures, custom-written software Partseg was used, described in [41]. At least 120 nuclei, from 3 independent experiments were analyzed for each experimental variant.

Real-time reverse transcriptase-PCR for Bdnf mRNA

Total cellular RNA was isolated from three independent hippocampal primary cultures using RNeasy Mini Kit (Qiagen) according to the manufacturer’s procedure. 1 μg of RNA was subjected to RT reaction using the SuperScript first-strand synthesis system for RT-PCR (Invitrogen) according to the manufacturer’s protocol. PCR was performed using SYBR Green PCR Master Mix (Thermo Fisher Scientific). Forward and reverse primers, were respectively: 5'-CCATAAGGACGCGGACTTGTAC and 5'-AGACATGTTTGCGGCATCCAGG.

Statistical analysis

The correlation between sprouting intensity and percentage of BDNF alleles on the nuclear border was calculated with Anaconda Software Distribution (2020) (Anaconda Inc) retrieved from https://docs.anaconda.com/, using SciPy library.

The data for RT-PCR experiments were obtained from 3–5 independent batches of neurons and normalized to the control in each experiment. Statistical analysis of normalized data was performed using the Kruskal-Wallis group comparison, and the One-Sample T-test or Welch...
Two Sample t-test for pairwise comparison, with the use of R [42] retrieved from https://docs.anaconda.com/.

For the FISH experiments, the differences in the percentage of the gene at the nuclear periphery were analyzed using chi-square (all 4 groups together) and Fisher’s exact test for post hoc pairwise comparison. Since the contingency tables show precise counts, error bars were calculated as a standard deviation from the binomial distribution. The details are given in the Supplementary S1 File. The analysis was performed using GraphPad Prism software.

**Results**

**Morphological traits of the kainate model of TLE and clinical implications of Bdnf repositioning in the hippocampal granule neurons**

To verify the kainate model of TLE in rats, animals were carefully observed for 6 hours after a kainate injection, and 4 hours/day in the subsequent 4 weeks. The intensity of seizures has been assessed according to modified 6-grade Racine’s scale (0—lack of seizures—5—fully developed tonic-clonic seizures with a loss of posture). Additionally, clinical traits such as fur ruffling, forelimb tonus, body and head tremor, loss of posture, immobility, and aggression have been rated. Moreover, hippocampal specimens from the aforementioned animals were analyzed for neuronal damage in the DG granule cell layer and CA3 pyramidal layer by Fluoro-jade B staining and mossy fiber sprouting in DG molecular layer by synaptoporin staining.

In our model, 60% of animals underwent status epilepticus (fully developed tonic-clonic seizures with a loss of posture, Fig 1A and Table 1 and S1 File), and the remaining animals showed moderate seizure symptoms like head tremors (wet dog shaking) and fur ruffling. In 4 weeks following administration of kainate 5 out of 10 animals showed fur ruffling, 5 out of 10 demonstrated forelimb tonus, all animals exhibited body and head tremor, 5 out of 10 showed loss of posture, 7 out of 10—immobility, and 5 out of 10—aggression (for details see Table 1).

Fluoro-jade B staining showed no neuronal damage in the DG granule cell layer and extensive cell death in the CA3 pyramidal layer in all animals (Fig 1B). Extend of mossy fiber

![Fig 1. Kainate induced seizures and neuronal damage. A) Percentage of animals showing seizures after kainite administration. The intensity of seizure was scored according to the 6-grade modified Racine’s scale (0—lack of seizures—5—fully developed tonic-clonic seizures with loss of posture). B) Fluoro-jade B staining (depicted in green) showing neuronal damage in the CA3 region, but not the DG region of the hippocampus of kainite treated animals. Scale Bar: 100 μm.](https://doi.org/10.1371/journal.pone.0239111.g001)
sprouting was estimated by the intensity of synaptoporin immunostaining (Figs 2A and 2B and S1). In 8 out of 10 animals, it was possible to distinguish mossy fibers stained against

### Table 1. Estimation of the clinical and morphological traits in the kainate model of TLE.

| animal ID  | 1B | 1C | 1G | 2B | 2C | 2G | 3G | 3R | 4G | 4R |
|------------|----|----|----|----|----|----|----|----|----|----|
| seizures (0–5) | 2  | 5  | 5  | 5  | 4  | 5  | 2  | 5  | 5  | 2  |
| % of nuclei with Bdnf at the periphery | 43 | 53 | 29 | 29 | 53 | 55 | 65 | 33 | 24 | 50 |
| sprouting level (0–4) | 2  | 0  | 4  | 2  | 1  | 3  | 0  | 1  | 4  | 1  |
| ruffling of fur (0–2) | 0  | 1  | 1  | 0  | 0  | 0  | 2  | 1  | 1  | 1  |
| forelimb tonus (0–6) | 0  | 0  | 0  | 1  | 0  | 3  | 1  | 6  | 2  | 0  |
| body/head tremor (0–5) | 3  | 4  | 1  | 2  | 1  | 3  | 2  | 4  | 5  | 2  |
| loss of posture (0–4) | 2  | 3  | 0  | 2  | 1  | 0  | 0  | 0  | 0  | 1  |
| inactivity (0–9) | 1  | 0  | 1  | 1  | 0  | 0  | 1  | 7  | 1  | 1  |
| agression (0–3) | 1  | 0  | 1  | 0  | 0  | 1  | 0  | 0  | 3  | 1  |

For all traits, scales are shown in brackets (see Materials and Methods for full details).

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**Fig 2.** Correlation between the intensity of sprouting and the percentage of the nuclei with Bdnf alleles at the nuclear periphery. A) Mossy fiber sprouting was verified by immunofluorescent staining for synaptoporin in the molecular layer of DG region of the hippocampus. Representative pictures of different levels of sprouting of animals at 4 weeks after administration of kainate are shown. B) Percentage of animals showing different levels of synaptoporin staining intensity scored in 5-grade scale (0-lack of sprouting- 4 very strong sprouting). C) Correlation between the levels of sprouting in the DG, 4 weeks from the administration of kainate, measured in 5- grade scale, and percentage of the nuclei with Bdnf alleles localized at the nuclear periphery. Scale Bar: 100 μm.

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synaptoporin (scored from 0—lack of sprouting, to 1—weak sprouting to 4—very strong sprouting, in Figs 2A and 2B and S1 and Table 1), where 30% showed moderate to very strong sprouting (scored ≥3). The level of sprouting was independent of the intensity of the initial seizures (right after kainate injection). All tested animals showed behavioral and morphological traits of epilepsy within 4 weeks from kainate treatment.

In our previous work [34] using the same model of neuronal activation upon kainate-induced seizures, we have observed repositioning of the $Bdnf$ alleles in hippocampal granule neurons. We have shown that transcriptionally inactive $Bdnf$ is attached to a nuclear lamina and localized at the nuclear periphery, after neuronal activation it has repositioned towards the nuclear center. Here we observed, that the level of sprouting correlated (R = -0.67, Pearson correlation) with the percentage of the nuclei with $Bdnf$ allele localized at the nuclear periphery (Fig 2C). In animals with the strongest level of sprouting, in less than 50% of nuclei $Bdnf$ alleles were present at the nuclear rim, while lack of sprouting correlated with the higher percentage of nuclei showing $Bdnf$ localized in proximity to the nuclear envelope. We have found the same correlation calculated for sprouting levels estimated visually by two independent observers (R = -0.70) Moreover, the intensity of sprouting correlated positively with the level of aggression (R = 0.75, Pearson correlation), animals with a higher level of sprouting tend to be more aggressive (supplementary S1 File). Unfortunately, we haven’t found any other correlations of behavioral traits neither with the level of sprouting nor the percentage of nuclei with $Bdnf$ alleles on the nuclear periphery.

**The causal relationship between $Bdnf$ allele transcriptional activity and repositioning**

To further investigate the cause of the intranuclear reposition of $Bdnf$ and its relationship with BDNF transcription, we used an *in vitro* model of neuronal excitation based on hippocampal dissociated cultures and a chemical model of long-term potentiation (cLTP) [43–45]. cLTP evoked by picrotoxin, forskolin, and rolipram was proven to be non-toxic for neurons and to induce a program of gene expression similar to the one observed in a brain upon stimulation. We observed that 2 hours after the initiation of long-term potentiation, $Bdnf$ expression was significantly (4 times) increased compared to the control (Fig 3A). At the same time, the $Bdnf$ allele had repositioned toward the center of a cell nucleus (Figs 3B–3D and S2). In the control cells (treated with a solvent alone) the $Bdnf$ alleles were most frequently positioned at a nuclear margin, with 76.4% of alleles located 350 nm or less from a nuclear border (Fig 3C and 3D, blue bars). This distance is an approximate microscope resolution limit, hence it has been chosen as an indicator of allele proximity to the nuclear border, as previously described [34]. In activated neurons, we observed a distinct repositioning of the $Bdnf$ gene from the nucleus periphery towards the nucleus center (Fig 3C and 3D, orange bars). The percentage of $Bdnf$ alleles localized closer than 350 nm to the nuclear margin was significantly lower than in the control group (62.9% Fisher’s exact tests). To verify whether $Bdnf$ allele repositioning is a cause or a consequence of transcriptional activity, we performed experiments with the use of Actinomycin D, a potent inhibitor of RNA polymerase II. Preincubation with Actinomycin D for 2 hours was sufficient to inhibit $Bdnf$ expression upon stimulation with cLTP (6 fold decrease compared to cLTP treatment, Fig 3A). Inhibition of transcription completely blocked $Bdnf$ repositioning upon cLTP treatment (Figs 3B–3D and S2). The percentage of $Bdnf$ alleles located at the nuclear periphery upon Actinomycin D and cLTP treatment was similar to control cells. Presented results show that transcriptional activity is a cause of $Bdnf$ repositioning.
Histone deacetylases are necessary for the attachment of Bdnf alleles to the nuclear lamina

Covalent modifications of chromatin were shown to be involved in the regulation of Bdnf gene expression [27,46,47]. Data indicate, that histone deacetylases (HDAC) play an important role in this process [48,49]. Therefore, we have raised a question of HDAC involvement in Bdnf repositioning observed after neuronal stimulation (Fig 4). Preincubation of hippocampal cultures with Trichostatin A, which is a commonly used HDAC paninhibitor, slightly increased Bdnf transcription (Fig 4A) and was sufficient to induce Bdnf allele repositioning toward the nuclear center (Figs 4B–4D and S3). The percentage of Bdnf alleles localized at the nuclear periphery was significantly lower upon TSA treatment compared to the control group. Induction of cLTP after Trichostatin A treatment did not decrease the percentage of alleles located at the nuclear periphery, in comparison to cLTP or TSA alone. The preincubation with a more specific HDAC inhibitor- romidepsin (Fig 5), selective for HDAC1 and HDAC2 deacetylases

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had no effect on Bdnf transcription (Fig 5A) and induced Bdnf allele repositioning only upon neuronal stimulation (Figs 5B–5D and S4). These results show again the causal relation between transcriptional activation of Bdnf and Bdnf allele’s repositioning towards the nucleus center.

Discussion

The three-dimensional organization of chromatin in the cell nucleus exhibits a higher-order regulation of gene expression [50–52]. The role of the nuclear lamina as a compartment regulating transcriptional activity is already quite well explored [53–55]. However, the knowledge of particular mechanisms, which are responsible for driving detachment of the genes from the nuclear lamina, is still vague [56].

To study the relationship between Bdnf repositioning, and morphological and clinical epileptic traits, we used the kainate model of epilepsy. This particular animal model was chosen...
because it resembles morphological traits of TLE such as mossy fiber sprouting in the dentate gyrus and neurodegeneration in CA1 and 3 regions of the hippocampus [57,58]. Further, to study mechanisms of Bdnf allele repositioning, we used in vitro model of neuronal stimulation. This model was chosen to avoid problems with the distribution of the Actinomycin D [59] through the blood-brain barrier and to decrease the number of sacrificed animals. Transcriptomic studies by Dabrowski and collaborators showed that the expression pattern in excited neurons in vivo is sustained in in vitro model [60]. Moreover, Szepesi and collaborators (2013) [43] showed that the induction of excitation by rolipram, forskolin, and picrotoxin induced a formation of dendritic spine protrusions, which resembled in vivo formation of new synapses.

The nuclear lamina is known as a transcriptionally repressive nuclear compartment [61]. Williams et al. (2007) [62] and Peric-Hupces et al. (2010) [63] showed a rearrangement of interactions between chromatin and the nuclear lamina, and an association of such phenomenon
with transcription during differentiation of embryonic stem cells into neurons. However, the number of reports investigating a relationship between the rearrangement of chromatin architecture and transcription in fully differentiated neurons is very limited. In the presented study, using in vitro model, we confirmed our previous finding [34], where we showed that after neuronal excitation Bdnf alleles had repositioned toward the nuclear center. However, in comparison to the aforementioned paper, in the in vitro model, the percentage of alleles localized at the nuclear periphery was higher in the control cells, and the difference to stimulated neurons was smaller than in in vivo studies. Such discrepancy might be a result of a more uniform environment in cell culture, compared to in vivo situations, where spontaneous neuronal activity is present. Additionally, the kainate treatment, used in the in vivo studies, induces much stronger neuronal excitation than chemically induced LTP applied in vitro. In the presented study we showed that inhibition of transcription prevents Bdnf repositioning in neuronal nuclei after stimulation. It suggests that depolarization of the neuronal membrane is not sufficient for the detachment of Bdnf alleles, but the transcriptional activity itself is necessary for full repositioning. Our finding is consistent with the expertise presented by Crepaldi and colleagues (2013), who showed that in cultured cortical neurons depolarization induced by KCl stimulation evoked repositioning of activity-induced genes, including Bdnf, into transcription factories [33]. Moreover, this study showed that repositioning is controlled by transcription factor TFIIIC. Our current finding complements the previous results [34], where we showed the association of Bdnf repositioning and changes in transcription level during epileptogenesis. Both of those studies support the hypothesis that Bdnf allele repositioning acts as a kind of molecular memory of the cell to prepare neurons for future activation. The finding is also in agreement with the report by Ito and colleagues (2014) showing, that loss of three-dimensional architecture in neuronal nuclei leads to impaired transcription of several genes [64].

Surely, the presented mechanism is not the only one involved in the Bdnf repositioning. Presumably, several pathways must be orchestrated to activate Bdnf transcription and detachment of gene from the nuclear lamina, as well as its reposition toward the nuclear center. One possible mechanism may involve cohesins, which are well-known genome organizers [65] (and references therein). In Cornelia de Lange Syndrome, which belongs to cohesinopathies and is associated with epileptic seizures [66,67] acetylation of cohesins is impaired due to mutation in the gene encoding histone deacetylase HDAC8 [68]. Our results obtained with the use of inhibitors of histone deacetylases speak for this scenario. It has been shown that HDAC inhibitors induce hyperacetylation of more than 1700 proteins, which included chromatin remodelers, transcription factors, and protein kinases [69]. The trichostatin A - less specific but potent HDAC inhibitor, induced Bdnf transcription and repositioning even in the absence of neuronal activation. On the contrary, the more selective romidepsin, which inhibits mainly HDAC1 and 2, had no impact on both processes in silent neurons and upon stimulation. The cohesin-dependent mechanism may be at least in part responsible for Bdnf transcriptional activation, detachment, and repositioning, as CTCF, which acts in concert with cohesins [70] was shown to regulate the transcription of Bdnf [71].

Also, transcription factors such as Serum Response Factor (SRF) seem to be good candidates responsible for Bdnf repositioning. It is known that SRF can regulate Bdnf transcription [72] and its deletion leads to increased epileptogenesis and differential expression of more than 370 activity-induced genes including reduced transcription of Bdnf [73]. The disturbed balance of expression of inhibitory/excitatory regulators may contribute to the increased epileptogenesis, which was manifested by more frequent and acute spontaneous seizures in KO animals. Furthermore, it was shown, that histone acetylation at the promoter region is necessary for activation of gene expression [74]. Histone deacetylases reverse this process and are taking part in creating a repressive zone by the nuclear lamina [75]. In a presented study we showed,
that inhibition of histone deacetylases with paninhibitor Trichostatnin A leads to Bdnf transcription and repositioning. Similar activation of Bdnf gene transcription upon TSA treatment was shown for the Hek293 cell line [76]. Also, HDACs’ involvement in Bdnf expression is well established [28,48,77–80]. However, the exact mechanisms of HDACs’ participation in the higher-order mechanisms of transcriptional regulation of BDNF are unknown. One possible mechanism might involve Methyl-CpG-Binding protein 2 (MeCP2). Activation of neurons leads to the decreased CpG methylation of the Bdnf promoter region and dissociation of the MeCP2-mSin3A-HDAC1 silencing complex [25]. Besides, MeCP2, which expression is reduced upon TSA treatment [81], binds to the inner nuclear membrane lamin B receptor [82]. Therefore the association of transcriptionally inactive Bdnf to the nuclear lamina and its detachment upon TSA treatment may be dependent on MeCP2 protein. Mutations of the MeCP2 encoding gene are responsible for most cases of Rett Syndrome, a neurodevelopmental disorder, in which patients develop seizures [83].

Additionally, the process of Bdnf repositioning might involve actin-based molecular motors, since studies by Serebryannyy et al. (2016) [84] reported that actin regulates the function of HDAC1 and HDAC2 and also can be involved in gene expression by association with RNA polymerase II.

Finally, in the presented study we attempted to investigate the involvement of Bdnf repositioning in the pathogenesis of TLE in rats. We observed that the percentage of nuclei with the Bdnf allele at the nuclear periphery is negatively correlated with the intensity of mossy fiber sprouting. This observation is consistent with an idea that BDNF takes part in sprouting events in epilepsy [17,18]. In addition the intensity of sprouting correlated positively with the increased level of aggression, which is one of the behavioral traits typical for TLE [85]. The Actinomycin D, which has been already used in treatments of several types of cancer [86–88], has blocked Bdnf repositioning. However, the potential use of Actinomycin D for the treatment of neurological disorders including epilepsy would be challenging due to difficulties of its distribution through the blood-brain barrier [59]. Taking into account all the results together, the presented study supports the idea of Simonato [89], that Bdnf can be a very good target for novel anti-epileptic therapies.

Conclusively, the presented results are consistent with the current trend in research on the pathogenesis of epilepsy and show that neuronal cell nuclei are interesting targets to search for mechanisms of epileptogenesis.

**Supporting information**

**S1 Fig. The representative images of mossy fiber sprouting and Bdnf alleles repositioning within neuronal nuclei in the TLE model.** Mossy fiber sprouting was verified by immunofluorescent staining for synaptoporin (left panels, depicted in green) in the molecular layer of DG region of the hippocampus. Representative pictures from animals at 4 weeks after administration of kainate are shown. The right panels show images of nuclei of granular neurons acquired from the same animals. Hoechst 3342 staining for chromatin is shown in a grey scale and segmentation of FISH signals for Bdnf gene are shown in magenta and cyan. (TIF)

**S2 Fig. The causal relationship between Bdnf transcriptional activity and Bdnf’s allele repositioning.** The representative pictures of the nuclei of hippocampal neurons incubated for 2 hours with DMSO vehicle (CTRL) or picrotoxin, forskolin, and rolipram (cLTP), incubated for 2 hours with Actinomycin D and 2 hours with DMSO (ActD) or picrotoxin, forskolin, and rolipram (ActD+cLTP). Hoechst 3342 staining for chromatin is shown in a grey scale and
segmentation of FISH signals for Bdnf gene are shown in magenta and cyan.

(TIF)

S3 Fig. The inhibition of histone deacetylases induces Bdnf repositioning independently from the neuronal stimulation. The representative pictures of the nuclei of hippocampal neurons incubated for 2 hours with DMSO vehicle (CTRL) or picrotoxin, forskolin, and rolipram (cLTP), incubated for 12 hours with Trichostatin A and 2 hours with DMSO (TSA) or picrotoxin, forskolin, and rolipram (cLTP TSA). Hoechst 3342 staining for chromatin is shown in a grey scale and segmentation of FISH signals for Bdnf gene are shown in magenta and cyan. (TIF)

S4 Fig. The inhibition of HDAC1/2 is not sufficient to induce Bdnf repositioning. The representative pictures of the nuclei of hippocampal neurons incubated for 2 hours with DMSO vehicle (CTRL) or picrotoxin, forskolin, and rolipram (cLTP), incubated for 2 hours with romidepsin and 2 hours with DMSO (romidepsin) or picrotoxin, forskolin, and rolipram (cLTP romidepsin). Hoechst 3342 staining for chromatin is shown in a grey scale and segmentation of FISH signals for Bdnf gene are shown in magenta and cyan. (TIF)

S1 File. Clinical correlates and statistical analysis for Figs 1–5. (XLSX)

Author Contributions

Conceptualization: Agnieszka Walczak, Grzegorz Marek Wilczynski, Joanna Dzwonek, Adriana Magalska.

Data curation: Grzegorz Marek Wilczynski, Adriana Magalska.

Formal analysis: Anna Skupien-Jaroszek, Agnieszka Walczak, Iwona Czaban, Katarzyna Karolina Pels, Błażej Ruszczycki, Joanna Dzwonek, Adriana Magalska.

Funding acquisition: Grzegorz Marek Wilczynski, Joanna Dzwonek, Adriana Magalska.

Investigation: Anna Skupien-Jaroszek, Agnieszka Walczak, Iwona Czaban, Andrzej Antoni Szczepankiewicz, Joanna Dzwonek.

Methodology: Anna Skupien-Jaroszek, Katarzyna Karolina Pels, Katarzyna Krawczyk.

Project administration: Grzegorz Marek Wilczynski.

Supervision: Grzegorz Marek Wilczynski, Joanna Dzwonek, Adriana Magalska.

Validation: Iwona Czaban, Adriana Magalska.

Visualization: Anna Skupien-Jaroszek, Agnieszka Walczak, Adriana Magalska.

Writing – original draft: Agnieszka Walczak, Adriana Magalska.

Writing – review & editing: Grzegorz Marek Wilczynski, Joanna Dzwonek, Adriana Magalska.

References

1. Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci. 2001; 24:677–736. Epub 2001/08/25. https://doi.org/10.1146/annurev.neuro.24.1.677 PMID: 11520916
2. Bramham CR, Messaoudi E. BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. Prog Neurobiol. 2005; 76(2):99–125. Epub 2005/08/16. https://doi.org/10.1016/j.pneurobio.2005.06.003 PMID: 16099088

3. Vignoli N, Battistini G, Melani R, Blum R, Santi S, Berardi N, et al. Peri-Synaptic GLIA Recycles Brain-Derived Neurotrophic Factor for LTP Stabilization and Memory Retention. Neuron. 2016; 92(4):873–87. Epub 2016/10/18. https://doi.org/10.1016/j.neuron.2016.09.031 PMID: 27746130

4. Costa A, Pepeo A, Carlesimo GA, Zabbaroni S, Scalici F, Caltagirone C, et al. Brain-derived neurotrophic factor serum levels correlate with cognitive performance in Parkinson’s disease patients with mild cognitive impairment. Front Behav Neurosci. 2015; 9:253. Epub 2015/10/07. https://doi.org/10.3389/fnbeh.2015.00253 PMID: 26441580

5. Zhang XY, Chen DC, Tan YL, Tan SP, Luo X, Zuo L, et al. BDNF polymorphisms are associated with schizophrenia onset and positive symptoms. Schizophr Res. 2016; 170(1):41–7. Epub 2015/11/26. https://doi.org/10.1016/j.schres.2015.11.009 PMID: 26603468

6. Webb C, Gunn JM, Potiridis M, Everall IP, Bousman CA. The Brain-Derived Neurotrophic Factor Val66Met Polymorphism Modulates the Effects of Childhood Abuse on Severity of Depressive Symptoms in a Time-Dependent Manner. Front Psychiatry. 2016; 7:151. Epub 2016/09/14. https://doi.org/10.3389/fpsyt.2016.00151 PMID: 27621711

7. Nassan M, Croarkin PE, Luby JL, Veldic M, Joshi PT, McElroy SL, et al. Association of brain-derived neurotrophic factor (BDNF) Val66Met polymorphism with early-onset bipolar disorder. Bipolar Disord. 2015; 17(6):645–52. Epub 2015/11/04. https://doi.org/10.1111/bdi.12323 PMID: 26528762

8. Shen N, Zhu X, Lin H, Li J, Li L, Niu F, et al. Role of BDNF Val66Met functional polymorphism in temporal lobe epilepsy. Int J Neurosci. 2016; 126(5):436–41. Epub 2015/05/23. https://doi.org/10.3109/00207454.2015.1026967 PMID: 26000807

9. Koyama R, Ikegaya Y. To BDNF or not to BDNF: that is the epileptic hippocampus. Neuroscientist. 2005; 11(4):282–7. Epub 2005/08/03. https://doi.org/10.1177/1073858105227857

10. Lin TW, Harward SC, Huang YZ, McNamara JO. Targeting BDNF/TrkB pathways for preventing or suppressing epilepsy. Neuropharmacology. 2019:107734. Epub 2019/08/05. https://doi.org/10.1016/j.neuropharm.2019.107734 PMID: 31377199

11. Wiebe S. Epidemiology of temporal lobe epilepsy. Can J Neurol Sci. 2000; 27 Suppl 1:S6–10; discussion S20-1. Epub 2000/06/01. https://doi.org/10.1017/s0317167100000561 PMID: 10830320

12. Takahashi M, Hayashi S, Kakita A, Wakabayashi K, Fukuda M, Kameyama S, et al. Patients with temporal lobe epilepsy show an increase in brain-derived neurotrophic factor protein and its correlation with neuropeptid Y. Brain Res. 1999; 818(2):579–82. Epub 1999/03/20. https://doi.org/10.1016/s0006-8993(99)01355-9 PMID: 10062852

13. Suzuki F, Junier MP, Guilhem D, Sorensen JC, Onteniente B. Morphogenetic effect of kainate on adult hippocampal neurons associated with a prolonged expression of brain-derived neurotrophic factor. Neuroscience. 1995; 64(3):665–74. Epub 1995/02/01. https://doi.org/10.1016/0306-4522(94)00463-f PMID: 7715779

14. Gall CM. Seizure-induced changes in neurotrophin expression: implications for epilepsy. Exp Neurol. 1993; 124(1):150–66. Epub 1993/11/01. https://doi.org/10.1006/exnr.1993.1186 PMID: 8528072

15. Murray KD, Isackson PJ, Blum R, Santi S, Berardi N, et al. Altered mRNA expression for brain-derived neurotrophic factor and type II calcium/calmodulin-dependent protein kinase in the hippocampus of patients with intractable temporal lobe epilepsy. J Comp Neurol. 2000; 418(4):411–22. Epub 2000/03/14. https://doi.org/10.1002/(sici)1096-9861(20000320)418:4<411::aid-cne4>3.0.co;2-f PMID: 10713570

16. Guillhem D, Dreyfus PA, Makriya Y, Suzuki F, Onteniente B. Short increase of BDNF messenger RNA triggers kainic acid-induced neuronal hypertrophy in adult mice. Neuroscience. 1996; 72(4):923–31. Epub 1996/06/01. https://doi.org/10.1016/0306-4522(96)0005-x PMID: 8735220

17. Danzer SC, Crooks KR, Lo DC, McNamara JO. Increased expression of brain-derived neurotrophic factor induces formation of basal dendrites and axonal branching in dentate granule cells in hippocampal explant cultures. J Neurosci. 2002; 22(22):9754–63. Epub 2002/11/13. https://doi.org/10.1523/JNEUROSCI.22-22-09754.2002 PMID: 12427830

18. Scharfmian HE, Goodman JH, Sollas AL, Croll SD. Spontaneous limbic seizures after intrahippocampal infusion of brain-derived neurotrophic factor. Exp Neurol. 2002; 174(2):201–14. Epub 2002/04/02. https://doi.org/10.1002/exnr.20016 PMID: 11922662

19. Saha RN, Wissink EM, Bailey ER, Zhao M, Fargo DC, Hwang JY, et al. Rapid activity-induced transcription of Arc and other IEGs relies on poised RNA polymerase II. Nat Neurosci. 2011; 14(7):848–56. Epub 2011/05/31. https://doi.org/10.1038/nn.2839 PMID: 21623364
20. Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T. Mouse and rat BDNF gene structure and expression revisited. J Neurosci Res. 2007; 85(3):525–35. Epub 2006/12/07. https://doi.org/10.1002/jnr.21139 PMID: 17149751

21. Timmusk T, Palm K, Metsis M, Reintam T, Paalme V, Saarma M, et al. Multiple promoters direct tissue-specific expression of the rat BDNF gene. Neuron. 1993; 10(3):475–89. Epub 1993/03/01. https://doi.org/10.1016/0896-6273(93)90335-o PMID: 8461137

22. Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME. Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron. 1998; 20(4):709–26. Epub 1998/05/15. https://doi.org/10.1016/s0896-6273(00)81010-7 PMID: 9581763

23. Timmusk T, Palm K, Lendahl U, Metsis M. Brain-derived neurotrophic factor expression in vivo is under the control of neuron-restrictive silencer element. J Biol Chem. 1999; 274(2):1078–84. Epub 1999/01/05. PMID: 9873054

24. Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC, et al. Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science. 2003; 302(5646):885–9. Epub 2003/11/01. https://doi.org/10.1126/science.1086446 PMID: 14593183

25. Martinowich K, Halford D, Wu H, Fouse S, He F, Hu Y, et al. DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science. 2003; 302(5646):890–3. Epub 2003/11/01. https://doi.org/10.1126/science.1090842 PMID: 14593184

26. Lubin FD, Roth TL, Sweatt JD. Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. J Neurosci. 2008; 28(42):10576–86. Epub 2008/10/17. https://doi.org/10.1523/JNEUROSCI.1786-08.2008 PMID: 18923034

27. Tsankova NM, Kumar A, Nestler EJ. Histone modifications at gene promoter regions in rat hippocampus after acute and chronic electroconvulsive seizures. J Neurosci. 2004; 24(24):5603–10. Epub 2004/06/18. https://doi.org/10.1523/JNEUROSCI.0589-04.2004 PMID: 15201333

28. Fukuchi M, Nii T, Ishimaru N, Minamino A, Hara D, Takasaki I, et al. Valproic acid induces up- or down-regulation of gene expression responsible for the neuronal excitation and inhibition in rat cortical neurons through its epigenetic actions. Neurosci Res. 2009; 65(1):35–43. Epub 2009/05/05. https://doi.org/10.1016/j.neures.2009.05.002 PMID: 19463867

29. Rowley MJ, Corces VG. Organizational principles of 3D genome architecture. Nat Rev Genet. 2018; 19(12):789–800. Epub 2018/10/28. https://doi.org/10.1038/s41576-018-0060-8 PMID: 30367165

30. Egecioglu D, Brickner JH. Gene positioning and expression. Curr Opin Cell Biol. 2011; 23(3):338–45. Epub 2011/02/05. https://doi.org/10.1016/j.ceb.2011.01.001 PMID: 21292462

31. Akhtar A, Gasser SM. The nuclear envelope and transcriptional control. Nat Rev Genet. 2007; 8(7):507–17. Epub 2007/06/06. https://doi.org/10.1038/nrg2122 PMID: 17549064

32. Finlan LE, Sproul D, Thomson I, Boyle S, Kerr E, Perry P, et al. Recruitment to the nuclear periphery can alter expression of genes in human cells. PLoS Genet. 2008; 4(3):e1000039. Epub 2008/03/29. https://doi.org/10.1371/journal.pgen.1000039 PMID: 18369458

33. Crepaldi L, Poliarcil C, Coatti A, Sherlock WT, Jongbloets BC, Down TA, et al. Binding of TFIIIC to sine elements controls the relocation of activity-dependent neuronal genes to transcription factories. PLoS Gen. 2013; 9(8):e1003699. Epub 2013/08/24. https://doi.org/10.1371/journal.pgen.1003699 PMID: 23966877

34. Walczak A, Szczepankiewicz AA, Ruszczycy B, Magalka A, Zamylnska K, Dzwonek J, et al. Novel higher-order epigenetic regulation of the Bdnf gene upon seizures. J Neurosci. 2013; 33(6):2507–11. Epub 2013/02/09. https://doi.org/10.1523/JNEUROSCI.1085-12.2013 PMID: 23392678

35. Hellier JL, Patrylo PR, Buckmaster PS, Dudek FE. Recurrent spontaneous motor seizures after repeated low-dose systemic treatment with kainate: assessment of a rat model of temporal lobe epilepsy. Epilepsy Res. 1998; 31(1):73–84. Epub 1998/08/08. https://doi.org/10.1016/s0920-1211(98)00017-5 PMID: 9696302

36. Skupien A, Konopka A, Trzaskoma P, Labus J, Gorlewicz A, Swiech L, et al. CD44 regulates dendrite morphogenesis through Src tyrosine kinase-dependent positioning of the Golgi. J Cell Sci. 2014; 127(Pt 23):5038–51. Epub 2014/10/11. https://doi.org/10.1242/jcs.154542 PMID: 25307955

37. Cremer M, Grassner F, Lanctot C, Muller S, Neusser M, Zinner R, et al. Multicolor 3D fluorescence in situ hybridization for imaging interphase chromosomes. Methods Mol Biol. 2008; 463:205–39. Epub 2008/10/28. https://doi.org/10.1007/978-1-59745-406-3_15 PMID: 18951171

38. Wilczynski GM, Konopacki FA, Wilczek E, Lasiecka Z, Gorlewicz A, Michaluk P, et al. Important role of matrix metalloproteinase 9 in epileptogenesis. J Cell Biol. 2008; 180(5):1021–35. Epub 2008/03/12. https://doi.org/10.1083/jcb.200708213 PMID: 18332222

39. Schmued LC, Albertson C, Slikker W Jr. Fluoro-Jade: a novel fluorochrome for the sensitive and reliable histochemical localization of neuronal degeneration. Brain Res. 1997; 751(1):37–46. Epub 1997/03/14. https://doi.org/10.1016/s0006-8993(96)01387-x PMID: 9098566
40. Ruszczycyki B, Pels KK, Walczak A, Zamlynska K, Such M, Szczepankiewicz AA, et al. Three-Dimensional Segmentation and Reconstruction of Neuronal Nuclei in Confocal Microscopic Images. Front Neuroanat. 2019; 13:81. Epub 2019/09/05. https://doi.org/10.3389/fnana.2019.00081 PMID: 31481881

41. Bokota G, Sroka J, Basu S, Das N, Trzaskoma P, Yushkevich Y, et al. PartSeg, a Tool for Quantitative Feature Extraction From 3D Microscopy Images for Dummies. BMC Bioinformatics. 2021, 22, 1–15. Epub 2021/02/17. https://doi.org/10.1186/s12859-020-03881-z PMID: 33388027

42. Team RC. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing 2020.

43. Szepesi Z, Bijata M, Ruszczycyki B, Kaczmarek L, Wlodarczyk J. Matrix metalloproteinases regulate the formation of dendritic spine protrusions during chemically induced long-term potentiation. PLoS One. 2013; 8(5):e33314. Epub 2013/05/23. https://doi.org/10.1371/journal.pone.0063314 PMID: 23696812

44. Niedringshaus M, Chen X, Dzakpasu R, Conant K. MMPs and soluble ICAM-5 increase neuronal excitability within in vitro networks of hippocampal neurons. PLoS One. 2012; 7(8):e42631. Epub 2012/08/23. https://doi.org/10.1371/journal.pone.0042631 PMID: 22912716

45. Otmakhov K, Khibnik L, Otmakhova N, Carpenter S, Riasli S, Asrani B, et al. Forskolin-induced LTP in the CA1 hippocampal region is NMDA receptor dependent. J Neurophysiol. 2004; 91(5):1955–62. Epub 2004/01/02. https://doi.org/10.1152/jn.00208.2003 PMID: 14702333

46. Schmidt HD, Sangrey GR, Darnell SB, Schassburger RL, Cha JH, Pierce RC, et al. Increased brain-derived neurotrophic factor (BDNF) expression in the ventral tegmental area during cocaine abstinence is associated with increased histone acetylation at BDNF exon l-containing promoters. J Neurochem. 2012; 120(2):202–9. Epub 2011/11/03. https://doi.org/10.1111/j.1471-4159.2011.07571.x PMID: 22043863

47. Koh DX, Sng JC. HDAC1 negatively regulates Bdnf and Pvalb required for parvalbumin interneuron maturation in an experience-dependent manner. J Neurochem. 2016; 139(3):369–80. Epub 2016/10/25. https://doi.org/10.1111/jnc.13773 PMID: 27534825

48. Kim J, Lee S, Choi BR, Yang H, Hwang Y, Park JH, et al. Sulforaphane epigenetically enhances neuronal BDNF expression and TrkB signaling pathways. Mol Nutr Food Res. 2017; 61(2). Epub 2016/10/14. https://doi.org/10.1002/mnfr.201600194 PMID: 27735126

49. Schmauss C. An HDAC-dependent epigenetic mechanism that enhances the efficacy of the antidepressant drug fluoxetine. Sci Rep. 2015; 5:8171. Epub 2015/02/03. https://doi.org/10.1038/srep08171 PMID: 25639887

50. Goetze S, Mateos-Langerak J, van Driel R. Three-dimensional genome organization in interphase and its relation to genome function. Semin Cell Dev Biol. 2007; 18(5):707–14. Epub 2007/10/02. https://doi.org/10.1016/j.semcdb.2007.08.007 PMID: 17905616

51. Goetze S, Mateos-Langerak J, Gierman HJ, de Leeuw W, Giromus O, Indemans MH, et al. The three-dimensional structure of human interphase chromosomes is related to the transcriptome map. Mol Cell Biol. 2007; 27(12):4475–87. Epub 2007/04/11. https://doi.org/10.1128/MCB.00208-07 PMID: 17420274

52. Eskiw CH, Cope NF, Clay I, Schoenfelder S, Nagano T, Fraser P. Transcription factories and nuclear organization of the genome. Cold Spring Harb Symp Quant Biol. 2010; 75:501–6. Epub 2011/04/07. https://doi.org/10.1101/sqb.2010.75.046 PMID: 21467135

53. Lund E, Collas P. Nuclear lamins: making contacts with promoters. Nucleus. 2013; 4(6):707–14. Epub 2007/10/02. https://doi.org/10.1111/jnc.13773 PMID: 27534825

54. Kim J, Lee S, Choi BR, Yang H, Hwang Y, Park JH, et al. Sulforaphane epigenetically enhances neuronal BDNF expression and TrkB signaling pathways. Mol Nutr Food Res. 2017; 61(2). Epub 2016/10/14. https://doi.org/10.1002/mnfr.201600194 PMID: 27735126

55. Schmauss C. An HDAC-dependent epigenetic mechanism that enhances the efficacy of the antidepressant drug fluoxetine. Sci Rep. 2015; 5:8171. Epub 2015/02/03. https://doi.org/10.1038/srep08171 PMID: 25639887

56. Goetze S, Mateos-Langerak J, van Driel R. Three-dimensional genome organization in interphase and its relation to genome function. Semin Cell Dev Biol. 2007; 18(5):707–14. Epub 2007/10/02. https://doi.org/10.1016/j.semcdb.2007.08.007 PMID: 17905616

57. Goetze S, Mateos-Langerak J, Gierman HJ, de Leeuw W, Giromus O, Indemans MH, et al. The three-dimensional structure of human interphase chromosomes is related to the transcriptome map. Mol Cell Biol. 2007; 27(12):4475–87. Epub 2007/04/11. https://doi.org/10.1128/MCB.00208-07 PMID: 17420274

58. Eskiw CH, Cope NF, Clay I, Schoenfelder S, Nagano T, Fraser P. Transcription factories and nuclear organization of the genome. Cold Spring Harb Symp Quant Biol. 2010; 75:501–6. Epub 2011/04/07. https://doi.org/10.1101/sqb.2010.75.046 PMID: 21467135

59. Lund E, Collas P. Nuclear lamins: making contacts with promoters. Nucleus. 2013; 4(6):707–14. Epub 2007/10/02. https://doi.org/10.1111/jnc.13773 PMID: 27534825

60. Zhao H, Sifakis EG, Sumida N, Millan-Arino L, Scholz BA, Svensson JP, et al. PARP1- and CTCF-Mediated Interactions between Active and Repressed Chromatin at the Lamina Promote Oscillating Transcription. Mol Cell. 2015; 59(6):984–97. Epub 2015/09/01. https://doi.org/10.1016/j.molcel.2015.07.019 PMID: 26321255

61. Dobrzynska A, Gonzalez S, Shanahan C, Askjaer P. The nuclear lamina in health and disease. Nucleus. 2016; 7(3):233–48. Epub 2016/05/10. https://doi.org/10.1089/nucle.2015.01094 PMID: 27158763

62. van Steensel B, Furlong EEM. The role of transcription in shaping the spatial organization of the genome. Nat Rev Mol Cell Biol. 2019; 20(6):327–37. Epub 2019/03/20. https://doi.org/10.1038/s41580-019-0114-6 PMID: 30886333

63. Wenzel HJ, Woolley CS, Robbins CA, Schwartzko Hirn PA. Kaicin acid-induced mossy fiber sprouting and synapse formation in the dentate gyrus of rats. Hippocampus. 2000; 10(3):244–60. Epub 2000/07/21. https://doi.org/10.1002/1098-1063(2000)10:3<244::AID-HIPO>3.0.CO;2-7 PMID: 10902894

64. Leite JP, Babar TL, Pretorius JK, Kuhiman PA, Yeoman KM, Mathern GW. Neuron loss, mossy fiber sprouting, and interictal spikes after intrahippocampal kainate in developing rats. Epileps Res. 1996; 26(1):219–31. Epub 1996/12/01. https://doi.org/10.1016/s0920-1211(96)00055-1 PMID: 8985702
59. Tattersall MH, Sodergren JE, Dengupta SK, Trites DH, Modest EJ, Frei E, 3rd. Pharmacokinetics of actinomycin D in patients with malignant melanoma. Clin Pharmacol Ther. 1975; 17(6):701–8. Epub 1975/06/01. https://doi.org/10.1002/cpt1975176701 PMID: 1139861

60. Dabrowski M, Aerts S, Van Hummelen P, Craessaerts K, De Moor B, Annaert W, et al. Gene profiling of hippocampal neuronal culture. J Neurochem. 2003; 85(5):1279–88. Epub 2003/05/20. https://doi.org/10.1046/j.1471-4199.2003.01753.x PMID: 12753086

61. Zuleger N, Robson MJ, Schirmer EC. The nuclear envelope as a chromatin organizer. Nucleus. 2011; 2(5):339–49. Epub 2011/10/06. https://doi.org/10.4161/ncl.2.5.17846 PMID: 21970986

62. Williams SK, Tyler JK. Transcriptional regulation by chromatin disassembly and reassembly. Curr Opin Genet Dev. 2007; 17(2):88–93. Epub 2007/02/20. https://doi.org/10.1016/j.gde.2007.02.001 PMID: 17307351

63. Peric-Hupke D, Meuleman W, Pagie L, Bruggeman SW, Solovei I, Brugman W, et al. Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. Mol Cell. 2010; 38(4):603–13. Epub 2010/06/02. https://doi.org/10.1016/j.molcel.2010.03.016 PMID: 20513434

64. Ito S, Magalska A, Alcaraz-Ibora M, Lopez-Atalaya JP, Rovira V, Contreras-Moreira B, et al. Loss of neuronal 3D chromatin organization causes transcriptional and behavioural deficits related to serotoninergic dysfunction. Nat Commun. 2014; 5:4450. Epub 2014/07/19. https://doi.org/10.1038/ncomms5450 PMID: 25034090

65. Merkenschlager M, Nora EP. CTCF and Cohesin in Genome Folding and Transcriptional Gene Regulation. Annu Rev Genomics Hum Genet. 2016; 17:17–43. Epub 2016/04/20. https://doi.org/10.1146/annurev-genom-083115-022339 PMID: 27089971

66. Verritti A, Agostinelli S, Prezioso G, Coppola G, Capovilla G, Romeo A, et al. Epilepsy in patients with Cornelia de Lange syndrome: a clinical series. Seizure. 2013; 22(5):356–9. Epub 2013/03/12. https://doi.org/10.1016/j.seizure.2013.01.017 PMID: 23473710

67. Pavlidis E, Cantalupo G, Bianchi S, Piccolo B, Pisani F. Epileptic features in Cornelia de Lange syndrome: case report and literature review. Brain Dev. 2014; 36(10):837–43. Epub 2014/01/28. https://doi.org/10.1016/j.braindev.2013.12.008 PMID: 24461912

68. Deardorff MA, Bando M, Nakato R, Watrin E, Itoh T, Minamino M, et al. HDAC8 mutations in Cornelia de Lange syndrome affect the cohesin acetylation cycle. Nature. 2012; 489(7415):313–7. Epub 2012/08/14. https://doi.org/10.1038/nature11316 PMID: 22885700

69. Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, et al. Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science. 2009; 325(5942):834–40. Epub 2009/07/18. https://doi.org/10.1126/science.1175371 PMID: 19608861

70. Dorsett D. Gene regulation: the cohesin ring connects developmental highways. Curr Biol. 2010; 20(20):R886–8. Epub 2010/10/26. https://doi.org/10.1016/j.cub.2010.09.036 PMID: 20971431

71. Chang J, Zhang B, Heath H, Galjart N, Wang X, Milbrandt J. Nicotinamide adenine dinucleotide (NAD)-regulated DNA methylation alters CCCTC-binding factor (CTCF)/cohesin binding and transcription at the BDNF locus. Proc Natl Acad Sci U S A. 2010; 107(50):21836–41. Epub 2010/11/26. https://doi.org/10.1073/pnas.1012031107 PMID: 21106760

72. Ramanan N, Shen Y, Sarsfeld S, Lemberger T, Schultz G, Linden DJ, et al. SRF mediates activity-induced gene expression and synaptic plasticity but not neuronal viability. Nat Neurosci. 2005; 8(6):759–67. Epub 2005/05/10. https://doi.org/10.1038/nn1462 PMID: 15880109

73. Kuznietska B, Nader K, Dabrowski M, Kaczmarek L, Kalita K. Adult Deletion of SRF Increases Epileptogenesis and Decreases Activity-Induced Gene Expression. Mol Neurobiol. 2016; 53(3):1478–93. Epub 2015/02/01. https://doi.org/10.1007/s12035-014-9089-7 PMID: 25636886

74. Forsberg EC, Bresnich EH. Histone acetylation beyond promoters: long-range acetylation patterns in the chromatin world. Bioessays. 2001; 23(9):820–30. Epub 2001/09/06. https://doi.org/10.1002/bies.1117 PMID: 11536294

75. Somech R, Shaklai S, Geller O, Amargil N, Simon AJ, Rechavi G, et al. The nuclear-envelope protein and transcriptional repressor LAP2beta interacts with HDAC3 at the nuclear periphery, and induces histone H4 deacetylation. J Cell Sci. 2005; 118(Pt 17):4017–25. Epub 2005/09/01. https://doi.org/10.1242/jcs.02521 PMID: 16129885

76. Bagheri A, Habibzadeh P, Razavipour SF, Volmar CH, Chee NT, Brothers SP, et al. HDAC Inhibitors Induce BDNF Expression and Promote Neurite Outgrowth in Human Neural Progenitor Cells-Derived Neurons. Int J Mol Sci. 2019; 20(5). Epub 2019/03/08. https://doi.org/10.3390/ijms20051109 PMID: 30841499

77. Sakharkar AJ, Vetreno RP, Zhang H, Kokare DM, Crews FT, Pandey SC. A role for histone acetylation mechanisms in adolescent alcohol exposure-induced deficits in hippocampal brain-derived neurotrophic factor expression and neurogenesis markers in adulthood. Brain Struct Funct. 2016; 221(9):4691–703. Epub 2016/03/05. https://doi.org/10.1007/s00429-016-1196-y PMID: 26941165
78. Stertz L, Fries GR, Aguiar BW, Pfaffenseller B, Valvassori SS, Gubert C, et al. Histone deacetylase activity and brain-derived neurotrophic factor (BDNF) levels in a pharmacological model of mania. Braz J Psychiatry. 2014; 36(1):39–46. Epub 2013/12/19. https://doi.org/10.1590/1516-4446-2013-1094 PMID: 24346357

79. Koppel I, Timmusk T. Differential regulation of Bdnf expression in cortical neurons by class-selective histone deacetylase inhibitors. Neuropharmacology. 2013; 75:106–15. Epub 2013/08/07. https://doi.org/10.1016/j.neuropharmac.2013.07.015 PMID: 23916482

80. Zocchi L, Sassone-Corsi P. SIRT1-mediated deacetylation of MeCP2 contributes to BDNF expression. Epigenetics. 2012; 7(7):695–700. Epub 2012/06/09. https://doi.org/10.4161/epi.20733 PMID: 22677942

81. Good KV, Martinez de Paz A, Tyagi M, Cheema MS, Thambirajah AA, Gretzinger TL, et al. Trichostatin A decreases the levels of MeCP2 expression and phosphorylation and increases its chromatin binding affinity. Epigenetics. 2017; 12(11):934–44. Epub 2017/11/04. https://doi.org/10.1080/15592294.2017.1380760 PMID: 29099289

82. Guarda A, Bolognese F, Bonapace IM, Badaracco G. Interaction between the inner nuclear membrane lamin B receptor and the heterochromatic methyl binding protein, MeCP2. Exp Cell Res. 2009; 315 (11):1895–903. Epub 2009/04/01. https://doi.org/10.1016/j.yexcr.2009.01.019 PMID: 19331822

83. Bienvenu T, Carrie A, de Roux N, Vinet MC, Jonveaux P, Couvert P, et al. MECP2 mutations account for most cases of typical forms of Rett syndrome. Hum Mol Genet. 2000; 9(9):1377–84. Epub 2000/05/18. https://doi.org/10.1093/hmg/9.9.1377 PMID: 10814719

84. Serebryannyy LA, Cruz CM, de Lanerolle P. A Role for Nuclear Actin in HDAC 1 and 2 Regulation. Sci Rep. 2016; 6:28460. Epub 2016/06/28. https://doi.org/10.1038/srep28460 PMID: 27345839

85. Devinsky O, Bear D. Varieties of aggressive behavior in temporal lobe epilepsy. The American journal of psychiatry. 1984; 141(5):651–6. Epub 1984/05/01. https://doi.org/10.1176/ajp.141.5.651 PMID: 6711685

86. D’Angio GJ, Evans A, Breslow N, Beckwith B, Bishop H, Farewell V, et al. The treatment of Wilms’ tumor: results of the Second National Wilms’ Tumor Study. Cancer. 1981; 47(9):2302–11. Epub 1981/05/01. https://doi.org/10.1002/1097-0142(19810501)47:9<2302::aid-cncr2820470933>3.0.co;2-k PMID: 6164480

87. Khatau S, Nair CN, Ghosh K. Immune-mediated thrombocytopenia following dactinomycin therapy in a child with alveolar rhabdomyosarcoma: the unresolved issues. J Pediatr Hematol Oncol. 2004; 26 (11):777–9. Epub 2004/11/16. https://doi.org/10.1097/00043426-200411000-00020 PMID: 15543019

88. Grimm RA, Muss HB, White DF, Richards F 2nd, Cooper MR, Stuart JJ, et al. Actinomycin D in the treatment of advanced breast cancer. Cancer Chemoother Pharmacol. 1980; 4(3):195–7. Epub 1980/01/01. https://doi.org/10.1007/BF00254018 PMID: 7397943

89. Simonato M. Gene therapy for epilepsy. Epilepsy Behav. 2014; 38:125–30. Epub 2013/10/09. https://doi.org/10.1016/j.yebeh.2013.09.013 PMID: 24100249