Figures and figure supplements

Instantaneous antidepressant effect of lateral habenula deep brain stimulation in rats studied with functional MRI

Gen Li *et al.*

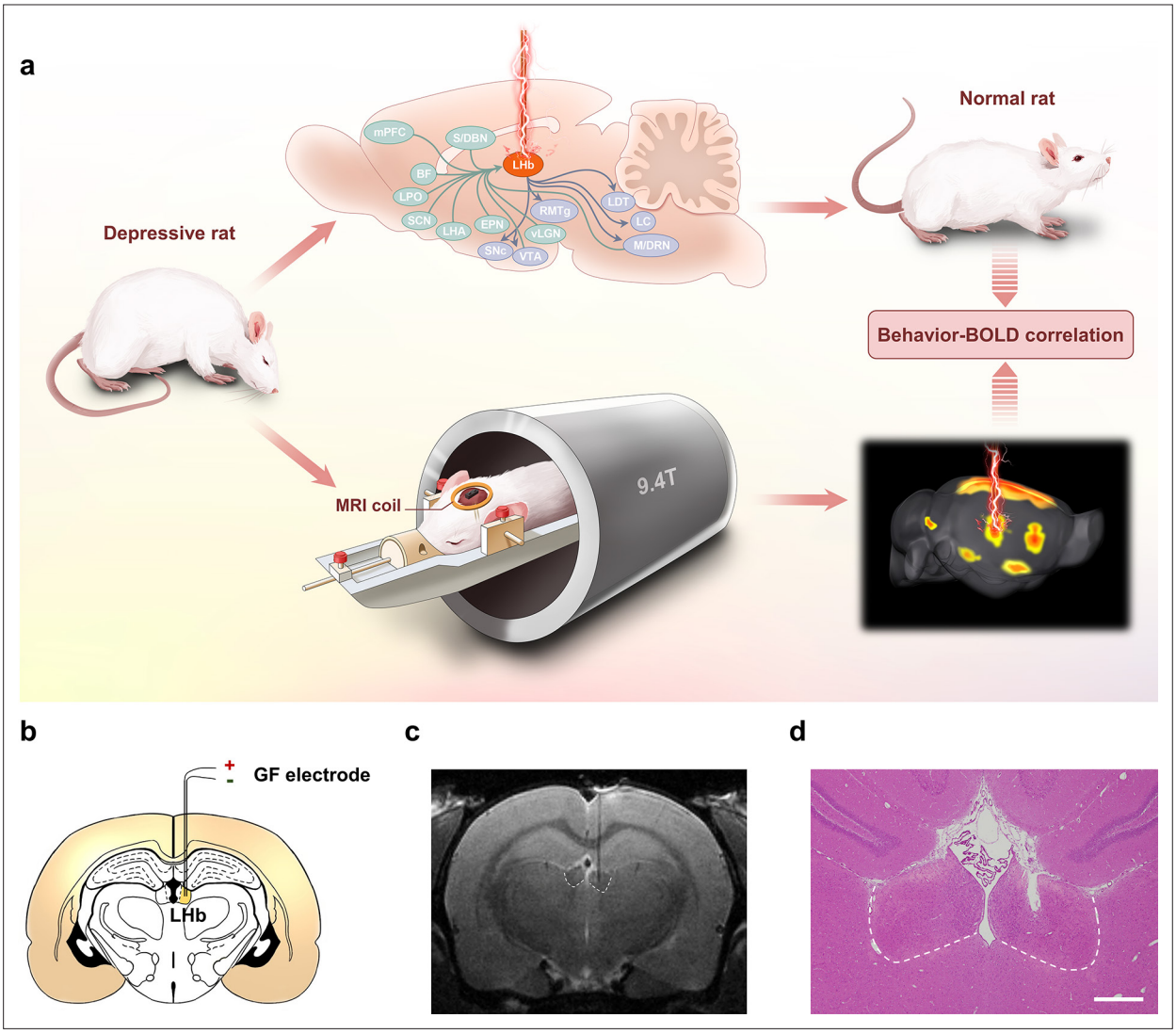

Figure 1. Lateral habenula (LHb)-deep brain stimulation (DBS) to rat models of depression studied by functional MRI (fMRI). (**a**) Schematics showing the immediate antidepressant effect of LHb-DBS and fMRI studies in rats. (**b**) A schematic brain section showing the placement of a graphene fiber (GF) bipolar electrode in the LHb of a rat. (**c**) A representative coronal section from the T2-weighted MRI scan of a rat brain with a GF bipolar microelectrode implanted in the LHb. (**d**) An image of a hematoxylin and eosin (H&E) stained brain section with the GF electrode implanted in the LHb from the same rat shown in c. Scale bar, 500 μm.

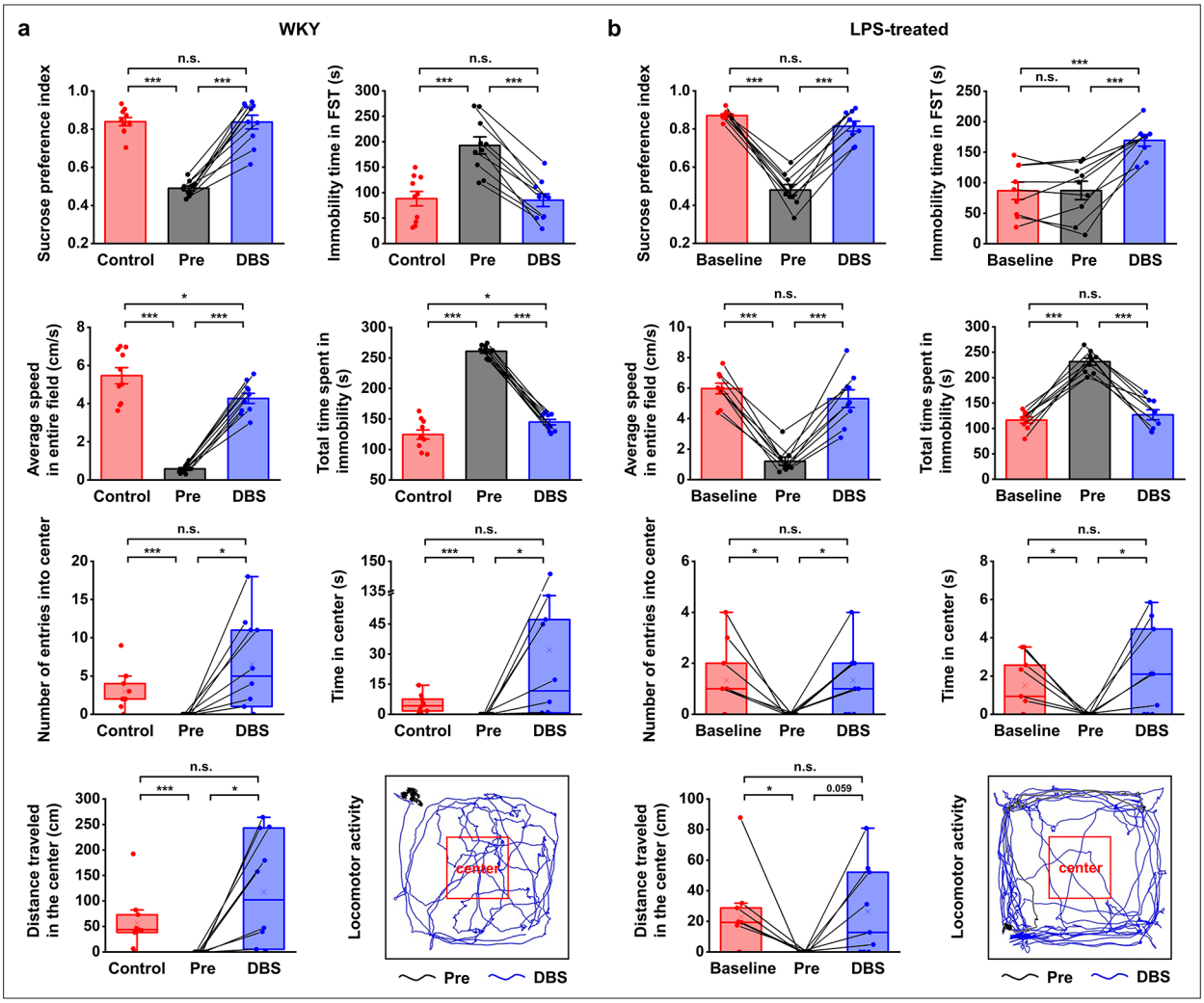

Figure 2. Lateral habenula (LHb)-deep brain stimulation (DBS) immediately alleviates depressive-like symptoms in Wistar Kyoto (WKY) and lipopolysaccharide (LPS)-treated rats (models of depression). Quantification of the behavioral responses of WKY (**a**) and LPS-treated (**b**) rats to LHb-DBS (DBS), including the sucrose preference index, the duration of immobility in the forced swim test (FST), average speed in entire field in the open field test (OFT), total duration of immobility in the OFT, number of entries into the center area of the OFT, time spent in the center area of the OFT, and distance traveled in the center area of the OFT. For WKY rats, the behavioral performances of normal Sprague–Dawley (SD) rats (Control) and WKY rats before DBS (Pre) were included for comparison. For LPS-treated rats, the behavioral performances of these rats before LPS injection (Baseline) and before DBS (Pre) were included for comparison. Data from the same animals are connected with black lines. Data are represented as the mean ± SEM (n=10 animals for WKY rats, n=9 animals for LPS-treated rats). The dots indicate data from each individual subject. To compare scores on the sucrose preference test (SPT), FST, and performance in total area of the OFT in WKY rats between the Pre and DBS groups, a two-tailed paired *t* test was used. A two-tailed unpaired *t* test was used for comparisons between the Control and Pre groups as well as between the Control and DBS groups. Because the data on variables from the center area of the OFT were not normally distributed in WKY rats, Wilcoxon's matched pairs signed-rank test was used to compare the Pre and DBS groups, and the Mann–Whitney *U* test was used to compare the Control and Pre groups as well as the Control and DBS groups. Data from the SPT, FST, and total area of the OFT in LPS-treated rats were compared by one-way repeated-measures ANOVA tests with Tukey post hoc tests. Because the data from the center area of the OFT of LPS-treated rats was nonnormally distributed, Friedman's tests were used for comparison. n.s.: not significant, *p<0.05, **p<0.01, and ***p<0.001. The last panels in (**a**) and (**b**) show examples of the locomotor activity of a WKY rat and an LPS-treated rat before (Pre, black line, 5 min) and during (DBS, blue line, 5 min) LHb-DBS with graphene fiber (GF) bipolar electrodes. The area outlined by the red square was defined as the center area, accounting for one-ninth of the total area size. See ***Figure 2—figure supplements 1 and 2*** for additional details. Results from detailed statistical tests are summarized in ***Supplementary file 1***.

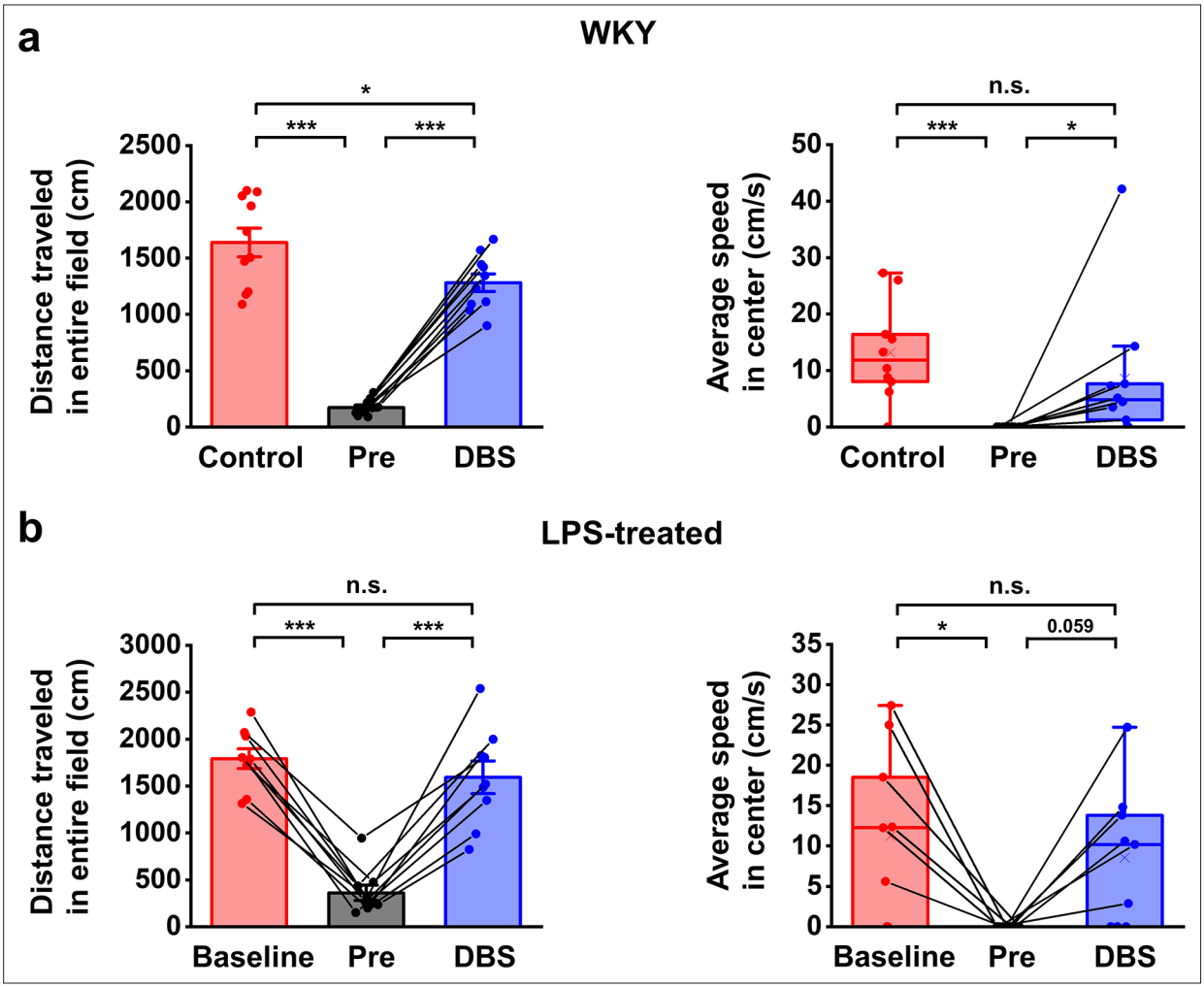

Figure 2—figure supplement 1. Additional behavioral response indicators in open field test (OFT) of Wistar Kyoto (WKY) and lipopolysaccharide (LPS)-treated rat models of depression.

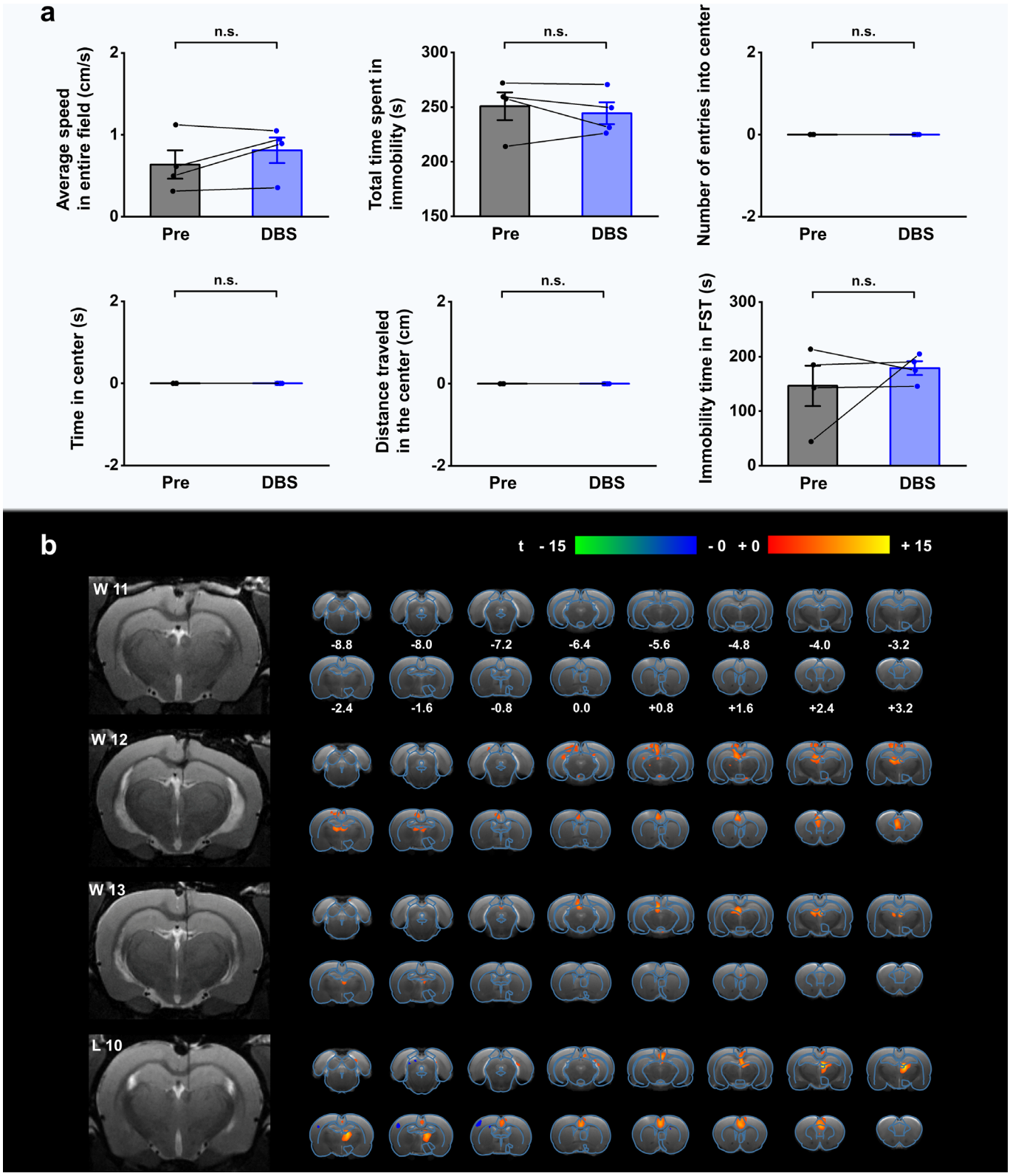

Figure 2—figure supplement 2. Behavior and functional MRI (fMRI) activation patterns of depressive rat models under deep brain stimulation (DBS) with stimulating graphene fiber (GF) electrodes implanted outside lateral habenula (LHb). Same stimulation parameters were used as those used in ***Figure 2***. The data was from four depressive rat models including three Wistar Kyoto (WKY) and one lipopolysaccharide (LPS)-treated rats, labeled as W11 to W13 and L10, respectively. (**a**) Behavioral response in open field test (OFT) and forced swim test (FST). The scattered dots indicate data from

Figure 2—figure supplement 2 continued on next page

Figure 2—figure supplement 2 continued

each individual subject. Data from the same animals are connected with black lines. Data represented as mean ± SEM. Same statistical analysis methods were used as those in ***Figure 2***. n.s: not significant. (**b**) Coronal brain sections from the T2-weighted MRI (left) and blood-oxygenation-level-dependent (BOLD) activation maps (right) from the rats implanted with graphene fiber (GF) microelectrodes outside the LHb. The T2 images were sectioned through the position of the implants. The electrodes were implanted to the dorsal third ventricle for W11–W12 rats and to the thalamus for L10 rat. The BOLD activation maps are overlaid onto averaged anatomical images. The numbers below slices denote the relative distance from bregma (in mm). The color bar denotes t-score values obtained by Generalized Linear Model (GLM) analyses, with a significance threshold of corrected $p<0.001$.

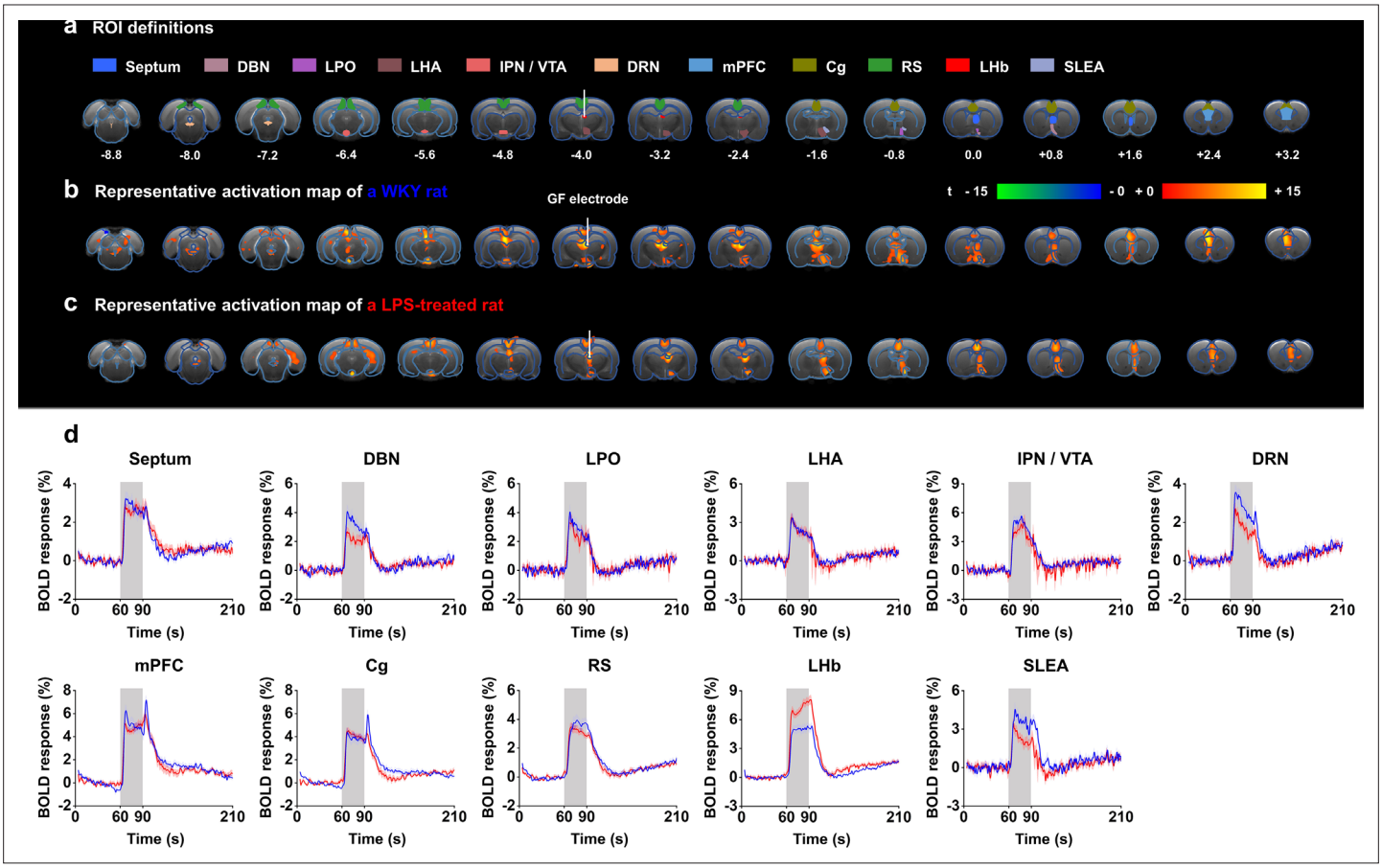

Figure 3. Functional blood-oxygenation-level-dependent (BOLD) activation evoked by lateral habenula (LHb)-deep brain stimulation (DBS) with graphene fiber (GF) stimulating electrodes in rat models of depression. The same sets of rats were used as those in the behavioral tests. (**a**) Definition of regions of interest (ROIs) for different brain regions. The numbers below slices denote the relative distance from bregma (in mm). The same set of distance numbers applies to the slices in **b and c**. (**b and c**) Representative BOLD maps of a Wistar Kyoto (WKY) rat (**b**) and a lipopolysaccharide (LPS)-treated rat (**c**). The BOLD activation maps are overlaid onto averaged anatomical images. The color bar denotes t-score values obtained by GLM analyses, with a significance threshold of corrected p<0.001. The vertical white lines in a–c indicate the graphene fiber (GF) bipolar electrode. (**d**) BOLD signal time series at anatomically defined ROIs evoked by LHb-DBS in WKY (blue) and LPS-treated (red) rats. The stimulation epoch is indicated by the gray-shaded band. The solid lines show the average signal, and the shaded regions represent the SEM, n=30 scans from 10 WKY, n=27 scans from 9 LPS-treated rats. DBN, diagonal band nucleus; LPO, lateral preoptic area; LHA, lateral hypothalamic area; IPN, interpeduncular nucleus; VTA, ventral tegmental area; DRN, dorsal raphe nucleus; mPFC, medial prefrontal cortex; Cg, cingulate cortex; RS, retrosplenial cortex; LHb, lateral habenula; SLEA, sublenticular extended amygdala. See ***Figure 3—figure supplements 1 and 2*** for additional details.

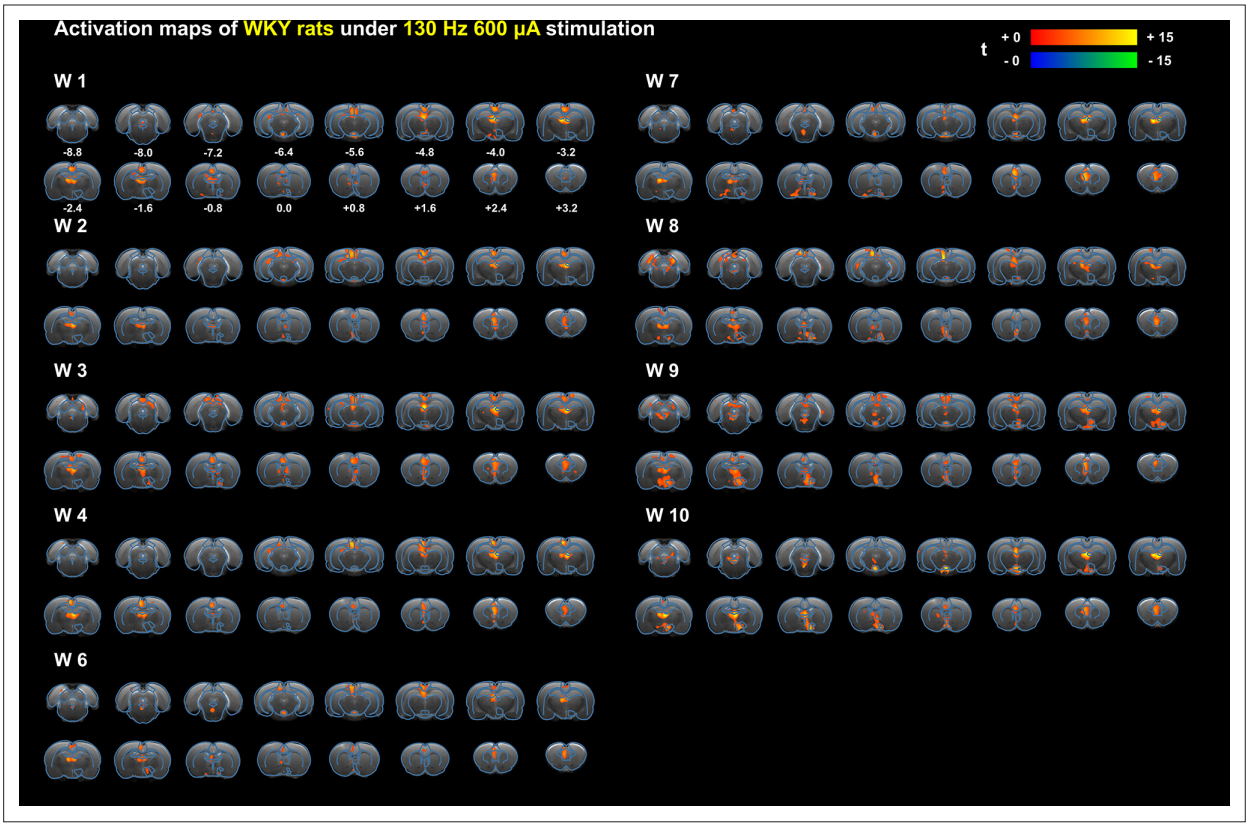

Figure 3—figure supplement 1. Individual blood-oxygenation-level-dependent (BOLD) activation maps evoked by lateral habenula (LHb)-deep brain stimulation (DBS) from the 10 Wistar Kyoto (WKY) depressive rats. The BOLD activation maps are overlaid onto averaged anatomical images. The numbers below slices denote the relative distance from bregma (in mm). The color bar denotes t-score values obtained by GLM analyses, with a significance threshold of corrected $p<0.001$.

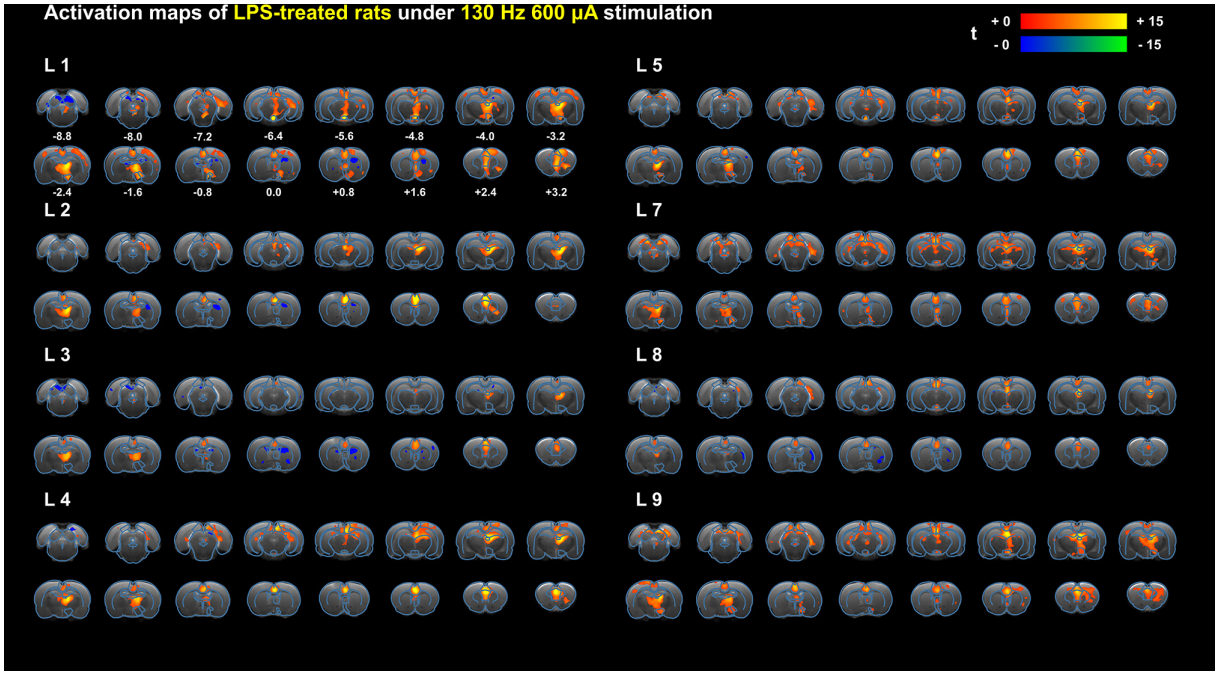

Figure 3—figure supplement 2. Individual blood-oxygenation-level-dependent (BOLD) activation maps evoked by lateral habenula (LHb)-deep brain stimulation (DBS) from the 9 lipopolysaccharide (LPS)-treated depressive rats. The BOLD activation maps are overlaid onto averaged anatomical images. The numbers below slices denote the relative distance from bregma (in mm). The color bar denotes t-score values obtained by GLM analyses, with a significance threshold of corrected $p<0.001$.

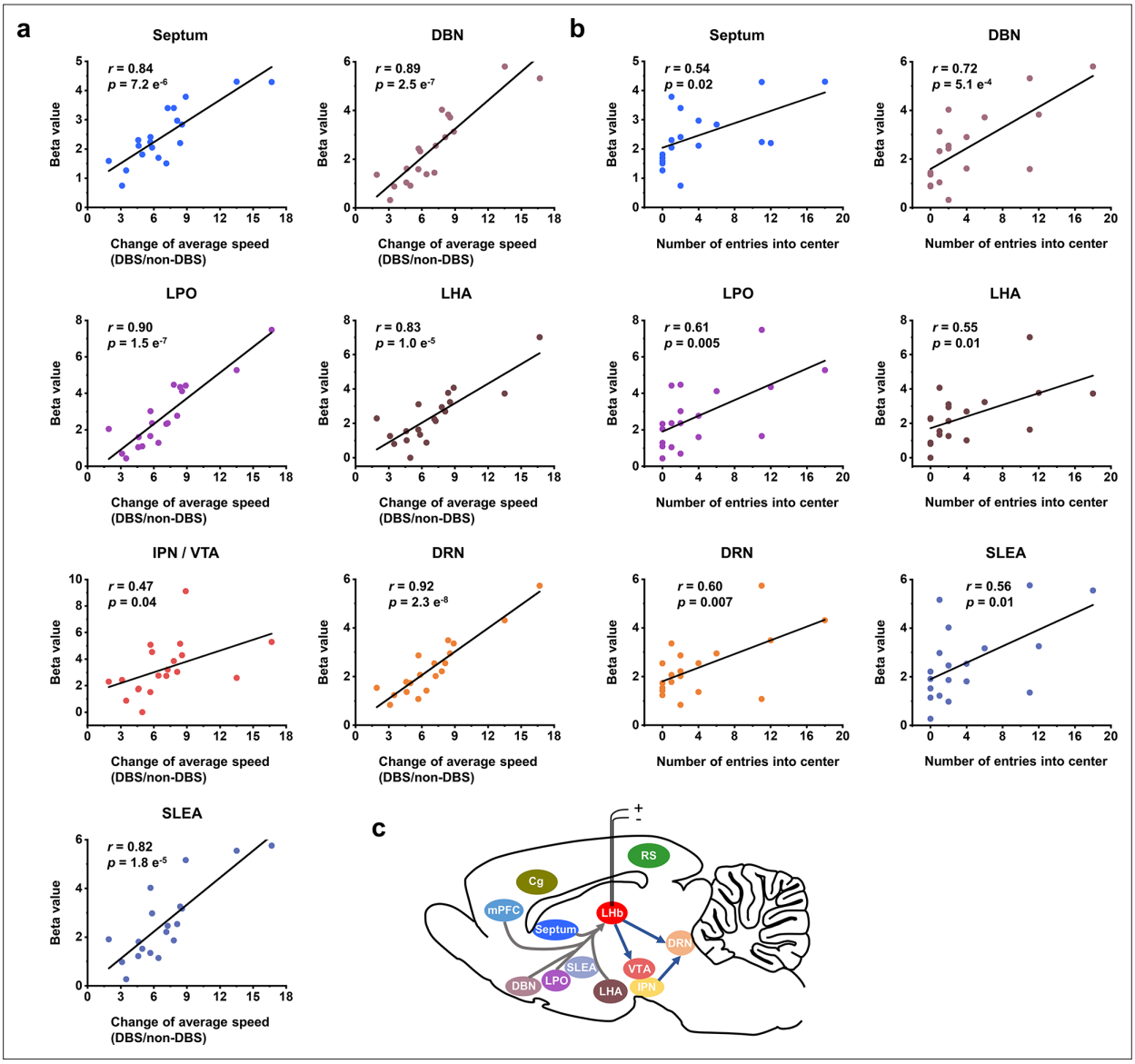

Figure 4. Correlation between functional MRI (fMRI) responses and behavioral improvement. Scatter plots of the regional beta values of blood-oxygenation-level-dependent (BOLD) responses and performance indicators on the open field test (OFT) from all the Wistar Kyoto (WKY) and lipopolysaccharide (LPS)-treated rats upon administration of lateral habenula (LHb)-deep brain stimulation (DBS), including (**a**) change of average speed, defined as the ratio of average speed with DBS (DBS) and without DBS (non-DBS), and (**b**) number of entries into the center. The Pearson's correlation coefficient *r* between behavioral improvement in the OFT and beta values of BOLD responses across rats were calculated for each regions of interest (ROI). (**c**) A schematic showing the placement of the stimulating electrode in LHb and summary of the activated brain areas. Arrows to and from LHb indicate afferent and efferent connections, respectively. See ***Figure 4—figure supplement 1*** and ***Figure 4—source data 1*** for additional details.

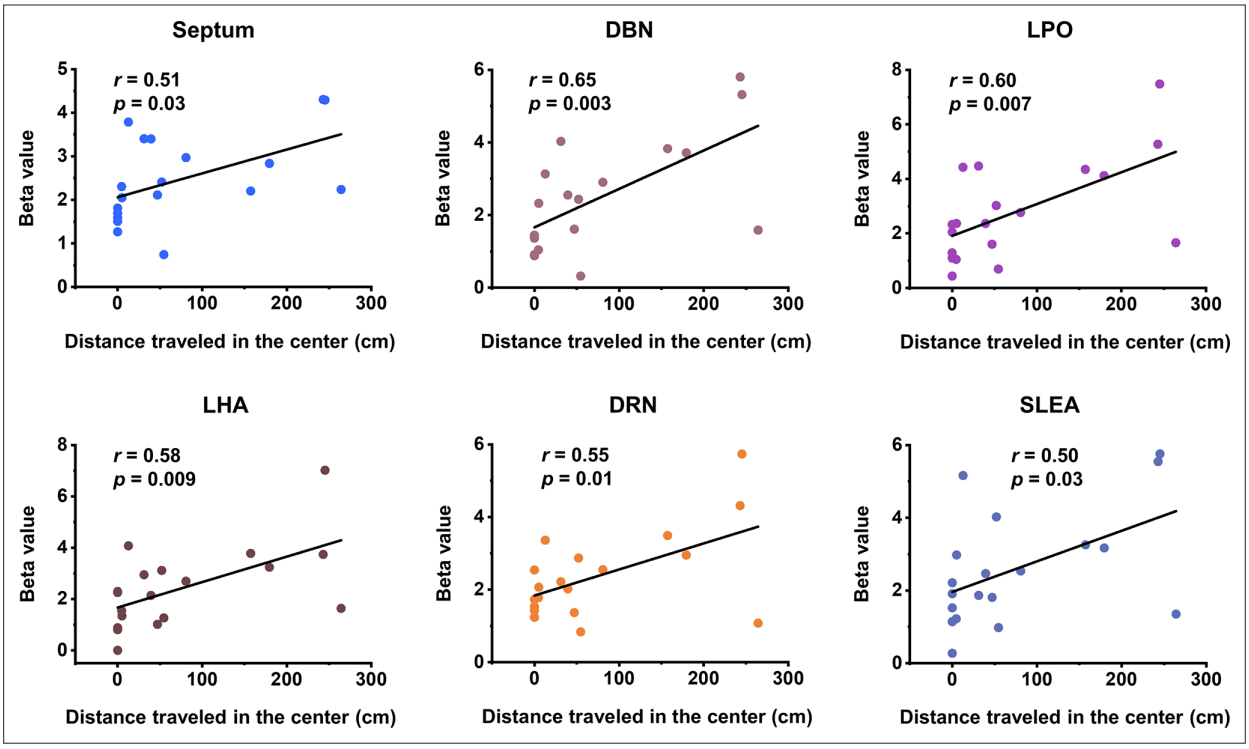

Figure 4—figure supplement 1. Correlation between functional MRI (fMRI) responses and distance traveled in center. Scatter plots of the regional beta values of blood-oxygenation-level-dependent (BOLD) responses and distance traveled in the center area of open field test (OFT) from all the Wistar Kyoto (WKY) and lipopolysaccharide (LPS)-treated depressive rats upon administration of lateral habenula (LHb)-deep brain stimulation (DBS). The Pearson's correlation coefficient *r* between distance traveled in center area of OFT and beta values of BOLD responses across rats was calculated for each regions of interest (ROI).

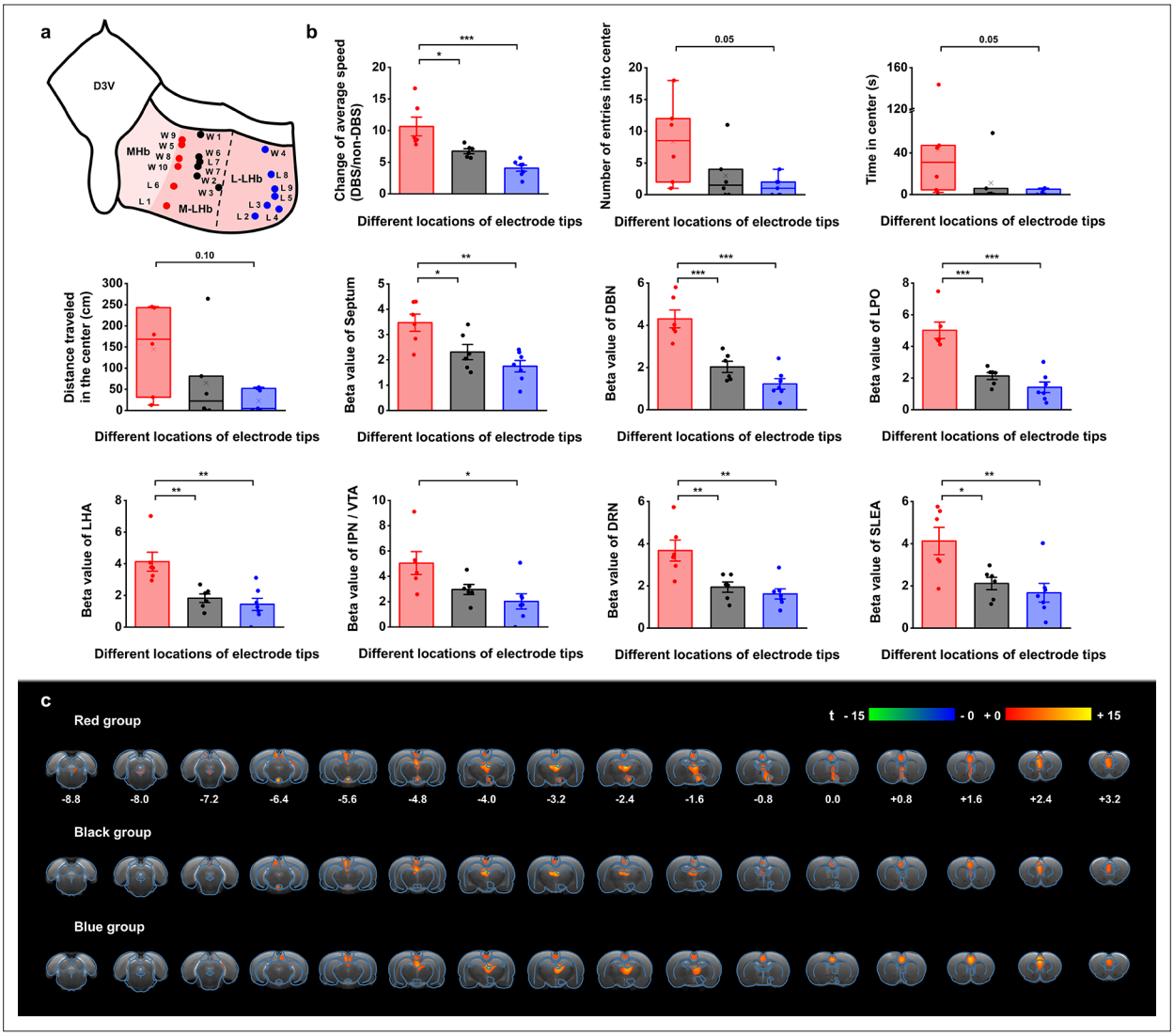

Figure 5. Stimulation position dependence of the antidepressant efficacy and blood-oxygenation-level-dependent (BOLD) responses. (**a**) A schematic diagram showing the positions of the graphene fiber (GF) electrode tips within the habenula from all the Wistar Kyoto (WKY) and lipopolysaccharide (LPS)-treated depressive rats. Each dot represents the electrode tip position in a depressive rat. Labels with "W" indicate WKY rats and those with "L" indicate LPS-treated rats. According to the positions in the medial-lateral direction, the depressive rats were divided into three groups, with red dots corresponding to the group with stimulation positions more medial, black dots corresponding to the group with stimulation positions in the middle, and blue dots corresponding to the group with stimulation positions more lateral in lateral habenula (LHb). The electrode tip position was delineated from the T2-weighted MRI images of each rat. The dashed line indicates the boundary between the medial LHb (M-LHb) and the lateral LHb (L-LHb). (**b**) Performance indicators in the open field test (OFT) and beta values of BOLD responses in several regions of interest (ROIs) from the three groups of rats with different stimulation locations. The change of average speed is defined as the ratio of average speed with deep brain stimulation (DBS) and without DBS (non-DBS). The number of entries into the center, time spent in the center and distance traveled in the center area of the OFT correspond to the behavioral performance with DBS. *p<0.05, **p<0.01, ***p<0.001, one-way ANOVA tests with Tukey post hoc analysis for change of average speed, Kruskall–Wallis ANOVA for indicators in the center of the OFT, one-way ANOVA tests with Tukey post hoc analysis for beta values of BOLD responses. Red, black, and blue histogram bars represent the three groups marked with red, black, and blue dots in (**a**). The dots represent the data of each individual rat. The error bars indicate the SEM. (**c**) BOLD activation maps averaged from the three groups of rats with stimulation positions marked with red, black, and blue dots in (**a**) respectively. The numbers below slices denote the relative distance from bregma (in mm). The color bar denotes t-score values obtained by GLM analyses, with a significance threshold of corrected p<0.001. See ***Figure 5—figure supplements 1 and 2*** for additional details. Results from detailed statistical tests are summarized in ***Supplementary file 1***.

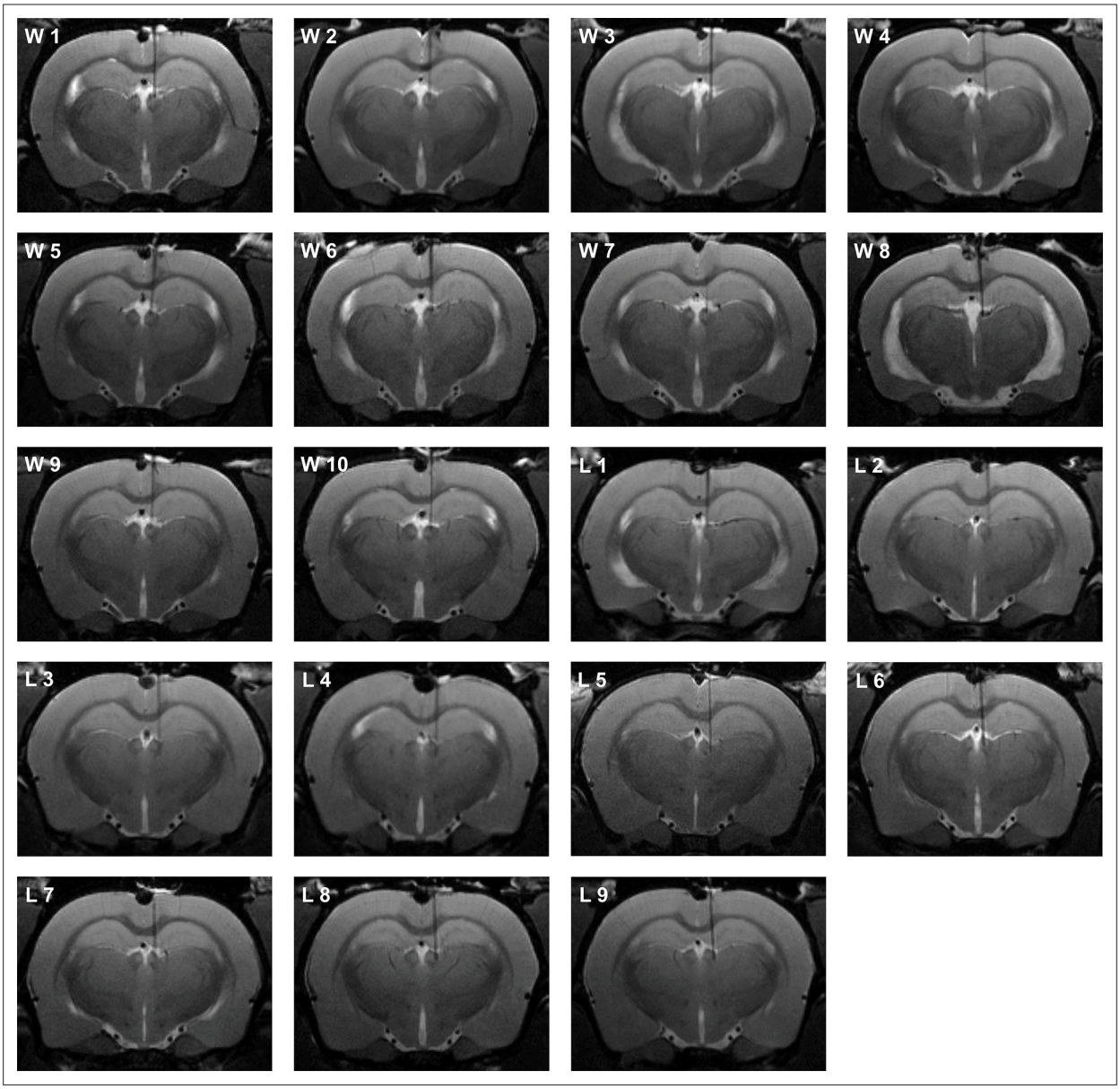

Figure 5—figure supplement 1. Individual coronal T2-weighted MRI images from all the Wistar Kyoto (WKY) and lipopolysaccharide (LPS)-treated depressive rats used in behavioral tests and functional MRI (fMRI) studies. The images show the implantation positions of the electrodes and the electrode tip locations in the lateral habenula (LHb). W1–W10 represents WKY depressive rats 1–10, L1–L9 represents LPS-treated depressive rats 1–9.

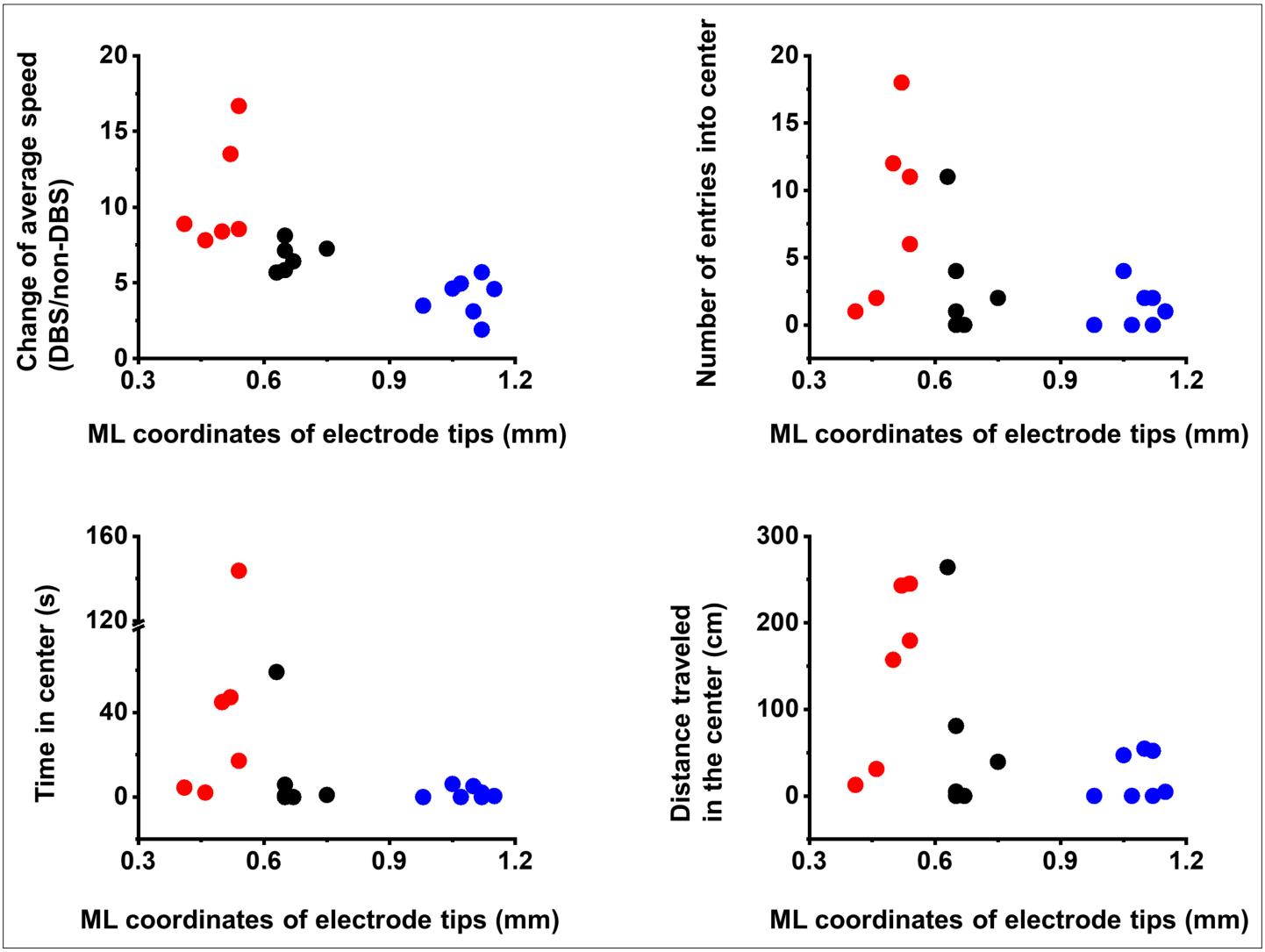

Figure 5—figure supplement 2. Color-coded scatter plots of medial-lateral (ML) positions of electrode tips and different behavioral response indicators. Red, black, and blue dots correspond to the three groups marked with red, black, and blue dots in ***Figure 5***.