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Effects of environmental noise exposure on DNA methylation in the brain and metabolic health

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Abstract

Environmental noise exposure is associated with adverse effects on human health including hearing loss, heart disease, and changes in stress-related hormone levels. Alteration in DNA methylation in response to environmental exposures is a well-known phenomenon and it is implicated in many human diseases. Understanding how environmental noise exposures affect DNA methylation patterns may help to elucidate the link between noise and adverse effects on health. In this pilot study we examined the effects of environmental noise exposure on DNA methylation of genes related to brain function and investigated whether these changes are related with metabolic health. We exposed four groups of male Wistar rats to moderate intensity noise (70-75dB with 20Hz-4000Hz) at night for three days as short-term exposure, and for three weeks as long-term exposure. Noise exposure was limited to 45 dB during the daytime. Control groups were exposed to only 45 dB, day and night. We measured DNA methylation in the *Bdnf*, *Comt*, *Crhr1*, *Mc2r*, and *Snca* genes in tissue from four brain regions of the rats (hippocampus, frontal lobe, medulla oblongata, and inferior colliculus). Further, we measured blood pressure and body weight after long-term noise exposure. We found that environmental noise exposure is associated with gene-specific DNA methylation changes in specific regions of the brain. Changes in DNA methylation are significantly associated with changes in body weight (between *Bdnf* DNA methylation and Δ body weight: $r = 0.59$, $p = 0.018$; and between LINE-1 ORF DNA methylation and Δ body weight: $r = -0.80$, $p = 0.0004$). We also observed that noise exposure decreased blood pressure ($p = 0.038$ for SBP, $p = 0.017$ for DBP and $p = 0.017$ for MAP) and decreased body weight ($\beta = -26$ g, $p = 0.008$). In conclusion, environmental noise exposures can induce changes in DNA methylation in the brain, which may be associated with adverse effects upon metabolic health through modulation of response to stress-related hormones.

Keywords: Environmental noise stress, brain, DNA methylation, epigenetics

Introduction

According to the World Health Organization (WHO), noise is the second highest environmental stressor from the public health perspective [1]. Continuous urbanization and transportation growth has resulted in a significant proportion of the general population, especially those living in urban areas, being chronically exposed to high levels of noise. Traffic noise primarily from roads, railways and aircraft is the main contributor to the overall burden of environmental noise, but with many additional sources. Subsequently, environmental noise is an inevitable pollutant that is linked with increased risk of a range of human diseases.

A recent European study revealed that at least 19.8 million adults feel irritation and annoyance due to traffic and industrial-derived noise. Environmental noise has been linked to important adverse health problems through studies in humans and animal models [2], such as cognitive deficits [3, 4], sleep disturbance, and physical change [5]. It has been estimated that environmental noise annually contributes to 900,000 additional cases of hypertension, 43,000 additional cases of hospital admission, and 10,000 premature deaths linked to heart disease and strokes [6]. Further, a variety of epidemiological studies have demonstrated that even low levels of noise exposure may induce brain dysfunction, cardiovascular disease, and hypertension [7-11].

Epigenetics is often considered to be a link between environmental exposures and human diseases. Epigenetics refers to changes that alter gene transcription in the absence of direct alterations to the genetic sequence [12]. Changes in DNA methylation have been widely reported in a variety of human diseases, and many environmental pollutants including particulate matter, toxic metals and pesticides can induce aberrant DNA methylation [13]. Furthermore, psychological and physiological stressors have been demonstrated to disrupt normal epigenetic patterns [14]. Aberrant DNA methylation patterns have been reported

following disruption of circadian rhythms [15], in adult mice suffering chronic social stress and following maternal care deficiency [16], and following prenatal famine stress from the Dutch Hunger Winter of 1944-45 [17]. However, to date there have been no studies of the effects of environmental noise stress on DNA methylation, especially in the brain.

In this study, we investigated DNA methylation changes in response to chronic low intensity environmental noise stress, including short- and long-term environmental noise exposure in rats. We also examined whether these changes are related with metabolic health. DNA methylation was analyzed in the frontal lobe, hippocampus, inferior colliculus, and medulla oblongata in five genes related with stress, hormonal response, nerve growth factors and brain function: brain-derived neurotrophic factor (*Bdnf*); catechol-O-methyltransferase (*Comt*); corticotropin releasing hormone receptor 1 (*Crhr1*); melanocortin 2 receptor (*Mc2r*); and synuclein Alpha (*Snca*). Further, we measured global DNA methylation through LINE-1 (LINE-1 untranslated region (UTR) and open reading frame (ORF)). The DNA methylation levels were compared with markers of metabolic health, including blood pressure and body weight.

Materials and Methods

Animals and Housing Conditions

Thirty-two adult male Wistar rats weighing between 250-280g were obtained from Dalian Medical University, China. The rats were housed in a controlled ambient environment, including temperature ($24\pm 2^{\circ}\text{C}$), humidity (60%), and a fixed 12h light/dark schedule (lights on at 7:00 AM). Rats had *ad libitum* access to food and water and were allowed to adapt to the laboratory environment for seven days before the start of the experiment. Blood pressure was measured with Rodent Blood Pressure Analysis System (BP-2000, Visitech Systems, Inc.). Procedures involving animals and their care were conducted in conformity with EC

Directive 86/609/EEC and was approved by Animal Care and Use Committee of Dalian Medical University.

Environmental Noise Exposure

The traffic noise was recorded at a busy crossroad in Shanghai, China. The recorded noise had a major octave band ranging from 20Hz to 4000Hz, composed of background noise (60dB) and several unpredictable noise events (Wave lab 3.0, Steinberg, Germany). The duration of noise events and inter-noise intervals was randomly distributed throughout the recording. The sound intensity was measured using a sound level meter and maintained at a range of 70-75dB.

Rats were randomly divided into four groups. One group of eight rats was exposed to the recorded traffic noise at night (7:00 PM-7:00 AM) each day for three consecutive days. The second group of eight rats was exposed to the noise recording for three weeks (21 days) with the same exposure protocol. Two control groups, each containing eight rats, were designated for both the three-day and three-week noise exposure groups. Both control groups were kept in a quiet room at night (7:00 PM-7:00 AM) and exposed to daily activity at 45dB sound intensity during daytime (Figure 1).

Figure 1

Sample Preparation and DNA Extraction

The animals were decapitated immediately after each experiment. Brains were removed from the skull and the frontal lobe, hippocampus, inferior colliculus, and medulla oblongata were rapidly dissected, frozen on dry ice, and kept at -80°C until processed. These four regions of the brain were selected upon their roles in the auditory pathway (inferior colliculus), memory (frontal lobe, hippocampus), and autonomic functions (medulla oblongata). DNA was extracted from the tissues using the Wizard Genomic DNA purification kit (Promega, Madison, WI) following the manufacturer's instructions. Purified DNA was stored at -20°C until analysis.

DNA Methylation Analyses

One µg of genomic DNA was bisulfite-converted using the EZ-96 DNA Methylation-Gold Kit (Zymo Research, Orange, CA, USA) according to the manufacturer's protocol. Converted

DNA was eluted in 30 µl M-Elution Buffer. DNA methylation was quantified by polymerase chain reaction (PCR) and pyrosequencing. Details of the primers and thermocycling conditions are shown in Table 1. In brief, a 30 µl-PCR was carried out using 15 µl GoTaq Hot Start Green Master Mix (Promega, Madison, WI), 10 pmol forward primer, 10 pmol reverse primer, 1 µl bisulfite-treated genomic DNA, and water. Pyrosequencing was performed using the PyroMark Q96 MD Pyrosequencing System (QIAGEN, Germantown, MD). The percentage of methylated and unmethylated cytosines in the gene promoter was quantified for three CpG sites for *Bdnf*, two CpG sites for *Comt*, five CpG sites for *Crhr1*, two CpG sites for *Mc2r*, five CpG sites for *Snca*, two CpG sites for LINE-1 UTR and one CpG site for LINE-1 ORF. These regions were selected on the basis of a literature search to identify genes relevant to stress-response and to memory. The degree of methylation was expressed as the percentage of methylated cytosines divided by the sum of methylated and unmethylated cytosines (5mC %) measured in each sample. The pyrosequencing reactions were replicated and the mean of the replicates taken forward for analysis. The correlation coefficient between replicate pyrosequencing runs ranged from 0.51 (*Snca*) to 0.97 (*Comt*). The correlation coefficient between replicate pyrosequencing runs for LINE-1 UTR was 0.41 and 0.45 for LINE-1 ORF.

Statistical Analyses

Analyses were performed using the SigmaPlot 12.5 and Stata/SE 12.0 software programme. The difference in significance between the levels of methylation in a given gene in different tissues was calculated by one-way ANOVA and non-parametric Krustal-Wallis test. Significance of the exposure-associated difference in methylation at each locus was calculated by t-test and non-parametric Mann-Whitney test. Spearman correlations were performed to determine the significance and degree of correlation. All p values were corrected for multiple

testing using the Bonferroni method.

Figure 2

Results

Short-term noise exposure and DNA methylation in the brain tissues

DNA methylation levels of five genes were measured in the brains of rats exposed to environmental noise (70-75 dB) for three days during night time: *Bdnf*; *Comt*; *Crhr1*; *Mc2r*; and *Snca*. The basal DNA methylation level of each gene varied between the four regions of the brain studied here (hippocampus, frontal lobe, medulla oblongata and inferior colliculus) (Figure 2). Following short-term noise exposure, *Comt* gene DNA methylation was significantly increased in medulla oblongata (mean $\beta = 6.425$, $p = 0.013$). *Comt* methylation in other brain regions increased with noise exposure but did not achieve statistical significance. Global DNA methylation measured by LINE-1 was not significantly changed by short-term noise exposure in any of the four brain regions (Figure 4, A and B).

Figure 3

Long-term noise exposure and DNA methylation in the brain tissues

DNA methylation levels were measured in the same five genes in the brains of rats exposed to environmental noise (70-75 dB) for 21 days during night time. After this long-term noise

exposure, *Comt* methylation in the inferior colliculus was significantly increased (mean $\beta = 3.553$, $P = 0.013$) (Figure 3). The *Mc2r* gene displayed significantly decreased methylation in the hippocampus (mean $\beta = -1.222$, $p = 0.039$). Global DNA methylation measured through interrogation of the LINE-1 UTR was significantly increased in the medulla oblongata (mean $\beta = -0.797$, $P = 0.013$), but not in the other analyzed regions of the brain (Figure 4 C and D).

Figure 4

DNA methylation changes in the brain between short-term and long-term noise exposures

We did not observe an increased effect upon DNA methylation following long-term noise

exposure (21 days) in comparison to short-term exposure (3 days) (Figure 5 A and B). The DNA methylation changes observed following short- and long-term noise exposures were sporadic between genes and between brain regions.

Figure 5

DNA methylation and metabolic health parameters

We measured systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) before and after long-term noise exposure (Figure 6 A). SBP, DBP and MAP were all significantly decreased following long-term noise exposure ($\beta = -16.00$ mmHg, $p = 0.038$; $\beta = -18.25$ mmHg, $p = 0.017$; and $\beta = -17.50$ mmHg, $p = 0.017$ respectively). Heart rate was not significantly affected by long-term noise exposures (Figure 6 B).

The control group and the exposed group were randomly selected, and the body weights at baseline (Day 0) were not significantly different (Figure 7 A). However, the body weights were significantly lower in exposed group after the long-term noise exposure ($\beta = -26$ g, $p = 0.008$). The changes in body weight following long-term noise exposure were significantly associated with *Bdnf* DNA methylation in frontal lobe ($r = 0.52$, $p = 0.039$) (Figure 7 B) and medulla oblongata ($r = 0.59$, $p = 0.018$) (Figure 7 C). In contrast, global DNA methylation measured through interrogation of LINE-1 ORF was inversely correlated with body weight ($r = -0.80$, $p = 0.0004$) (Figure 7 D).

Figure 6

Figure 7

Discussion

This is the first time that DNA methylation changes have been observed in the brain in response to short- and long-term environmental noise exposure. We report an association between DNA methylation of the *Comt* gene with short-term noise exposure in rat medulla oblongata. The longer-term noise exposure at night was associated with aberrant methylation of *Comt* (inferior colliculus), *Mc2r* (hippocampus) and LINE-1 UTR (medulla oblongata) in the rat brain. Changes in *Bdnf* and LINE-1 ORF DNA methylation in the brain following noise exposure were associated with changes in blood pressure and body weight.

BDNF is a member of the neurotrophin growth factor family which is implicated in the survival of neurons and plays a role in the growth, differentiation, and maintenance of new neurons and synapses [18, 19]. It is active in brain regions associated with cognitive functions [20] and may have a role in the regulation of stress response [21]. *BDNF* is expressed in the regions of the brain involved in the regulation of homeostatic and hedonic food intake, and is known to be implicated in appetite suppression [22]. In our study, we found a strong association between decreased *Bdnf* methylation in the rat brain and decreased body weight after long-term noise exposure. Although, *Bdnf* methylation decreased in each of the four analyzed regions of the brain with noise exposure, it did not reach statistical significance in any of them. We speculate that the changes in *Bdnf* methylation may be implicated in the observed decrease in body weight, although further study is required to analyse this correlation in detail.

Catechol-O-methyltransferase (*Comt*), a key enzyme for inactivation of prefrontal dopamine (DA), has been found to be closely related with stress response, cognition, anxiety, and pain sensitivity [23-26]. Previous studies have identified associations between mutations and polymorphisms within the *Comt* gene and cognition impairment, working memory, depression, and early life experiences, thereby demonstrating its direct functional role in the response to stress [27-32]. Furthermore, it has been shown that SNPs can influence the epigenetic regulation of this gene. The rs4680 SNP (G to A substitution) influences the thermostability and activity of COMT and also removes a CpG site within exon 4, and Ursini *et al.* found that under stress this CpG site is methylated and that this leads to silencing of the gene [25]. *Comt* expression and activity is therefore regulated by both genetic and epigenetic mechanisms, but only the latter modifiable in response to exogenous factors, such as noise pollution exposure. In our study, we identified hypermethylation of *Comt* in the inferior colliculus after long-term noise exposure, suggesting a mechanism by which chronic

nocturnal noise exposure could affect brain functions such as cognition and memory, and increase the risk of depression. Future studies are warranted to determine whether *Comt* hypermethylation in the inferior colliculus is associated with cognition impairment, working memory, or depression.

As a receptor specific to the adrenocorticotrophic hormone (ACTH), *Mc2r* (melanocortin type 2 receptor) is abundantly expressed in the brain [33, 34]. Repeated or chronic stressors often result in increased sensitivity of the adrenals to ACTH [35-37]. Mutations in *Mc2r* have led to hereditary unresponsiveness to ACTH and are responsible for approximately 25% of familial glucocorticoid deficiency (FGD) cases, also termed FGD type I [38]. Previous studies have shown that polymorphisms within the *Mc2r* gene, which helps to regulate adrenal cortisol secretion, is important in the physiological response to stressors by mediating the response to ACTH stimulation [39, 40]. In our study, for the first time, we have demonstrated changes in methylation of the *Mc2r* gene in the hippocampus after chronic noise exposure. As *Mc2r* is the sole receptor for ACTH, this observation may provide insight into how environmental noise exposure may modulate the stress response of the hippocampus. Studies elsewhere have reported that chronic environmental noise exposure is associated with disrupted function of the hippocampus, an extra-auditory structure of the central nervous system, thereby inducing memory deficit and cognition impairment [41-47]. Indeed, Jáuregui-Huerta *et al.* showed that chronic exposure to environmental noise in the early stages of life produces a long-lasting reduction in cell proliferation in the hippocampus in the neurogenic and non-neurogenic hippocampal regions [48]. Further studies are required to determine whether chronic nocturnal environmental noise exposure-induced *Mc2r* hypomethylation in the hippocampus is associated with memory deficit and cognition impairment.

LINE-1 comprises approximately 20% of mammalian genomes [49-51], and therefore measurements of LINE-1 DNA methylation has been used to estimate global genomic DNA

methylation levels [52]. Aberrant methylation of repetitive elements accelerates genomic instability and may alter gene expression [53-55]. In previous studies, aberrant LINE-1 methylation has been associated with the risk or development of hypertension and cardiovascular disease [56, 57]. In the present study, we observed LINE-1 hypermethylation in the medulla oblongata after long-term environmental noise exposure, but not in other parts of the brain. Accumulation of global hypermethylation in the medulla oblongata, a region of the brain that is related to the development and maintenance of elevated arterial pressure and hypertension [58, 59], suggests chronic environmental noise exposure could significantly affect the expression of cardiovascular-related genes. In this context, our results support the hypothesis that long-term nocturnal environmental noise exposure could increase the risk of cardiovascular disease and hypertension [60, 61]. LINE-1 methylation was also significantly associated with body weight after long-term noise exposure.

Previous studies have suggested that the consequences of noise exposure depend upon the context and characteristics of the acoustic insult, including stimulus duration, intermittency, frequency content, intensity, and predictability [5, 62-64]. Aircraft noise, which has a higher intensity than traffic noise, has a stronger association with the risk of hypertension [65]. In noise-induced sleep disturbance, sleep stages are affected differently by unpredictable, intermittent or continuous noise exposure [66, 67]. Additionally, with the same intensity of exposure (70dB), low frequency noise (0.1 kHz) can cause impaired balance in contrast to high frequency noise (16 kHz) in mice [68]. Due to lack of standardization criteria for noise exposure in current research, studies comparing noise exposure are becoming increasingly difficult; therefore, we recommend providing a precise description of noise characteristics in future studies.

It has been suggested that noise exposure might contribute to the prevalence of cardiovascular disease, based upon evidence from epidemiological studies [65]. However, the

effect of noise exposure upon blood pressure is still inconclusive as both hypertension and hypotension has been reported [69-71]. This inconsistency may be the product of limitations in adjustment for confounders such as air pollution exposure, which is a key risk factor for increased blood pressure. In this rat study, we found a significantly decreased blood pressure with noise exposure without having air pollution exposure as a potential confounder, as the rats were exposed to air of the same quality.

Although we have been able to identify differentially methylated genes in response to noise exposure, our study does contain some limitations. Firstly, by taking a candidate gene approach we are unlikely to have identified all genes that are affected by the exposure. A microarray-based approach may enable further targets to be identified for functional analysis. Secondly, while the performance of the work in the rat model has enabled us to tightly control noise exposure, the small number of rats within each exposure group has limited the statistical power of this study, and we therefore cannot exclude the possibility that some associations may have been missed.

In summary, we have identified gene-specific changes in DNA methylation in the rat brain following short- and long-term noise exposures. Long-term environmental noise exposure was associated with changes in blood pressure and body weight, which were significantly associated with *Bdnf* methylation. Together, our study may provide insight into the link between environmental noise exposures and metabolic health, through modulation of response to stress-related hormones.

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Figure legends

Figure 1. Experimental model of noise exposure in Wistar rats

For short-term noise exposure, rats were exposed to 70-75dB of noise during the night-time and 45dB during day-time for three days (n=8). Control rats were exposed to 45dB day and night (n=8). For long-term noise exposure, rats were exposed to 70-75dB of noise during the night-time and 45dB during day-time for 21 days (n=8). Control rats were exposed to 45dB day and night for 21 days (n=8).

Figure 2. DNA methylation changes by short-term noise exposure in rat brains

DNA methylation changes in *Bdnf* (A), *Mc2r* (B), *Comt* (C), *Snca* (D) and *Chr1* (E) in the hippocampus, frontal lobe, medulla oblongata and inferior colliculus of controls and exposed rats. Box: median, 25th and 75th percentiles. Bars: min and max values. C: control and E: exposed group.

Figure 3. DNA methylation changes by long-term noise exposure in rat brains

DNA methylation changes in *Bdnf* (A), *Mc2r* (B), *Comt* (C), *Snca* (D) and *Chr1* (E) in the hippocampus, frontal lobe, medulla oblongata and inferior colliculus of controls and exposed rats. Box: median, 25th and 75th percentiles. Bars: min and max values. C: control and E: exposed group.

Figure 4. LINE-1 DNA methylation by noise exposure

DNA methylation changes in LINE-1 UTR (A) and LINE-1 ORF (B) in the hippocampus, frontal lobe, medulla oblongata and inferior colliculus of controls and exposed rats with short-term noise exposure, and in LINE-1 UTR (C) and LINE-1 ORF (D) with long-term noise

exposure. Box: median, 25th and 75th percentiles. Bars: min and max values. C: control and E: exposed group.

Figure 5. Comparison of DNA methylation changes following short- and long-term noise exposure in rat brains

Differential DNA methylation in *Bdnf*, *Mc2r*, *Comt*, *Snca*, and *Chr1* in the hippocampus, frontal lobe, medulla oblongata and inferior colliculus (A), and LINE-1 UTR and ORF in the same regions (B). Grey circles: differential DNA methylation (% change) between short-term exposed rats and the control group. White circles: differential DNA methylation (% change) between long-term exposed rats and the control group. * represents $p < 0.05$.

Figure 6. Blood pressure and heart rate changes after long-term noise exposure

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) in the controls and long-term noise-exposed group (A). Heart rates among the controls and long-term noise-exposed group (B). Box: median, 25th and 75th percentiles. Bars: min and max values.

Figure 7. Body weight changes after long-term noise exposure

Body weight before (Day 0) and after long-term noise exposure (Day 21) (A). C: control and E: exposed group. Box: median, 25th and 75th percentiles. Bars: min and max values.

Correlation of changes in body weight and *Bdnf* DNA methylation (%) in the frontal lobe (B) and medulla oblongata (C). Correlation of changes in body weight and LINE-1 ORF DNA methylation (%) in the medulla oblongata (D).

Table 1. PCR and pyrosequencing primer sequences

Gene name	Primer	Sequence
<i>Bdnf</i>	Forward Reverse (biotin) Sequencing Entry	TTTTTAGTTTTTGTTTAGATTAAATGGAGT CAACAAAAAAATTAAATTATTAATAATAAA GTTTTTTATTGAAGG C/TGTGC/TGAGTATTATTTTC/TGTTATG
<i>Comt</i>	Forward Reverse (biotin) Sequencing Entry	GTTTGTGTGTAGTTTGGAGTTAGGT CTCATCCTCCCCCATTACCT TATGGTTTAGTGTGG C/TGGTTGTGGGGTGTAGGGGGC/TGGGGGGGT
<i>Crhr1</i>	Forward Reverse (biotin) Sequencing Entry	ATTTTAGGTGTGTTTAGGTTAAAGG CCTACAATCATCCAAAAAATTCAC GTGTTTAGGTTAAAGG C/TGC/TGC/TGGAGTAAGGGGAGGGGGTTC/TG GATTC/TGG
<i>Mc2r</i>	Forward Reverse (biotin) Sequencing Entry	TGTTTGTTTTTAGTATTTAAAAATTTAGA AATAACCTTTAATATCACCCACATC ATTTAATTAAAATTT GAC/TGTAGTTTAC/TGTTTTAAAA
<i>Snca</i>	Forward Reverse (biotin) Sequencing Entry	TTTTGTTTTATAGATGGGGGAAAA ATTCACACAACCTAAAAATTCCATT TTTTGTTTTATAGATGGGGGAAAA AAAAATTAGC/TGTTTGGTAGTC/TGTTGATTGG TGGAAAC/TGA GAGTTTTTTTTTGT TTTC/TGATTTGTAATTTTTTTTTTAGTC/TGGTTT TTT
LINE-1UTR	Forward Reverse (biotin) Sequencing Entry	GGTGTATAGGTTTTTTTTGGTTGTTG AAATTCACCAAACAACCTTTCTTACAA TTTTTTTGGTTGTTGT C/TGTTGTAGAGAGTTC/TGTGGTAGTATTTTA
LINE-1ORF	Forward Reverse (biotin) Sequencing Entry	AGAAAGAATATTAAAAATAGTAAGGGAAAA ATCAATCCAAAATCTTCTAACCTTC TTATATTAGATTTTT C/TGTTAGAAATTAT