



**Extended Data Figure 6. Ablation of POA Calb1+ neurons delays ejaculation but does not affect the initiation of mounting or intromission during male mating**

**a.** Serial montage of representative images from anterior (A) to posterior (P) showing the elimination of *Calb1*+ *in situ* signals in the POA (outlined) after AAVs encoding Cre-On Caspase3 (Casp3) were injected into the POA of *Calb1-Cre* male mice to ablate *Calb1*+ neurons. Control males were injected with AAVs encoding Cre-On EYFP. Scale bar, 200  $\mu$ m.

**b.** Quantification of the number of POA *Calb1*+ neurons in control (EYFP-injected) and Casp3-injected males.  $n = 7$  Casp3 males and 9 EYFP males.

**c.-e.** Casp3-injected males were comparable to control males in the initiation of mounting (**c**) and intromission (**d**) during male mating tests but the total duration of intromission was extended in the Casp3 group. Moreover, the percentage of males that ejaculated at a given time after introduction of a female (time '0') was reduced in Casp3-injected males and the latency to ejaculation following the first intromission (Post-intro latency) was significantly increased.  $n = 17$  Casp3 and 16 EYFP males..

Values are presented as mean  $\pm$  SEM. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .