Shastri, Mythreyi D., and Jack R. Girton. Department of Zoology and Genetics, Iowa State University, Ames, Iowa. Prolonged exposure to carbon dioxide does not affect the electroretinograms permanently.

In this study, we examined the effects of prolonged exposure to carbon dioxide on the electrical activity in the visual system of *Drosophila.* Electroretinograms (ERG) were measured in *Drosophila* after exposure to carbon dioxide followed by a lengthy time period for recovering from anaesthesia. Previous studies on the effect of anaesthetics on electroretinograms have focused primarily on ether. The studies noted that ether and carbon dioxide cause temporary alterations in ERGs during exposure (Stark, 1972). We show statistical evidence that the amplitude and the shape of the electroretinogram is not altered permanently by prolonged exposure to carbon dioxide.

The electroretinograms in this study have been characterized in terms of amplitude and shape. The normal ERG has quick responses to the beginning and ending of the light stimulus. These are the "on" and "off" transients due to electrical activity in the synaptic junctions with the L1 and L2 cells of the lamina. The sustained corneal negative is due to the electrical activity in the retina (Hotta and Benzer, 1969). The amplitude or height of the ERG is the distance between the tips of the "on" and "off" transients. The amplitude of the ERG is measured as mV/Division. In the figure, one division is seen as one large square with twenty five small squares.

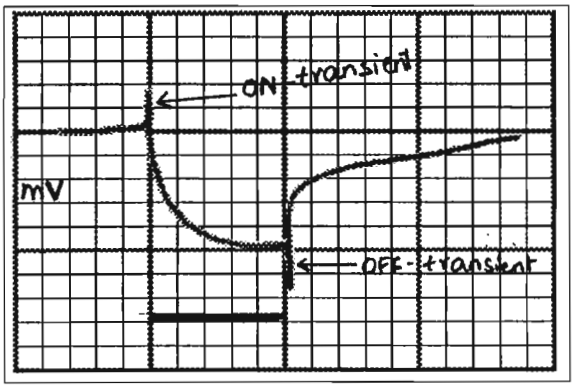

Figure 1.

The ERGs were measured in *y w sn* flies. Flies with the *white* mutation are used instead of wild type flies to measure ERGs because the white eyes do not 'light adapt' as quickly as the red eyes. ERGs were also measured from w^a fa^g and w^a fa^g*;Bpt* flies. w^a *fa* flies have apricot coloured eyes and rough facets. In w^a fa^g*;Bpt* flies, the rough eyes have a black patch on the retina. This is due to death and degeneration in the retina and the underlying optic lobes (Duus *et al.*, 1992).

Flies were prepared for measuring ERG, in batches. Flies in each batch were prepared for the experiment by anesthetizing with CO_2 and gluing to a coverslip with nail polish. Each batch contained flies exposed to 3 min, 5 min, and 8 min of carbon dioxide. The coverslip was laid on a block of agar, prepared with 0.9% NaCl, and the flies were connected to the block with small strips of agar. A silver reference electrode was inserted into the block of agar, and the signals from the bodies of the flies (background noise) were grounded. A fiber optic illuminator provided a white light stimulus that was focussed onto the eyes of the fly by using a mirror and a convex lens. Wavelength filters of 470 nm and 568 nm were used to change the wavelength of the stimulus. The light stimulus always lasted for one second and is shown as a black bar in the figure.The average amplitude of the ERG was measured at two wavelengths, 470 nm and at 568 nm. Electroretinograms were measured from each fly by inserting a recording electrode filled with 0.9% NaCl into the eye. Signals from the recording electrode were amplified 10x by a preamplifier, Dagan corporation (Minneapolis, MN) and then displayed on the screen of an oscilloscope. The recording electrodes were made by using glass capillaries with filament, No. TW100F-3, World Precision Instruments, (Sarasota, FL).

Statistical analyses were done on the amplitudes of the ERGs recorded from *y w sn* flies. Seven flies, exposed to 3 min of carbon dioxide show an average amplitude of 8.21 mV (470 nm) and 8.14 mV (568 nm). Five flies, when exposed for 5 min have average amplitudes of 6.1 mV (470 nm) and 5.9 mV (568 nm). Even after exposure to carbon dioxide for 8 min, ten flies show amplitudes of 5.8 mV (470 nm) and 5.6 mV (568 nm). The differences between these means is not significant ($P > 0.05$). The electroretinograms always showed the on and off transients (Figure 1). Electroretinograms measured in the flies with other genotypes were not affected by prolonged exposure to carbon dioxide.

In our study, we have conclusively shown that prolonged exposure to carbon dioxide does not alter the amplitude and shape of the ERGs permanently. Previous studies have measured the effects of anaesthetics on electroretinograms. Prolonged exposure to ether for more than 3 min permanently blocked the transients and sometimes, even lowered the corneal negative. Measurement of ERGs during short exposure to carbon dioxide showed reversible changes. The off-transient disappeared in less than 5 sec and the on-transient disappeared in less than 10 sec (Stark, 1972). These studies, taken together, indicate that the metabolic and synaptic effects of these two anaesthetics on

electroretinograms are very different. We suggest that carbon dioxide should be the anaesthetic of choice when measuring electroretinograms.

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References: Duus, K. M., W. J. Welshons, and J. R. Girton 1992, Dev. Biol. 151:34-47; Hotta, Y., and B. Benzer 1969, Nature 222:354-356; Stark, W. S. 1972, Dros. Inf. Serv. 48:82.

Goode, S. Department of Genetics, Harvard Medical School. Additional gain of function phenotypes associated with the *Ocellarless* gene of *Drosophila melanogaster*.

Ocellarless (*Oce*, 1-5.7)/+ females are missing 60-80% of ocellar and 90-95% of postvertical head bristles and sometimes show incised margins on the wings (Lindsley and Zimm, 1992). We report additional phenotypes of *Oce*/+ females. We find that ocelli of *Oce*/+ females are usually moved closer together, or fused, and that additional head bristles are often missing or absent (Figure 1, A-C). The wings of *Oce*/+ flies typically have a gap in the fifth longitudinal wing vein and less frequently in the posterior cross vein (Figure 2, A-C). *Oce*/+ phenotypes do not result from haplo-insufficiency, since females heterozygous for *Df(1)HC244*, which removes DNA spanning the 3E to 4F region (approximate meiotic map positions 1-5 to 1-11.5), are completely wild type.

Both *Oce* wing vein gap and ectopic bristle phenotypes resemble phenotypes associated with loss and gain of function mutations of Notch receptor and the *Drosophila* EGF receptor (DER; Clifford and Schüpbach, 1989; Diaz-Benjumea and Hafen, 1994; Schellenbarger and Mohler, 1978; unpublished observations). *brainiac* (*brn*) maps within 0.2 map units of *Oce*, at position 5.9, and *brn* mutant animals display phenotypes common to both the Notch and EGF receptor signaling pathways (Goode *et al.*, 1992, 1996). We ruled out the possibility that *Oce* mutations are gain of function *brn* alleles. $Df(1)rb^{33}$, which was synthesized on an *Oce* chromosome (Banga *et al.*, 1986), fails to complement *brn* mutations, but still retains dominant *Oce* phenotypes.

Oce phenotypes are completely penetrant in *Oce*/*w v* l^{14ts} or *Oce*/*FM3* females reared at 29°C (n > 1400), making a simple F_1 reversion screen for rearrangements in the *Oce* gene easy. These rearrangements should be useful for isolation of *Oce* DNA sequences, since a genomic walk spanning the 3F-4A region has been completed (Goode *et al.*, 1996). Elucidation of the *Oce* molecular structure and function may add to our knowledge of Notch and/or DER signaling pathways.

References: Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, Inc., San Diego; Clifford, R.J., and T. Schüpbach 1989, Genetics 123: 771-787; Diaz-Benjumea, F.J., and E. Hafen 1994, Development 120: 569-578; Shellenbarger and Mohler 1978, Dev. Biol. 62: 432-446; Goode, S., D. Wright, and A.P. Mahowald 1992, Development 116: 177-192; Goode, S., M. Morgan, Y-P. Liang, and A.P. Mahowald 1996, Dev. Biol., 178: 35-50; Banga, S.S., B.T. Bloomquist, R.K. Brodberg, Q.N. Pye, D.C. Larrive, J.M. Mason, J.B. Boyd, and W.L. Pak 1986, Chromosoma 93: 341-346.

Figure 1 (next page). *Oce* head phenotypes. Scanning electron micrographs of the dorsal side of wild type (A), and *Oce* (B,C) adult heads. Arrows point to the ocellar bristles and stars demarcate the postvertical bristles of wild type flies (A). These bristles are frequently missing in *Oce*/+ flies (B, C; Lindsley and Zimm, 1992). Further, the ocelli (arrows, A) are either moved closer together (B), or fused (C). Other head bristles are often missing or misplaced in *Oce*/+ flies. The "wild type" fly in (A) has an extra microchaete (x).