Neuron, Volume *103*

Supplemental Information

Temporally and Spatially Distinct Thirst Satiation Signals

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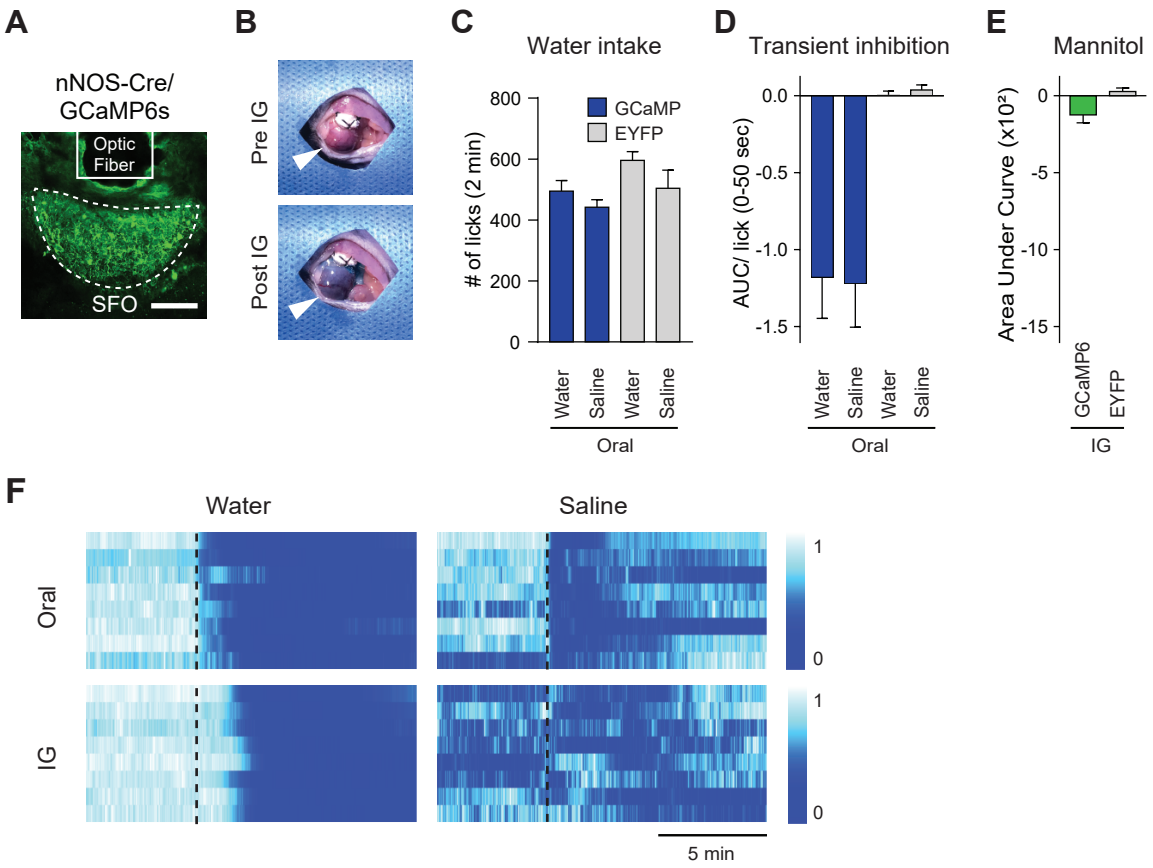

Figure S1. Characterization of SFO^{nNOS} neurons after oral or IG administration of fluid, related to Figure 1. A, A representative image of GCaMP6s expression and optic fiber placement in the SFO. **B,** Confirmation of IG surgery. Blue dye was infused into the stomach to ensure successful IG surgery. Before (top) and after (bottom) IG infusion from the same animal are shown. **C,** The number of licks for Figures 1D and 1E. Liquid intake for 2 min was quantified while recording calcium dynamics of SFO^{nNOS} neurons. **D,** Activity change per lick for SFO^{nNOS} neurons (n = 8 mice for GCaMP6s, n = 6 mice for EYFP). All data were reanalyzed from Figure 1E. **E,** Hypoosmotic stimulus is required for persistent inhibition of SFO^{nNOS} neurons. IG infusion of isotonic mannitol (308 mM) had no effect on the activity of SFO^{nNOS} neurons (n = 8 mice for GCaMP6s, n = 4 mice for EYFP). **F,** Normalized fluorescence changes of SFO^{nNOS} neurons from individual mice during oral ad lib drinking or IG infusion. Data presented as mean ± s.e.m. Scale bar, 100 μm.

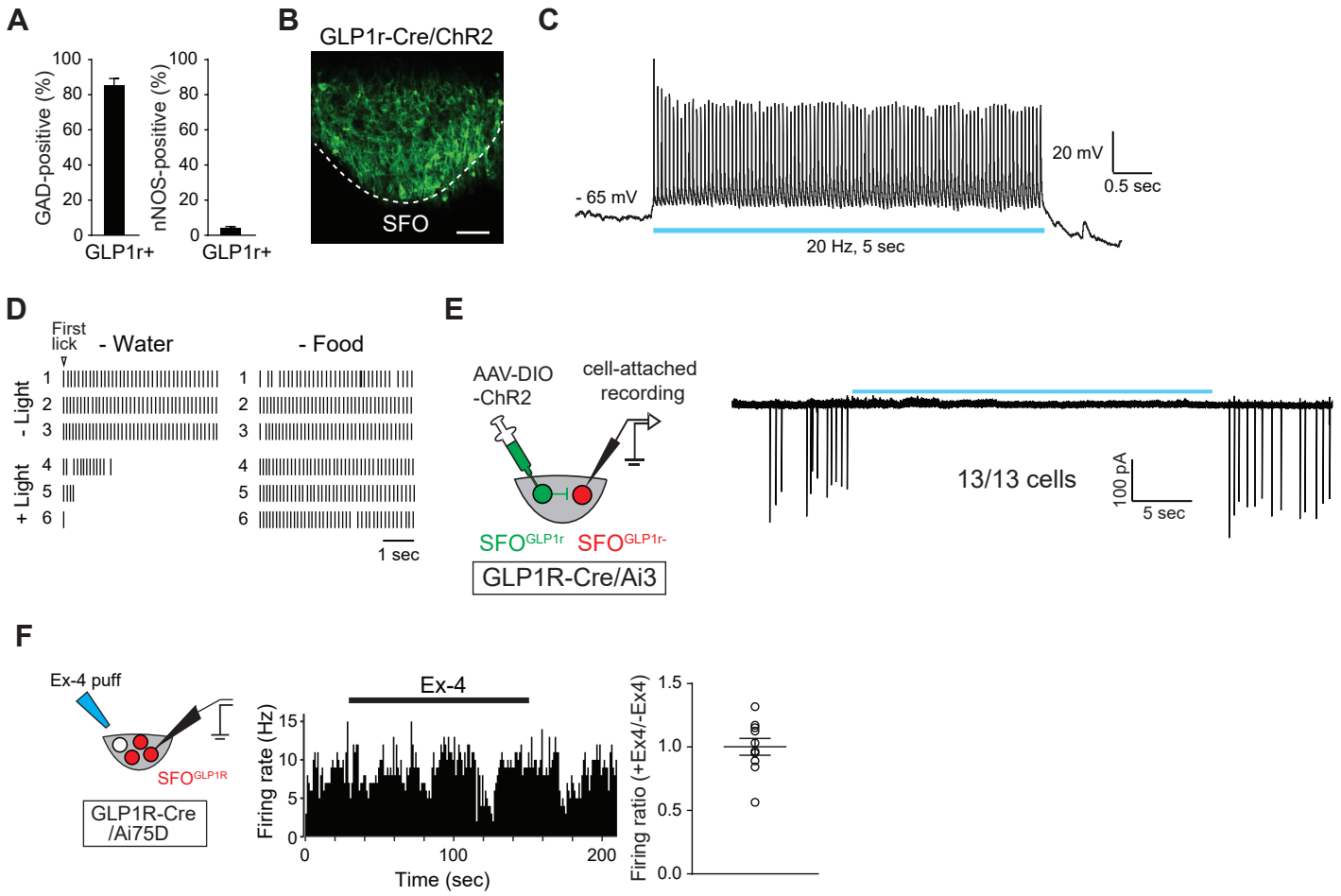

Figure S2. Gain-of-function of SFO^{GLP1r} neurons, related to Figure 2. A, Cell count for Figure 2A. Quantification of the percentage of GAD- or nNOS-positive neurons that coexpressed GLP1r. **B,** A representative image of ChR2-expressing SFO^{GLP1r} neurons (1 out of 5 mice). **C,** Electrophysiological recording in fresh brain slices. Illumination of 475 nm light at 20 Hz activates SFO^{GLP1r} neurons infected with AAV-DIO-ChR2-EYFP (8 out of 8 neurons from 2 mice). **D,** Photostimulation of SFO^{GLP1r} neurons inhibited water intake under water-restricted conditions. However, the same stimulation did not affect Ensure intake under food-restricted conditions. Each black bar indicates a lick event. Representative raster plots from 1 out of 5 mice are shown. **E,** The SFO^{GLP1r} → SFO$^{non\text{-}GLP1r}$ monosynaptic connection. Spontaneous firing of all GLP1r-negative neurons tested (13/13 cells) were suppressed by optogenetic activation of SFO^{GLP1r} neurons under cell-attached recording conditions. **F,** Application of an agonist for GLP1r did not induce firing. Electrophysiological recording in SFO^{GLP1r} neurons upon brief application of Exendin-4, a GLP1r-agonist (20 μM), did not affect the firing rate (10/10 cells). Data presented as mean ± s.e.m. Scale bar, 50 μm.

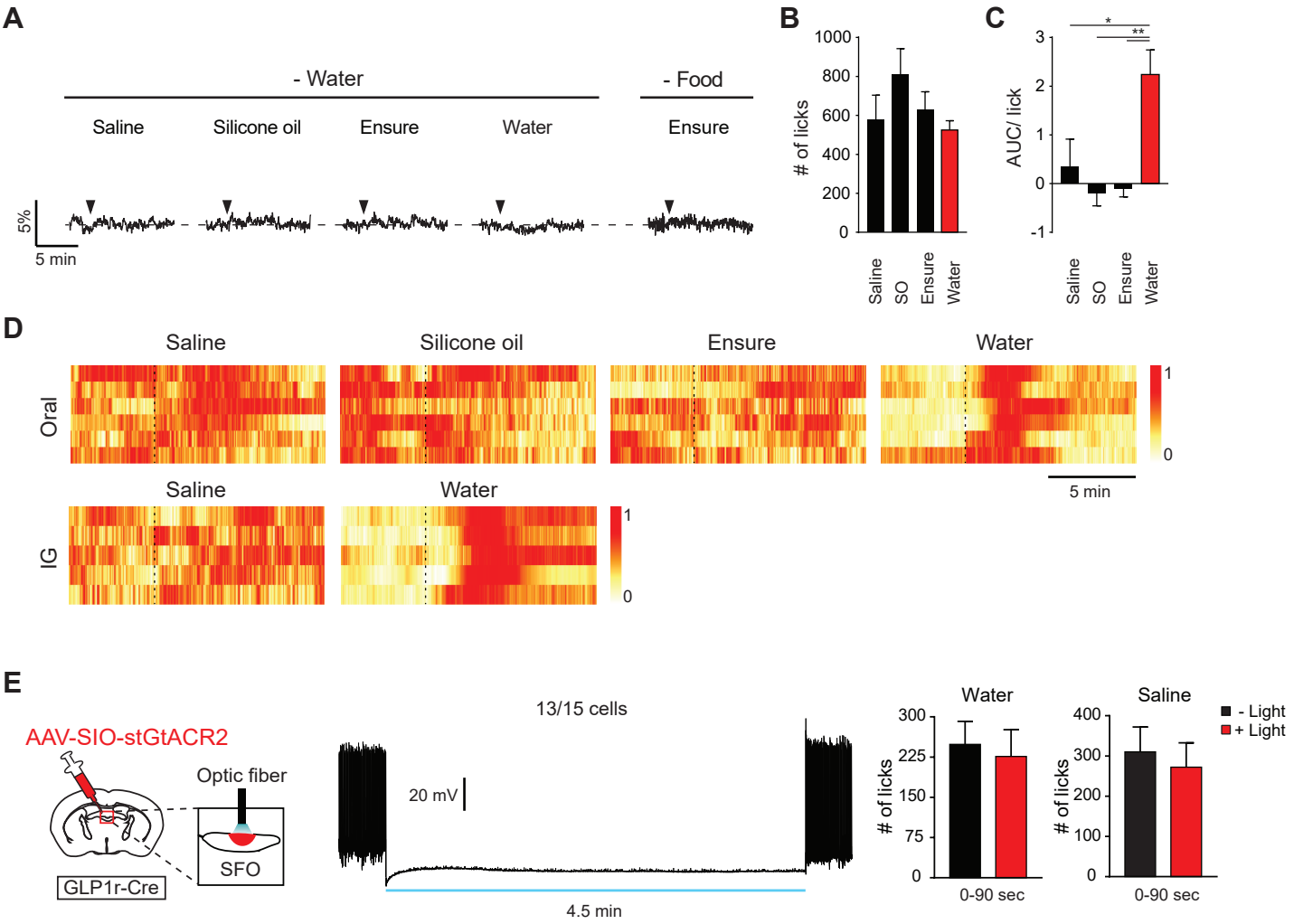

Figure S3. SFO^{GLP1r} neurons are activated by hypoosmotic stimuli in the gut, related to Figure 3. A, Control experiments for Figure 3A. Representative responses of SFO^{GLP1r} neurons infected with AAV-DIO-EYFP to different fluids. **B,** The amount of liquid intake for the first 5 min was quantified for Figure 3B. **C,** Fluorescence change per lick for SFO^{GLP1r} neurons (n = 6 mice for GCaMP6s). All data were reanalyzed from Figure 3B. **D,** Normalized fluorescence change of SFO^{GLP1r} neurons from individual mice during oral ad lib drinking or IG infusion. **E,** A diagram for optogenetic inhibition of SFO^{GLP1r} neurons (left). Electrophysiological recording in brain slices. Illumination of 475 nm light inhibits action potential firing in SFO^{GLP1r} neurons infected with AAV-SIO-stGtACR2 (middle, 13 out of 15 neurons). Number of licks before optogenetic inhibition of SFO^{GLP1r} neurons (right, 0-90 sec, Figure 3D). *P<0.05 and **P<0.01 by one-way repeated measures ANOVA (Dunnett's multiple comparisons). Data presented as mean ± s.e.m.

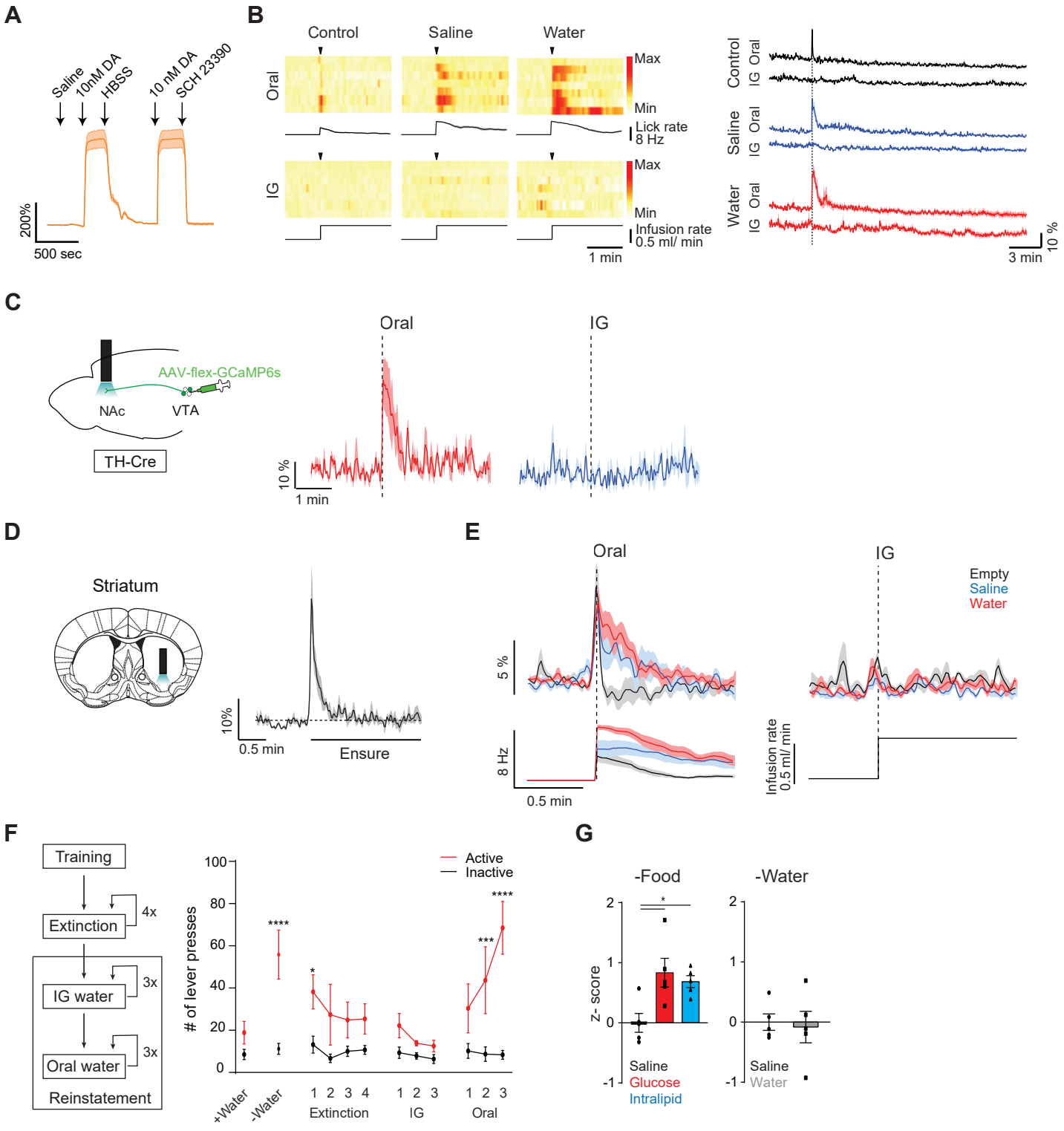

Figure S4. dLight fluorescence change upon fluid administration, related to Figure 4. A, dLight is sensitive and photostable in response to low concentration of dopamine during continuous imaging (~30mins, n = 5 cells). dLight did not respond to saline but showed increased fluorescence to 10 nM dopamine before the signal was abolished by DRD1 specific antagonist SCH 23390 (right, 5nM). **B,** dLight fluorescence changes from individual mice are shown during oral ad lib intake and IG infusion (left, n = 7 mice). For oral access, animals were given an empty bottle (control), isotonic saline, or water. For IG infusion, air, isotonic saline, or water was infused at a speed of 0.5 mL/min for 2 min. Mean traces of dLight fluorescent signals during oral ad-lib drinking or IG infusion (right, n = 7 mice). **C,** A diagram of GCaMP6s recording from the projections of VTATH neurons in the NAc. Water was given either orally or via IG infusion. Spontaneous drinking induced robust activation in the NAc when the animal drank water (middle) compared to IG infusion of water (right, n = 3 mice). **D,** A diagram of optical recording of dLight fluorescence in the dorsal striatum. DA release is induced by rewarding stimulus (Ensure, n = 6 mice). **E,** Peristimulus time histogram of dLight responses to empty, saline, and water. Similar to the NAc, only spontaneous drinking induced DA release in the dorsal striatum (n = 6 mice). **F,** A training paradigm for operant conditioning. Mice underwent training and extinction sessions, followed by reinstatement sessions. In reinstatement sessions, animals were first subjected to IG sessions followed by oral sessions. The data for IG and oral reinstatement sessions are from Figure 4H (n=6 mice). **G,** Quantified data of dLight responses to intragastric infusion of nutrients or water (n = 5 mice). Isotonic saline, 45% glucose or 20% Intralipid was infused into food-deprived mice. Saline or water was infused into water-deprived mice. Post infusion DA release was observed in food-deprived animals (left), but not in water-deprived animals (middle). *P<0.05, ***P<0.001 and ****P<0.0001 by one-way repeated measures ANOVA (Dunnett's multiple comparisons) or two-way repeated measures ANOVA (Bonferroni's multiple comparisons). Data presented as mean ± s.e.m.