Supplementary Material

Movie 1. Full red flash warning display in an animal missing iridescence on one side due to stellate connective cut.

Movie 2. An animal with a stellate connective cut exhibits lack of iridescent, but retains fin motility.

Movie 3. Electrical stimulation of the Fin Iridescence nerve (FI nerve), results in a dramatic iridescence increase and in fin muscle contractions, but the chromatophores remain relaxed. The video is shown x4 faster than real time.

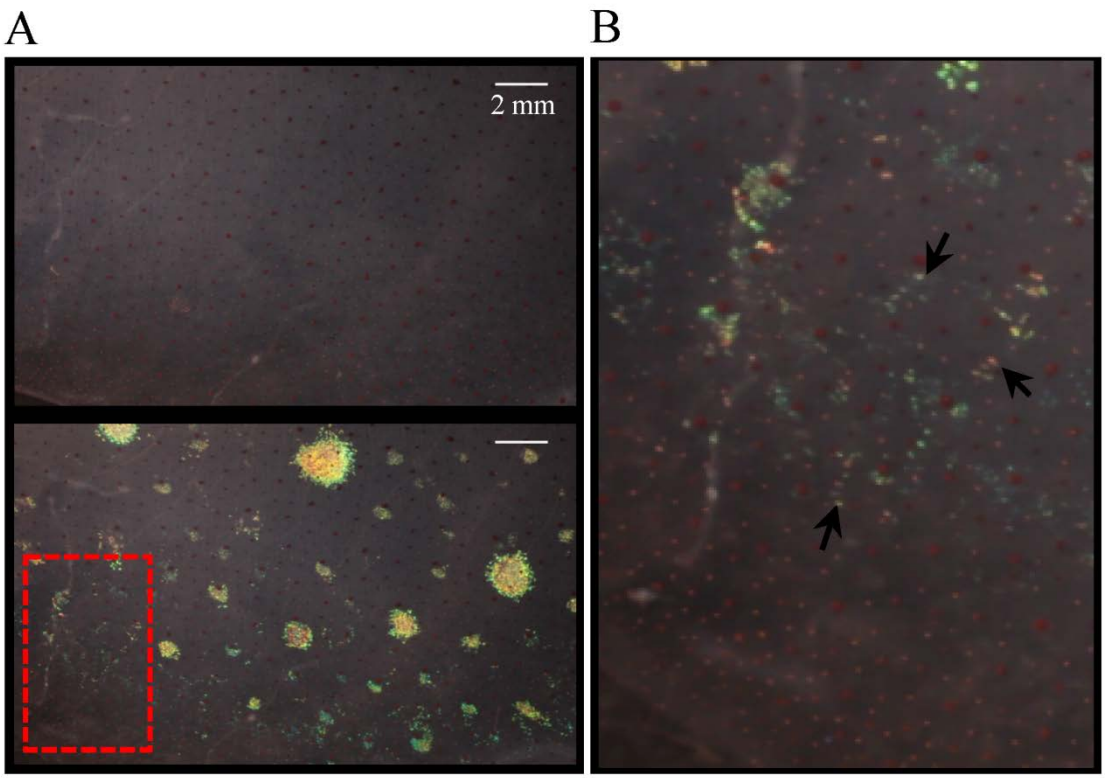

Fig.S1. The iridescence of single iridocytes is also increased in response to electrical stimulation of the FI nerve. (A) Fin tissue before and after electrical stimulation of the FI nerve. (B) Zoom in on region shown by red square in a. Arrows point to individual iridocytes.

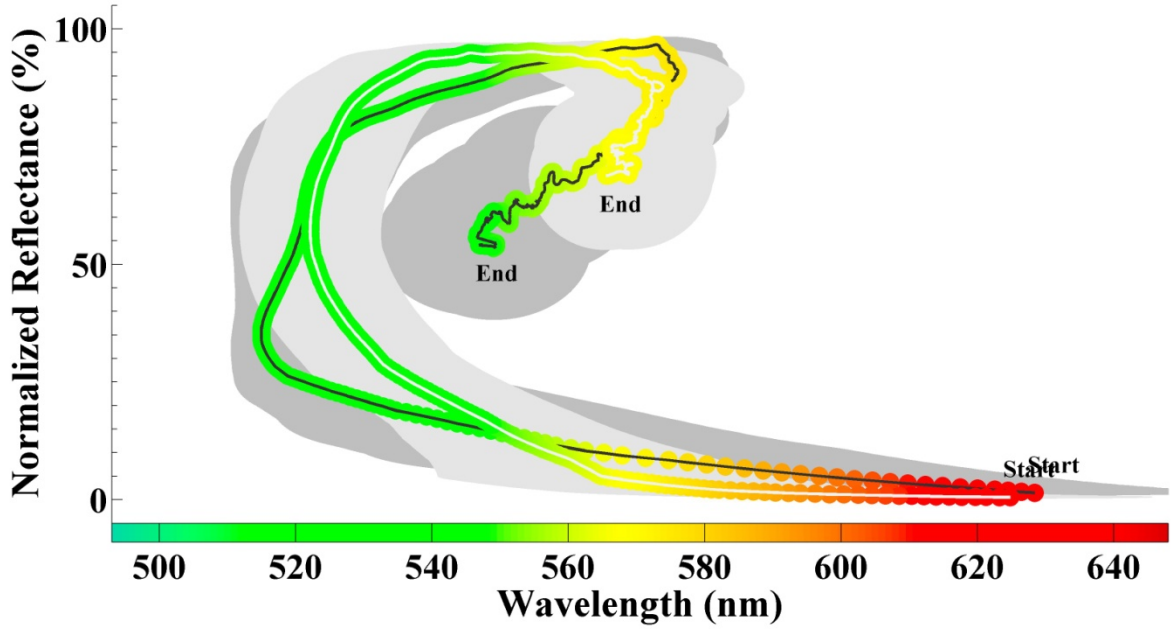

Fig.S2. Iridescence coloration and brightness dynamics due to nerve stimulation do not differ 7 or 15 days post-denervation. At maximum excitation, the iridophore color in animals whose stellate connective was severed 7 (light grey) and 15 (dark grey) days previous to the experiment was not significantly different. Grey shading represents the standard deviation. Note how the standard deviations overlap throughout the response, indicating the 1 and 2 weeks post denervation treatments are not significantly different.

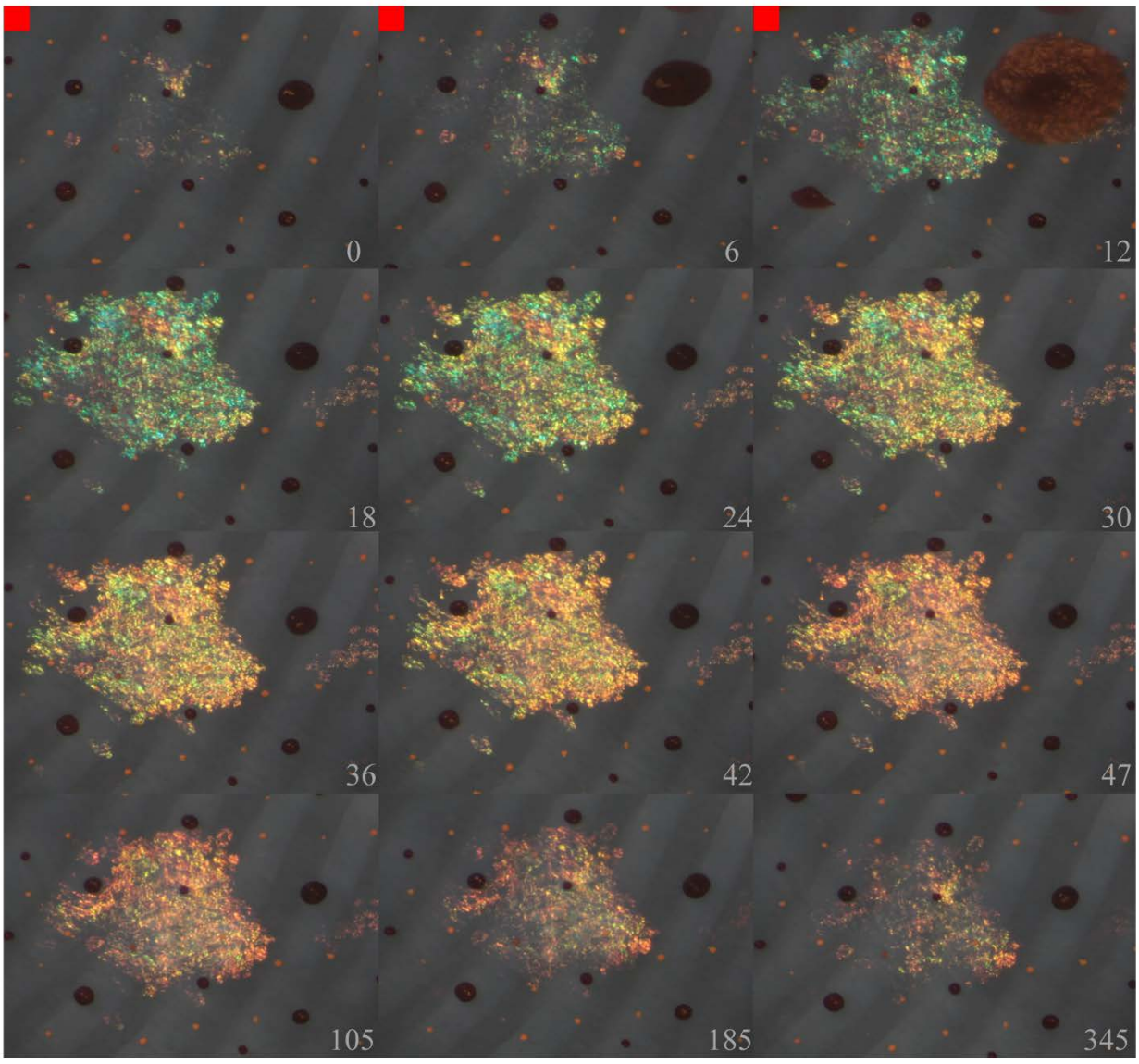

Fig.S3. Iridescence change in a denervated animal due to electrical stimulation. Electrical stimulation of a small dermal nerve in a denervated animal reveals that an iridophore is located within a patch of skin where only a few iridocytes were visible at rest. The increase in intensity and the color change can be appreciated. Numbers on the lower corners show the time after stimulation had started. The red square indicates when electrical stimulation was given (stimulation lasted 15 seconds).

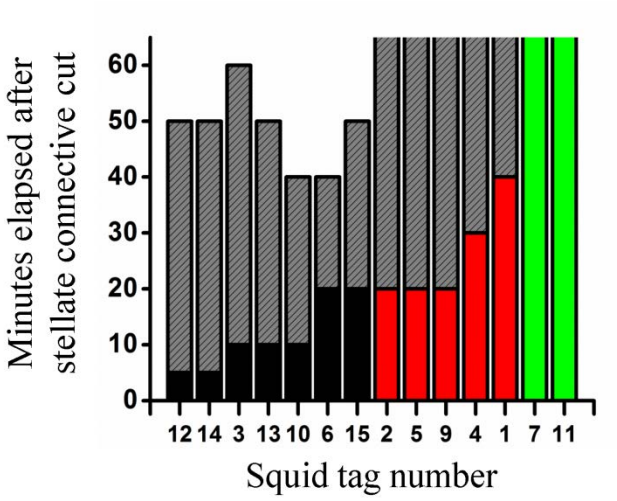

Fig.S4. Decline in iridescence after the stellate connective is cut. Each squid in the experiment was given a number. They are plotted in the x-axis according to the time taken until a substantial decline in iridescence was noticed (solid bars). The distribution of the results from our sampled population show that the majority of the population would exhibit a decline in iridescence within 30 minutes, but that only 50 % of the population would have complete absence of iridescence within one hour.

MATERIALS AND METHODS

Animals

Healthy animals were put into a separate tank. Only the animals that had fed well for at least one day, a sign of strength and drop in stress levels, were used for surgical experiments.

Denervation

For denervation, the squid was held out of the water by gently wrapping the left hand around the squid mantle, between the fins and the head. Squids usually maintained their heads firmly in a straight line with the body, which allowed the experimenter to look into the mantle cavity and visually identify the location of the stellate ganglion. Next, micro-scissors held with the right hand were used to cut the descending fibers. The complete process takes less than 20 seconds.

Nerve fills

To fill inputs and outputs of the stellate ganglion with fluorescent dyes, the stellate ganglia were removed and bathed in a Ca^{2+} free solution (Strathmann 1987) for 10 min at room temperature. Then, they were transferred to a petridish with a bed of Sylgard 184 (Dow Corning). While the dish was dry, small cubes of Sylgard were cut out, forming a well that was lined with Vaseline applied through a blunted syringe tip. The nerve of interest was pinned with an insect pin into the inside of the well. The rest of the ganglion was pinned down to avoid movement. Next, the Vaseline syringe was used to make the walls of the well taller, trapping the nerve between the Vaseline layers. Subsequently a few microliters of dye were delivered into the well. After confirming that the tip of the nerve was well submerged, The Vaseline syringe was used to close up the well in the shape of an igloo, the preparation was covered with Trish buffered sea water and left in the fridge (at 4°C) overnight. We followed this protocol for all the nerves of interest, up to 3 for one ganglion. Most ganglia were double filled through application of Lucifer yellow to the output of the stellate ganglion and MicroRuby to the descending inputs. We backfilled the fin iridescence nerve with Lucifer yellow and forward filled the Stellate connective with MicroRuby.

Iridescence 7 and 15 days after denervation

Because the timing taken for motorneurons to go through apoptosis depends on the temperature of the water, we monitored the denervated squids as they went though the described sequence, which consisted of relaxed chromatophores for approximately 2 days, expanded chromatophores on day 3-4, and the development of “passing clouds” on day 5 which form perfect waves by day 7. Thus, according to Young (1972) at day 7 post denervation the cut neurons should have died.