

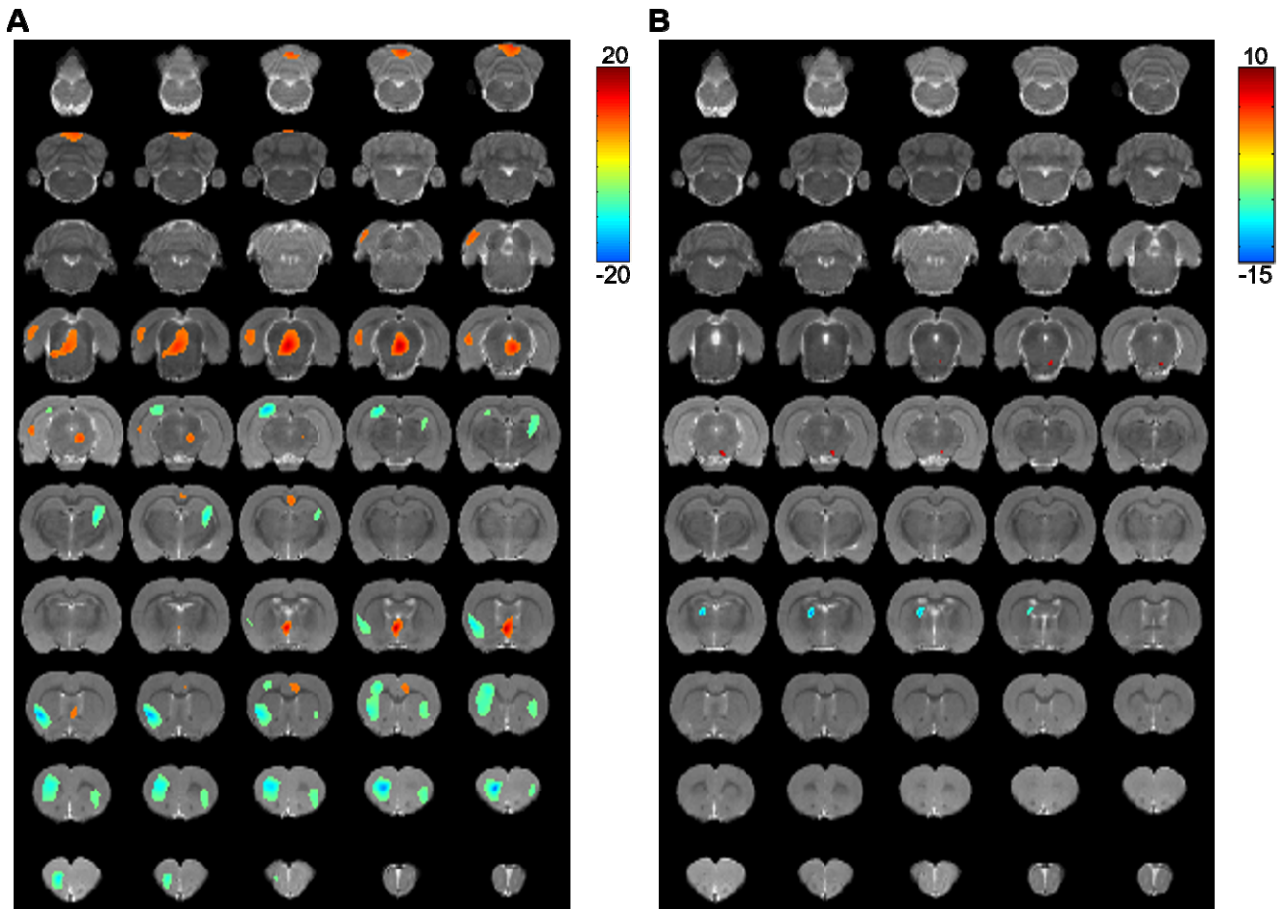
Supplemental materials

Optogenetic stimulation procedures

Rats were habituated to the Plexiglas box for three sessions before the PET scan. After placement in the Plexiglas box, blue (473 nm) laser stimulation, pulsed at 50 Hz, was applied through the fiber at 30 s intervals (30 s on/30 s off) for 30 min to induce panic-like behaviors. The laser power of the fiber was measured by using an optogenetic power meter (Sanwa, LP1) and limited below 10 mW. The intensity of the laser power directly illuminating the brain was converted into mW/mm^2 .

Immunostaining

One week after the stimulation PET scan, rats were given 50 Hz blue laser stimulation to induce panic-like behaviors again. In this study, we performed c-Fos and CaMKII α immunofluorescent staining at 90 minutes after optogenetic stimulation according to the previous study by Thanos PK, et al., in which they did the staining after the same time interval ^[1]. Free floating brain sections were incubated with primary antibodies: rabbit anti-CaMKII α (1:250; Abcam), rabbit anti-c-Fos (1:100; SY SY), followed by incubation with secondary antibodies conjugated with Alexa Flour 488 dye (1:500; Gibco). Immunofluorescence was imaged on a Zeiss microscope (Axio Observer 3).



Supplemental figure S1. *In vivo* ^{18}F -FDG PET images of the rat brain. Serial coronal sections in ChR2⁺ (A) and ChR2⁻ (B) rats demonstrated glucose metabolic changes after dPAG stimulation (n = 6 in each group; $P < 0.001$). Brain regions with significant differences were color-coded and superimposed on MRI template.

Supplemental video 1. Representative video of galloping and jumping performance induced by the optogenetic stimulation on the excitatory neurons of the dPAG.

Supplemental video 2. Representative video of rotation performance induced by the optogenetic stimulation on the excitatory neurons of the dPAG.

Reference

[1] Thanos PK, Robison L, Nestler EJ, Kim R, Michaelides M, Lobo MK and Volkow ND. Mapping brain metabolic connectivity in awake rats with muPET and optogenetic stimulation. *J Neurosci* 2013;

33(15): 6343-9