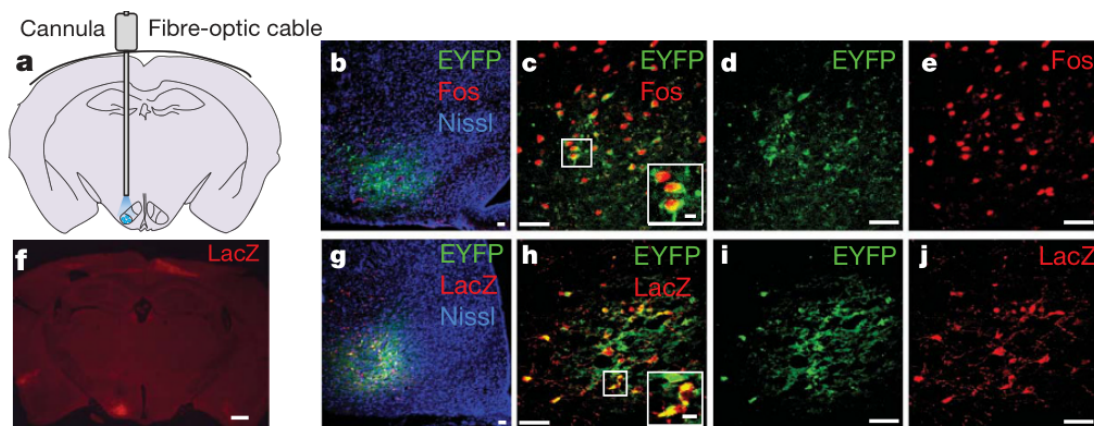


## Description of Techniques and Explanation of Concepts

### Optogenetic Stimulation Induced Attack

- “We next tested whether functional manipulations of VMHvl would affect mating or fighting<sup>1</sup>. Although VMHvl overlaps the rat HAA, extensive attempts to elicit attack by conventional electrical stimulation of this region in mice were unsuccessful. As an alternative, therefore, we expressed channelrhodopsin-2 (ChR2) in VMHvl neurons unilaterally<sup>1</sup>, using stereotactic co-injection of adeno-associated viral vectors (AAV2)<sup>2</sup> expressing Cre recombinase and a Cre-dependent form of ChR2 fused with enhanced yellow fluorescent protein (ChR2–EYFP) and selectively illuminated cells<sup>2</sup> in this region using an implanted fibreoptic cable (Fig. 4a)<sup>3</sup>.



- Hypothesis in question<sup>1</sup>: this section deals with figure 4 in the paper, where the authors are testing whether functional manipulations of VMHvl are affect behavior, but had an issue of selectively activating the area.
- ChR2<sup>2</sup>: use of channelrhodopsin (light gated ion channels), specifically channelrhodopsin-2, allowed the researcher to select particular neurons<sup>2</sup> to analyze cell responses in VMHv1 directly.
- Co-injection of AAV2<sup>3</sup>: AAV2 infects neurons preferentially, allowing only neurons whose cell bodies are local to the injection site express the CHR2. Co-injection ensures that cells observed are exactly the neurons in question, which blue region under **a** in the above image.
- Selectively illuminated cells<sup>2</sup> using an implanted fibreoptic cable<sup>3</sup>: “results showed that c-fos could be strongly induced in VMHvl on the infected, but not the contralateral control side after repeated blue light stimulation in awake animals.”
  - The actual analysis of how the authors determined this result is something I’m struggling to understand. I get the idea of injecting and selectively controlling regions that respond to the optogenetic light, allowing for functional testing of behavior, but I don’t **b–e** and **g–h**.
  - **f**: does use LacZ to identify infected cell bodies, which I assume is to verify effectiveness of AAV2.