Supporting Materials and Methods

Antibody staining

*apmd544-Gal4* flies were crossed to flies containing a *UAS-GFPnls* allele. Adult males and females carrying both the Gal4 and UAS alleles were collected. Brains and VNCs were dissected in PBS, fixed in 4% paraformaldehyde and washed in PBS and PBST. We used a 1:50 concentration of anti-GFP in an overnight incubation. After more PBST washes, a 1:1200 concentration of secondary antibody was used.

EcR antibody staining

*apmd544-Gal4* flies were crossed to flies containing a *UAS-lac\textsubscript{Z}nls* allele. Brains and VNCs were dissected out of adult males carrying both the Gal4 and UAS alleles. The tissues were fixed in 4% paraformaldehyde and washed in PBS and PBST. We used a 1:10,000 concentration of rabbit anti-beta-galactosidase and a 1:5 concentration of anti-EcR AG10.2 (Talbot et al. 1993) in an overnight incubation. The tissues were washed with PBST and a 1:1500 concentration of each secondary antibody was used for fluorescent detection.

EcR behavioral assay

To address whether or not the courtship defect seen in *apmd544-Gal4 eghR\textsubscript{nah}i* mutants was due to decreased *egh* expression or disruption in *ap*-expressing neural signaling, we reduced expression of EcR in Ap neurons. Crosses between *UAS-Dicer2;apmd544-Gal4* to *tubulin-Gal80ts;UAS-EcRR\textsubscript{NAI}-97* (Colombani et al. 2005) were maintained at 20°C. Virgin males were collected and housed at 29°C for 5 days. Female courtship objects were collected as virgins and aged for 4 to 5 days at 25°C. Behavioral assays were performed under red light at 29°C as previously described and CI values were analyzed by ANOVA and Tukey’s post-hoc analysis.

Supporting References
