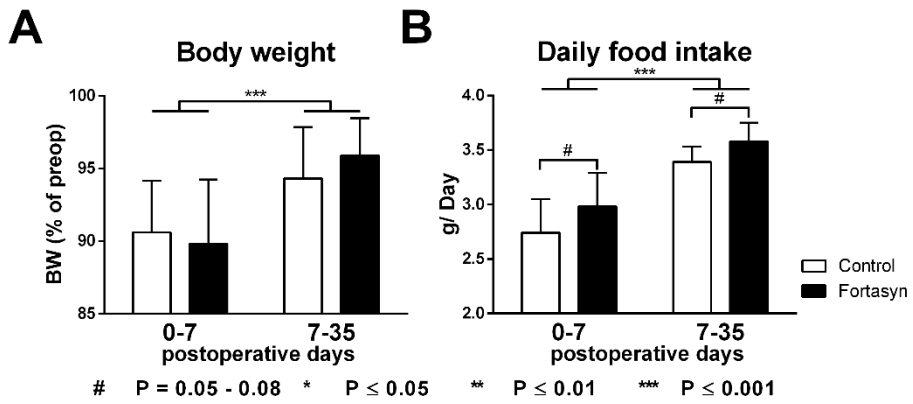
Supplementary Materials

Results

Food intake and body weight (Nijmegen, The Netherlands)

Body weight (*Supplemental figure 1A*) of both dietary groups decreased poststroke (-9.8±1.3 %, $F(1,13)=54.0$, $p<.001$), while it increased significantly over time, comparing the first with the second to fifth week poststroke in all mice (4.9±1.0 %, $F(1,13)=26.6$, $p<.001$).

The daily food intake (*Supplemental figure 1B*) increased over time on both diets in all mice poststroke ($F(1,12)=53.3$, $p<.001$). Fortasyn diet tended to increase food intake compared to Control diet ($F(1,12)=4.7$, $p<.052$).



Supplemental fig. 1. Body weight (BW) and daily food intake measured in the first week and second to fifth week after tMCAo in mice fed either Fortasyn or Control diet. Values represent mean±SD (#, 0.05 < p < 0.08 (tendency); *, p ≤ 0.05; **, p ≤ 0.01; ***, p ≤ 0.001). (A) Relative BW of both dietary groups decreased after tMCAo (-9.8±1.3 %, p<.001). Thereafter, BW increased significantly over time in all mice (4.9±1.0 %, p<.001). (B) Food intake increased over time on both diets in all mice (p<.001). Mice on Fortasyn diet tended to eat more than mice on Control diet (p<.052).

Rotarod (Nijmegen, The Netherlands)

The rotarod is used to measure motor coordination. Mice performed a motor task on a rotating rod at fixed speeds or acceleration at 8 and 21 days poststroke. The latency to fall was recorded. No significant effects between both dietary groups (data not shown) were revealed 8 and 21 days poststroke.

Prepulse inhibition (Nijmegen, The Netherlands)

Sensorimotor integration as measured with the prepulse inhibition test demonstrated no significant differences between both dietary groups (data not shown) 15 days poststroke. Moreover, both animal groups also did not demonstrate a habituation to the 120 dB pulses (data not shown).

Novel object recognition test (Nijmegen, The Netherlands)

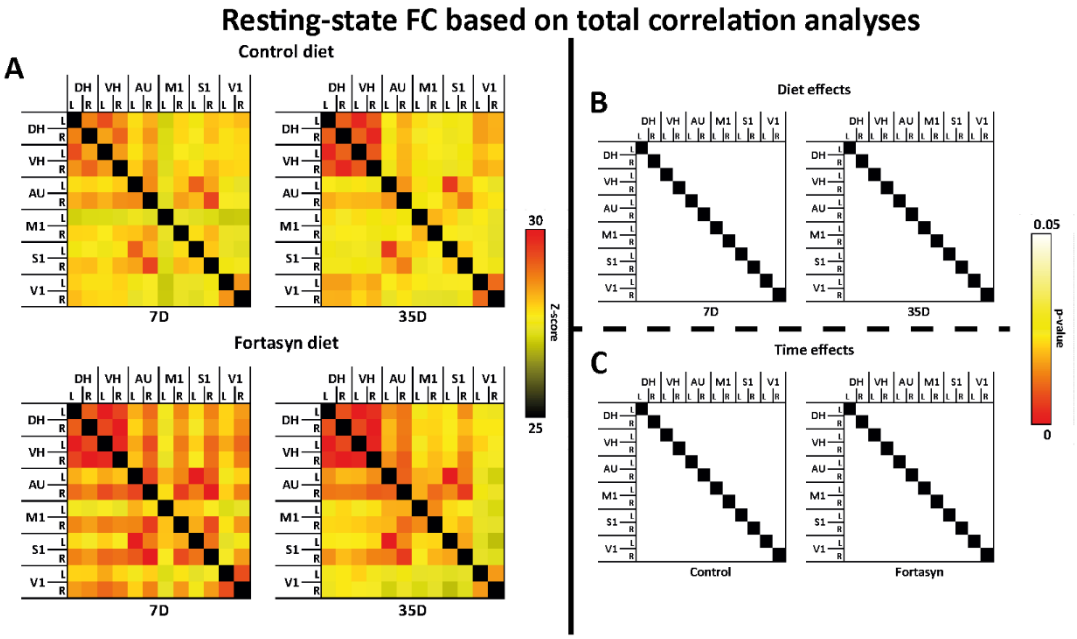
We evaluated changes in cognitive function after 22 to 24 days poststroke. Therefore, mice were tested in the novel object recognition test. No diet effects were observed for the preference for the novel object (data not shown).

Resting state fMRI (Nijmegen, The Netherlands)

To compare functional connectivity (FC) patterns at 7 and 35 days poststroke and on different diets, rsfMRI data were statistically analyzed based on total (*Supplemental figure 2A-C*) in twelve ROI including: left and right dorsal hippocampus (DH), left and right ventral hippocampus (VH), left and right auditory cortex (AU), left and right motor cortex (M1), left and right somatosensory cortex (S1), and left and right visual cortex (V1).

Total correlation analyses

With total correlations no significant diet effects nor time effects (*Supplemental figure 2B,C*) were measured.

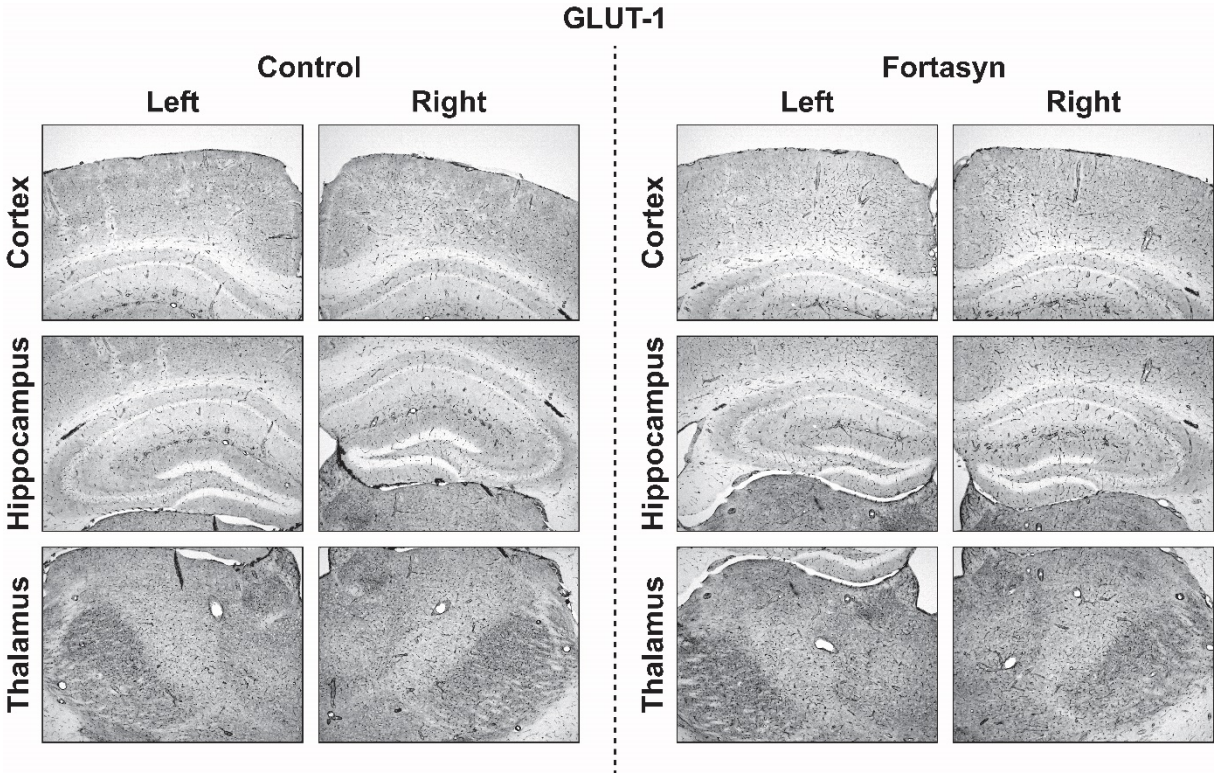


Supplemental fig. 2. Resting-state functional connectivity (FC) based on total correlation analyses (A) in the brains of mice fed Fortasyn or Control diet 7 and 35 days poststroke. FC was measured between 12 ROI: dorsal hippocampus (DH), ventral hippocampus (VH), auditory cortex (AU), motor cortex (M1), somatosensory cortex (S1), and visual cortex (V1). (A-C) No significant diet effects were found with total correlations 7 and 35 days poststroke.

Immunohistochemical stainings (Nijmegen, The Netherlands)

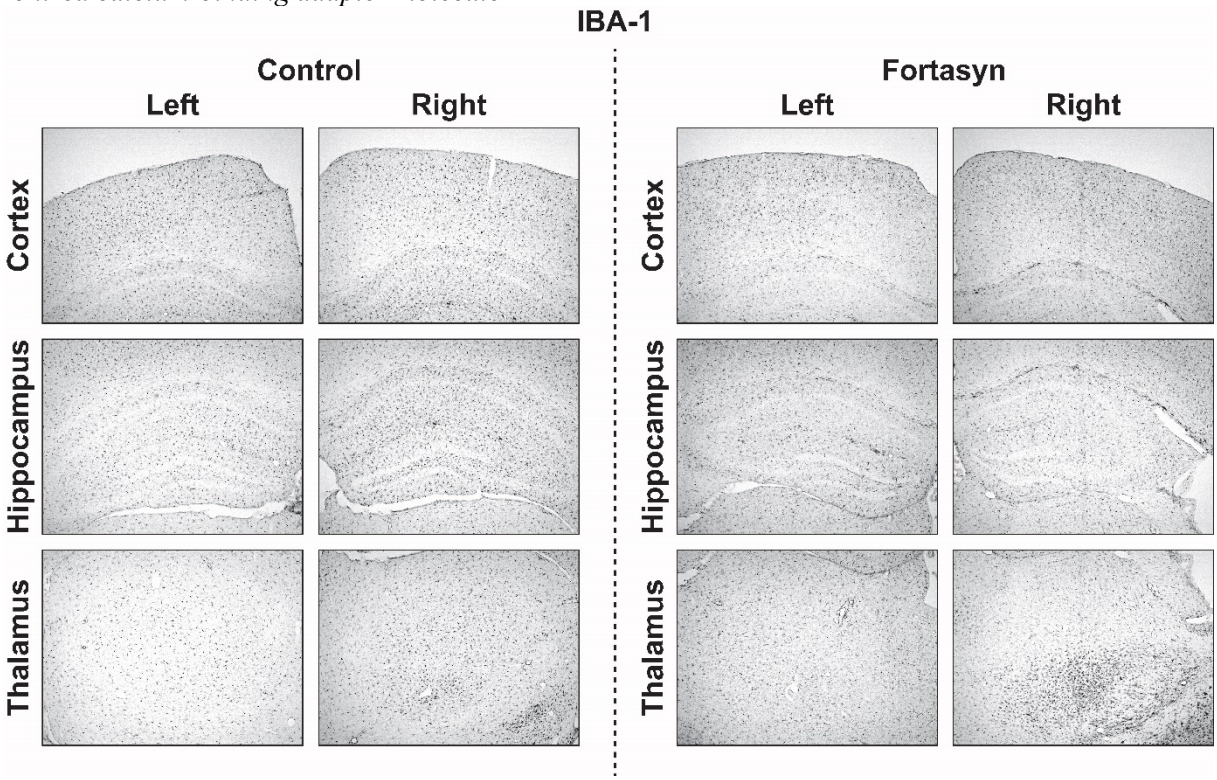
For each treatment and ROI a photo is given for each immunohistochemical staining: Glucose transporter-1 (*supplemental figure 3*), ionized calcium-binding adapter molecule 1 (*supplemental figure 4*), doublecortin (*supplemental figure 5*), synaptophysin (*supplemental figure 6*), postsynaptic Density-95 Protein (*supplemental figure 7*).

Glucose transporter-1



Supplemental fig. 3. Immunohistochemical stainings for glucose transporter-1 on brains of Fortasyn and Control fed mice 35 days after tMCAo. One representative photo for each single condition (Control or Fortasyn) for each left and right region of interest (cortex, hippocampus, thalamus) is shown.

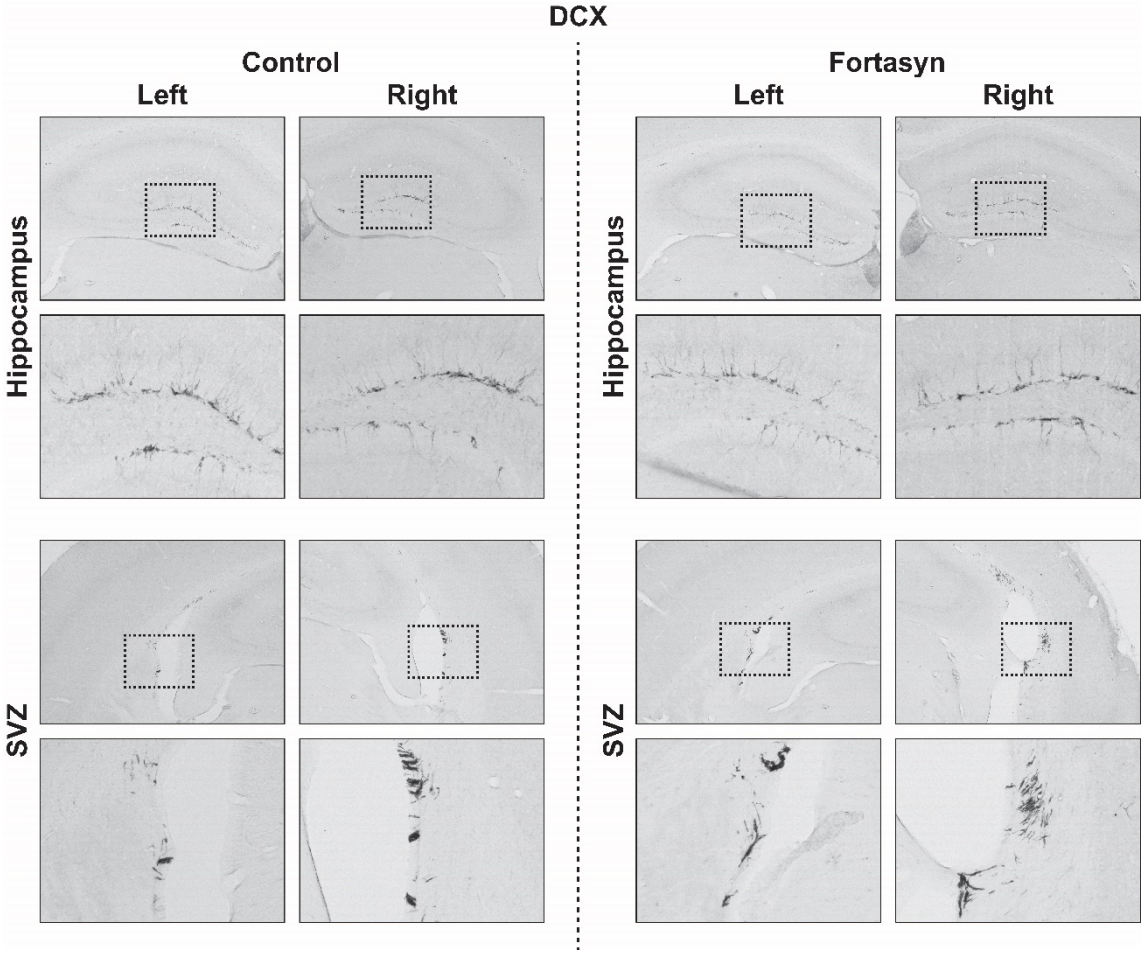
Ionized calcium-binding adapter molecule 1



Supplemental fig. 4. Immunohistochemical stainings for ionized calcium-binding adapter molecule 1 (IBA-1) on brains of Fortasyn and Control fed mice 35 days after tMCAo. One

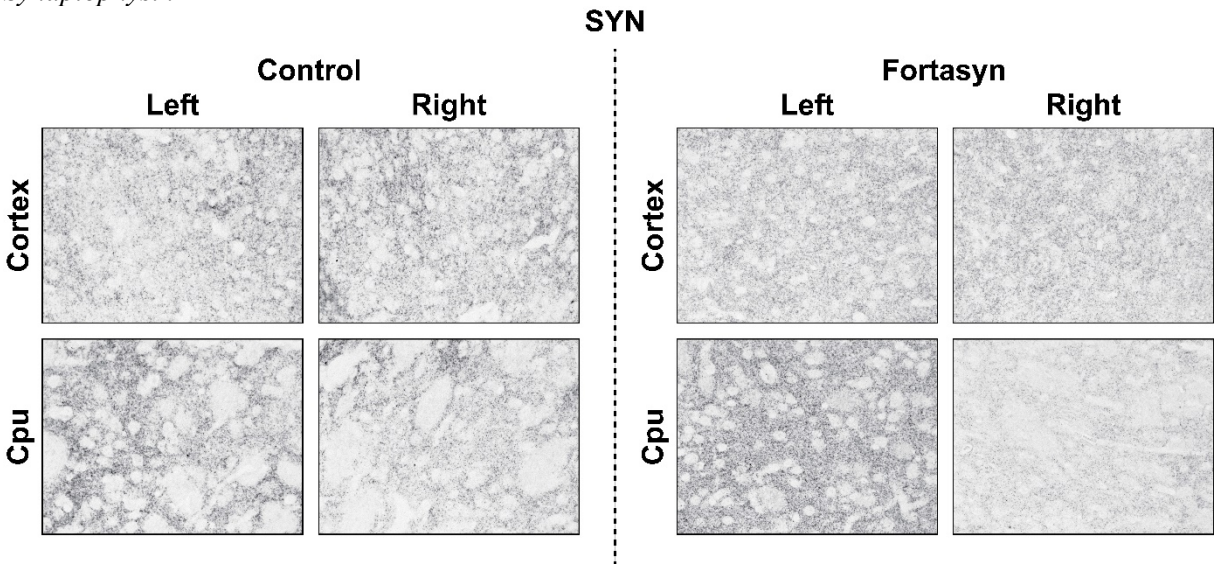
representative photo for each single condition (Control or Fortasyn) for each left and right region of interest (cortex, hippocampus, thalamus) is shown.

Doublecortin



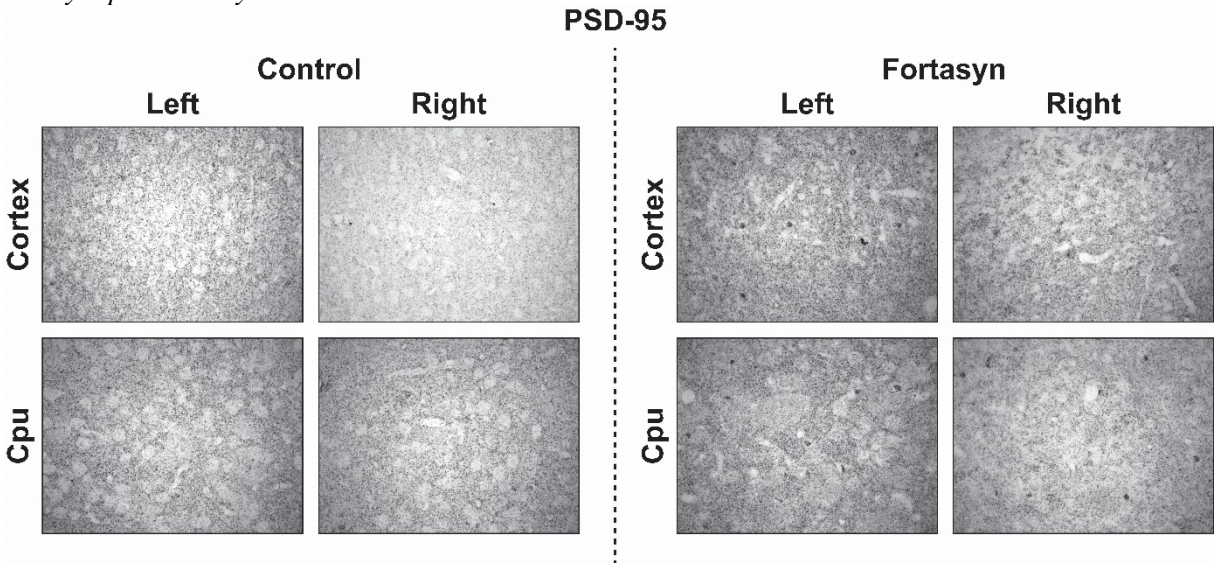
Supplemental fig. 5. Immunohistochemical stainings for doublecortin (DCX) on brains of Fortasyn and Control fed mice 35 days after tMCAo. One representative photo for each single condition (Control or Fortasyn) for each left and right region of interest (hippocampus, subventricular zone (SVZ)) is shown.

Synaptophysin



Supplemental fig. 6. Immunohistochemical stainings for synaptophysin (SYN) on brains of Fortasyn and Control fed mice 35 days after tMCAo. One representative photo for each single condition (Control or Fortasyn) for each left and right region of interest (hippocampus, caudate putamen (Cpu)) is shown.

Postsynaptic Density-95 Protein



Supplemental fig. 7. Immunohistochemical stainings for postsynaptic Density-95 Protein (PSD-95) on brains of Fortasyn and Control fed mice 35 days after tMCAo. One representative photo for each single condition (Control or Fortasyn) for each left and right region of interest (hippocampus, caudate putamen (Cpu)) is shown.

Materials and methods

Sample size calculation

A sample size of minimal 5 mice per subgroup (Fortasyn & Control) was chosen based on power calculation based on results from our previous study (18 months old ApoE4: Control vs Fortasyn), in which we calculated functional connectivity averages and standard deviations of the Control (35.0±1.4) and Fortasyn mice (37.6±1.5) between right auditory and right somatosensory cortex being needed for the effect size calculation for this recent study (d=1.79). A power analysis has been performed with alpha level (or the Type I error rate, α=0.05), statistical power (1-β, 0.80), an assumption of equal sized sample groups (N1 to N2 is 1), and the calculated effect size (1.79): minimal n=5 per group (actual power: 0.93) after experimental stroke. Due to exclusion criteria or unexpected mortality after the induction of an experimental stroke, minimal n=12 per surgical group.

Rotarod

In order to study motor coordination Rotarod was performed prior to the tMCAo (training), and also 8 and 21 days poststroke (for a more detailed description see [1]. Mice were placed on a rotating rod (3.18 cm in diameter; IITC Inc., Woodland Hills, CA, USA) and their ability to stay on the drum was recorded as latency to fall (s). Trials were performed at both fixed speed (10, 15 and 20 rpm) and accelerated speed (4–40 rpm).

Prepulse inhibition

To examine sensorimotor integration, as previously described [2, 3], startle reactivity was measured in a startle response system, the SR-LAB (San Diego Instruments, San Diego, CA, USA), at 16 days

poststroke. The whole-body startle response of the mouse was calculated during three blocks of startle pulses. A detailed description of the testing session can be found in [2].

Novel object recognition test (ORT)

All mice underwent ORT to measure short term memory at 22 to 24 days poststroke. First, all mice acclimatized for 10 minutes to the open field box. At the next acquisition day, each mouse started with the familiarization trial. Before this trial, two identical objects equidistant from the center were placed in the open field. The mouse was then placed into the open field for four minutes to freely explore the two identical objects (F1 and F2). After 30 minutes delay (first acquisition day) respectively 60 minutes delay (second acquisition day) after the familiarization trial, another copy of the previous familiar objects (F3) and one novel (N1) object of the previous trial was placed in the open field. Now again, the mouse had 4 minutes to explore the two different objects (F3 and N1). Using EthoVision XT10.1 (Noldus, Wageningen, The Netherlands), exploratory behaviour was measured as direct contact with the object or in a zone around the object at distance less than 2 cm. For the results, recognition memory was measured using a recognition index being calculated as the time spent exploring N1 minus the time spent exploring F3: [N1-F1] [4].

References

1. Janssen CIF, Zerbi V, Mutsaers MPC, de Jong BSW, Wiesmann M, Arnoldussen IAC, et al. Impact of dietary n-3 polyunsaturated fatty acids on cognition, motor skills and hippocampal neurogenesis in developing C57BL/6J mice. The Journal of Nutritional Biochemistry. 2015; 26: 24-35.
2. Janssen CIF, Zerbi V, Mutsaers MPC, Jochems M, Vos CA, Vos JO, et al. Effect of perinatally supplemented flavonoids on brain structure, circulation, cognition, and metabolism in C57BL/6J mice. Neurochemistry International. 2015; 89: 157-69.
3. Streijger F, Oerlemans F, Ellenbroek BA, Jost CR, Wieringa B, Van der Zee CEEM. Structural and behavioural consequences of double deficiency for creatine kinases BCK and UbCKmit. Behavioural Brain Research. 2005; 157: 219-34.
4. Muñoz PC, Aspé MA, Contreras LS, Palacios AG. Correlations of recognition memory performance with expression and methylation of brain-derived neurotrophic factor in rats. Biological Research. 2010; 43: 251-8.