Supporting Information

Kuperman et al. 10.1073/pnas.1003969107

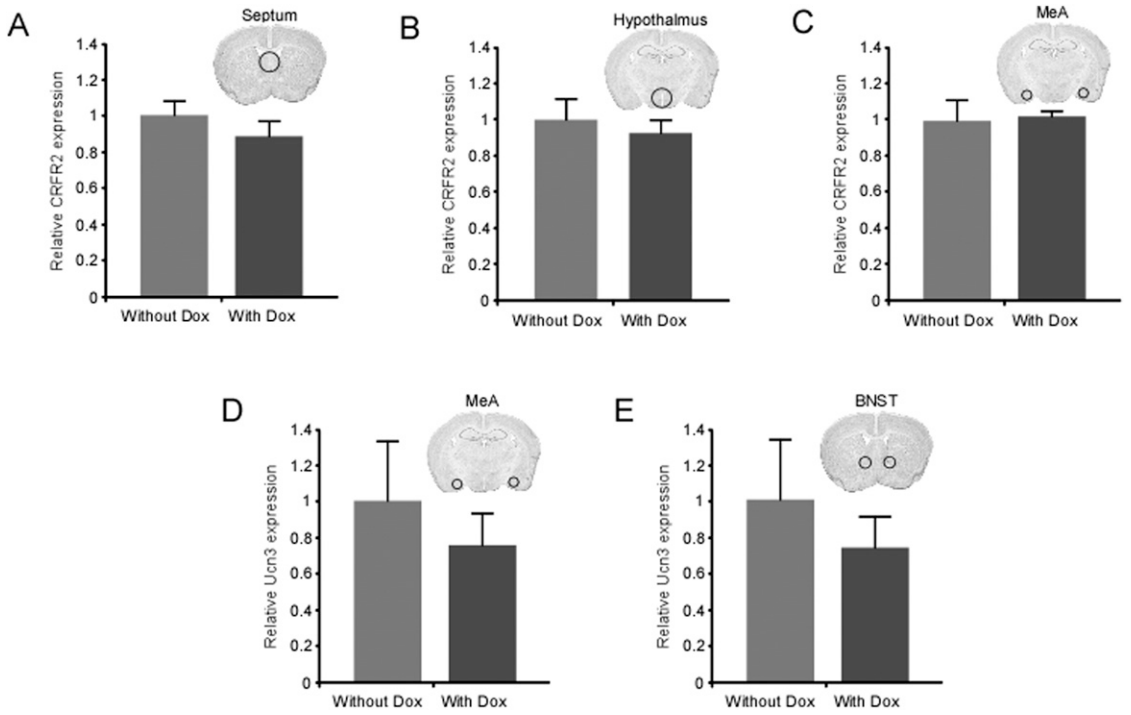

Fig. S1. Corticotropin-releasing factor receptor type 2 (CRFR2) and Urocortin-3 (Ucn3) mRNA levels in specific brain regions of the rostral perifornical area (rPFA)-Ucn3–overexpressing mice. CRFR2 mRNA levels in the septum (*A*), the hypothalamus (*B*), and the medial amygdala (MeA) (*C*) of rPFA-Ucn3–overexpressing mice were not different from the control group. Ucn3 mRNA levels in the MeA (*D*) and the bed nucleus of the stria terminalis (BNST) (*E*) also were unchanged. $n = 7–9$ mice in each group. CRFR2 and Ucn3 mRNA levels were quantified relative to hypoxanthine–guanine phosphoribosyltransferase-1 (HPRT1) mRNA. (*Inset*) The punch location (modified from ref. 1).

1. Paxinos G, Franklin KBJ (2001) *The Mouse Brain in Stereotaxic Coordinates* (Academic, San Diego).

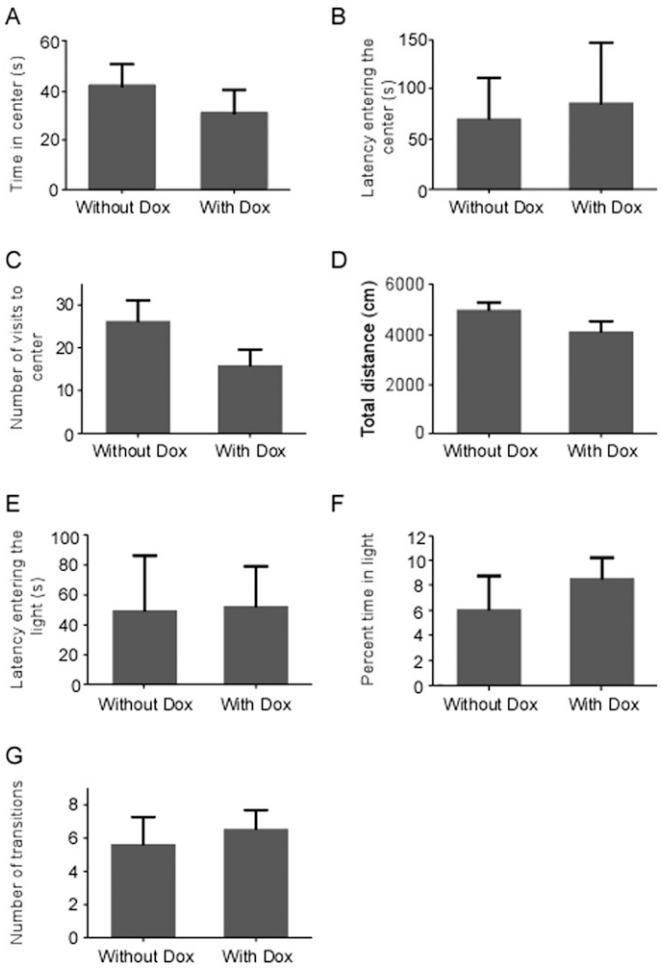

Fig. S2. Doxycycline (Dox) treatment in wild-type mice does not affect anxiety-like behavior. (*A*–*D*) Anxiety-like behavior of Dox-treated wild-type animals as measured by the open-field test. No significant differences were found in time spent in the center of the arena (*A*), latency entering the center (*B*), visits to the center (*C*), and distance traveled during the test (*D*). (*E*–*G*) Anxiety-like behavior as measured by the dark–light transfer test. No significant differences were found in the latency entering the light compartment (*E*), percentage of time spent in the light compartment (*F*), and number of transitions into the light compartment (*G*). Values are expressed as the mean ± SEM (n = 8 mice per group).

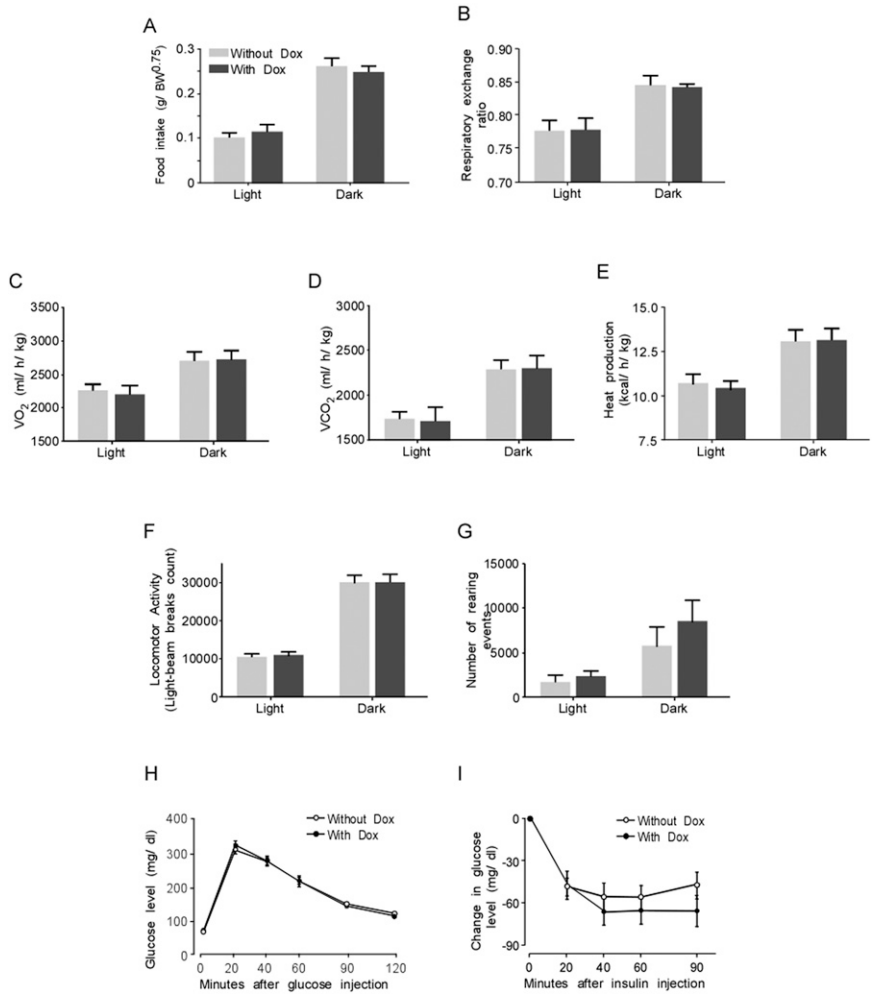

Fig. S3. Dox treatment in wild-type mice does not affect metabolic parameters. (*A*) Food intake, (*B*) respiratory exchange ratio (RER), (*C*) oxygen consumption (VO_2), (*D*) carbon dioxide production (VCO_2), (*E*) heat production, (*F*) locomotor activity, and (*G*) rearing events were all measured continuously and simultaneously and were not significantly different between the two experimental groups. (*H and I*) Glucose-tolerance test (*H*) and insulin-tolerance test (*I*) profiles determined in mice treated with or without Dox, showed no significant differences between the two experimental groups. Mice were injected i.p. with 2 g glucose/kg body weight (BW) (*H*) or with 0.75 U insulin/kg BW (*I*), and plasma glucose was measured at the indicated time points. Values are expressed as the mean ± SEM. No significant differences were observed (n = 8 mice per group).

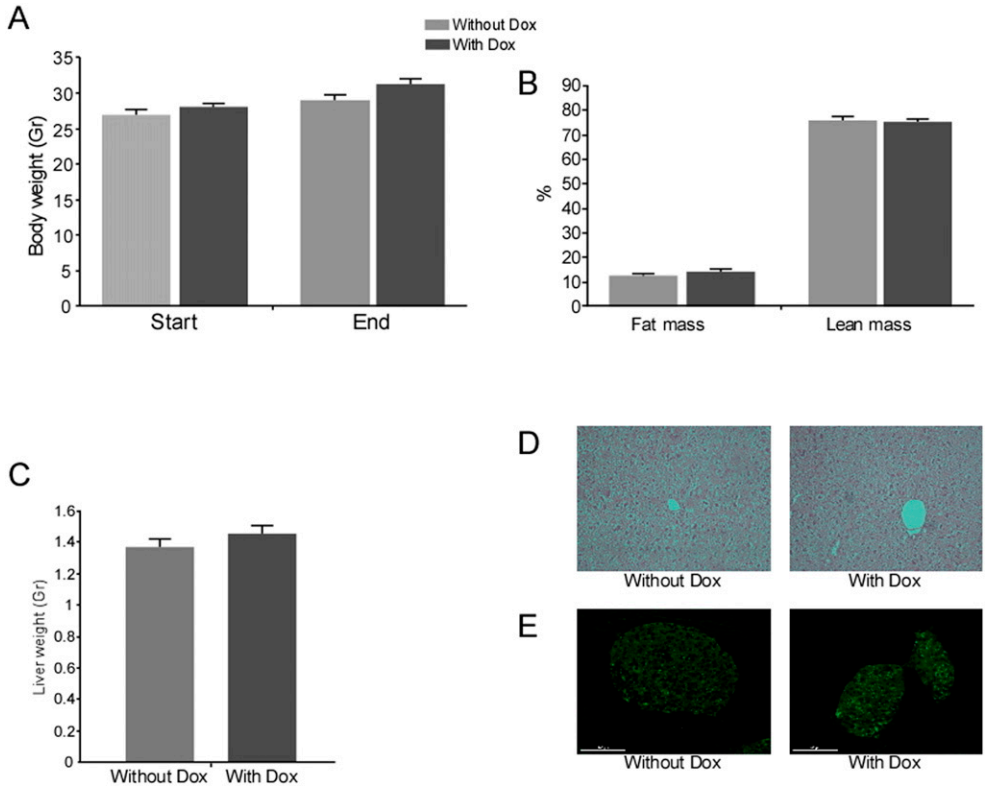

Fig. S4. BW and composition of the rPFA-Ucn3–overexpressing mice. (*A*) Mice overexpressing Ucn3 for 20 days tended to gain more weight ($P = 0.071$). (*B*) Body composition analysis shows no significant differences in fat or lean mass. (*C*) Liver mass and (*D*) hepatic H&E staining show no overt differences in liver histology and no sign of fatty liver. (*E*) Pancreatic staining for insulin shows comparable β-islet morphology. Values are expressed as the mean ± SEM ($n = 5$–9 mice per group).