

Deficits in sexual and aggressive behaviors in *Cnga2* mutant mice

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Odors detected by the vomeronasal organ or the main olfactory epithelium (MOE) trigger social behaviors in many animals. It is unknown whether MOE neurons detect cues that initiate mating or aggression. We demonstrate that mice lacking functional CNGA2 (cyclic nucleotide-gated channel $\alpha 2$), which is required for odor-evoked MOE signaling, fail to mate or fight, suggesting a broad and essential role for the MOE in regulating these behaviors.

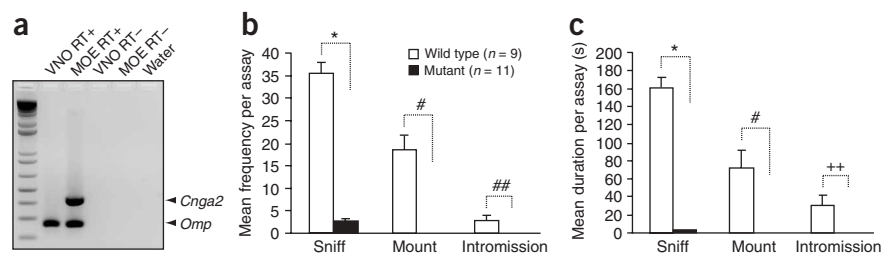
Activation of the MOE or the vomeronasal organ (VNO) by specific odors can trigger appropriate social behaviors in rodents and many other vertebrates. The MOE is thought to be involved in detecting cues that initiate nursing and suckling^{1,2}. The VNO is required for aggression in mice. Targeted deletion of transient receptor potential cation channel c2 (*Trpc2*), a gene expressed in the VNO, attenuates the responsivity of VNO neurons to mouse urine and abolishes aggression^{3,4}. The chemosensory regulation of mating may be more complex. Olfactory bulbectomy, which eliminates transmission of MOE and VNO signals, abolishes sexual behavior². *Trpc2*^{-/-} males, however, mate normally with females^{3,4}. Taken together with additional studies^{2,5}, these experiments suggest that the MOE and the VNO may mediate mating in a redundant fashion. Alternatively, mating and aggression may be segregated such that the MOE regulates mating, whereas the VNO initiates fighting. To distinguish between these models, we examined mice deficient in odor-evoked activity in the MOE. Although there may be redundancy in the early steps of odor-evoked signaling^{1,6}, CNGA2 (also referred to as CNG $\alpha 2$ or OCNC1) is the only cyclic nucleotide-binding subunit of the CNG channel

expressed in most MOE neurons⁷. CNGA2 is essential for odor-evoked activity in the vast majority of MOE neurons⁸. Because CNGA2 is not expressed in vomeronasal neurons⁷ (Fig. 1a), mice lacking the *Cnga2* gene permit analysis of the MOE's contribution to mating independent of the VNO.

We examined male mating in *Cnga2*^{-Y} mice (*Cnga2* is X-linked) and their wild-type siblings. Male mating consists of several sequential subroutines, including chemoinvestigation, mounting and intromission³. We observed a striking deficit in each of these components of sexual behavior in *Cnga2*^{-Y} mice compared to wild-type males (Fig. 1b,c). The latency to first sniff the female was 64-fold longer in *Cnga2*^{-Y} mice (mean \pm s.e.m.: 419.4 \pm 90.0 s, $n = 11$) compared to wild-type male mice (6.5 \pm 1.4 s, $n = 9$, $P = 6.0 \times 10^{-4}$). All wild-type males (9 of 9) mounted in these assays, whereas none of the mutants (0 of 11) were observed to mount. Such deficits in sexual behavior persisted in a mating assay lasting several days (Supplementary Note online). These data demonstrate a profound reduction in many components of sexual behavior in *Cnga2* mutants.

Male mice chemoinvestigate males as well as females⁹. We asked whether the sniffing deficit exhibited by *Cnga2*^{-Y} mice toward females would also extend to male intruders. Wild-type resident males typically sniff and attack intruder males⁹. *Cnga2*^{-Y} residents showed a substantial reduction in sniffing and aggression compared to the wild-type mice (*Cnga2*^{-Y}: 5 of 11 sniffed and 1 of 11 attacked; wild-type: 9 of 9 sniffed and 6 of 9 attacked; Fig. 2). The latency to first sniff the intruder was 32-fold longer for *Cnga2*^{-Y} mice (mean \pm s.e.m.: 364.0 \pm 165.8 s, $n = 11$) compared to wild-type male mice (11.4 \pm 3.0 s, $n = 9$, $P = 1.0 \times 10^{-3}$). Together with the reduction in chemoinvestigation of females, these results suggest that *Cnga2* mutant males show a deficit in sniffing conspecifics during mating and aggression. Unlike *Trpc2*^{-/-} males^{3,4}, *Cnga2*^{-Y} mice did not mount intruder males. The mounting of male intruders by *Trpc2*^{-/-} residents has led to a model suggesting that in the absence of fighting, male mice revert to a 'default' mode of mating indiscriminately with all conspecifics⁴. Our data provide a dissociation

Figure 1 Loss of sexual behavior in *Cnga2*^{-Y} mice. (a) Reverse transcriptase–polymerase chain reaction (RT-PCR) experiments reveal expression of olfactory marker protein (*Omp*) and *Cnga2* in the MOE but only of *Omp* in the VNO. Shown is a typical gel run from an RT-PCR (35 cycles). The lowest band of the ladder is 200 bp with ~ 100 bp increments up to 600 bp. RT+, reaction with reverse transcriptase; RT-, reaction without reverse transcriptase; water, reaction without cDNA. Resident males were exposed to a female for 30 min. (b,c) The frequency (b) and duration (c) of mating subroutines are significantly diminished in mutants compared to wild-type mice. Animal care and handling was done in accordance with Institutional Animal Care and Use Committee guidelines. * $P, 2.0 \times 10^{-4}$; # $P, 4.6 \times 10^{-5}$; ## $P, 7.0 \times 10^{-4}$; ++ $P, 2.3 \times 10^{-3}$. Frequency represents the number of times a particular behavioral subroutine was observed in the assay. Error bars represent s.e.m.



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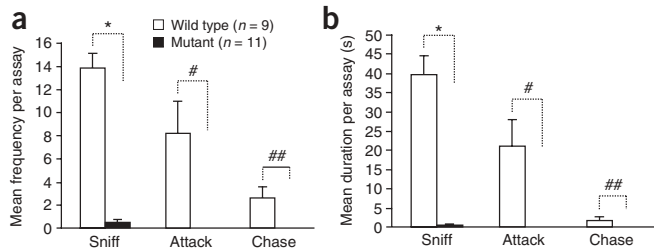


Figure 2 Loss of aggressive behavior in *Cnga2*^{-Y} mice. Resident males were exposed to a wild-type intruder male for 15 min. (**a,b**) The frequency (**a**) and duration (**b**) of sniffs, attacks and chases directed toward the intruder were significantly reduced in mutants compared to wild-type mice. **P*, 2.0×10^{-4} ; #*P*, 5.7×10^{-3} ; ##*P*, 2.3×10^{-3} . Frequency represents the number of times a particular behavioral subroutine was observed in the assay. Error bars represent s.e.m.

between sexual behavior and the absence of aggression, suggesting that mating may involve a sensory contribution from the MOE.

The deficits in *Cnga2* mutants could result from a general avoidance of conspecifics. Alternatively, *Cnga2*^{-Y} mice may be unable to recognize conspecific odors that initiate social behaviors. *Cnga2* mutants groomed males and females in a manner similar to that of the wild-type residents, suggesting that these mutants did not avoid conspecifics entirely (Supplementary Fig. 1 online). Moreover, we found that *Cnga2*^{-Y} mice fail to sniff male or female odors even when these were presented on a neutral substrate. We provided socially naïve wild-type and mutant males with female and male urine simultaneously on separate cotton pads. *Cnga2*^{-Y} mice sniffed these odors significantly less than did the wild-type mice (Fig. 3a). As expected from previous studies¹⁰, wild-type males showed a preference for female urine. By comparison, *Cnga2*^{-Y} mice failed to sniff either pad preferentially. In addition to sniffing, wild-type and mutant males interacted with the pads by carrying them in their mouths, tearing them and pushing them around. Although *Cnga2*^{-Y} mice engaged in such non-sniff interactions with greater frequency than wild-type mice, the total duration of these interactions was similar between the two genotypes (Fig. 3b). Taken together, these results suggest that the mating and aggression deficits in *Cnga2*^{-Y} mice are unlikely to result solely from an avoidance of conspecifics or their odor cues.

Our data suggest an essential role for the MOE in mating and aggression. One explanation for the dual requirement for the MOE and the VNO in aggression is that these epithelia function in a parallel fashion to regulate fighting. In another model, attractant volatiles from conspecifics detected by the MOE may provoke chemoinvestigation involving physical contact, thereby permitting the VNO to access odorant cues. Diminished sniffing in *Cnga2* mutants may prevent the VNO from processing cues that initiate aggression. In support of such a sequential model of activation of first the MOE and then the VNO, conspecific volatiles activate synaptic target neurons of the MOE, whereas physical contact and chemoinvestigation seems essential for the activation of synaptic target neurons of the VNO^{11,12}. Finally, the VNO may also detect volatile odors^{2,13}. Thus, the reduced sniffing by *Cnga2*^{-Y} mice may prevent the VNO from gaining access to aggression-modulating volatile cues. It will be interesting to determine the mechanisms underlying the diminished sniffing in these mutants. In any event, an intact VNO is not required for mating^{3,4}, suggesting that the MOE also processes cues that regulate sexual behavior. In future studies, it will be important to determine whether prior social experience bypasses the requirement for a functional MOE in

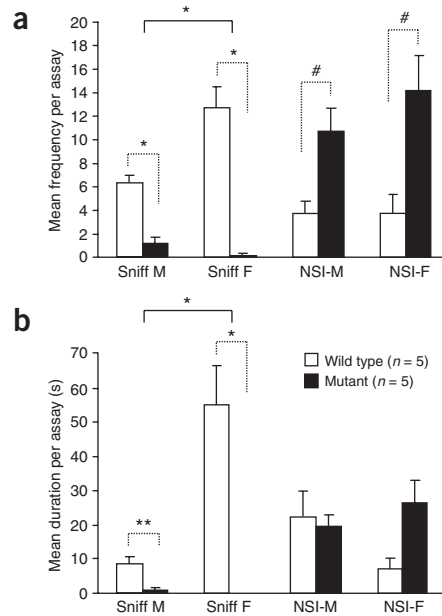


Figure 3 Loss of preference for female urine odors in *Cnga2*^{-Y} mice. Resident mutant and wild-type males were exposed to two cotton pads wetted with male (M) or female (F) urine for 5 min. (**a,b**) Wild-type mice sniffed female urine more frequently (**a**) and for longer duration (**b**) than did the mutants. *Cnga2*^{-Y} mice displayed more non-sniff interactions (NSI) with urine-wetted pads compared to the wild-type mice, although the total duration of NSI was similar between the two genotypes. **P*, 7.9×10^{-3} ; ***P*, 1.6×10^{-2} ; #*P*, 3.2×10^{-2} . NSI-F and NSI-M, NSI with female urine- and male urine-wetted swabs, respectively. Frequency represents the number of times a particular behavioral subroutine was observed in the assay. Error bars represent s.e.m.

chemoinvestigation, mating and aggression. Finally, some MOE neurons do not express CNGA2 and use a distinct odor-evoked signaling pathway². Our data suggest that this subpopulation cannot initiate mating or fighting in the absence of MOE neurons expressing functional CNGA2.

We cannot exclude the possibility that central deficits, including aberrant connectivity, produce the behavioral phenotypes we observe in *Cnga2*^{-Y} mice. CNGA2 is also expressed in several brain regions¹⁴. Nevertheless, *Cnga2*^{-Y} mice resemble wild-type mice in many behavioral tests, including grooming (Supplementary Fig. 1) and operant conditioning². Finally, *Cnga2*^{-Y} mice have circulating levels of testosterone that are similar to those in wild-type mice (Supplementary Note), suggesting that the behavioral phenotypes are unlikely to arise from testosterone deficits in adults. The behavioral deficits observed in *Cnga2*^{-Y} mice resemble the phenotypes resulting from adult bulbectomy¹⁵. These surgically lesioned males fail to chemoinvestigate conspecifics and do not mate or fight. Our results are therefore consistent with a role for CNGA2-expressing MOE neurons in regulating chemoinvestigation, mating and aggression.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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1. Belluscio, L., Gold, G.H., Nemes, A. & Axel, R. *Neuron* **20**, 69–81 (1998).
2. Restrepo, D., Arellano, J., Oliva, A.M., Schaefer, M.L. & Lin, W. *Horm. Behav.* **46**, 247–256 (2004).
3. Leypold, B. *et al. Proc. Natl. Acad. Sci. USA* **99**, 6376–6381 (2002).
4. Stowers, L., Holy, T.E., Meister, M., Dulac, C. & Koentges, G. *Science* **295**, 1493–1500 (2002).
5. Del Punta, K. *et al. Nature* **419**, 70–74 (2002).
6. Wong, S.T. *et al. Neuron* **27**, 487–497 (2000).
7. Berghard, A., Buck, L.B. & Liman, E.R. *Proc. Natl. Acad. Sci. USA* **93**, 2365–2369 (1996).
8. Brunet, L.J., Gold, G.H. & Ngai, J. *Neuron* **17**, 681–693 (1996).
9. Miczek, K.A., Maxson, S.C., Fish, E.W. & Faccidomo, S. *Behav. Brain Res.* **125**, 167–181 (2001).
10. Pankevich, D.E., Baum, M.J. & Cherry, J.A. *J. Neurosci.* **24**, 9451–9457 (2004).
11. Lin, Y., Zhang, S.Z., Block, E. & Katz, L.C. *Nature* **434**, 470–477 (2005).
12. Luo, M., Fee, M.S. & Katz, L.C. *Science* **299**, 1196–1201 (2003).
13. Sam, M. *et al. Nature* **412**, 142 (2001).
14. Kingston, P.A., Zufall, F. & Barnstable, C.J. *Synapse* **32**, 1–12 (1999).
15. Devor, M. & Murphy, M.R. *Behav. Biol.* **9**, 31–42 (1973).