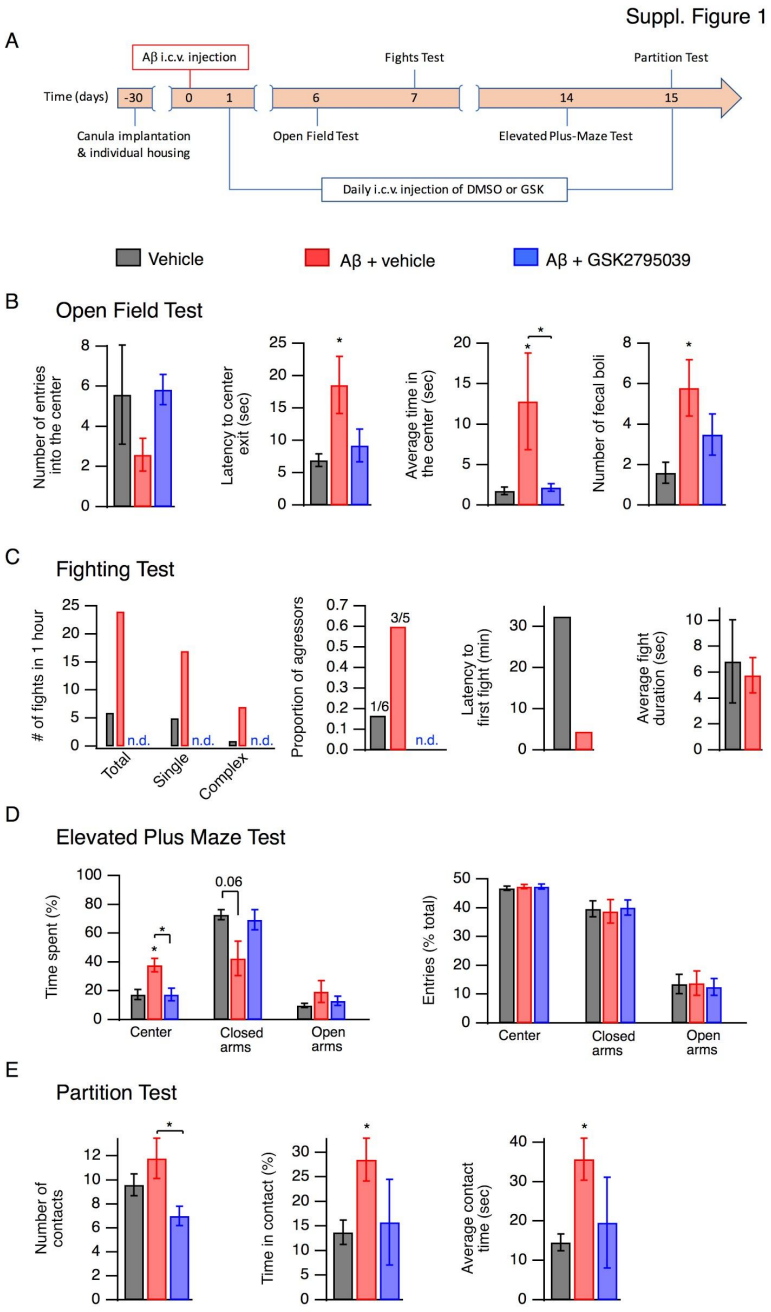
Behavioral and psychological symptoms induced by Aβ are prevented by NOX2 inhibition.

To assess changes in mouse behavior following $A\beta_{1\text{-}42}$ i.c.v. injection, we utilized performance tests evaluating anxiety and aggression, as these tendencies are clearly evident in the Aβ-injected mice. Tests were performed following 1 week (Open field and Fights) and 2 weeks (Elevated plus-maze and Partition) of i.c.v. administration of β-amyloid (Fig. S1A).



Supplementary Figure 1. ***NOX2 blockade prevents Aβ$_{1\text{-}42}$ –induced neuropsychiatric-like behavioral abnormalities.***

A. Timeline diagram of experimental design. **B. Open field test.** Animals were placed in the center of the OF and latent time (sec) of the first run from the central square of 20 × 20 cm was recorded. Such indicators of anxiety as the number of entries into the center (n), the average time in the center (sec), and the number of fecal boli (n) are also presented. **C. Fighting Test.** Animals were placed on a round field for 1 hour and the number of fights (n), the number of aggressors (n), the latent time of the first attack (min), the average duration of fights (sec), are presented. Fights were separated by the number of participants: two mice in a simple fight, three mice and more in a complex fight. The number of simple and complex fights increased sharply in the Aβ group, while the duration of each fight did not significantly differ from the control values. Surprisingly, in the group with daily GSK2795039 injection, not a single fight was recorded during the entire hour of registration. Mice actively interacted, but elements of aggression were absent. **D. Elevated Plus Maze Test**. Such indicators of anxiety as the number of entries into the center or arms (n) and average time in the center or arms (sec) are presented. In all groups, mice preferred close arms to open ones. However, mice from the Aβ group spent much less time in closed arms compared with control mice. All parameters of the group with daily GSK2795039 injection did not differ from the control one. **E. Partition Test**. As direct contact with the partition correlates with the level of aggressiveness in mice, there were analyzed a number of contacts (n), time in contact (%), and average contact time (sec). All parameters in group Aβ significantly increased in comparison with the control, indicating increased aggressiveness. All parameters of the group with daily GSK2795039 injection did not differ from the control one.

In the Open field test *(1)*, Aβ-injected mice spent twice as long in the central part of the field compared to control mice, and the increased anxiety was further demonstrated by a higher level of defecation (Fig. S1B). A similar behavior trend was confirmed in the Elevated plus-maze test *(2),* in which the Aβ-injected mice were more likely than controls to stay in the central area and less likely to stay in the closed arms (Fig. S1D). Meanwhile, the mice in Aβ-group displayed a much higher level of aggression as indicated by the Fights test. In the control group, there was only one initiator of fights (1 of 6; 17%), whereas in the Aβ-group, this indicator rose to 60% (3 of 5), and the number and total duration of fights increased sharply (Fig. S1C). The increased aggression in Aβ-injected mice was also confirmed in the modified Partition test *(3)*, where these mice spent a significantly longer time in proximity to the partition with the reference mouse trying to reach it (Fig. S1E). Altogether, these tests demonstrated anxiety behavior in mice following i.c.v. $A\beta_{1\text{-}42}$ injection (see also *(4, 5)*), and revealed a marked trend to aggression in male mice. Importantly, all the behavioral abnormalities were prevented by daily i.c.v. injections of GSK2795039 (Figs.1S, B-E), indicating that these neuropsychiatric-like disturbances were caused by NOX2-induced oxidative stress.

Methods

Experiments were performed on BALB/c mice (25–33 g) obtained from the Laboratory Animal Nursery "Pushchino" (http://spf-animals.ru/, Pushchino, Russia). Animals were housed individually with food and water ad libitum. Animals were maintained with a 12-h light/dark cycle (lights on from 9 AM to 9 PM) in temperature (22°C ± 1°C) -controlled room. The protocol was approved by the Committee on the Bioethics of Animal Experiments of the Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences.

Surgery

Experimental animals underwent a neurosurgical operation under general anesthesia: mixture ketamine (200 mg/kg, i.m.) and Xylazine hydrochloride (10 mg/kg, i.p.). Premedication with atropine (0.04 mg/kg) was performed subcutaneously 15 minutes before surgery. The body temperature was

maintained by a heating pad and the cardiopulmonary parameters were monitored during the surgery by an Oxy9Vet Plus pulse oximeter (Bionet, South Korea). Guide cannula (stainless steel, 21 gauge) was implanted above the left lateral brain ventricle (AP = –0.7; L = 1.37; H = 1.25) according to the mice brain atlas (Paxinos and Watson 2007).

Drug administration

Drugs were administered 1-month post-surgery to avoid surgery side effects. Mice were allocated to three groups: control (n = 6), Aβ (n = 5), and Aβ+GSK2795039 (n = 6). Injections were made i.c.v. through the guide cannula (1 μl/min) to awake mice. All substances were injected in equal volumes (1 μl) and the guide cannula was closed by a plunger. Mice in the Aβ+GSK2795039 group received GSK2795039 (MedChemExpress Europe; 4.5 mg/ml in DMSO) and Aβ1-42 (Sigma-Aldrich, 1 mg/ml in 1.0% NH4OH) on the first day, and daily GSK2795039 further for 14 days. Mice in the Aβ group received Aβ1-42 on the first day, and daily DMSO further for 14 days. Mice in the control group received daily DMSO for 15 days.

Behavioral tests

Experiments were carried out at 18:00–21:00. The EthoVision system (Noldus Information Technology, Wageningen, the Netherlands) and RealTimer (Open Science, Moscow, Russia) were used for video registration and subsequent analysis.

Open field test

To evaluate anxiety, the animals' behavior was observed in an "open field", which was a square, black area 60 × 60 cm in size with sides 40 cm high *(1)*. The central area was defined as a 20 cm × 20 cm square, and the other region was defined as the peripheral area. Animals were placed initially in the center of the "field".

Fights test

To evaluate aggressive interaction in a group of animals accustomed for 5 weeks to social isolation, the "fights" test was used. Animals of the same experimental group were placed in the round field (diameter 50 cm) for 1 hour. Each mouse was marked with a colored spot on its back for identification. An attack was defined as the rushing and leaping at a partner with bites and kicks *(6)*. The latent time of the first attack, the number of episodes of fights, the average duration of fights, the number of participants in fights, and the number of aggressors were analyzed.

Partition test

The Partition test *(3)* allows evaluation of the behavior of individual animals in neighboring compartments of a common chamber, separated by a transparent partition with holes, and directly correlates with the level of aggressiveness in mice. An experimental mouse was placed in a large compartment (24.1 × 17.8 × 14.0 cm) and a reference mouse in a smaller compartment (6.4 × 17.8 × 14.0 cm). For 5 minutes of the test, the number of approaches to the partition and the total time spent near the partition, when the mouse touches it with its paws or nose, reacting to a partner in the neighboring compartment, as well as the average time of approaches to the partition, were analyzed.

Elevated plus-maze test

The elevated plus-maze test is widely used in neurobiological research on anxiety *(2)*. It is based upon the natural aversion of rodents to heights and open spaces. The classical measures taken in this test are entries into and time spent on the aversive open arms. The test was performed for 5 minutes in a dimly lit room. The maze was raised 50 cm above the floor and consists of two open and two closed arms. A mouse was placed in the center of the open arm, facing the closed one and the spontaneous behavior was recorded. The closed and open arm entries and the duration spent in closed and open arms were calculated.

1. L. Prut, C. Belzung, The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review, *Eur. J. Pharmacol.* **463**, 3–33 (2003).

2. R. G. Lister, The use of a plus-maze to measure anxiety in the mouse, *Psychopharmacology* **92**, 180–185 (1987).

3. N. N. Kudryavtseva, An experimental approach to the study of learned aggression, *Aggressive Behavior: Official Journal of the International Society for Research on Aggression* **26**, 241–256 (2000).

4. S. Sharma, S. Verma, M. Kapoor, A. Saini, B. Nehru, Alzheimer's disease like pathology induced six weeks after aggregated amyloid-beta injection in rats: increased oxidative stress and impaired long-term memory with anxiety-like behavior, *Neurol. Res.* **38**, 838–850 (2016).

5. S. Sharma, N. Sharma, A. Saini, B. Nehru, Carbenoxolone Reverses the Amyloid Beta 1-42 Oligomer-Induced Oxidative Damage and Anxiety-Related Behavior in Rats, *Neurotox. Res.* **35**, 654–667 (2019).

6. S. Mertens, M. A. Vogt, P. Gass, R. Palme, B. Hiebl, S. Chourbaji, Effect of three different forms of handling on the variation of aggression-associated parameters in individually and group-housed male C57BL/6NCrl mice, *PLoS One* **14**, e0215367 (2019).