Pharmacology, Biochemistry and Behavior 127 (2014) 21–26

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Maternal methyl-enriched diet in rat reduced the audiogenic seizure proneness in progeny

I.I. Poletaeva [a,*], N.M. Surina [a,1], V.V. Ashapkin [b,2], I.B. Fedotova [a,1], I.B. Merzalov [c,3], O.V. Perepelkina [a,1], G.V. Pavlova [c,3]

[a] Biology Faculty, Moscow State University, Leninskie Gori, 1, build. 12, Moscow 119992, Russia
[b] Belozersky Institute of Physical-Chemical Biology, Moscow State University, Leninskie Gori, 1, build. 40, Moscow 119992, Russia
[c] Institute of Gene Biology, Russian Academy of Sciences, Vavilova str., 34/5, Moscow 119334, Russia

ARTICLE INFO

Article history:
Received 26 January 2014
Received in revised form 23 September 2014
Accepted 26 September 2014
Available online 5 October 2014

Keywords:
Audiogenic epilepsy
Methyl enriched diet
DNA methylation

ABSTRACT

Audiogenic epilepsy proneness was analyzed in the progeny of rats from two strains (audiogenic seizure prone—strain "4"—and audiogenic seizure non-prone, strain "0"). Females were fed by a diet which contained substances enriched with methyl-groups during 1 week before mating (MED), during pregnancy period and 1 week after the delivery. This MED treatment resulted in a decrease of audiogenic seizure fit intensity, which was more evident in rats of strain "0". Control rats of strain "4" displayed intense seizures (tonic seizure, 3.85 arbitrary units). Med "4" rats seizures were less intense (3.23, tonic seizure of lower intensity), control "0" strain rats demonstrated the seizure with mean 3.09 arbitrary units, "0" MED rats only 2.03 arbitrary unit intensity (only clonic seizures, significantly, $p < 0.05$, different from controls). Methyl-enriched diet resulted in the significant changes in methylation status of several genes (Cpne6, Gtf2i, Sctr,1 Sfmbt, Phe2). These genes among others were chosen for analysis as their expression was analyzed in other methylation study. These genes were hypermethylated after "epileptic tolerance". Due to this procedure, the intensity of status epilepticus, produced by kainate in mice, decreased (Miller-Delaney et al., 2012). The modulation of audiogenic seizure intensity as the result of methyl-enriched diet during prenatal and early postnatal ontogeny was demonstrated for the first time.

1. Introduction

The genetic predisposition to seizure development was described both in humans (see EPICURE Consortium and EMINet Consortium, 2012; Helbig and Lowenstein, 2013) and in animals (Coppola and Moshé, 2012). As it was demonstrated the processes of brain development are also under epigenetic control, and it is now possible to analyze the role of epigenetic processes and namely the DNA methylation in epileptogenesis (Schatz et al., 1983; Lester et al., 2011; Hwang et al., 2013; Kobow et al., 2009; Kobow and Blümcke, 2011).

The role of DNA methylation in epileptogenesis was investigated in animal experiments (see Sellinger et al., 1986; Tsankova et al., 2004; Miller-Delaney et al., 2012) and in clinic (Kobow and Blümcke, 2012). Gene MBD5 (the product -*methyl-CpG binding domain protein 5*) is the member of methyl-binding group of genes, and it probably participates in brain DNA methylation (Noh and Graham, 2012). The deletion of this gene, found in humans, was accompanied by severe CNS dysfunction, including seizures and developmental delay (Motobayashi et al., 2012). The patterns of gene expression, which are presumably determined (among other factors) by changes in epigenetic processes, vary in animal models of epilepsy (Kobow et al., 2013).

Human brain DNA methylation and epilepsy are shown to be connected. Miller-Delaney et al. (2012) analyzed the effects of seizure preconditioning using status epilepticus (SE) model (seizures induced in C57BL/6 mice by intra-amygdala microinjections of kainic acid). The preconditioning procedure included i.p. injection of kainic acid before SE induction, which promoted seizure tolerance. Based on genome-wide DNA methylation analysis authors discovered changes in methylation status of 288 genes (in hippocampal tissue), and 15 genes from this group were differentially hypermethylated (in comparison to SE group) in the case when pre-conditioning + SE procedures were used. Most of these genes were "novel" and not known previously to be implicated in SE tolerance mechanisms.

The study of epigenetic mechanisms involved in seizure development includes usually the comparison of brain DNA methylation patterns during seizures (or immediately after seizures) vs a normal state. Using this approach changes were found in the methylation level of several genes (Doyle and Sellinger, 1980; Sellinger et al., 1986). At the same time practically no data exist concerning the role of DNA

* Corresponding author at: Biology Faculty, Moscow State University, Leninskie Gori, 1, build. 12, Russia. Tel.: +7 495 9394468; fax: +7 495 9392837.
E-mail addresses: ingapoletaeva@mail.ru (I.I. Poletaeva), ashapkin@genebee.msu.su (V.V. Ashapkin).
[1] Tel.: +7 495 9394468; fax: +7 495 9392837.
[2] Tel.: +7 495 939 53 59.
[3] Tel.: +7 499 1352541.

http://dx.doi.org/10.1016/j.pbb.2014.09.018
0091-3057/

methylation in determining seizure proneness in genetic animal models of seizure states.

The adequate and easily reproducible generalized seizure fit model is rodent audiogenic epilepsy (Feller et al., 1994; Faingold, 1999; Misawa et al., 2002; Prieto-Martín et al., 2012). The genetic models used are mainly KM strain (Russia), GEPR strains (USA) and WAR strain (Brazil) (Poletaeva et al., 2011). The proneness to these types of seizures has demonstrated genetic components (Poletaeva et al., 2011; McMillan and White, 2004). The physiological mechanisms of audiogenic seizure fit (ASF) are different from those involved in limbic seizure development (Faingold, 1999; Poletaeva et al., 2011).

The DNA methylation level could be modulated using special methyl-enriched diet (Prasolova et al., 2006, 2009; Van den Veyver, 2002; McGowan et al., 2008). In these types of experiments changes in the gene methylation were described as well as some phenotypic changes induced by this treatment (Pliusnina et al., 2007; Gerbek et al., 2010; McGowan et al., 2008; Zhang et al., 2013). These data were the prerequisite for the suggestion that brain DNA methylation level could be analyzed in rats with different proneness for seizures when methylation "load" was induced by means of special diet. In the present study the intensity of ASF intensity, as well as development of audiogenic myoclonic seizures was analyzed in rats of two strains which differed in audiogenic seizure proneness. Special maternal diet should presumably induce changes in brain DNA methylation. The methylation status (using methylation-dependent DNA polymerase chain reaction, PCR) was analyzed in those genes, which were found to be hypermethylated in mice in case of preconditioning + SE experiments, mentioned above (tolerance, by Miller-Delaney et al., 2012).

2. Material and methods

2.1. Subject and selection procedure

Rats of strains "0" and "4" (18^{-th} selection generation) were used. These strain are selected for the absence of ASF in response to loud sound strain "0" (for "null" reaction to sound) and for its maximal intensity (strain "4", as "4" is the maximal arbitrary unit of this trait intensity, adopted in the laboratory). Selection started using animals of hybrid population between rats of the inbred strain with high audiogenic seizure proneness (Krushinsky–Molodkina or KM strain: see Poletaeva et al., 2011) and Wistar rats. From the group of 60 Wistar rats 4 males and 3 females were chosen that did not develop ASF after 3 successive sound exposures, presented after 1 week interval (Fedotova et al., 2012). Animals from the hybrid population, which did not develop seizures from sound testing, were chosen as parents for strain "0"; animals with maximal ASF intensity were the founders for strain "4". The offspring of these matings were behaviorally tested to sound at 3 months of age using the same protocol. Thus animals used in the present study differed in audiogenic seizure proneness yet to a large extent share a common genetic background. As the selection for "0" degree of ASF slowly proceeded (Fedotova et al., 2012) an absence of ASF was found only in a portion of "0" strain rats (in the control group of "0" rats used in present study there were around 20% of rats without any signs of audiogenic epilepsy), although the ASF intensity of rats from "0" strain was low (see below). Five females from strain "0" and 7 females from strain "4" after mating to males of the respective strains gave birth to a total of 67 pups (see Table 1). Part of them were controls, as their mothers received the normal food (without methyl enrichment, see below), while others were "experimental" (i.e. after maternal methyl enriched diet -MED).

Table 1
Numbers of control and experimental (MED) animals of both strains, which participated in the experiment.

Strain	Group	Male	Female	Total
"0"	"Experiment"	12	6	18
	"Control"	5	6	11
"4"	"Experiment"	6	5	11
	"Control"	10	17	27

2.2. Bioethical standards

Animals used in research have been bred in the laboratory and cared for in accordance with the guidelines published in NIH *Guide for the Care and Use of Laboratory Animal* and the principles presented in the "Guidelines for the Use of Animals in Neuroscience Research" by the Society for Neuroscience. The experimental protocol was approved by Moscow State University Ethical Committee and was in accordance to general bioethical principles (namely to EC Convention 2010 rules).

2.3. Methyl-enriched diet (MED)

The chemical ingredients, which were added (per 1 kg of food) in order to enrich rat food with methyl-group radicals, were the following: choline—5 g, betain—15 g, folic acid—15 mg, vitamin B12—1.5 mg, L-methionine—7.5 g, and zink (as ZnSO4)—150 mg (by Prasolova et al., 2006). These ingredients were mixed with boiled buckwheat, cottage cheese and fresh eggs. Females received this food for 1 week before mating, during the entire pregnancy period and 1 week after the delivery. Control females received the same food without methyl-group enriching ingredients.

2.4. Testing the audiogenic seizure proneness

The testing of audiogenic seizures was performed twice at the age of 3 and 4.5 months. Animal was placed inside the sound attenuating box and the sound (auditory-bell, 120 dB) was switched on. After the development of seizure fit (or after 90 s in cases, when no seizure developed) the sound was switched off. The audiogenic seizure intensity was evaluated according to the arbitrary scale, with "0"—for the absence of fit, "1"—for the stage of "clonic" run (or "wild run stage"), "2"—clonic seizure, "3"— tonic seizure, and "4"— maximal intense tonic seizure of the trunk muscles and extremities and animal falling on the side (Poletaeva et al., 2011). After weaning, at 28 days animals were separated from mother and kept in the female or male groups of 3–4 individuals in A4 cages with water and standard rodent food (Laboratorkorm firm) ad lib. At the age of 3 months all rats were tested for audiogenic proneness for the first time. At the age of 4.5 month a second similar test and the procedure of audiogenic kindling took place. The latter included daily repetitive sound exposure (during 25 days) to test the development of audiogenic myoclonic seizures (Fedotova and Semiokhina, 2002; Galvis-Alonso et al., 2004).

2.5. DNA extraction

DNA extraction was performed from the whole brain tissue (for technical reasons) by means of standard phenol extraction technique. The tissue samples were taken after three to four weeks after the end of audiogenic seizures and audiogenic kindling testing; thus the previous sound exposure presumably could not affect the methylation status of the DNA.

2.6. Methylation level assays

A 200–400 bp segment containing one to five CCGG sequences recognized by restriction endonucleases *Hpa*II and *Msp*I was chosen for each gene in study. These restriction endonucleases are well known isoschizomers having different sensitivity to cytosine methylation. Namely *Hpa*II is inhibited by internal cytosine methylation (Cm5CGG sites) whereas *Msp*I cleaves DNA irrespective of such methylation. Both endonucleases are inhibited by external cytosine methylation

(m5CCGG sites) but such methylation is a rather rare event in mammalian DNA and is known to occur in embryonic stem cells primarily. The oligonucleotide primers for the PCR amplification of the gene segments chosen were designed with Primer3 (http://frodo.wi.mit.edu/primer3/) and synthesized by Evrogen (Moscow) (see Table 2).

The relative quantity of DNA-target was measured with real-time SYBR Green PCR (qPCR-HS SYBR + ROX kit, Evrogen, Moscow) on a DT-322 (DNA-Technologiya, Moscow) thermocycler. The relative quantities of each DNA-target DNA segment were calculated in samples C, H and M. Since the C sample represents an intact DNA the quantity of each DNA-target DNA segment in this sample (C) was regarded as a full gene dosage and a value of 1 was ascribed to it. The intact DNA segments in sample H correspond to those methylated at the internal cytosines of CCGG sites (all unmethylated ones were hydrolyzed by HpaII and therefore did not participate in PCR); thus the quantity of respective DNA targets (H) was regarded as a measure of DNA methylation. Last but not least, the quantity of each DNA-target segment in sample M was regarded as a background noise (*Msp*I is not sensitive to internal cytosine methylation and therefore all DNA-target DNA segments must be hydrolyzed). The methylation level of each DNA-target DNA segment in % was calculated as $100 \times (H - M) / (C - M)$. Each level was calculated from three independent PCR experiments carried out in parallel.

2.7. Statistics

Group differences in audiogenic seizure proneness were evaluated using ANOVA-MANOVA (Statistica 6) with *post hoc* Fisher *LSD test*, which was considered the most adequate for the goal of the present study. The significance of differences in DNA methylation for the pair comparisons was evaluated be Kolmogorov–Smirnov criterion which fits for this type of data processing.

3. Results

3.1. General observations

Rats of both strains from the MED groups did not differ from controls in body weight (around 160–170 g), in general appearance and activity, although other special behavioral tests were not performed.

Table 2
The primers for PCR (genes chosen for analysis of methylation level, see text, www.evrogen.ru).

Oligo	Sequence 5′–3′	L
Cpne6F	GCCCAGTCTCCATAACAACATC	20
Cpne6R	ATTCTCTCTGTGCCTGTCCA	20
Gtf2iF	TTTATTCGACACTCTGCCCC	20
Gtf2iR	AGACCATCCCTCTCAAACAC	20
Hmga2F	GGTCCCACGGATTAAAAGCG	20
Hmga2R	GCTTTTAAAGATCCCGAGGA	20
Hspa1bF	ACGGCAAGGTGGAGATCATCG	20
Hspa1bR	TCGTTGAAGTAGGCGGGCAC	20
Ifr4F	AGCTATTGGGACTGTGGTGT	20
Ifr4R	GGGAATAAGGGGTATTGTGG	20
Mta1F	GCAGTCAGGGGACATAGATA	20
Mta1R	CCAAACATAGGGAATCGCAG	20
Phc2F	GGATAGCCCCCACTTTAAAC	20
Phc2R	TTATCCAGTAATGCCCCAGG	20
Scrt1F	ACGCCTTCTTCATCACTGAC	20
Scrt1R	TTTTGCCACACTCTCCGCAC	20
Sfmbt2F	GGTTTTCATCTTGGATTGGC	20
Sfmbt2R	GACCTCCTTCCTTTCTCCTT	20
Tcfap2eF	AGGAGTTGGTAATCGTCCAG	20
Tcfap2eR	TAGAAGAGGATGGCAGTGAG	20

3.2. Audiogenic epilepsy proneness in “0” and “4” rats in control and MED groups. ASF and kindling

Three factor ANOVA (strain, sex, treatment) revealed no effect of “sex” factor ($F166 = 0.8816$, $p = 0.351593$), so data on males and females of the same group were pooled for further processing. As mentioned above, the trait “non-proneness to audiogenic seizure” in the course of strain “0” selection (Fedotova et al., 2012) was always far from 100%. It means that certain proportion of strain “0” rats (in intact population in general, and in control group of this study in particular) developed seizures in response to sound although of low intensity (Table 3). The proportion of strain “0” animals, which showed no reaction to sound was in the range of 30–60% in previous selection generations (Fedotova et al., 2012).

The mean fit intensity values (Table 3) in rats of strain “4” at ages of 3 and 4.5 months were lower than maximal values in rats of their progenitor strain (KM) at the same age, these rats demonstrating stable 4 arbitrary units (Poletaeva et al., 2011).

Two factor ANOVA (factors—“strain” and “treatment”) with *post hoc* Fisher *LSD* analysis revealed no significant factor influence for ASF intensity at the age of 3 months.

The similar analysis for the fit intensity and latencies values in the same animals at the age of 4.5 months (Table 3) revealed the significant effects of both factors (strain—$F1–2 = 7.5912$, $p = 0.007659$ and treatment—$F1–2 = 5.6260$, $p = 0.020764$). *Post hoc* LSD criterion showed significant differences between control and MED groups.

The fit latency, being very brief in KM rats (usually 2–4 s), was longer in rats of both strains tested (Table 3), and two factor ANOVA showed the significant effect of the factor “treatment” ($F1–2 = 4.29$, $p = 0.042338$) on the scores of this variable. The fit latency in groups after MED was longer, which also indicated a decrease of audiogenic seizure proneness.

The data demonstrated the unidirectional influence of MED on seizure fit characteristics decrease of seizure proneness in rats treated with MED. The proportions of animals which did not develop audiogenic fit after sound exposure in control and MED groups of strain “0” were 18.2% and 33.3% respectively, while in strain “4” the respective values were 18.2% and 18.5%. This fact indicated that MED decreased the fit intensity mainly in “0” selected rats, although as shown in Table 3 the decrease of mean fit intensity and the increase of fit latency in the “4” strain occurred also in values not significantly different from controls ($p > 0.05$).

Audiogenic kindling scores in rats of both strains did not reveal the influence of MED. Regular daily sound exposure during 24 days resulted in the development of myoclonic hyperkinetic partial seizures in all rats of “4” strain, similar to those described for rats of KM strain (Poletaeva et al., 2011) and rats of other audiogenic epilepsy prone strains as well. These partial seizures involved head and forepaw muscles which developed in the course of ASF or after it. No signs of audiogenic kindling both in control and MED groups were noticed in strain “0” rats. No differences in kindling parameters in strain “4” groups (MED vs controls) were found.

3.3. Changes in methylation of DNA regions

The differences in methylation of genes chosen for analysis are shown in the Table 4.

The methylation levels of genes *Irf4*, *Mta1*, *Hspa1b* and *Tcfap2e* in control and MED groups were similar. Other genes demonstrated both inter strain differences and differences between MED and control groups. The gene *Cpne6* gene was significantly highly methylated ($p < 0.01$) in the MED group of strain “4” in comparison to “0” MED, and it was significantly ($p < 0.05$) more methylated in strain “0” controls in comparison to MED “0” group. The methylation of *general transcription factor II I* (*Gtf2i*) gene was significantly higher in control group of strain “0” in comparison to strain “4” controls, although MED

Table 3
Audiogenic fit intensities and latencies in rats of "0" and "4" groups in control and "Med" conditions.

Genotype	Group	Fit intensity, age 3 months[&]	Fit intensity, age 4.5 months[&]	Fit latency, age 3 months, s	Fit latency, age 4.5 months, s
	"0" "MED"	1.76 ± 0.41	2.03 ± 0.32[*]	49.83 ± 12.21	44.66 ± 9.34[#]
	"0" "control"	2.59 ± 0.51	3.09 ± 0.41	28.09 ± 15.62	17.18 ± 11.95
	"4" "MED"	3.09 ± 0.51	3.23 ± 0.26	24.45 ± 15.62	24.36 ± 11.95
	"4" "control"	2.76 ± 0.32	3.85 ± 0.26	35.33 ± 9.97	8.81 ± 7.63

[&] In arbitrary scores, see Methods.
[*] Significantly different from scores of control group, $p < 0.05$.
[#] Different from scores of control group (tendency, $p < 0.075$).

induce significant ($p < 0.01$) increase in methylation in strain "4" only. The methylation of *Sctr1* gene was different ($p < 0.05$) in two control groups. It was also different in two MED groups as well ($p < 0.05$), being higher in "4". The methylation levels were also different between the control and MED groups in both "0" and "4" rats. At the same time in strain "0" MED group methylation was higher than in controls, while in strain "4"—the pattern of differences was the reverse.

Gene *Sfmbt* (its product -*Scm-like with four mbt*) *domains*) (Lee et al., 2013) is highly methylated in both control groups (60% and 70%, Table 3); its methylation did not change as the result of MED in strain "4", and it was significantly ($p < 0.05$) *higher* in MED "0" brains in comparison to strain "4" sample. The methylation levels of *Phe2* gene (*polyhomeiotic homolog 2* product) were significantly ($p < 0.05$) different between control groups of two strains with higher values for strain "4".

4. Discussion

4.1. Possible explanations of data

No differences in methylation levels in control and MED groups of *Irf4*, *Mta1*, *Hspa1b* and *Tcfap2e* genes were found as the result of MED. The hypomethylation of these genes was reported for SE and hypermethylation—for SE + tolerance procedure in Miller-Delaney et al. (2012) study. These data could indicate rather large differences in molecular mechanisms of seizures in ASF and kainic acid–SE models. It should be also mentioned that *Hspa1b* gene, which codes for the respective heat-shock protein 70 kD was in this group is of certain interest. It was shown earlier that infusion of heat-shock inducible protein (*heat shock* 70 kDi) into the brain of KM rats decreased the intensity of ASF in these animals (Khudik et al., 2011).

Gene *Cpne6* is coding for the *copine VI (neuronal) phosphatidylserine binding* protein. This protein provides the calcium-dependent binding of phospholipids in association with membranes. This gene was frequently found in amplified state in cases of malignant malformations (Nakayama et al., 1999). The methylation of this gene was higher in MED "4" brains, significantly lower in "0" MED brains and could be presumably associated with the decrease in the ASF intensity in "0" MED group (it should be reminded that group MED of "0" strain revealed the low ASF intensity). Thus the pattern of differences for this gene methylation was in a way opposite to that which could be expected (as methylation of DNA is thought to reduce gene expression). This phenomenon could be explained by the complicated nature of relationships between brain cells membrane processes and ASF proneness. It should be mentioned that in KM rats the ASF development was influenced by the intensity of oxidative stress processes (see Poletaeva et al., 2011). *The general transcription factor II I* (*Gtf2i*) gene is expressed during early ontogeny (Bayarsaihan et al., 2012) and thus could participate in the development of brain regions which influence brain excitability levels.

Scrt1 gene is coding for zinc-finger protein (*scratch homolog 1, zinc finger protein SCRATCH1*), which participates in the ontogenetic processes and has homologues practically in all taxons (Bastid et al., 2010). The levels of *Sctr1* methylation were significantly different ($p < 0.05$) in all four groups and it is difficult to find the explanation for these results, as the function of this gene is not well known. This gene belongs to the neural-specific Snail family transcriptional repressor (Nakakura et al., 2001) and was shown to be predominantly confined to the brain and spinal cord, appearing in newly differentiating, post-mitotic neurons and persisting into postnatal life.

The intergroup differences in methylation of gene *Sfmbt* followed mainly the differences in ASF (the decrease of ASF in MED "0" group). Mice with null mutation in this gene showed defects which resembled the symptoms of Williams–Beuren syndrome, i.e. the dysfunction of this gene affects CNS development rather seriously (Schneider et al., 2012).

4.2. General discussion

The study of epileptogenesis mechanisms is very important for clinic, and it requires different approaches and various experimental designs. The interference into brain development, which was different by type from that of MED, could affect the ASF as well, as was shown in our previous work (Poletaeva et al., 2012). In this study KM and

Table 4
The genes in which the methylation level in the brain tissue changed as the result of MED.

Gene	Gene product	Animal group, the level of methylation (in %)			
		"4", control.	"4", MED.	"0", control.	"0", MED.
Cpne6	*Copine VI (neuro-nal) phosphatidyl-serine binding*	35%	50%	30%	Not methylated
Gtf2i	*General transcription factor II I*	Not methylated	40%	30%	32%
Hmga2	*High mobility group AT-hook 2chromo-somal architecture protein*	Nor methylated	10%	10%	10%
Hspa1b	*Heat shock 70 kD protein 1B*	20%	20%	20%	20%
Ira4	*Interferon regulatory factor 4*	Not methylated	Not methylated	Not methylated	Not methylated
Mta	*Metastasis associated 1*	Not methylated	Not methylated	Not methylated	Not methylated
Phc2	*Polyhomeotic homolog 2*	30%	20%	10%	10%
Scrt1	*Scratch homolog 1, zinc finger protein*	50%	30%	30%	35%
Sfmbt2	*Scm-like with four mbt domains*	60%	60%	70%	80%
Tcfap2e	*Transcription factor AP-2 epsilon*	40%	40%	40%	40%

Wistar rat pups of the first days of life were injected with neuropeptide Semax (synthetic analogue of ACTH 4–10 fragment) during either the first or the second weeks of life and tested for ASF proneness at 3 months of age. The decrease in ASF intensity was found in experimental animals as remote effects of that treatment, similarly to MED which was also a mild intervention into brain development processes and there was a change in ASF intensity.

The finding of significant inter strain differences in methylation levels between control "0" and "4" groups for 3 genes among 10 chosen for analysis (*Gtf2i*, *Phc2*, *Scrt1*) is of interest, although interpretation of these results is rather difficult. The three genes mentioned are the participants of signaling cascades active during early ontogeny. Audiogenic prone rats (KM, GEPR and WAR strains) have numerous neurochemical deviations in neurotransmitter systems (monoaminergic, glutamatergic, GABAergic etc.). The involvement of early developmental events in the genesis of ASF pathological trait could be connected to the epigenetic regulation of these genes.

Gene analysis did not reveal a pattern of differences in methylation parallel to those with audiogenic proneness (with the probable exception of gene *Sfmbt*). Different interpretations of these results could be given. The pattern of methylated–demethylated status of genes involved in the epileptogenesis could depend on the type of seizures, i.e. on the experimental model chosen for study. In case of ASF the epileptic discharge starts in the medulla, quickly spreads to the midbrain and afterwards to forebrain structures. ASF is the generalized seizure state which develops quickly and is uniform in form across all rodents species analyzed. ASF is thus different "structurally" from limbic seizures (Pallud et al., 2011) and the pattern of genetic regulation could also be not identical. The differences of temporal lobe epilepsy and the generalized seizures in human are also well known (de Lanerolle and Lee, 2005; Bartolomei et al., 2008).

The plausible differences in epigenetic regulation of seizure proneness are of course not restricted to 10 genes chosen for analysis in this work. And it is also possible that the increase of methylation in genes which promote the seizure inhibition could also occur (inducing thus the increase in fit intensity). Nevertheless, we should stress that changes (decrease of fit severity) were demonstrated in audiogenic epilepsy as the result of MED, which acted during pre- and early postnatal ontogeny. In the present work changes in audiogenic seizures scores (the trait, which has the definite genetic component) were demonstrated for the first time which were the result of increased methylation "load" during the prenatal and early postnatal ontogeny. Changes in the methylation levels of several genes, which were described, prove that the MED technique is effective in modulation brain DNA methylation. The data also demonstrate the high phenotypic complexity of the audiogenic seizures, and we think that these results show that MED could be rather powerful tool for modulation seizure proneness in other seizure models.

5. Conclusions

1. Methyl enriched diet during prenatal development and first days of postnatal ontogeny of pups induced a significant decrease in the intensity of audiogenic seizure fit in rats of the strain, less prone to audiogenic seizures.
2. The maternal food enrichment with ingredients, which presumably can increase DNA methylation, resulted in changes of methylation pattern in genes (*Cpne6*, *Gtf2i*, *Sctr*,*1 Sfmbt*, *Phe*) of their progeny. According to present knowledge some of these genes are members of signaling cascades which are active during early ontogeny.

Acknowledgments

This study was supported by the Russian Foundation for Fundamental research, grants nos. 09-04-00481 and 12-04-00360.

References

Bartolomei F, Chauvel P, Wendling F. Epileptogenicity of brain structures in human temporal lobe epilepsy: a quantified study from intracerebral EEG. Brain 2008;131:1818–30.
Bastid J, Bouchet BP, Ciancia C, Pourchet J, Audoynaud C, Grelier G, et al. The SNAIL family member SCRATCH1 is not expressed in human tumors. Oncol Rep 2010;23:523–9.
Bayarsaihan D, Makeyev AV, Enkhmandakh B. Epigenetic modulation by TFII-I during embryonic stem cell differentiation. J Cell Biochem 2012;113:3056–60.
Coppola A, Moshé SL. Animal models. Handb Clin Neurol 2012;107:63–98.
de Lanerolle NC, Lee TS. New facets of the neuropathology and molecular profile of human temporal lobe epilepsy. Epilepsy Behav 2005;7:190–203.
Doyle RL, Sellinger OZ. Differences in activity in cerebral methyltransferases and monoamine oxidases between audiogenic seizure susceptible and resistant mice and deermice. Pharmacol Biochem Behav 1980;13:589–91.
EPICURE Consortium, EMINet Consortium. Genome-wide association analysis of genetic generalized epilepsies implicates susceptibility loci at 1q43, 2p16.1, 2q22.3 and 17q21.32. Hum Mol Genet 2012;21:5359–72.
Faingold CL. Neuronal networks in the genetically epilepsy-prone rat. Adv Neurol 1999;79:311–21.
Fedotova IB, Semiokhina AF. Developmental changes in audiogenic epilepsy and myoclonus in KM rats. Zh Vyssh Nerv Deiat Im I P Pavlova 2002;52:261–5. [Russian].
Fedotova IB, Kostyna ZA, Surina NM, Poletaeva II. Laboratory rat selection for the trait "the absence of audiogenic seizure proneness". Russ J Genet 2012;48:685–91.
Feller DJ, Bassir JM, Crabbe JC, Le Fevre CA. Audiogenic seizure susceptibility in WSP and WSR mice. Epilepsia 1994;35:861–7.
Galvis-Alonso O, Cortes De Oliveira JA, Garcia-Cairasco N. Limbic epileptogenicity, cell loss and axonal reorganization induced by audiogenic and amygdala kindling in Wistar audiogenic rats (WAR strain). Neuroscience 2004;125:787–802.
Gerbek IuE, Os'kina IN, Gulevich RG, Pliusnina IZ. Effects of maternal methyl-supplemented diet on glucocorticoid receptor gene expression in the hippocampal of rats selected for behavior. Tsitol Genet 2010;44:45–52. [Russian].
Helbig I, Lowenstein DH. Genetics of the epilepsies: where are we and where are we going? Curr Opin Neurol 2013;26:179–85.
Hwang JY, Aromolaran KA, Zukin RS. Epigenetic mechanisms in stroke and epilepsy. Neuropsychopharmacology 2013;38:167–82.
Khudik KA, Pastukhov IuF, Guzhova IV. Effects of thermal preconditioning on convulsive activity in rats with inheritable form of audiogenic epilepsy. Ross Fiziol Zh Im I M Sechenova 2011;97:1237–46. [Russian].
Kobow K, Blümcke I. The methylation hypothesis: do epigenetic chromatin modifications play a role in epileptogenesis? Epilepsia 2011;52(Suppl. 4):15–9.
Kobow K, Blümcke I. The emerging role of DNA methylation in epileptogenesis. Epilepsia 2012;53(Suppl. 9):11–20.
Kobow K, Jeske I, Hildebrandt M, Hauke J, Hahnen E, Buslei R, et al. Increased reelin promoter methylation is associated with granule cell dispersion in human temporal lobe epilepsy. J Neuropathol Exp 2009;68:356–64.
Kobow K, Kaspi A, Harikrishnan KN, Kiese K, Ziemann M, Khurana I, et al. Deep sequencing reveals increased DNA methylation in chronic rat epilepsy. Acta Neuropathol 2013;126:741–56.
Lee K, Na W, Maeng JH, Wu H, Ju BG. Regulation of DU145 prostate cancer cell growth by Scm-like with four mbt domains 2. J Biosci 2013;38:105–12.
Lester BM, Tronick E, Nestler E, Abel T, Kosofsky B, Kuzawa CW, et al. Behavioral epigenetics. Ann N Y Acad Sci 2011;1226:14–33.
McGowan PO, Meaney MJ, Szyf M. Diet and the epigenetic (re)programming of phenotypic differences in behavior. Brain Res 2008;1237:12–24.
McMillan DR, White PC. Loss of the transmembrane and cytoplasmic domains of the very large G-protein-coupled receptor-1 (VLGR1 or Mass1) causes audiogenic seizures in mice. Mol Cell Neurosci 2004;26:322–9.
Miller-Delaney SFC, Das S, Sano T, Jimenez-Mateos EM, Bryan K, Buckley PG, et al. Differential DNA methylation patterns define status epilepticus and epileptic tolerance. J Neurosci 2012;32:1577–88.
Misawa H, Sherr EH, Lee DJ, Chetkovich DM, Tan A, Schreiner CE, et al. Identification of a monogenic locus (jams1) causing juvenile audiogenic seizures in mice. J Neurosci 2002;22:10088–93.
Motobayashi M, Nishimura-Tadaki A, Inaba Y, Kosho T, Miyatake S, Niimi T, et al. Neurodevelopmental features in 2q23.1 microdeletion syndrome: report of a new patient with intractable seizures and review of literature. Am J Med Genet A 2012;158A:861–8.
Nakakura EK, Watkins DN, Schuebel KE, Sriuranpong V, Borges MW, Nelkin BD, et al. Mammalian Scratch: a neural-specific Snail family transcriptional repressor. Proc Natl Acad Sci U S A 2001;98:4010–5.
Nakayama T, Yaoi T, Kuwajima G, Yoshie O, Sakata T. Ca2(+)-dependent interaction of N-copine, a member of the two C2 domain protein family, with OS-9, the product of a gene frequently amplified in osteosarcoma. FEBS Lett 1999;453:77–80.
Noh GJ, Graham Jr JM. 2q23.1 microdeletion of the MBD5 gene in a female with seizures, developmental delay and distinct dysmorphic features. Eur J Med Genet 2012;55:354–7.
Pallud J, Häussler U, Langlois M, Hamelin S, Devaux B, Deransart C, et al. Dentate gyrus and hilus transection blocks seizure propagation and granule cell dispersion in a mouse model for mesial temporal lobe epilepsy. Hippocampus 2011;21:334–43.
Pliusnina IZ, Os'kina IN, Shchepina OA, Prasolova LA, Trut LN. Maternal methylcontaining dietary supplementation alters the ability to learn in adult rats in swimming Morris test. Neurosci Behav Physiol 2007;37:425–8.
Poletaeva II, Fedotova IB, Sourina NM, Kostina ZA. Audiogenic seizures. Biological phenomenon and experimental model of human epilepsies. In: Afawi Z, editor.

Clinical and genetic aspects of epilepsy. Rijeka, Croatia: InTech Publ 978-953-307-700-0; 2011. p. 115–48.
Poletaeva II, Perepelkina OV, Boyarshinova OS, Lil p IG, Markina NV, Timoshenko TB, et al. Neonatal injections of pharmacological agents and their remote genotype dependent effects in mice and rats. Rus J Dev Biol 2012;43:319–31.
Prasolova LA, Trut LN, Os'kina IN, Gulevich RG, Pliusnina IZ, Vsevolodov EB, et al. The effect of methyl-containing supplements during pregnancy on the phenotypic modification of offspring hair color in rats. Genetika 2006;42:78–83. [Russian].
Prasolova LA, Os'kina IN, Pliusnina IZ, Trut LN. Methyl-containing diet of mothers affects the AGOUTI gene expression in the offspring of rats with various behavioral types. Genetika 2009;45:670–6. [Russian].
Prieto-Martín AI, Llorens S, Pardal-Fernández JM, Muñoz LJ, López DE, Escribano J, et al. Opposite caudal versus rostral brain nitric oxide synthase response to generalized seizures in a novel rodent model of reflex epilepsy. Life Sci 2012;90:531–7.
Schatz RA, Wilens TE, Tatter SB, Gregor P, Sellinger OZ. Possible role of increased brain methylation in methionine sulfoximine epileptogenesis: effects of administration of adenosine and homocysteine thiolactone. J Neurosci Res 1983;10:437–47.
Schneider T, Skitt Z, Liu Y, Deacon RM, Flint J, Karmiloff-Smith A, et al. Anxious, hypoactive phenotype combined with motor deficits in Gtf2ird1 null mouse model relevant to Williams syndrome. Behav Brain Res 2012;233(2):458–73. http://dx.doi.org/10.1016/j.bbr.2012.05.014. [Aug 1, Epub 2012 May 28].
Sellinger OZ, Schatz RA, Gregor P. Cerebral methylations in epileptogenesis. Adv Neurol 1986:465–73.
Tsankova NM, Arvind KA, Nestler EJ. Histone modifications at gene promoter regions in rat hippocampus after acute and chronic electroconvulsive seizures. J Neurosci 2004;24:5603–10.
Van den Veyver IB. Genetic effects of methylation diets. Annu Rev Nutr 2002;22:255–82.
Zhang TY, Labonté B, Wen XL, Turecki G, Meaney MJ. Epigenetic mechanisms for the early environmental regulation of hippocampal glucocorticoid receptor gene expression in rodents and humans. Neuropsychopharmacology 2013;38:111–23.