doi:10.1038/nature13169

SUPPLEMENTARY NOTE 1

Our finding that optogenetic activation of VMHvl Esr1$^+$ neurons can evoke mounting is consistent with the observations that VMHvl contains neurons that are activated during male-female encounters[31], and that knockdown of Esr1 mRNA or genetic ablation of PR$^+$ neurons in this structure partially reduces male-female mounting[32,33]. However, we did not observe any reduction of male-female mounting by optogenetic inhibition of Esr1$^+$ neurons in VMHvl. Assuming that Esr1$^+$ and PR$^+$ neurons are equivalent[33], there are several possible explanations for this discrepancy. First, since the earlier studies utilized loss-of-function manipulations that were carried out days[32] or weeks[33] before behavioral tests were conducted, they could have interfered with processes that are a pre-requisite for male-female mounting, such as detection of female pheromones. By contrast, our time-resolved optogenetic inhibition experiments were carried out just before or after the onset of male-female mounting. The failure to observe any inhibition could indicate that the function of these neurons is no longer required once mounting is initiated.

A second possibility follows from the "intensity coding" model of social behavioral control by Esr1$^+$ neurons in VMHvl (see Extended Data Figure 10b and Supplementary Note 2). If indeed a smaller number of Esr1$^+$ neurons must be active for mounting than for attack to occur, then a more complete inhibition of the Esr1 population may be required to completely suppress mounting than attack. Yang *et al*. achieved a virtually complete genetic ablation of the PR$^+$ population, which could be verified by staining for PR expression[33]. By contrast, there is no easy way to determine the fraction of Esr1$^+$ VMHvl neurons that were inhibited by expression of eNpHR3.0, nor the extent to which each neuron was inhibited. Therefore, the likelihood of residual activity among these

neurons is greater in our experiments than in the case of genetic ablation. Such residual activity may be sufficient to permit mounting behavior. This interpretation could also explain why we failed to see any inhibition of mounting using the IVM/GluCl system to inhibit neuronal activity in VMHvl in our earlier study[31]: in that case, the inhibition of attack was less complete than was observed here using halorhodopsin, implying an even less efficient inhibition of the relevant VMHvl population.

An "intensity coding" model could also explain why optogenetic activation of VMHvl failed to promote mounting in our previous experiments[31]. In that case, activation of a Cre-dependent rAAV encoding ChR2 was achieved by co-infection with another rAAV encoding Cre recombinase under the control of the strong cytomegalovirus promoter-enhancer (CMV-Cre). The level of Cre expression under those conditions is likely to be much higher than the level obtained from our single-copy *Esr1* knock-in cassette, particularly in males where the level of *Esr1* expression is lower than in females[34]. This interpretation is supported by the fact that attack could be elicited from mice that received this constitutive virus combination as early as 2 weeks after infection[31], while in the current studies robust attack was typically not elicited until 4-5 weeks after injection. If mounting can only be elicited by optogenetic stimulation of a relatively small population of $Esr1^{+}$ neurons, the high and widespread level of ChR2 expression achieved in our earlier study might explain why only attack and not mounting was observed. However, the difference could also be due to a difference in the genetic background and/or rearing conditions of the commercially supplied vs. locally bred, gene-targeted animals used in the two studies.

SUPPLEMENTARY NOTE 2

Two alternative models (not mutually exclusive) may explain why activation of $Esr1^+$ neurons in VMHvl can promote either mounting or attack (or both), depending on conditions. One possibility is that deterministically different subsets of $Esr1^+$ neurons normally control mounting vs. attack, in response to signals from conspecific males vs. females, respectively (Extended Data Fig. 10a). In that case, optogenetic activation of both populations simultaneously could lead to a mixed behavioral outcome of mounting plus attack. Consistent with this model, our earlier electrophysiological recordings and fos catFISH experiments revealed that VMHvl contains some neurons that were selectively activated during male-male vs. male-female social encounters[31].

The simplest version of this model does not, however, easily account for our observation that optogenetically evoked mounting is typically seen under conditions when smaller numbers of $Esr1^+$ neurons are activated, or are stimulated at lower intensities. Nevertheless, this model can be reconciled with those observations if additional *ad hoc* assumptions are made. For example, the mount-specific subpopulation could be maintained under a lower level of tonic inhibition than the attack-specific subpopulation. In that case, a lower intensity of optogenetic stimulation would be required to activate the mount-specific subpopulation, than the attack-specific subpopulation. Furthermore, the putative mount-specific subset of $Esr1^+$ neurons may be more easily infected by rAAV, or expresses a higher level of Cre recombinase, than the putative attack-specific subset. In that case, when a smaller amount of virus is injected, the putative mount-specific subset of $Esr1^+$ neurons would be more likely to express

ChR2, and therefore be more likely to be activated during photostimulation, than the attack-specific subset.

A second model, that requires fewer *ad hoc* assumptions to explain the scalable effects of optogenetic stimulation on social behavior, postulates that whether mounting or attack is evoked depends on the number of $Esr1^+$ neurons that are activated, with attack triggered only when larger numbers of neurons are activated at high levels (Fig. 4v and Extended Data Fig. 10b). This model is consistent with our observation that larger numbers of c-fos^+ neurons (~2-fold) are detected in VMHvl following attack vs. mounting (Fig. 4u and ref. [31]). More importantly, it is supported by our previous fos catFISH data showing that when an episode of fighting is followed 30 minutes later by an episode of mating, almost 50% of the cells activated during mating had also been active during the preceding fighting episode[31]. By contrast, when the order of the two behaviors was reversed, <25% of the cells activated during the second episode (fighting) were also active during the first episode (mating). These data suggest that many of the cells activated during mating are a nested, smaller subset of those activated during fighting. Such an "intensity coding" model could, moreover, explain our failure to observe optogenetically evoked mounting in previous experiments using constitutively expressed ChR2[31], as well as our inability to optogenetically inhibit mounting in the present experiments (see Supplementary Note 1).

The simplest version of this model, however, does not easily explain why mounting can be repeatedly evoked towards a male intruder, especially when such behavior does not typically accompany the escalation of male-male social interactions from investigative to attack behavior[35]. If the only factor determining the type of behavioral

output (mounting vs. attack) were the total number of active Esr1$^+$ neurons (and the level of activity per neuron), then any additional increase in activity caused by optogenetic stimulation would be expected simply to promote an increased likelihood of attack. Why, then, can mounting be evoked by weaker optogenetic stimulation of Esr1$^+$ neurons? One possibility is that when the extent and intensity of optogenetic activation of Esr1$^+$ neurons is limited, either by a small number of ChR2-expressing cells, or by a low intensity of illumination, the overall level (or pattern) of activity among Esr1$^+$ neurons may never achieve the threshold (or pattern) necessary to evoke attack. From a psychological point of view, if the quantitative level of Esr1$^+$ neuron activity indeed encodes the intensity of a state of social arousal, then the abortive mounting towards males may represent a form of appetitive behavior that is engaged in when the animal experiences persistent social arousal at a low level, that fails to escalate to the threshold necessary for attack.

More complex explanations are also possible, in which overlapping populations of Esr1$^+$ neurons can exist in alternative, stable "attractor" configurations that promote mounting vs. attack[36]. Resolving this issue will require further anatomic, molecular and physiological dissection of the Esr1$^+$ population.

SUPPLEMENTARY NOTE 3

Most (~65%) ChR2-expressing males occasionally exhibited a brief episode of an apparent defensive behavior during photostimulation, in which they ran to a corner of the cage and remained immobile for a short period of time, averaging ~3 s. This "cornering"

behavior tended to occur near the onset of photostimulation, and typically was rapidly replaced by a social behavior such as sniffing, mounting or attack (See Supplementary Video 9). This phenotype was not dependent upon photostimulation intensity, and was most often observed at initial stages of testing performed at shorter times following viral injection. It was no longer observed in most animals when photostimulation evoked robust aggression.