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Matrix Metalloproteinase-9 and Synaptic Plasticity in the Central Amygdala in Control of Alcohol-Seeking Behavior

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ABSTRACT

BACKGROUND: Dysfunction of the glutamatergic system has been implicated in alcohol addiction; however, the molecular underpinnings of this phenomenon are still poorly understood. In the current study we have investigated the possible function of matrix metalloproteinase-9 (MMP-9) in alcohol addiction because this protein has recently emerged as an important regulator of excitatory synaptic plasticity.

METHODS: For long-term studies of alcohol drinking in mice we used IntelliCages. Dendritic spines were analyzed using Diolistic staining with DiI. Whole-cell patch clamp was used to assess silent synapses. Motivation for alcohol in human subjects was assessed on the basis of a Semi-Structured Assessment for the Genetics of Alcoholism interview.

RESULTS: Mice devoid of MMP-9 (MMP-9 knockout) drank as much alcohol as wild-type animals; however, they were impaired in alcohol seeking during the motivation test and withdrawal. The deficit could be rescued by overexpression of exogenous MMP-9 in the central nucleus of the amygdala (CeA). Furthermore, the impaired alcohol seeking was associated with structural alterations of dendritic spines in the CeA and, moreover, whole-cell patch clamp analysis of the basal amygdala to CeA projections showed that alcohol consumption and withdrawal were associated with generation of silent synapses. These plastic changes were impaired in MMP-9 knockout mice. Finally, C/T polymorphism of MMP-9 gene at position –1562, which upregulates MMP-9 expression, correlated with increased motivation for alcohol in alcoholics.

CONCLUSIONS: In aggregate, our results indicate a novel mechanism of alcohol craving that involves MMP-9–dependent synaptic plasticity in CeA.

Keywords: Alcohol addiction, Central amygdala, Matrix metalloproteinase-9, MMP-9, Motivation, Silent synapses

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Drug addiction, a psychiatric disorder characterized by uncontrolled drug taking and seeking, is thought to be driven by aberrant glutamatergic transmission in the brain reward system (1–4). This hypothesis is supported by the observation that chronic alcohol consumption leads to elevated levels of extracellular glutamate and alterations in the expression and localization of various glutamate receptors, including alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors (5,6). The role of the glutamatergic system in the development of addiction is also strengthened by new therapies of alcoholism that are focused on glutamate receptors (2,5,7). Despite the great effort to understand the regulation of drug-evoked plasticity of the glutamatergic system, the molecular details are still largely missing.

Recently, matrix metalloproteinase-9 (MMP-9) has emerged as an important controller of the synaptic plasticity of excitatory synapses (8). MMP-9 was found to affect surface diffusion of NMDA receptors (NMDARs) (9), NMDAR function (10), NMDAR-dependent long-term potentiation of synaptic efficacy, activity-driven alterations of morphology of dendritic spines (11), and different forms of learning (8,12,13). Moreover, an important insight into the role of MMP-9 in addiction has recently been provided by Smith *et al.* (14), who found transient increases in MMP-9 activity in the nucleus accumbens during cue-induced cocaine relapse. Increased MMP activity was required for both cocaine- and cue-induced relapse and relapse-associated synaptic plasticity (14). Furthermore, general inhibitors of MMPs attenuated escalating ethanol self-administration (15). The possible function of MMP-9 in alcohol addiction was also supported in human studies by Samochowiec *et al.* (16), who found the polymorphism of the MMP-9 gene (at position –1562),

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producing higher protein expression, to be more frequent in alcoholics' families than in control subjects' families. Despite the growing body of evidence implicating MMP-9 in addiction, the detailed understanding of the function of this metalloproteinase in addiction-related processes is still missing.

The aim of this study was to examine the role of MMP-9 in the regulation of alcohol addiction–related behaviors. First, we performed a longitudinal study in IntelliCages (NewBehavior AG, Zurich, Switzerland) to test whether depletion of MMP-9 in MMP-9 knockout (MMP-9 KO) mice affects different aspects of alcohol addiction, such as alcohol consumption, motivation to obtain alcohol, and alcohol seeking during withdrawal (17). Our experiment showed that MMP-9 KO mice drank as much alcohol as wild-type (WT) animals; however, they were impaired in alcohol seeking during motivation test and withdrawal, suggesting the role of MMP-9 in the regulation of alcohol craving. The impaired alcohol seeking was rescued by the local overexpression of exogenous MMP-9 in the central nucleus of the amygdala (CeA). Furthermore, the lack of MMP-9 prevented functional synaptic plasticity in the CeA, observed after alcohol withdrawal (i.e., an increase in the frequency of silent synapses). Moreover, after distinguishing the animals into groups of low- and high-motivation drinkers, we demonstrated that whereas in the highly motivated WT mice alcohol drinking resulted in an increase of dendritic spine size in CeA, such differences could not be seen in MMP-9 KO mice. Finally, we performed a validation study of the Semi-Structured Assessment for the Genetics of Alcoholism questionnaire and found that in the population of alcoholics MMP-9 gene –1562C/T polymorphism (rs3918242) correlates with increased motivation for alcohol.

METHODS AND MATERIALS

The experiments were performed on adult C57BL/6J KO (MMP-9 KO) mice and their WT siblings (Supplemental Figure S1). The animals were generated and genotyped as described in the Supplemental Methods and Materials and in Vu *et al.* (18). Strain colony was maintained in the animal house of the Nencki Institute of Experimental Biology of Polish Academy of Sciences. All procedures were performed in accordance with the Animal Protection Act in Poland, Directive 2010/63/EU, and were approved by the first Local Ethics Committee (Permission No. 257/2012).

All details of the experimental procedures are in the Supplemental Methods and Materials.

IntelliCage Training

The alcohol trainings in the IntelliCages were performed according to previously published protocols with some minor modifications (17,19).

Dendritic Spines Analysis

After behavioral training, mice were sacrificed and brain tissue was collected. Brains were cut into 130-μm sections on vibratome (Leica VT 1000S, Leica Biosystems Nussloch GmbH, Wetzlar, Germany) and processed for DiI (Life Technologies, Warsaw, Poland) staining. Z-stacks of dendrites in the CeA were acquired using LSM780 confocal system (Zeiss, Poznan, Poland). Maximum intensity projections of images were analyzed using semiautomatic SpineMagick! Software (20). Data analysis was performed using custom scripts written in Python, using NumPy and SciPy (21), IPython (22), scikit-learn (23), and Matplotlib (24).

Injection of Lentiviral Vector Expressing MMP-9 Into the CeA

Lentiviral vectors expressing autoactive MMP-9 driven under synapsin-1 promoter (LV-MMP-9) and green fluorescent protein (LV-GFP) were used for bilateral stereotactic injections into the CeA. Two weeks after the surgery mice underwent training in IntelliCages.

Electrophysiology Experiments

Mice were housed separately in their home cages, receiving food and water ad libitum. Mice exposed to long-term alcohol protocol received additional bottle containing alcohol (4% and 8% for 2 days each, 10% for 3 weeks). Mice were sacrificed 1 day after the termination of 3-week alcohol exposure (1–day withdrawal group) or after 7 days of withdrawal. Control mice were drinking water at all times. At the end of the training mice were anesthetized with isoflurane and decapitated. The whole-cell patch-clamp analysis of silent synapses was performed on the basal amygdala to CeA projections as previously described (25,26).

Human Subjects and Assessment

In the present study the Semi-Structured Assessment for the Genetics of Alcoholism interview (27) was used to assess alcohol and family history of alcoholism and more precisely selected addiction criteria, as described by Samochowiec *et al.* (16). The protocol of the study was approved by the local institutional review board, and all the participants provided their written informed consent.

Statistical Analysis

All results are expressed as mean ± SEM. The appropriate tests were chosen, taking into account whether data had normal distribution and equal variation. All analyses were conducted using GraphPad Prism, version 7.02 (GraphPad Software, Inc., La Jolla, CA) or SPSS (version 9, SPSS Inc., Chicago, IL). Differences between the experimental groups were considered significant if the type 1 error was less than 5%.

RESULTS

MMP-9 KO Mice Have Decreased Motivation for Alcohol and Are Less Persistent in Alcohol Seeking

To assess the role of MMP-9 in the regulation of alcohol addiction-related behaviors we used MMP-9 KO mice along with their WT littermates (18). The mice underwent a long-term alcohol addiction training in the IntelliCages as described in detail before by Radwanska and Kaczmarek (17). In this apparatus, the animals are housed in large groups and are