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The dependence of Ca^2 concentration on photocurrent and light-induced Ca^2 release in UV-sensitive zebrafish cones.

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The UV-sensitive cones of the zebrafish are insensitive to visible light and can therefore be used for Ca^2 measurements with fluo dyes and an argon ion laser without undue bleaching of the cone photopigment. This has made it possible for the first time to make multiple Ca^2 measurements from the same vertebrate photoreceptor (Leung et al., 2002). We have used this technique to explore in detail the relationship between outer segment Ca^2 concentration (Ca^2_i) and photocurrent during a flash of light and in the presence of backgrounds. Dark-adapted zebrafish were stunned by concussion, then killed by decapitation and pithing. Cones were isolated from the retina and loaded for 30 min wtih 10 μM fluo 4-AM; fluorescence was measured as described previously (Matthews & Fain 2001, 2002). Simultaneous photocurrent measurements were made with a suction pipette.A single cone was stimulated repeatedly with a flash of constant intensity from the optical bench; after each light flash a single 50 ms laser exposure was used to evoke dye fluorescence before and at four times during the ensuing flash response. Response waveforms to the light flash alone before and after the fluorescence measurements were nearly identical, indicating that this protocol bleached a minimal amount of photopigment. Comparison of photocurrent with fluorescence indicated that Ca^2_i declined after the flash and then recovered with a time course that was indistinguishable from that of the photocurrent. This indicates that Ca^2_i in the cone outer segment is primarily determined by Ca^2 flux through the light-dependent channels (Sampath et al., 1999).We also exposed cones to steady background light and compared the change in sensitivity and photocurrent with the change in Ca^2_i. For dim backgrounds, photocurrent and Ca^2_i declined in proportion, but for brighter backgrounds, Ca^2_i was considerably greater than expected, probably as the result of light-induced Ca^2 release (Leung et al., 2002; Brockerhoff et al., 2003). This release of Ca^2 seemed, however, to have little effect on the sensitivity of the cell which declined monotonically according to the Weber-Fechner relation in spite of the anomalous increase in Ca^2_i, suggesting that changes in Ca^2_i may have only a small effect on light adaptation in cones apart from the modulation of guanylyl cyclase.

Brockerhoff SE et al. (2003) *J. Neurosci.* **23** 470-480.
Leung Y-T et al. (2002) *J. Physiol.* **539P**, 142-143.
Matthews HR & Fain GL (2001) *J. Physiol.* **532**, 305-321.
Matthews HR & Fain GL (2002) *J. Physiol.* **542**, 829-841.
Sampath AP et al. (1999). *J. Gen. Physiol.* **113**, 267-277.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Use of a complex vocalisation to study processing in the guinea pig auditory cortex

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An impressive aspect of the human brain is its ability to process the thousands of different combinations of phonemes that are used in the sentences of everyday speech. Although verbal speech is unique to humans, the initial neural processing of communication sounds by the cortex is likely to be similar in other mammalian species. In the guinea pig cortex there are at least 7 auditory areas: two core areas, the primary area AI and a dorsocaudal area (DC) which are almost entirely surrounded by 5 *belt* areas. We presented a digitised example of a guinea pig vocalization called *chutter* to animals while recording with glass insulated tungsten electrodes from cortical units in 4 areas. Guinea pigs were terminally anaesthetised with a mixture of ketamine and xylazine and the cortex was exposed as described in Wallace et al. (2002) Hearing Research 172:160-171. Stimuli were presented binaurally in the closed field. Peri stimulus time histograms were constructed from the responses to the chutter presented at a level equivalent to 20 dB above the pure tone threshold. The chutter used has a series of irregular noisy bursts of sound of different duration and loudness. When the responses to this call were compared across all 412 units, specific responses were found at 17 temporal positions. We then classified our units in terms of whether they responded or not within windows set to correspond to each of these 17 temporal components. No one unit responded during all of the windows, but each had a distinct combination of responses at as many as 9 different windows. Within each area there was a wide range of different response combinations. In AI there were 65 different combinations of response amongst 260 units while in DC there were 31 distinct combinations among 62 units (14 of these combinations were not found in AI). In the ventrorostral belt (VRB, 75 units) there were 38 distinct response combinations (18 of these were not found in AI or DC) and in the dorsocaudal belt (DCB) all 15 units responded differently: 2 of them in a way unique to DCB. Thus out of 412 recorded units there were 99 (24%) distinct temporal response patterns to the chutter. This is a remarkably low rate of redundancy within the cortex and implies that the cortex is sensitive to the sequence of salient sound features in the environment. To further illustrate the differences between cortical areas the mean response was plotted for each area. Mean responses were very similar in AI and DC with large responses at several temporal windows, but very different in DCB which gave its largest response at a different window. The mean response in VRB was smaller than the core areas except for the second last window.

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The role of nitric oxide in the submanibular salivary function of sheep

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Experiments were performed on weaned lambs and adult sheep of various breeds (15-52 kg body weight)in the U.K. Experimental procedures were performed as previously described (Buckle et al. 1995), comparing stimulation of the chorda-lingual nerve continuously at 4Hz with the equivalent intermittent pattern of bursts of 40 Hz for 1 second in every 10 (40Hz 1:10). Anaesthesia was induced and maintained with sodium pentobarbitone (15-30mg kg^{-1} I.V. then 0.1-0.3 mg min^{-1} kg^{-1} I.V.). Animals were eventually killed by lethal injection of barbiturate (ca. 15ml 20% I.V.). Data are given as mean±S.E.M. and analysed by paired and unpaired Students t-test; n = number of animals. The fall in submandibular vascular resistance was unaffected by the pattern of stimulation, (−69.4±2.7% at 4Hz; −71.4%±1.7% at 40Hz 1:10, n=6). Blockade of de novo synthesis of nitric oxide attenuated the vascular response at 4Hz, (−69.4±2.7%; −44.4±6.2% before & after L-NAME, $P<0.05$), and at 40Hz 1:10, (−71.4%±1.7%; −46.6±8.4% before & after L-NAME, $P<0.05$, n=6) to a similar degree. Subsequent administration of atropine did not attenuate the vascular response further, (42.5±6.0% at 4Hz, 38.2±5.36 at 40Hz 1:10, n=3). Continuous stimulation caused a significantly greater fluid secretion than intermittent, (0.047±0.004; 0.038±0.005 ml min^{-1} [g gland]$^{-1}$ at 4Hz and 40Hz 1:10 respectively, $P<0.05$, n=6). Blockade of de novo synthesis of nitric oxide did not affect the volume of secretion at 4Hz, (0.047±0.004; 0.040±0.005 ml min^{-1} [g gland]$^{-1}$ before & after L-NAME) or 40Hz 1:10, (0.038±0.005; 0.037±0.005 ml min^{-1} [g gland]$^{-1}$ before & after L-NAME). Subsequent administration of atropine reduced the salivary flow to only a few drops in a ten minute period. Administration of L-NAME significantly reduced the protein output at 4Hz, (50.9±5.6; 42.2±5.3 μg min^{-1} [g gland]$^{-1}$ before & after L-NAME, $P<0.05$, n=6), but had no effect at 40Hz 1:10, (55.0±10.8; 59.1±12.6 μg min^{-1} [g gland]$^{-1}$ before & after L-NAME). With continuous stimulation at 4Hz the secretion of fluid is known to be nitric oxide-independent and almost entirely cholinergic. Secretion of protein can be achieved by both nitric oxide-dependent and -independent mechanisms, demonstrated by the difference in protein output between continuous and intermittent patterns of stimulation. We suggest either that the secretion of protein is predominantly mediated by the release of a neurotransmitter with a nitric oxide-dependent 2nd messenger pathway, or that nitrergic facilitation of neurotransmitter release occurs, when stimulation is continuous, but not when it is intermittent. The vascular response is partially nitric oxide dependent.

Buckle AD et al. (1995) Exp. Physiol. 80, 1019-1030.

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