Title: Neural control of tuneable skin iridescence in squid

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Running head: Squid control their iridophores neurally

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Supplemental Figures and their Legends

Figure S1. Branching structure of squid fin nerves. A. Diagram of D. pealeii used for the study. Red rectangle indicates area shown in B. B. Once the ventral mantle is removed and the preparation pinned dorsal side down, the fin nerve is visible (arrow head). The fin nerve divides into smaller branches that travel towards the distal part of the fin. C. To gain access to the branches that innervate the skin, the overlying tissue must be removed (double arrow head points at the location where the proximal portion of the fin nerve is still visible). This higher magnification shows the neural branches and chromatophores in the dorsal skin. The tissue was obliquely illuminated from the dorsal side. D. Close-up of preparation, dorsal side down, showing a small branch departing from the nerve, making a second branch that travels across the iridophore (dotted oval). Multiple arrow heads show the branching locations. Scale bars: B. 2 mm, C. 1 mm, D. 200 µm.
Figure S2. Fin cross-section diagrams showing the different preparations employed to elucidate iridophore activation. A. Intact tissue preparation, where only a small portion of tissue was opened to access skin nerves. With this preparation, only the green iridophore phenotype (see inset) was observed after neural activation. B. Removal of all tissue above and below the iridophore layer, (still containing nerves and connective), resulted in red phenotype responses after stimulation. C. Opening the surface the fin skin (i.e. removing the epidermis, hyaline and chromatophore layer) also resulted in the red phenotype. D. Removal of the fin muscle and ventral skin also resulted in the red phenotype.
Figure S3. Activated iridocytes flicker locally, independent of chromatophore activity. A. Close up of an activated iridophore, in an intact fin, immediately after electrical stimulation, B. 200 ms later and C. 400 ms later. Circle in (B) indicates a group of iridocytes that “flickered” and resulted in a momentary reduction in iridescence (see also movie S4). Scale bars: A-C 50 µm.

Supplemental Movie Legends

Movie S1. Neurally activated iridescence in squid iridophores. “Intact fin” preparation yields a green iridophore phenotype when a nearby nerve branch is stimulated through a proximal suction pipette. Fin iridophores at rest have low peak reflectance. After electrical stimulation of a skin nerve in the fin, a field of yellow-green iridophores is clearly visible (see also figure 1). Playback speed is four times faster than real time. Red square indicates electrical stimuli being applied.

Movie S2. Tissue connectivity is essential for structural coloration. A preparation, with the layers below and above the iridophore layer removed, such that only the nerves, iridophores and connective tissue remain. In this preparation, neural stimulation activates iridophores, but the responses now show a red phenotype (see also figure 2). Playback speed is four times faster than real time. Red square indicates electrical stimuli being applied.

Movie S3. Structural coloration and peak reflectance have separate mechanisms. Neurally stimulating tissue where the fin muscle and ventral skin (both below the iridophore layer) had been removed, results in a red phenotype. Different response dynamics of peak reflectance and colour change can be observed between red and green iridophore responses (see also figure 3). Playback speed is four times faster than real time. Red square indicates electrical stimuli being applied.

Movie S4. Iridocyte flickering alters iridophore appearance. Patches of iridocytes within some iridophores were often observed to “flicker”. In this video, the group of iridocytes in the top part of this iridophore flicker, although the neighbouring chromatophores remained unchanged (see also figure S3). Playback speed is ten times slower than real time.