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Genetic background contributes to the co-morbidity of anxiety and depression with audiogenic seizure propensity and responses to fluoxetine treatment

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ABSTRACT

Background: Anxiety and depression are the most frequent comorbidities of different types of convulsive and non-convulsive epilepsies. Increased anxiety and depression-like phenotype have been described in the genetic absence epilepsy models as well as in models of limbic epilepsy and acquired seizure models, suggesting a neurobiological connection. However, whether anxiety and/or depression are comorbid to audiogenic epilepsy remains unclear. The aim of this study was to investigate whether anxiety or depression-like behavior can be found in rat strains with different susceptibility to audiogenic seizures (AS) and whether chronic fluoxetine treatment affects this co-morbidity.
Methods: Behavior in the elevated plus-maze and the forced swimming test was studied in four strains: Wistar rats non-susceptible to AS; Krushinsky–Molodkina (KM) strain, selectively bred for AS propensity from outbred Wistar rats; and a selection lines bred for maximal AS expression (strain "4") and for a lack of AS (strain "0") from KM × Wistar F2 hybrids. Effects of chronic antidepressant treatment on AS and behavior were also evaluated.
Results: Anxiety and depression levels were higher in KM rats (with AS) compared with Wistar rats (without AS), indicating the comorbidity with AS. However, in strains "4" and "0" with contrasting AS expression, but with a genetic background close to KM rats, anxiety and depression were not as divergent as in KMs versus Wistars. Fluoxetine treatment exerted an antidepressant effect in all rat strains irrespective of its effect on AS.
Conclusions: Genetic background contributes substantively to the co-morbidity of anxiety and depression with AS propensity.

1. Introduction

Epilepsies are complex heterogeneous disorders with substantial (30–35%) comorbidity with anxiety and depression [1,2]. Numerous clinical data demonstrate the association between epilepsy and different psychiatric diseases [3–6]. However, these comorbidities are not uniform, indicating the heterogeneity of epilepsy [7–9], on the one hand, and the complexity of the brain substrates involved in these pathologies [3], on the other. Interestingly, the comorbidity of depression and anxiety with non-epileptic seizures has been noted as well [10]. In some cases, psychiatric comorbidities can be related with patient's personality characteristics [11].

Consistent with the clinical evidence that anxiety and depression are the most frequent comorbidities of different types of convulsive and non-convulsive epilepsies, similar behavioral impairments have been reported in different epilepsy models [12]. Increased anxiety and depression-like phenotype have been described in the rat genetic absence epilepsy models [13–16] as well as in models of limbic epilepsy [17] and in acquired seizure models [18], suggesting a neurobiological connection. In a genetic absence epilepsy model, comorbidity between epilepsy and depressive-like symptoms has been confirmed by the data indicating that chronic and early pharmacological treatment with the anti-absence drug ethosuximide aimed to prevent epileptogenesis resulted in antidepressant-like effect (suppression of comorbid depression-like symptoms) [19,20]. However, there are also conflicting data, indicating that behavioral comorbidities are not associated with epilepsy conditions per se but may represent a consequence of seizure-induced brain damage and could also be explained by model-dependent dysregulation of HPA axis [21]. In one seizure model, a status epilepticus model of temporal lobe epilepsy [16] as well as a model of absence epilepsy [17], no correlation between depression-like behavior and seizure severity was previously reported, while in another model, the rapid kindling model of limbic epilepsy [22], a significant positive correlation between immobility time in the forced swimming test

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(behavioral symptom of depression) and seizure score was found. Pharmacological studies in a genetic absence epilepsy model indicate that long-term treatment with drugs, which demonstrate antiseizure effects, can exert diverse effects of comorbid depressive-like symptoms [23–25]. This means that the relationship between seizures and comorbidities is presumably multifactorial and can be regulated by many variables, including specificity of epilepsy model and strain (genotype) differences.

The genetic traits, namely the seizure propensity, and the genetic background of strains are also important factors that might affect the expression of epileptic phenotypes as well as behavioral comorbidities, and anxiety and depression in particular [7,26–31]. Importantly, rats selectively bred for seizure propensity demonstrate comorbid increased anxiety and depression-like phenotype [13,31], while rats selectively bred for the depression-like phenotype (increased immobility in the forced swimming test) exhibit a high seizure propensity [26]. However, high seizure propensity (Fast strain) compared with seizure resistance (Slow strain) can be also associated with reduced behavioral symptoms of anxiety and depression, as evidenced by reduced startle response and an increased, not decreased, sucrose consumption, respectively [32]. This discrepancy could be partly explained by differences in the seizure type and/or localization of seizure foci, i.e. in the initial focus of increased brain excitability, and in the ability of the particular seizure to spread through the brain [7]. At the same time, the comorbidity of depression with increased anxiety is rather frequent [33].

Audiogenic refractory epilepsy represents one of the convulsive epilepsy forms. Rats of the Krushinsky–Molodkina (KM) strain were selectively bred for high audiogenic seizure propensity [34], and their epileptic phenotype is in many aspects comparable with the Genetically Epilepsy-Prone Rats (GEPRs), also selectively bred for susceptibility to audiogenic generalized tonic–clonic seizures (AS) [35]. This model includes two strains — the GEPR-3 and GEPR-9. The GEPR-9s exhibit more intense seizures (score of 9) compared with GEPR-3s (score of 3). The GEPRs also show depression-like phenotypes compared to their parental strain, Sprague–Dawley rats, indicating that GEPRs can be regarded as a genetic model of audiogenic seizure predisposition and depression comorbidity [36]. However, whether anxiety and/or depression comorbidity are commonly found in other audiogenic seizure-prone genotypes is unknown. The presence or absence of this comorbidity needs to be investigated in other rat models of different genetic origin to clarify whether an association between AS susceptibility and anxiety/depression is universal.

Abnormality in the serotonergic brain system is implicated in the pathophysiology of audiogenic epilepsy and behavioral comorbidities, along with other neurochemical alterations [18,37,38]. The selective serotonin reuptake inhibitor (SSRI) antidepressant drug fluoxetine has been found to decrease AS severity [39–41]. It has also been shown [39] that the combined treatment with fluoxetine and 5-HT1A receptor antagonists (pindolol and LY 206130), which affect somato-dendritic autoreceptors, was more effective in AS suppression in GEPR-9s compared with fluoxetine only treatment.

The aim of the present study was to investigate whether anxiety and depression are co-morbid to AS propensity and whether chronic fluoxetine treatment can diversely affect this comorbidity in rats of different genotypes, that display the extremes of AS expression. To achieve this aim, anxiety and depression-like behavior were studied in rats of different genotypes with different AS propensity before and after chronic fluoxetine treatment.

Four different genotypes were used: 1) outbred Wistar rats non-susceptible to AS, 2) Krushinsky–Molodkina (KM) inbred strain derived from outbred Wistar rats at the end of the 1940s [34], and 3) strain "0" and 4) strain "4", two recently bred selection lines. The selection of strain "0" and "4" started from F2 hybrids of KM × sound non-prone Wistars [42]. Strain "0" was selected for not having AS, strain "4" for maximal intensity of AS. The aim of this selection program was to create strains that would be non-prone and highly prone to AS, being genetically more close to the KM strain than to Wistars. For this purpose two backcrosses to the KM parental strain were performed at the initial generations of selection [42]. Rats of KM and "4" strains exhibited AS of maximal intensity (robust tonic extension of trunk and extremities), while in Wistar and "0" strain rats there were no AS in response to a loud sound. These differences in AS expression allowed us to evaluate the possible association between the AS severity and behavioral indices of anxiety and depression. The rat strains used in this experiment differed not only by AS susceptibility but also by the genetic background. It was expected that the use of this set of strains that are different not only in AS susceptibility but also in the genetic background can help reveal the association between AS expression and behavioral comorbidities. Chronic fluoxetine treatment was used to reduce AS expression and, herewith, to examine whether it diversely affect anxiety and depression-like behaviors in rats of different genotypes.

2. Material and methods

2.1. Animals

Six-month-old male rats from the KM strain (n = 8), susceptible to AS, KM-derived strain selectively bred for maximal expression of AS (line "4", n = 10), KM-derived strain selectively bred for the absence of AS (line "0", n = 9), and age-matched male Wistar rats (n = 9, breeding company "CrolInfo", Russia) non-susceptible to AS were used. The KM, "0", and "4" strains have been bred in the Laboratory of Physiology and Genetics of Behavior (Chair of Higher Nervous Activity, Biology Department, Lomonosov Moscow State University), and were maintained in this laboratory. The selection of KM strain for AS proneness was initiated more than 60 years ago, and at the end of the 1980s, the inbreeding of this strain started with a strict brother–sister mating schedule. Rats used in the present study represented the 68th inbred generation. Strains "0" and "4" were selectively bred for the lack of AS and for the maximal intensity of AS, respectively. The mark "4" corresponds to the maximal AS intensity, according to the arbitrary scale [34]. The F2 hybrids of KM × Wistar cross were used as the initial population for this selection. In order to obtain these hybrids, the parental Wistar rats (males and females) were chosen from a group of animals (n = 60) according to the following requirement: no AS in response to a loud sound in three consecutive sound exposures, 5–7 days apart. Rats of strains "0" and "4" used in this study represent the 28–29th generations of selection (the inbreeding started at the level of F13–F14).

All rats were kept under a natural light–dark regimen (about 10 h of day-time light). Animals were housed in plastic cages (T4) in groups of 3–4 animals per cage with tap water and food (Chara firm) ad lib. Experiments were performed in accordance with the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes. Animal care and use confirmed to the institutional policies and guidelines. Experimental protocols were approved by the Ethical Committee of Moscow State University (from 12.11.2015). All efforts were made to minimize the number of animals used in experiments and their suffering from experimental procedures.

2.2. Audiogenic susceptibility

Audiogenic susceptibility was examined in a sound-attenuated plastic transparent box by exposing rats to 120 dB "auditorium bell" sound, as used in previous studies [34]. The AS pattern includes: wild running, clonic, and tonic seizures. The intensity of sound-induced seizures was evaluated, using a 7-degree scale. The seizure-rating scale assigns a score from 0 (no seizure) to 7 (generalized tonic–clonic seizure with complete hind limb extension). Each score above 0 represents seizures with increasing severity. The scale used here is more convenient for quantitative estimation in comparison to the more robust 4-degree scale traditionally used [34]. According to a 7-degree scale, score "0" means no response, score "1" means wild running with "two waves"