Supporting Information

Sanchez-Cardenas et al. 10.1073/pnas.1010849107

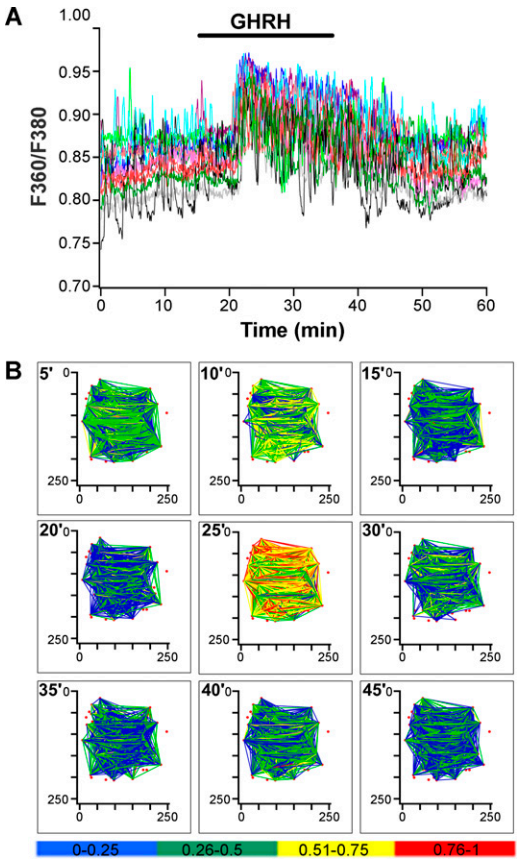


Fig. S1. GHRH triggers calcium responses, but not calcium waves, in lateral wing extremities of male pituitary slices. (*A*) Monitoring of calcium responses to GHRH (10 nM) in GH-GFP cells loaded with the fluorescent calcium dye fura-2. (*B*) Correlation maps, calculated at 5 min intervals, identifying significantly correlated GHRH-responsive GH-GFP cell pairs (Pearson's *R*). Red dots indicate the location of all GHRH-responsive GH cells. Significantly correlated cell pairs are connected with a straight line, the color of which represents the correlation strength; the bottom bar provides the color code.

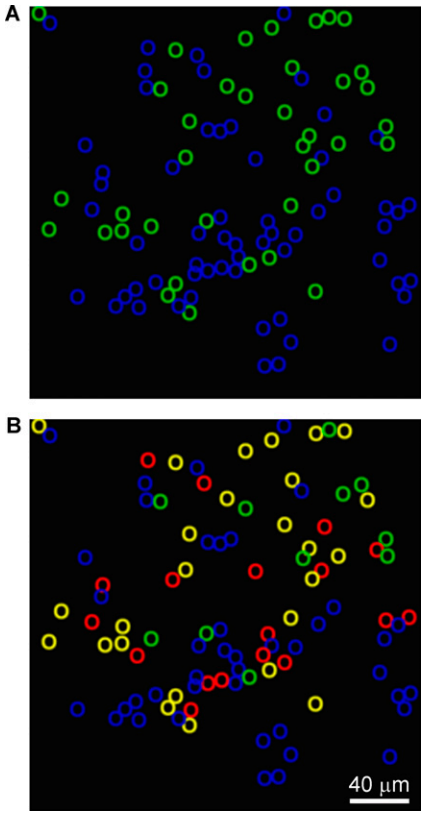


Fig. S2. Subpopulations of GH cells respond to successive GHRH stimuli. Calcium imaging in a pituitary slice from female adult mice exposed to a first GHRH stimulus (*A*) and to a second GHRH stimulus given 60 min later (*B*). Circles identify GH-eGFP cells in the imaged field. In *A*, green circles represent GH cells responding to GHRH with increased calcium spike activity (Fig. 1), and blue circles represent nonresponding cells. In *B*, green circles represent cells that responded to the first, but not to the second, GHRH stimulus; yellow circles represent cells that responded to both stimuli; red circles represent cells that responded only to the second GHRH application; and blue circles represent cells that did not respond to either GHRH stimulus.

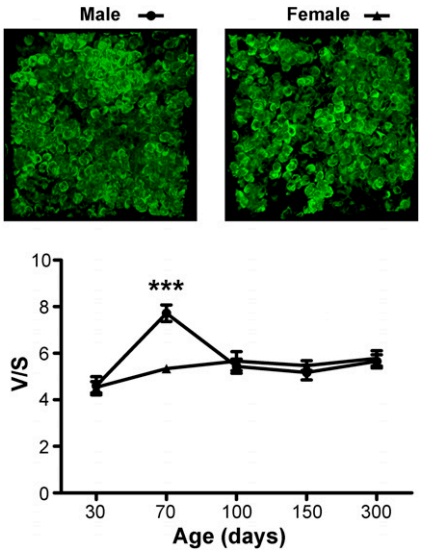


Fig. S3. Postnatal GH cell network architecture in male and female GH-eGFP mice. (*Lower*) The graph depicts the effect of age on the volume-to-surface (V/S) ratio of the GH cell network in male (●) and female (▲) GH-eGFP mice. Data are mean ± SEM. ***$P < 0.001$. (*Upper*) Representative 3D reconstructions of two-photon image stacks of the GH cell network in both male (*Left*) and female (*Right*) 60-d-old GH-GFP mice.

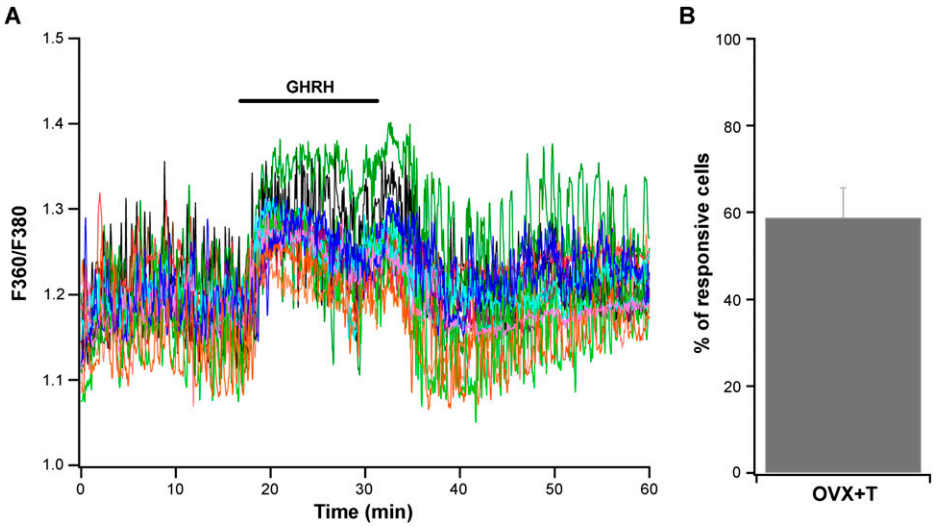
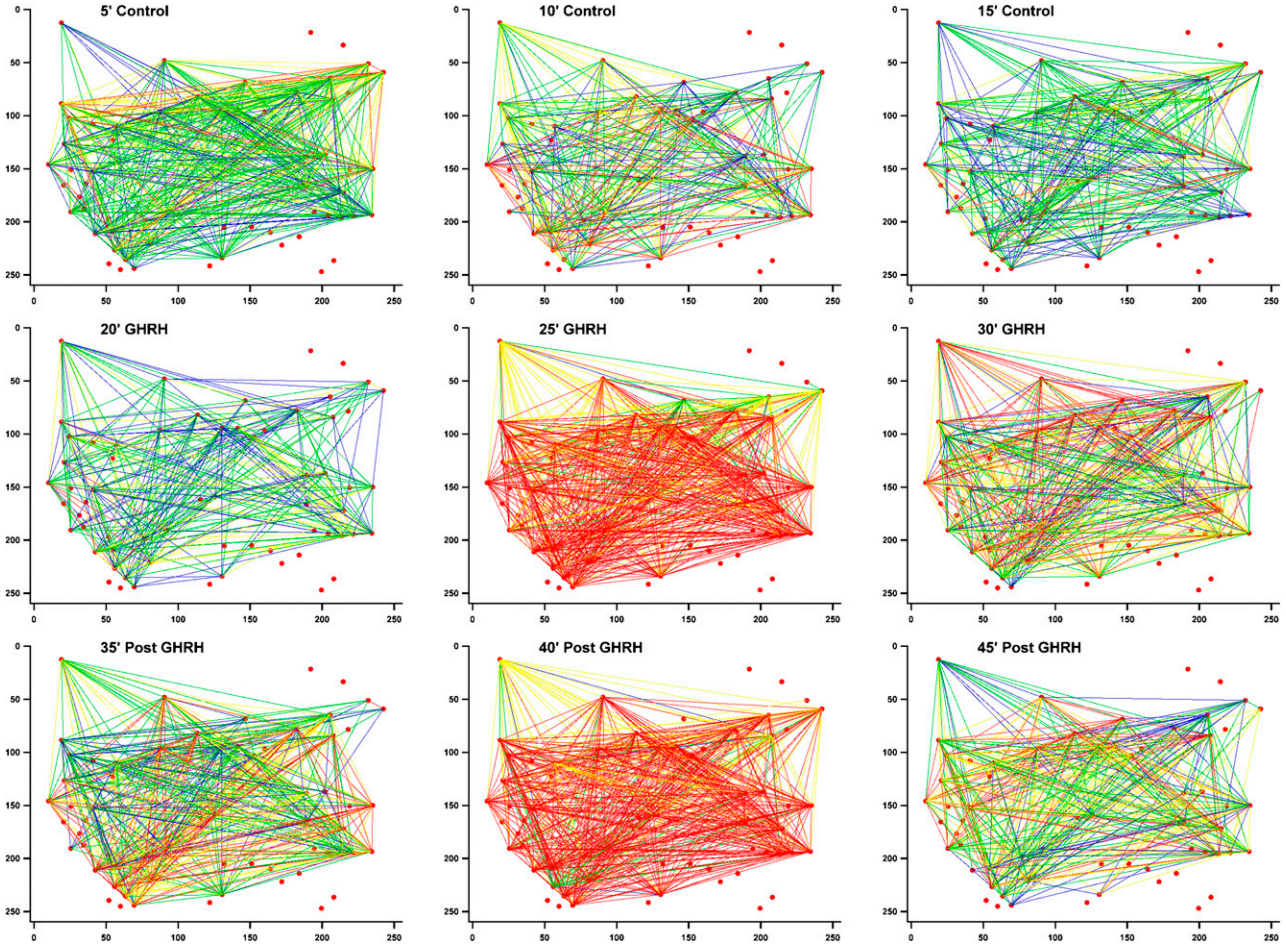
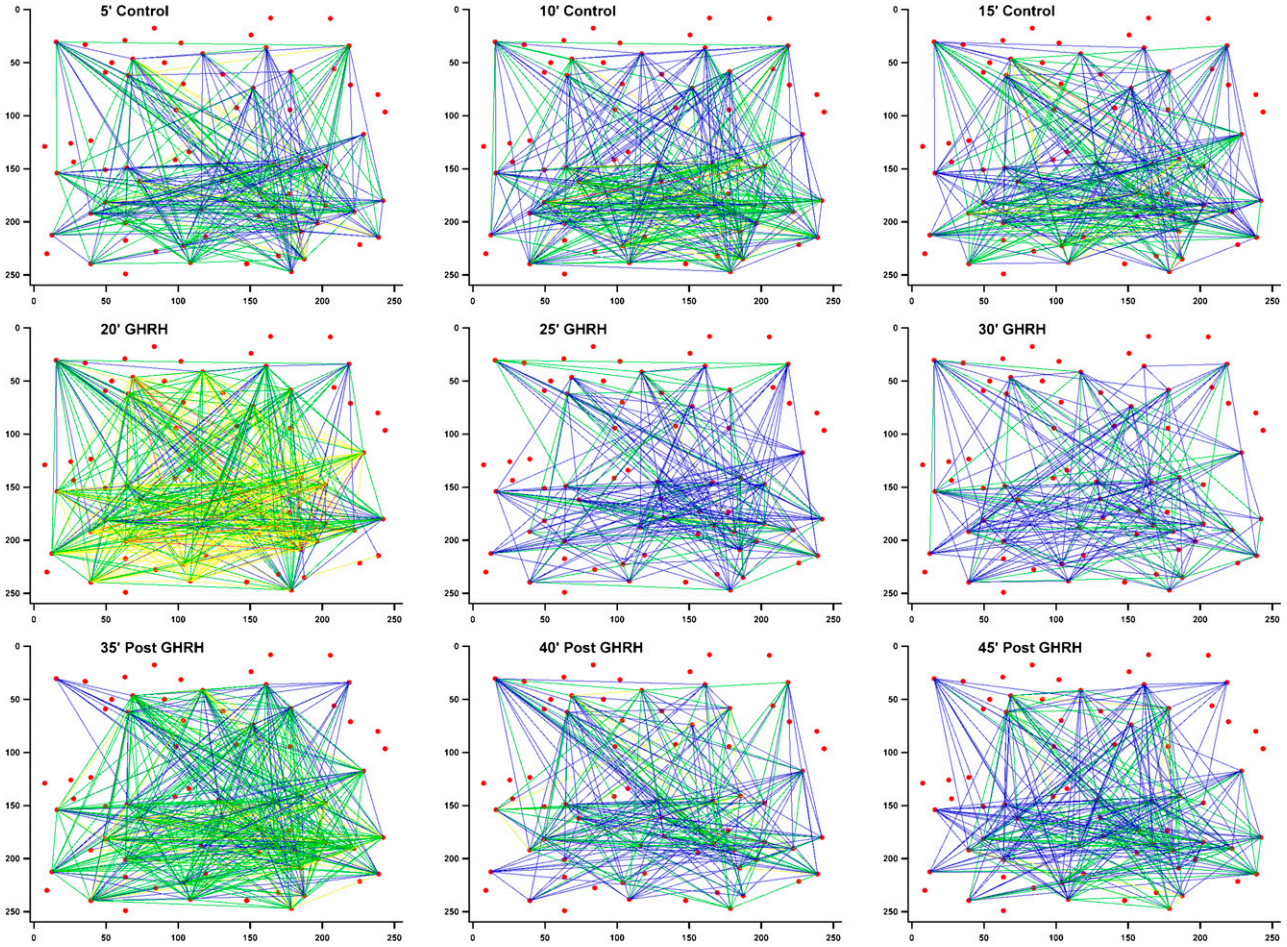


Fig. S4. Testosterone supplementation in ovariectomized females partially restores a male-like pattern of GHRH calcium responses. (*A*) Monitoring of calcium responses to GHRH (10 nM) in GH-eGFP cells in a pituitary slice prepared from an ovariectomized female supplemented with testosterone. (*B*) Proportion of GHRH-responsive cells in ovariectomized females supplemented with testosterone. Data are mean ± SD.



Movie S1. Image stacks of the heat maps of correlated activity in GH cell pairs in males, shown in Fig. 1*Ab*.

Movie S1



Movie S2. Image stacks of the heat maps of correlated activity in GH cell pairs in females, shown in Fig. 1*Bb*.

Movie S2