Supplementary material

S1.a Justification of the applied electrode montage

As we aimed at examining the impact of the right anodal tDCS on elemental and neurochemical in both hemispheres separately, we choose the epicranial montage of the active electrode. This technique allows for more precise and stable location of the electrode on the cranium because the smaller electrodes could be fixed and maintained for longer time more effortlessly (1, 2). Skin electrodes, used for animal studies, are about two times larger than our electrode (3, 4), and whenever both of them are placed on the head, it increases the risk for bicephalic stimulation. Furthermore, epicranial montage allows an animal free movement in a cage. Therefore skin electrode montage in the animal head do not replicate the human tDCS application. It is noteworthy that, in the achieved results, a stable increase in the body weight gain and feed intake in Sham (over the stimulation period) indicates that the potential inhibitory action of active tDCS on energy homeostasis far overweights the role of stress secondary to surgical manipulation and sham application.

S1.b Detailed description of the stimulation procedure

Under general anesthesia (10 % ketamine and xylocaine; 10 and 3 mg/kg i.m.), the circular electrode (contact area of 7.1 mm^2), composed of a tubular plastic jacket, was placed over the right frontal cortex: 1.5 mm anterior to the coronal fissure and 1.5 mm right to the sagittal fissure, using a glass ionomer cement (Ketac Cem, ESPE Dental AG, Seefeld, Germany). The treatment target was the dorsolateral prefrontal cortex (DLPFC). The active electrode was remained fixed for the entire experiment. The counter electrode, composed of a conventional rubber plate electrode (10.5 cm^2, Vermed, Graphic Controls, Poland), was placed on the animal's back using a corset. An unipolar setting of the epicranial electrode was chosen to prevent the bypassing brain currents that would occur when two head electrodes are close to each other. Additionally, asymmetric electrode sizing was used to achieve the highest current density directly beneath the active electrode.

A jacket electrode was filled with 0.9 % NaCl before stimulation. Both the epicranial electrode and larger back electrode were connected to the DC stimulator that was controlled by a dedicated software. The procedure for tDCS was applied for conscious rats. Each animal was subjected to a 20-minute session either active (400 μA, 20 minutes on) or sham (40 μA, 30 s on next off) stimulation every other day for the whole time period covering two weeks (in total=7 sessions). The current intensity was automatically ramped for 10 seconds to avoid the abrupt on/off switching. For the stimulation, the rats were placed in separate plastic cages and carefully observed. Stimulation parameters (the current intensity, time and number of stimulation as well as the diameter of active and counter electrodes) were chosen based on the previous established safe and not harmful protocols (5, 6). No macroscopic abnormalities of the brain tissue as well as neither abnormal behavior nor discomfort were observed in the animals subjected to the stimulation procedure.

S1.c Study limitations

Nevertheless, despite the wealth of neurochemical information drawn, there are also several limitations in our study: all of the experiments were carried out for male rats; therefore, additional studies are warranted to determine if our findings can be extrapolated to female rats. Another important consideration is the equivalence of the stimulation procedure in animal vs human. Due to small surface of the animal head the size of cranial electrode has to be much smaller to replicate human montage. This is important as the stimulated area might be different in rats and in humans. On the other hand, as the size of the electrode decreases the current density increases limiting safety parameters used for animal studies. All in all, neither long-lasting effects of tDCS on energy homeostasis nor elemental and biochemical brain properties were examined, and, therefore, future studies are needed to determine the minimal and most effective currents as well as possible long-lasting effects of tDCS.

S1.d Detailed description of the data quantification procedure

The Lavenburg-Marquard iterative fitting procedure along with the Hypermet fitting model was applied to separate out the net peak areas of Kα lines. The background was modeled and subtracted by the fifth order polynomial. The quantification procedure was based on the external standard method (7–9)□. Notably, due to significant self-absorption of P, S, and Cl characteristic radiation in a sample, their quantification was carried out by the intermediate sample approach. For the elements ranging from K upwards the quantitative analysis was based upon a simplified thin sample quantification approach. The effective energy approximation was used to account for polychromacity of the X-ray source applied (10)□□.

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