Supporting Information

Wu et al. 10.1073/pnas.1314137110

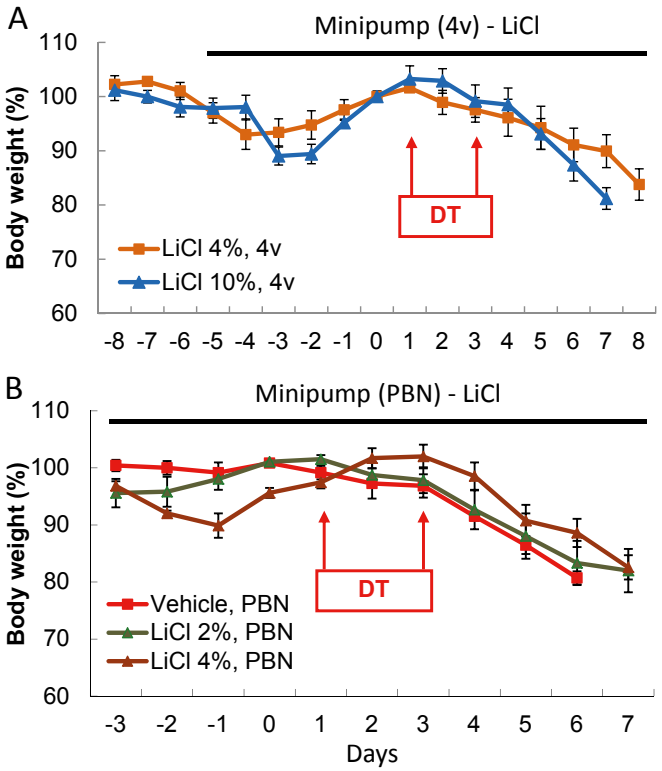

Fig. S1. Central administration of LiCl fails to rescue aphagia in mice in which agouti-related protein (AgRP) neurons have been ablated. (*A*) Body weight of $Agrp^{DTR/+}$ mice after chronic infusion of LiCl into the fourth ventricle (4v) ($n = 8$ per group). Diphtheria toxin (DT)-mediated ablation of AgRP neurons was initiated 6 d after implantation of minipumps loaded with 4% (wt/vol) or 10% (wt/vol) LiCl. (*B*) Body weight of $Agrp^{DTR/+}$ mice after chronic infusion of LiCl bilaterally into the parabrachial nucleus (PBN) ($n = 8$ per group). DT-mediated ablation of AgRP neurons was initiated 4 d after implantation of minipumps loaded with vehicle, 2% (wt/vol) LiCl, or 4% (wt/vol) LiCl. Results are shown as means ± SEM.

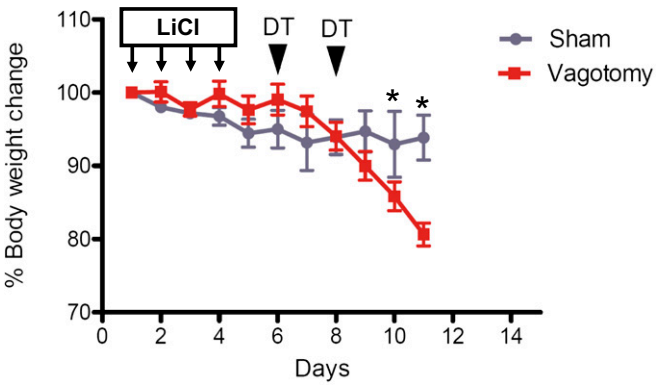

Fig. S2. Effects of systemic administration of LiCl on aphagia are abolished by gastric vagotomy in AgRP neuron-ablated mice. The graph shows the body weight of $Agrp^{DTR/+}$ mice treated with vagotomy or sham surgery. After recovery, LiCl (0.25 M, 10 $\mu L \cdot g^{-1} \cdot d^{-1}$) was administered i.p. for 5 d before DT-mediated ablation of AgRP neurons (arrowheads). *$P < 0.05$, ANOVA; $n = 8$ mice per group. Results are shown as means ± SEM.

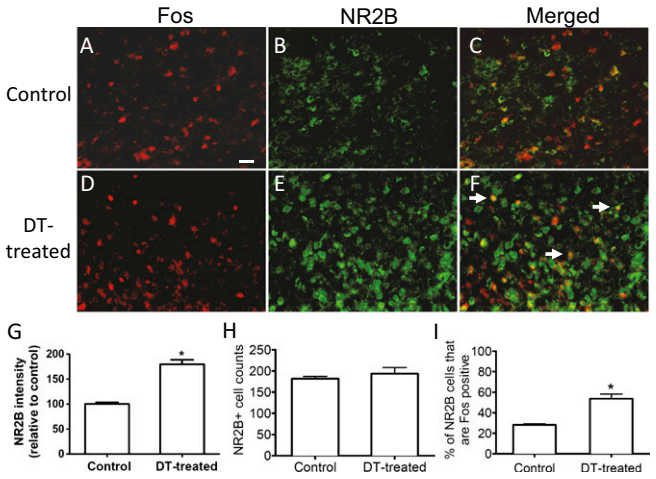

Fig. S3. Enhanced colocalization of Fos and the NMDA subunit NR2B in the PBN after ablation of AgRP neurons. Representative images show immunostaining results of anti-Fos (*A* and *D*), anti-NR2B (*B* and *E*), and merged images (*C* and *F*) in the dorsal PBN area of $Agrp^{DTR/+}$ mice 5 d after treatment with saline (control) or DT. Arrows indicate neurons coexpressing Fos and NR2B. (Scale bar in *A*: 30 μm for *A*–*E*.) (*G*–*I*) Quantitative analysis if the relative fluorescence intensity of $NR2B^{+}$ cells (*G*), the number of $NR2B^{+}$ cells (*H*), and the percentage of NR2B cells that are Fos^{+} (*I*). $^{*}P < 0.05$, ANOVA; $n = 8$ sections per group. Results are shown as means ± SEM.

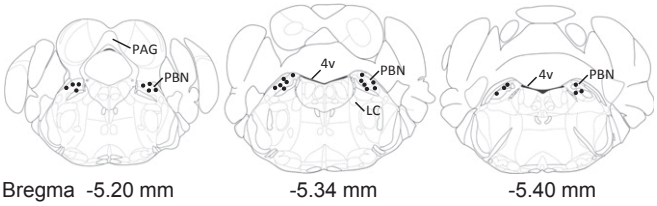

Fig. S4. Coronal illustrations of mouse hindbrain regions spanning the whole PBN. The dots represent effective infusion sites in the PBN area that received various doses of RO25-6981 and/or bretazenil as shown in Fig. 6. LC, locus coeruleus; PAG, periaqueductal gray region.