

# Supporting Information

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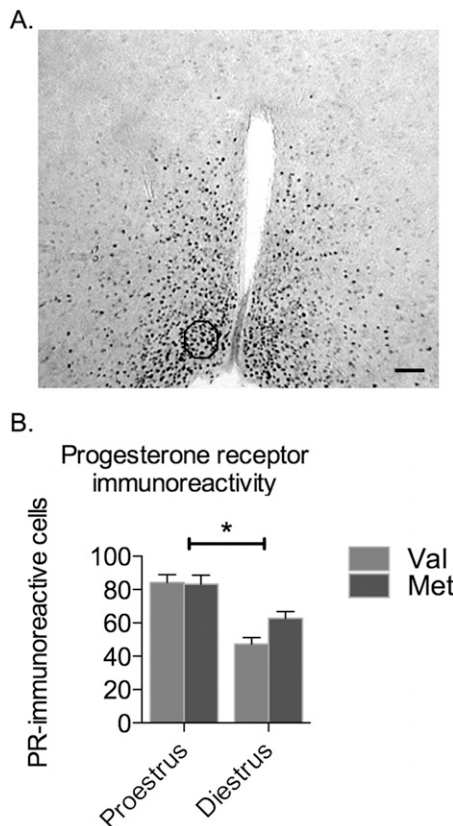
Met mice had estrous cycles that were indistinguishable from wild-type Val cycles with characteristic length of 6 days (1) and typical vaginal cytology. As a basic molecular measure of HPG axis function in these mice, we measured expression of the progesterin receptor (PR), which is induced by circulating estradiol in the medial preoptic area (MPO) of the mouse hypothalamus (2, 3). Immunocytochemistry was performed on sections through the MPO using a polyclonal rabbit anti-progesterone receptor (1:500) purchased from Dako North America. Specificity of this antibody was demonstrated by using knockout-mice and peptide preadsorption (4). PR-positive nuclei were counted in a fixed polygon of area 6,556.91  $\mu\text{m}^2$  from each hemisphere, using NeuroLucida

software. Consistent with estradiol induction of PR, the number of PR-positive nuclei in the MPO was higher in proestrus than diestrus for both wild-type Val and variant Met females (Fig. S1).

## SI Methods

**Immunocytochemistry and Densitometry.** Serial dilution tests of each antibody established that the labeling intensity was linear, and antibody dilutions were chosen that produced slightly less than half-maximal labeling intensity to optimize the detection of intensity variations (5). Specificity of the Akt antibody was confirmed by Western blot and peptide preadsorption in acrolein/paraformaldehyde-fixed tissue (6).

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2. Kudwa AE, Gustafsson JA, Rissman EF (2004) Estrogen receptor beta modulates estradiol induction of progesterin receptor immunoreactivity in male, but not in female, mouse medial preoptic area. *Endocrinology* 145:4500–4506.
3. Milner TA, et al. (2008) Angiotensin II-induced hypertension differentially affects estrogen and progesterin receptors in central autonomic regulatory areas of female rats. *Exp Neurol* 212:393–406.
4. Waters EM, Torres-Reveron A, McEwen BS, Milner TA (2008) Ultrastructural localization of extranuclear progesterin receptors in the rat hippocampal formation. *J Comp Neurol* 511:34–46.
5. Chang PC, Aicher SA, Drake CT (2000) Kappa opioid receptors in rat spinal cord vary across the estrous cycle. *Brain Res* 861:168–172.
6. Znamensky V, Akama KT, McEwen BS, Milner TA (2003) Estrogen levels regulate the subcellular distribution of phosphorylated Akt in hippocampal CA1 dendrites. *J Neurosci* 23:2340–2347.



**Fig. S1.** Progesterin receptor expression increases during proestrus in BDNF Val66Met mice. (A) Cells containing nuclear PR immunoreactivity were counted from a fixed area in the medial preoptic area of the hypothalamus. (B) The number of PR-immunoreactive cells was higher in proestrus than diestrus for both Val and Met mice. Two-way ANOVA showed a significant overall effect of cycle stage ( $F_{(1,16)} = 32.42$ ,  $P < 0.0001$ ). Post hoc tests revealed that for both genotypes, the density of PR-labeled cells increased significantly during proestrus ( $P < 0.05$  for both). \*,  $P < 0.05$ . PR, progesterone receptor; MPO, medial preoptic area.  $n = 6$  for Val proestrus, 4 for Val diestrus, 5 for Met proestrus, and 4 for Met diestrus. Error bars, SEM. (Scale bar: 100  $\mu\text{m}$ .)