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## Seizures in a colony of genetically obese mice

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Mice containing a targeted deletion of the basic helix-loop-helix transcription factor *Nhlh2* were originally developed in 1997 (ref. 1). These animals show adult-onset obesity characterized by reduced spontaneous physical activity levels and a lack of overt hyperphagia<sup>2</sup>. Several different institutions, including the National Institutes of Health, the University of Massachusetts-Amherst and our institution, Virginia Polytechnic Institute, have bred these mice. Recently, several individuals in our breeding colony of *Nhlh2*-knockout mice developed recurring seizures. The condition is not a known phenotype of either of the two known variants of *Nhlh2*-knockout mice<sup>1,3</sup>. During the previous 11 years of breeding, animal care staff reported only two to four mice in the colony to have had seizures. This condition was so infrequent that no further attention seemed necessary at the time. The affected mice were euthanized and excluded from testing or breeding. The condition became a concern when six mice in the colony were described as having seizures in one day. **Figure 1** shows the sequence of events leading to a full seizure in one of these animals.

*Nhlh2*-knockout mice, like most knockout mouse models, are on a mixed 129Sv/J × C57Bl/6 background. The 129 background strain in particular has a high susceptibility to testicular teratomas, which we have occasionally encountered in our colony. Increased susceptibility to seizure is not a characteristic in either of the two parental lines<sup>4,5</sup>. In fact, the C57Bl/6 line has a lower generalized seizure threshold than other inbred lines<sup>6</sup>.

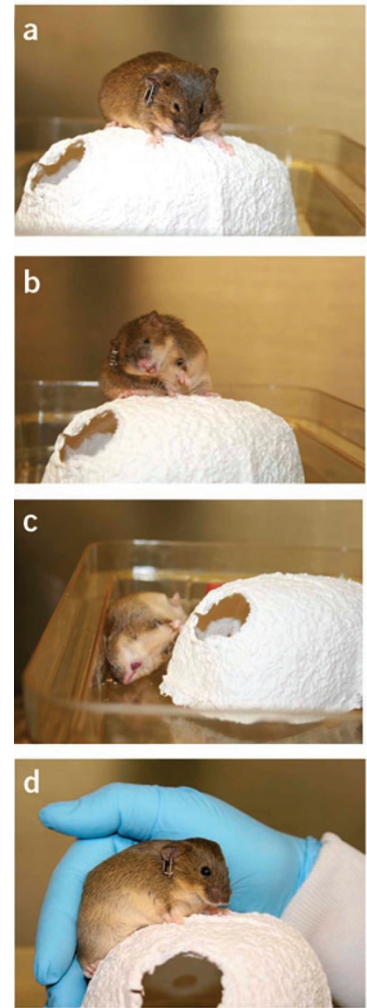
Environmental conditions in the mouse room are kept constant. The temperature range is 22–23 °C (± 0.5 °C). Humidity is not monitored directly in the room but is measured elsewhere in the facility; it does

not fluctuate significantly and is generally maintained within the recommended range of 30–70%. Mice in our colony consume two different diets: we feed breeders Teklad 7004 S-2335 Mouse Breeder Diet and non-breeders, or holding individuals, an irradiated Teklad 2918 diet. Because diet, temperature and humidity are not different from those in other mouse colonies in the facility, it is not likely that these contributed to the seizure phenotype.

Animal care technicians first observed the seizures during routine, weekly sanitation procedures. After handling, the mice began convulsing, shaking or chewing and then either circled rapidly around the cage or experienced momentary paralysis (**Fig. 1**). All episodes lasted less than 1 min, and none of the observed episodes ended in death. There seemed to be a range of severity of seizures among individuals; those who had more severe seizures would occasionally urinate during episodes. Furthermore, even though handling seemed to trigger the seizures, the condition did not occur every time the mouse was handled. In many cases, it was impossible to induce another seizure by handling a mouse that had previously had a seizure. Without direct observation of an epileptic episode, it was not possible to distinguish the affected mice from normal, healthy individuals in the colony.

We believed a spontaneous dominant mutation caused the seizure phenotype. Do you agree with our conclusion? Are there other documented cases of spontaneous seizures arising in breeding colonies? What should be the follow-up diagnosis and identification plan for our breeding colony?

### What's your diagnosis?



**FIGURE 1** | Sequence of events leading to full seizure in an affected individual. (a) After handling, the mouse is placed on the top of a Shepherd Shack (Shepherd Specialty Papers, Portage MI) and starts to experience paralysis. (b) Paralysis worsens, and the animal's mouth opens. The animal twists and shakes. (c) The animal falls from the Shepherd Shack and is convulsing with its mouth open. (d) The animal is fully recovered after approximately 5 min.