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ACE2 GENE TRANSGENESIS ENHANCES MEMORY OF PSYCHOPHYSIOLOGICAL TRAUMA IN MOUSE MODELS OF POST-TRAUMATIC STRESS DISORDER

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Introduction. The development of symptoms in post-traumatic stress disorder (PTSD) is determined by a set of factors, which are not limited to classical neurotransmitter systems in the brain or stress hormones. In particular, the brain renin-angiotensin-aldosterone system may be involved in the mechanisms of PTSD.
Objective. To study the effect of *hACE2* expression, angiotensin-converting enzyme 2 (ACE2) gene, on anxiety and susceptibility to psychophysiological stress in mice in the foot electroshock (FS) model of PTSD.
Materials and methods. The experiments were conducted using 4–5-month-old male C57Bl/6N and k18-hACE2-KI mice. C57Bl/6N mice were divided into three groups: control (n = 7); the foot shock (FS) (n = 7); FS + lisinopril (n = 7). k18-hACE2-KI mice were divided into two groups: control (n = 7) and the FS (n = 8). Pavlovian fear conditioning was performed using FS as an unconditioned stimulus. Mice in the FS + lisinopril group received lisinopril at a dose of 10 mg/kg per day with drinking water for 28 days after psychophysiological trauma. The expression of fear, reflecting the memory of psychophysiological trauma, was assessed on day 7 and day 28 after FS exposure. The magnitude of the fear response was assessed by evaluation of the relative time of freezing. The open field test was used to assess general locomotor activity. The tail suspension test was used to assess the stress-coping strategy, while the light-dark box test and the elevated plus maze test were used to measure anxiety. The Barnes maze test was used to explore spatial navigation and spatial learning dynamics. Behavior was analyzed using the ANY-maze Video-Tracking Software. Statistical analysis was performed using the Prism GraphPad v.10.0 software.
Results. k18-hACE2-KI mice with expression of humanized *ACE2* gene under the control of the cytokeratin gene promoter showed a more pronounced ability to remember and retain the memory about the conditioned stimulus/context of the traumatic event in the PTSD-model when compared to C57Bl/6N mice. Anxiety measured in the light-dark box test was lower in k18-hACE2 mice than C57Bl/6N mice after FS. At the same time, there was a decrease in the open-field motor activity and there were no changes in spatial memory in the Barnes maze test. Lisinopril, an ACE inhibitor (28 days after FS), did not reduce traumatic memory in C57Bl/6N mice, indicating that the promnestic effect of *hACE2* gene expression is not a result of systemic hypotension and pointing at the involvement of the central mechanisms in the realization of *hACE2* gene effect in the pathological phenotype development.
Conclusions. The data indicate that the *hACE2* gene affects the stress response in mice. Specifically, the expression of *hACE2* gene in mice leads to increased memory of psychophysiological trauma and reduced extinction of traumatic memory compared to wild-type mice. This may be due to the modulation of the ACE2-dependent renin-angiotensin-aldosterone system in the brain. The decreased RAAS activity under the action of the ACE inhibitor lisinopril with a hypotensive effect did not affect memory in wild-type mice.

Keywords: post-traumatic stress disorder; *hACE2*; anxiety; memory; lisinopril

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ТРАНСГЕНЕЗ ПО ГЕНУ *ACE2* УСИЛИВАЕТ ПАМЯТЬ О ПСИХОФИЗИОЛОГИЧЕСКОЙ ТРАВМЕ В МОДЕЛИ ПОСТТРАВМАТИЧЕСКОГО СТРЕССОВОГО РАССТРОЙСТВА У МЫШЕЙ

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Введение. Развитие симптомов посттравматического стрессового расстройства (ПТСР) определяется комплексом факторов, которые не ограничиваются принадлежностью к классическим нейротрансмиттерным системам мозга или стрессовым гормонам. В частности, в механизмы ПТСР возможно вовлечение ренин-ангиотензин-альдостероновой системы мозга.
Цель. Изучение влияния экспрессии гена *hACE2* ангиотензинпревращающего фермента 2-го типа (ACE2) на тревожность и восприимчивость к психофизиологическому стрессу при моделировании ПТСР-подобного состояния у мышей, осуществленному с применением электрошока (ЭШ) конечностей.

Материалы и методы. Эксперименты были проведены на самцах мышей линий C57Bl/6N и k18-hACE2 возрастом 4–5 месяцев. Было сформировано три группы мышей линии C57Bl/6N: группа «контроль» (n = 7); группа «электрошок (ЭШ)» (n = 7); группа «ЭШ + лизиноприл» (n = 7); две группы мышей линии k18-hACE2: группа «контроль» (n = 7); группа «ЭШ» (n = 8). Проведено обусловливание реакции страха по Павлову с использованием ЭШ конечностей в качестве безусловного стимула. Мыши группы «ЭШ + лизиноприл» в течение 28 дней после психофизиологической травмы получали лизиноприл в дозе 10 мг/кг в сутки с питьевой водой. Оценку экспрессии реакции страха, отражающей память о психофизиологической травме, проводили на 7-е и 28-е сутки после воздействия ЭШ. Величину экспрессии реакции страха оценивали по относительному времени замирания. Для оценки общей локомоторной активности использовали тест «открытое поле». Оценку стратегии стресс-зависимого поведения изучали в тесте подвешивания за хвост; оценку тревожности — в тестах «светло-темная камера» и «приподнятый крестообразный лабиринт». Оценку пространственной навигации и динамики пространственного обучения проводили в тесте «лабиринт Барнса». Поведенческие параметры оценивали при помощи программного обеспечения ANY-maze Video-Tracking Software. Статистический анализ проведен с помощью пакета ПО Prism GraphPad 10.0.

Результаты. При моделировании ПТСР-подобного состояния с помощью ЭШ конечностей у мышей линии k18-hACE2 с экспрессией гена гуманизированного *ACE2* под контролем промотора гена цитокератина выявлена более выраженная способность, по сравнению с мышами линии C57Bl/6N, к запоминанию и удержанию памяти об условном стимуле/контексте травмирующего события. После воздействия ЭШ у мышей линии k18-hACE2 тревожность в тесте «светло-темная камера» была ниже по сравнению с мышами линии C57Bl/6N. При этом наблюдали снижение двигательной активности в тесте «открытое поле» и не обнаруживали изменений в пространственной памяти в тесте «лабиринт Барнса». Применение лизиноприла, ингибитора АСЕ, у мышей линии C57Bl/6N в течение 28 дней после ЭШ не приводило к снижению травматической памяти, что свидетельствует о том, что промнестический эффект экспрессии гена *hACE2* не является следствием системной гипотензии, и указывает на участие центральных механизмов в реализации эффекта гена *hACE2* при формировании патологического фенотипа.

Выводы. Полученные данные свидетельствуют о влиянии гена *hACE2* на формирование реакции на стресс у мышей, а именно, экспрессия *hACE2* у мышей сопровождается усилением памяти о психофизиологической травме и снижением экстинкции памяти о травме по сравнению с мышами дикого типа, что может определяться модуляцией активности ACE2-зависимого каскада ренин-ангиотензин-альдестероновой системы в мозге. Уменьшение регулирования активности РААС при применении ингибитора АСЕ лизиноприла с гипотензивным действием не оказывало влияния на память у мышей дикого типа.

Ключевые слова: посттравматическое стрессовое расстройство; *hACE2*; тревожность; память; лизиноприл

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INTRODUCTION

The significance of post-traumatic stress disorder (PTSD) among other stress-related diseases is determined by the high probability of health and life threats, the high incidence of subsequent mental disturbances, and the ineffectiveness of existing prophylactic and treatment means [1].

The recent discovery of previously unknown factors of maladaptive changes in the brain has intensified the search for approaches to overcoming the consequences of high-intensity acute stress and the problem of PTSD therapy ineffectiveness. For example, when studying COVID-19 outcomes, Hoffmann et al. [2] found that angiotensin-converting enzyme 2 (ACE2), which acts as the main receptor for the S1 subunit of the SARS-CoV-2 spike protein, can determine not only infectivity, but also may increase anxiety and lead to the development of depressive symptoms in the setting of viral infections [3].

ACE2 protein is an important element of the renin-angiotensin-aldosterone system (RAAS), whose components largely determine the systemic blood pressure. ACE2 degrades the pressor angiotensin II (AngII) and thus functionally balances the activity of the ACE-dependent pro-hypertensive RAAS cascade [4, 5]. According to Yang et al., mice with overexpression of the humanized *ACE2* gene (*hACE2*) under control of the cytokeratin k18 gene promoter can be considered as a model of viral infection of high neuroinvasiveness [6], which attributes a range of negative changes in the central nervous system [7]. However, upon overexpression of the *ACE2* gene or its pharmacological activation, the balance between AngII and its derivative with hypotensive activity — Ang1-7, may change in favor of the latter. Thus, Lima et al. and Meng et al. showed that reduced functional activity of the pro-hypertensive RAAS cascade in the absence of infection can have a positive effect on brain processes [8, 9].

Literature data also suggests that the local brain RAAS is involved in the mechanisms of specific activity of the nervous tissue [10]. In particular, under acute stress, the extracellular level of cathepsin D in the prefrontal

cortex increases [11]. Since cathepsin D is one of the endopeptidases that determine the conversion of angiotensinogen to AngI [12, 13], acute stress increases the likelihood of AngII formation from AngI and enhances the RAAS activity in the brain. It has also been shown that memory consolidation is impaired by the administration of AngII in the CA1 region of the hippocampus in the active avoidance test. This effect is mediated by angiotensin II type 1 receptor (AGTR1) and involves the ERK1/2 intracellular signaling cascade [14]. In turn, the reduction of characteristic anxiety in mice with total overexpression of *hACE2* is associated with an Ang1-7-mediated activation of Mas receptors and the related changes in the activity of GABAergic neurons in the basolateral amygdala [15, 16].

Assuming susceptibility to stress depending on the level of ACE2 expression or on its functional activity and/or the RAAS activity, it is of interest to study the stress-induced behavior of mice under conditions of *hACE2* gene expression [15] or under chronic administration of lisinopril, an ACE inhibitor, with permeability of the blood–brain barrier.

The aim of this study was to explore the effect of *hACE2* gene expression on anxiety and susceptibility to psychophysiological stress in k18-hACE2-KI mice in a foot electroshock (FS) model of PTSD.

MATERIALS AND METHODS

The experiments were conducted using male mice of the C57Bl/6N and k18-hACE2-KI lines (Andreevka Nursery of the Scientific Center for Biomedical Technologies and the Centre for Strategic Planning and Management of Biomedical Health Risks) aged 4–5 months. The animals were kept in cages with artificial ventilation, 5–7 animals per cage, at a temperature of 24°C and a 12-hour light/12-hour dark cycle (light on at 7:00 a.m. and light off at 7:00 p.m.). Water and standard feed were *ad libitum*.

Studies were conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 and approved by the Bioethics Commission of Centre for Strategic Planning and Management of Biomedical Health Risks of FMBA (Protocol No. 2 dated 15.02.2024).

Experimental design

For the study, the animals were randomly divided into groups of 7–8 individuals.

Three groups of C57Bl/6N mice were formed:

1. Control group (n = 7);
2. FS group (n = 7);
3. FS + lisinopril group (n = 7);

Two groups of k18-hACE2 mice:

4. Control group (n = 7);
5. FS group (n = 8).

Given that lisinopril was used for pharmacological modeling of possible hypotensive effects of *hACE2* expression, an additional comparison group of k18-hACE2 transgenic mice of the “FS + lisinopril” line was not introduced.

PTSD model. After habituation of mice to the housing conditions and to each other in the formed groups, a modeling of PTSD-like endophenotype was performed using the method of foot electroshock (FS), as described earlier in [17, 18]. Two or three days before the experiment, the animals were habituated for 3 min to a test plexiglass chamber (16×16×32 cm^3) placed in a soundproof box and equipped with an electrode grid floor connected to a DC generator and with a video camera (Fear Conditioning System, UgoBasil, Italy). During the test session, after a 1-min rest period, two 1.5 mA 2 s pulses were applied to the floor, one after the other, with a 1 min interval. After the second pulse, the animal was left in the chamber for an additional 1 min before being returned to its home cage. The mice from the control group remained in the FS chamber for 5 min.

Drug administration. Mice in the FS + lisinopril group received lisinopril at a dose of 10 mg/kg per day with drinking water for 28 days after psychophysiological trauma. The used dose of 10 mg/kg per day corresponds to the doses recommended for treatment of arterial hypertension in humans. Before the experiment, daily water consumption was monitored for a week to assess background water consumption and calculate the working concentrations of the lisinopril solution. The average daily fluid intake per animal of 4.46 mL/day accords with the data on water intake in adult mice known from numerous literature sources. Based on the preliminary assessment of water intake, a solution of lisinopril (Alsi Pharma, Russia) was prepared with the working concentration such that each animal received an average daily dose of 10 mg/kg of the drug. The lisinopril solution was renewed every other day. During the 28 days of therapeutic exposure, the daily water intake was assessed to monitor the received dose of the drug. However, minor deviations from the average consumption value could have an impact on the final effect severity, thus affecting the experiment outcome.

FS memory assessment. On days 7 and 28 after FS exposure, mice were placed in the test chamber for 3 min and the time of freezing (absence of any movements, except for those caused by the respiratory excursion of the chest for two or more seconds) was measured using the ANY-maze Video-Tracking Software. The magnitude of the fear response was expressed as a relative freezing time.

Behavior such as locomotor activity, anxiety, stress coping strategy, and spatial navigation/spatial learning were evaluated in a series of tests on days 29–32 after FS.

To assess the overall locomotor activity, the open field (OF) test was used. For this purpose, the animals were placed in the arena (41×41×33 cm^3) of the motor activity measurement system (Multiple Activity Cage, UgoBasil, Italy). The locomotor activity and verticalization (the total number of stands with support on the test arena walls and without support on the test arena walls) were assessed based on the total number of intersections between the beams of the photodetectors, which were located 2 cm apart on the panels on both sides of the arena at two horizontal levels. The test was conducted for 30 min under 300 lux.

The tail suspension test (TST) was used to evaluate the stress coping strategy. The immobility time was assessed by evaluating the freezing time in the first 3 min and second 3 min of the test separately.

The light/dark box test (LDB) was used to assess the anxiety level. The test was conducted in a chamber (42×40×40 cm^3) divided into equal-sized open and closed compartments (Light/Dark Box for Mice, UgoBasil, Italy). The open space avoidance behavior (latent period from the moment the experimental animal was placed in the center of the light compartment (LC) to the moment of the first entry into the dark compartment (DC), the number of entries into the compartments, the time spent in the compartments, and the total distance traveled) was assessed over a 10-min period. The illumination in the LC was 400 lux.

The elevated plus maze (EPM) test was also used to measure anxiety. The maze was located at a height of 60 cm from the floor and consisted of two open (OA) (80 cm×5 cm) and two closed (CA) (80 cm×5 cm) arms intersected at a right angle (Elevated Plus Maze for Mice, UgoBasil, Italy). The number of glances into the OA, the number of entries into the OA and CA, the time spent in the OA and CA, and the total distance traveled were evaluated over a 5-min period. The OA illumination was 400 lux. According to the test results, the anxiety index (AI) was calculated using the following formula: AI = 1 — [(time spent in the OA)/total test duration) + (number of entries to the OA/total number of entries to the arms)]/2 [17].

Spatial navigation and spatial learning dynamics were assessed in the Barnes maze test (BMT) using an open circular arena with a diameter of 100 cm (Barnes Maze for Mice, UgoBasil, Italy) with an illumination of the central area of 600–700 lux. The maze was divided into four segments, with a shelter placed under the surface of one of them. The training sessions (the first and second days of the test, “day 1” and “day 2”) and the test trial (the third day of the test, “test”) each lasted 3 min.

Behavior in the LDB, EPM, and BMT tests was analyzed using the Any-maze Video-Tracking Software, and the video was recorded using the DMK 22AUC03 video camera (IMAGINSOURCE, Germany) with the Computar A4Z2812CS-MPIR lens (Megapixel, China).

The experimental design is shown in Fig. 1.

Statistical analysis was performed using the PrismGraphPad v10.0+ software. The data are presented as the mean value ± the standard error of the mean value (M ± SEM). Given the sample sizes determined by the availability of transgenic animals, the normality of the data distribution was not assessed. However, all data were checked for outliers using an algorithm based on a nonlinear regression model [19]. A two-factor analysis of variance (ANOVA) was used to compare the estimated effects in the groups. When a significant influence of any main factor was detected, between-group comparisons were conducted using the Tukey test.

Depending on the parameter being evaluated and the specifics of the test, the following pairs of principal factors were established:

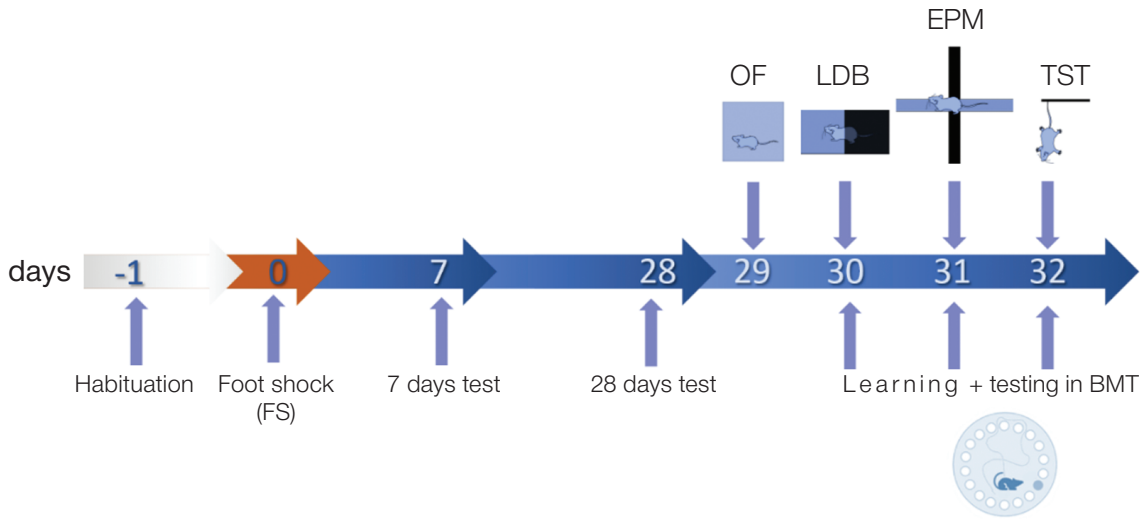

Figure prepared by the authors using their own data

Fig. 1. Design of experiment on modeling and phenotyping of PTSD-like state in mice: OF — open field test; LDB — light/dark box test; EPM — elevated plus maze; TST — tail suspension test; BMT — Barnes maze test

(1) FS (single FS exposure or no FS) × genotype (presence or absence of *hACE2*);

(2) genotype × time (minutes in the OF, days in the freezing assessment);

(3) FS × time (days in the freezing assessment and BMT parameters);

(4) groups (control, FS, or FS + lisinopril) × time (days in the freezing assessment and BMT and TST parameters).

In the case of assessing the lisinopril effect in all behavioral tests, except for assessing freezing and behavior in BMT and TST, a one-way analysis of variance was used to compare the groups ("no FS" *vs* "FS" *vs* "FS + lisinopril"). The differences were considered statistically significant at a p value of < 0.05.

RESULTS

Expression of *hACE2* gene was found to affect the behavioral activity of k18-hACE2-KI mice when compared to C57Bl/6N. A 1.2-fold decrease in the distance traveled in the OF was observed in intact k18-hACE2 mice compared to intact C57Bl/6N mice (genotype: $F(1.12) = 16.83$, $p = 0.0015$); the corresponding data are presented in Fig. 2a. In the LDB test, mice of the k18-hACE2-KI line showed a statistically significant 15.4-fold increase in the latent period before the first entry to the DC and a 1.9-fold increase in the time spent in the LC ($Fs > 7.98$, $p < 0.017$), which is confirmed by the results of post-hoc analysis ($p < 0.0205$); the corresponding data are presented in Figs. 2e and 2g.

The k18-hACE2 mice exposed to a single FS reacted more strongly to shock compared to wild-type mice (comparison of averages in the Student's t-test, $t = 3.561$, $df = 13$, $p = 0.004$). In addition, they remembered the conditioned stimulus/context of the traumatic event better, which was expressed as an increased freezing time after psychophysiological trauma in transgenic mice compared to C57Bl/6N mice on days 7 and 28 after FS exposure (genotype: ($F(1.12) = 226.98$, $p < 0.001$; time: ($F(1.12) = 24.79$, $p = 0.0003$; genotype × time: ($F(1.12) = 8.113$, $p = 0,015$). Moreover, these mice did not show signs of fear extinction (post-hoc: 7 days vs. 28 days; $p = 0.157$) (Fig. 3b), whereas fear expression in C57Bl/6N mice decreased over 28 days (post-hoc: 7 days vs. 28 days; $p = 0.0001$) (Fig. 3a).

Psychophysiological trauma caused delayed behavioral changes in k18-hACE2 mice, which differed from those observed in C57Bl/6N mice. The immobility time in the TST in k18-hACE2 mice was 2.4 times less than in C57Bl/6N mice in the first 3 min ($p = 0.049$) and 1.5 times less in the second 3 min ($p = 0.0137$) in the 6-min test (genotype: ($F(1.13) = 6.268$, $p = 0.029$) one month after FS; the corresponding data are shown in Fig. 2c.

Transgenic mice exposed to FS demonstrated a 14.4-fold increase in the latency period before the first entry into DC in the LDB test compared to wild-type mice (genotype: ($F(1.11) = 43.91$, $p < 0.001$; FS: ($F(1.12) = 9.201$, $p = 0.010$; genotype × FS: ($F(1.11) = 7.276$, $p = 0.021$) (Fig. 2e). In both experimental groups, a comparable decrease in the number of entries to the LC and DC after the FS was observed (FS: $F(1.13) = 3.134$, $p = 0.101$). However, only the transgenic mice showed a decrease in the time spent in the DC, which was not symmetrical to the increase in the time spent in the LC (FS: ($F(1.13) = 7.486$, $p = 0.017$; genotype: $Fs > 38.47$, $p < 0.001$; genotype × FS: ($F(1.11) = 7.704$, $p = 0.017$) (Figs. 2f, 2g).

The FS effect was generally not selective towards k18-hACE2-KI mice when assessing anxiety in EPM (FS: $Fs < 3.279$, $p > 0.097$, genotype × FS: $Fs < 2.137$, $p > 0.16$) (Fig. 2h, i, j), although a decrease in the distance traveled in the closed arms of the maze was found in k18-hACE2-KI mice, relative to that found in FS exposed C57Bl/6N mice (post-hoc: $p = 0.028$) (Fig. 2k). The average AI values for the C57Bl/6N control mice, for the C57Bl/6N mice that received FS, for the k18-hACE2-KI control mice, and for k18-hACE2-KI mice with FS were 0.933 ± 0.019, 0.954 ± 0.017, 0.996 ± 0.004, and 0.991 ± 0.008, respectively. Two-factor analysis of the AI variance revealed the genotype effect: $F(1.12) = 15.52$, $p = 0.002$; the FS effect: $F(1.13) = 0.345$, $p = 0.567$; the effect of factor interaction: $F(1.12) = 1.063$, $p = 0.323$. The subsequent post-hoc test indicated a statistically significant difference between control groups ($p = 0.005$).

Assessment of locomotor activity in the OF test revealed the genotype ($F(1.12) = 16.830$, $p = 0.002$) and FS ($F(1.13) = 5.810$, $p = 0.032$) effects of *hACE2* expression (Fig. 2a). In addition, a selective decrease in verticalization after FS was found in k18-hACE2 mice (genotype × FS: ($F(1.12) = 5.362$, $p = 0.039$ (post-hoc: $p = 0.007$); FS and genotype: $FS < 3.7495$, $p > 0.075$) (Fig. 2b).

Evaluation of spatial navigation/spatial learning in BMT showed that in C57Bl/6N mice, not in k18-hACE2 mice, the strategy of finding shelter after FS is optimized during three consecutive days of the test, which is expressed in an increase in the proportion of visited holes in the target segment (FS: ($F(1.12) = 2.150$, $p = 0.168$; day: ($F(1.12) = 4.434$, $p = 0.028$, FS × day: ($F(2.24) = 1.693$, $p = 0.205$ (post-hoc: day 1 compared to the third day ("test"), $p = 0.049$) (Fig. 2n). This was accompanied by a decrease in the time spent to find shelter (FS: ($F(1.12) = 1.011$, $p = 0.335$; day: ($F(1.12) = 6.561$, $p = 0.010$, FS × day: ($F(2.24) = 0.266$, $p = 0.768$ (post-hoc: day 1 compared to the third day ("test"), $p = 0.018$) (Fig. 2l). In terms of the proportion of entries to the shelter segment, the FS effect was not statistically significant ($Fs < 0.285$, $p > 0.466$) (Fig. 2m).

The lisinopril effect was separately analyzed in C57Bl/6N mice in a PTSD model. Lisinopril, when administered chronically orally (at a dose of 10 mg/kg per day), did not affect contextual memory in the PTSD model (post-hoc: 7 days $p = 0.609$ and 28 days $p = 0.341$) (Fig. 3a). The average AI values for mice of the

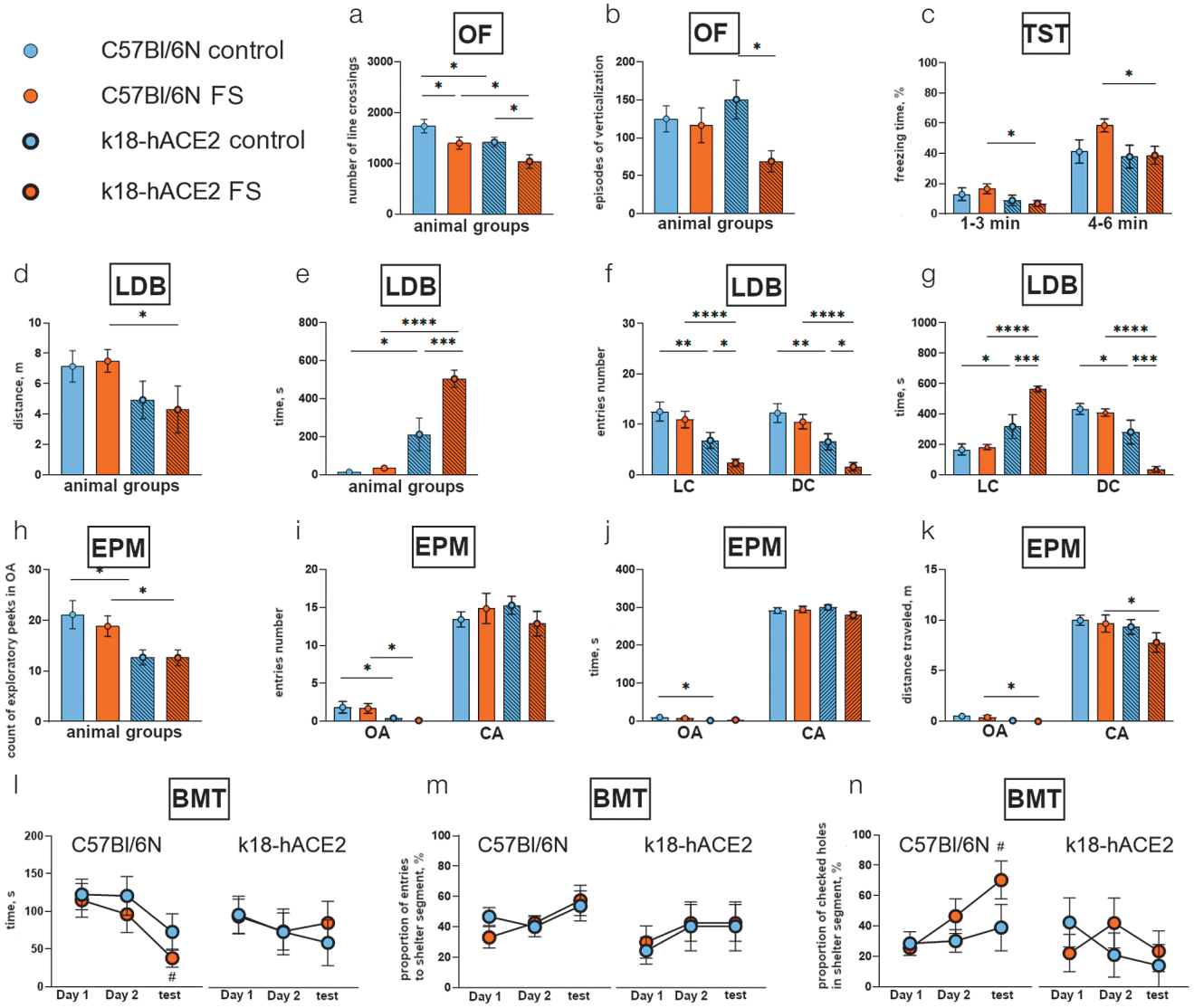

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Fig. 2. Comparison of the behavioral phenotype of C57Bl/6N and k18-hACE2 mice in the paradigm of assessing the delayed (29–32 days) effect of limbs electroshock (ES): a — locomotor activity; b — verticalization in open field (OF) test; c — freezing time in tail suspension test (TST); characteristic anxiety in light/dark box (LDB) test : d — total distance traveled in light compartment (LC) and dark compartment (DC); e — latent period before first visit to DC; f — number of entries to LC or DC; g — time spent in LC or DC; characteristic anxiety in elevated plus maze (EPM) test: h — count of exploratory peeks in open arm (OA); i — number of entries in OA or closed arm (CA); j — time spent in OA or CA; k — distance traveled in OA or CA; spatial navigation dynamics in Barnes maze test (BMT): l — time to find shelter; m– proportion of entries to shelter segment relative to number of entries to all segments; n — proportion of checked holes in shelter segment relative to total number of checked holes

Note: hatching — transgenic genotype; results of post-hoc comparison between groups: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; post-hoc comparison between day 1 and test when evaluating spatial navigation: # $p < 0.05$.

control group of the C57Bl/6N mice, for the C57Bl/6N mice who received FS, and mice of the C57Bl/6N line who received lisinopril after FS were 0.933 ± 0.019, 0.954 ± 0.017, and 0.927 ± 0.026, respectively, with the one-factor analysis of variance having revealed no differences between the groups F (2.18) = 0.446, $p = 0.647$.

No effect of lisinopril was found in the OF, TST, LDB, EPM, and BMT tests (Figs. 4a–j). There may be a possible effect on the dynamics of spatial navigation/spatial learning in LB; however, it was not confirmed by statistical analysis (post-hoc: $p > 0.073$) (Figs. 4h, 4i, 4j).

DISCUSSION

The obtained data indicate a complex profile of the *hACE2* gene expression effect on mouse behavior. On

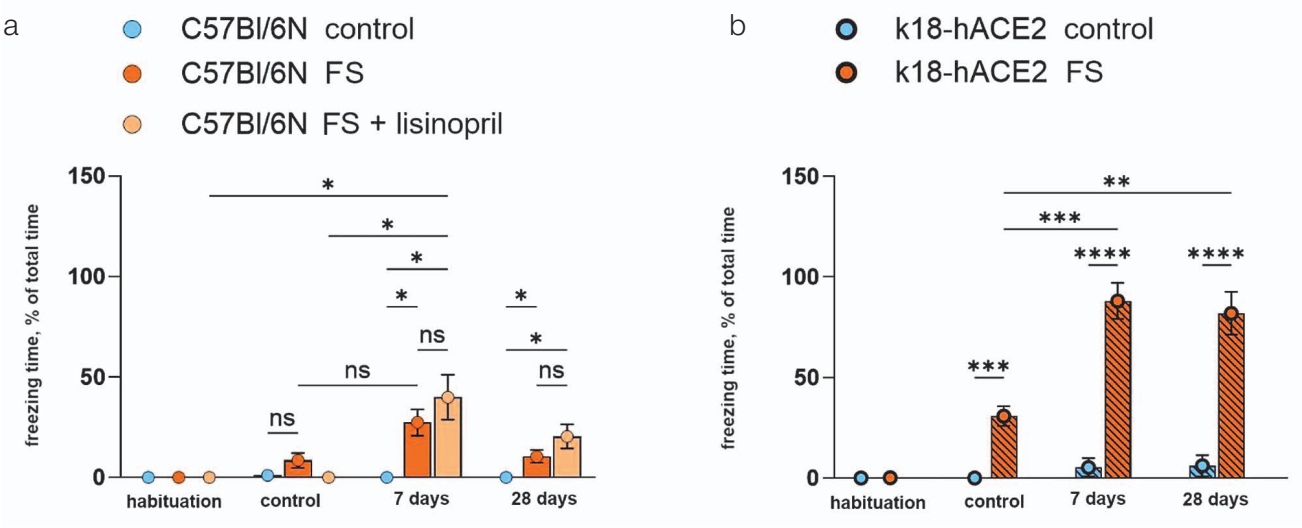

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Fig. 3. Freezing time dynamics in C57Bl/6N and k18-hACE2 mice: a — freezing time dynamics and effect of ACE inhibitor lisinopril (10 mg/kg per day) on the freezing time in C57Bl/6N mice; b — preservation of traumatic memory in k18-hACE2 mice

Note: hatching — transgenic genotype; post-hoc comparison results between groups: ns — not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; FS — foot shock.

the one hand, intact transgenic mice demonstrated an increase in resistance to an open illuminated space in the LDB test, which confirms the previously described anxiolytic effect of humanized *ACE2* gene overexpression in intact mice [15, 16]. On the other hand, our study established the effect of *hACE2* gene expression on the delayed consequences of PTSD-modeled acute stress. Thus, a longer exploration of LC, which is dangerous only potentially, in the LDB test and an increase in immobility, potentially of an adaptive nature, in the TST were observed. At the same time, the expression of the *hACE2* gene contributed to the strengthening and retention of traumatic memory, which was expressed in an increase in the freezing time and the absence of fear extinction (i.e., the inability to relearn the actual safety of the test chamber) within a month after psychophysiological trauma.

The enhancement of FS memory may be mediated by an increase in the FS perception since the immediate response of the transgenic mice to FS during conditioning was higher. It should be noted that this is likely to be determined by the perception mechanisms of the psychological component of stress rather than by an increase in the pain sensitivity, since a decrease in nociception was reported upon a decrease in efficiency of the ACE2-mediated signaling [20] and the transgenic model we used, on the contrary, involves an increase in the ACE2 function. Indeed, a comparison of our results with the characteristics of the behavioral endophenotype described in mice with *hACE2* gene overexpression [15, 16] indicates an increase in enzymatic activity of the ACE2 in k18-hACE2-KI mice, which may be mediated either by overexpression of the humanized gene or by the increased activity of *hACE2* compared to the wild type. The increase in enzymatic activity of the *hACE2* in k18-hACE2 mice may, in turn, lead to an increase in Ang1-7 production and an increase in the activity of the ACE2/Ang1-7-dependent RAAS cascade. The corresponding facilitation of MasR-dependent signaling in the mouse brain acts as a mechanism supporting neuroplasticity and enhancing memory [21, 22], as well as mediating anxiolytic and antidepressant action [23, 24].

Correa et al. and Fontes et al. considered RAAS regulators as potential targets in stress therapy [5, 25]. ACE inhibitors, AGTR1, as well as beta-blockers, have shown good results in the clinical setting of PTSD treatment. Preclinical studies revealed different effects. In the study by Marvar et al., the selective AGTR1 inhibitor losartan decreased traumatic memory, increased fear extinction, and did not affect the characteristic anxiety of the animals [26]. However, Braszko, Raghavendra et al. [27, 28] reported an increase in traumatic memory in a PTSD model. In our experiment, we studied the effect of ACE inhibitor lisinopril, an antihypertensive drug, on behavioral responses of C57Bl/6N mice in a PTSD model. We predicted that, at the selected dose of 10 mg/kg per day, which corresponds to the therapeutic doses used in clinical practice, lisinopril would exhibit an anxiolytic effect engaging its antihypertensive activity. However, chronic administration of lisinopril for 28 days did not affect either contextual memory or anxiety in mice. Earlier, Cohen et al. and Kao et al. observed no therapeutic effect of the

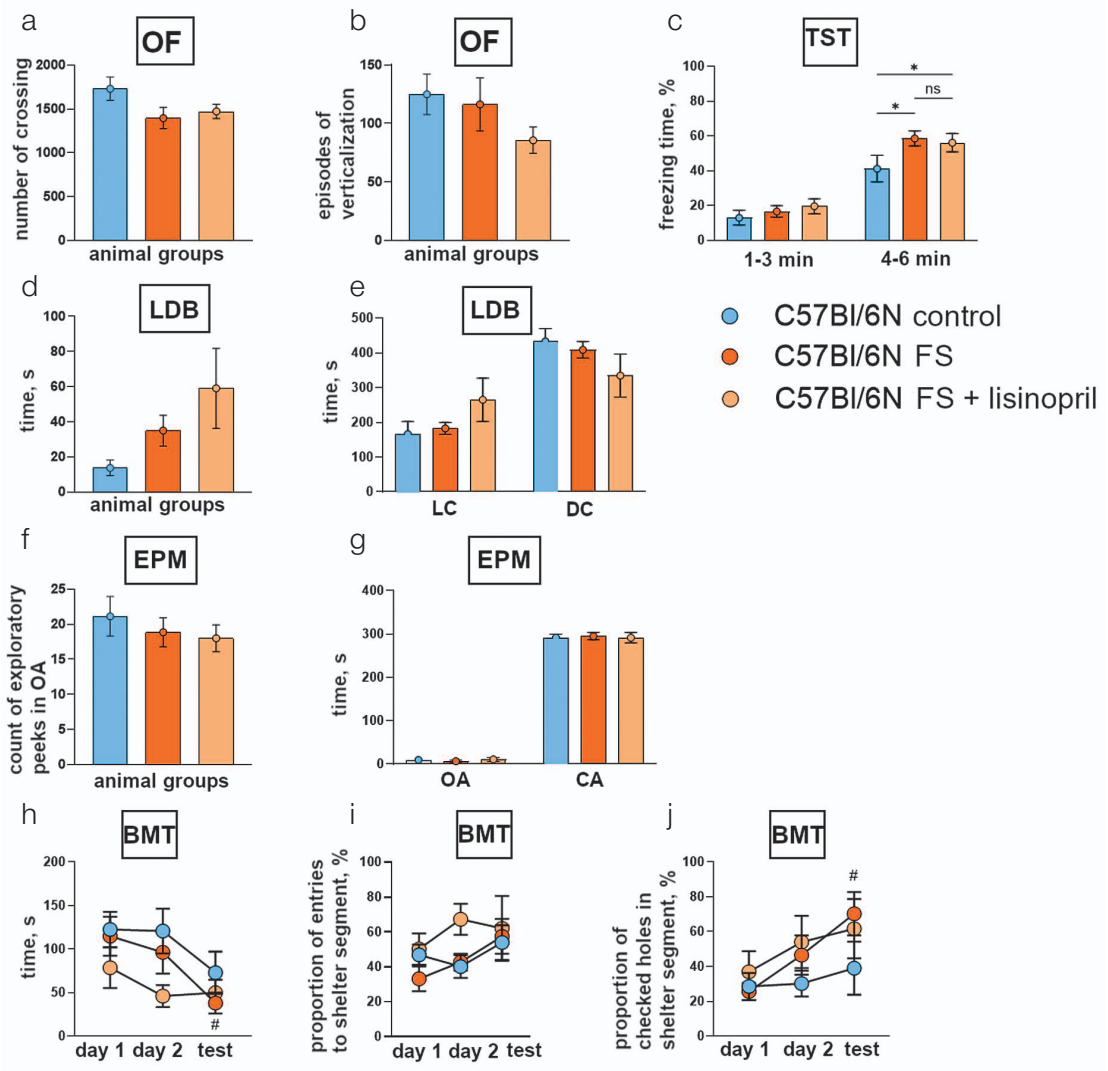

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Fig. 4. Effect of ACE inhibitor lisinopril on locomotor activity, anxiety, emotional coping, and spatial navigation in C57Bl/6N mice exposed to foot shock (FS): a — locomotor activity; b — verticalization in open field (OF) test; c — freezing time in tail suspension test (TST); d — latent period before the first entire to the dark compartment (DC); e — time spent in the light compartments (LC) and DC in the light/dark box (LDB) test; f — count of exploratory peeks in open arm (OA); g — time spent in open or closed arms (CA) in elevated plus maze (EPM) test; dynamics of spatial navigation in the Barnes maze test (BMT): h — time to find shelter; i — proportion of entries to shelter segment relative to number of entries to all segments; j — proportion of checked holes in shelter segment relative to total number of checked holes

Note: results of post-hoc comparison between groups: ns — not significant, $*p < 0.05$, $**p < 0.01$; results of a post-hoc comparison between data from day 1 and day 3 ("test") when evaluating spatial navigation: # $p < 0.05$.

beta-blocker propranolol in a PTSD model in mice [17, 29]. In combination, these data may point to the limited effect of normalizing systemic blood pressure with beta-blockers, as well as ACE inhibitors and AGTR1, in PTSD treatment. The ineffectiveness of lisinopril in our study may also be determined by the tertiary structure of the human enzyme. It is known that therapeutic effects of ACE inhibitors in PTSD depend on the *ACE* polymorphism, specifically determining resistance to therapy in the presence of the rs4311 TC nucleotide variant [30]. The probable increase in *ACE2* gene expression in response to chronic lisinopril use, which has already been described in the literature [31–35], was not sufficient to induce brain-specific ACE2/Ang1-7/MasR-dependent mechanisms.

CONCLUSION

The data obtained indicate the effect of the *hACE2* gene expression on mouse susceptibility to psychophysiological stress, which may be mediated by changes in the activity of the ACE2-dependent cascade of brain RAAS, and point to the significance of ACE2 in the traumatic memory acquisition in the PTSD model. Thus,

the *hACE2* expression in mice is accompanied by an increase in traumatic memory and by a decrease in extinction of traumatic memory compared to wild-type mice. The decreased RAAS activity under the action of the ACE inhibitor lisinopril with a hypotensive effect did not affect memory in wild-type mice.

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Authors' contributions. All authors confirm that their authorship meets the ICMJE criteria. Yulia A. Timoshina — modeling of a PTSD-like condition in mice, primary analysis, manuscript creation, and editing; Tatiana S. Deinekina — assessment of general locomotor activity and conducting the Barnes maze test, administration of drugs, and manuscript editing; Elena V. Savinkova — conducting the PCL, STC, and tail-suspension tests; Vladimir S. Yudin — material and technical support; Anton A. Keskinov — manuscript editing; Valentin V. Makarov — conceptualization and approval of the final manuscript version; Elmira A. Anderzhanova — conceptualization, methodology, data analysis, manuscript creation and editing, approval of the final manuscript, and research management.

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