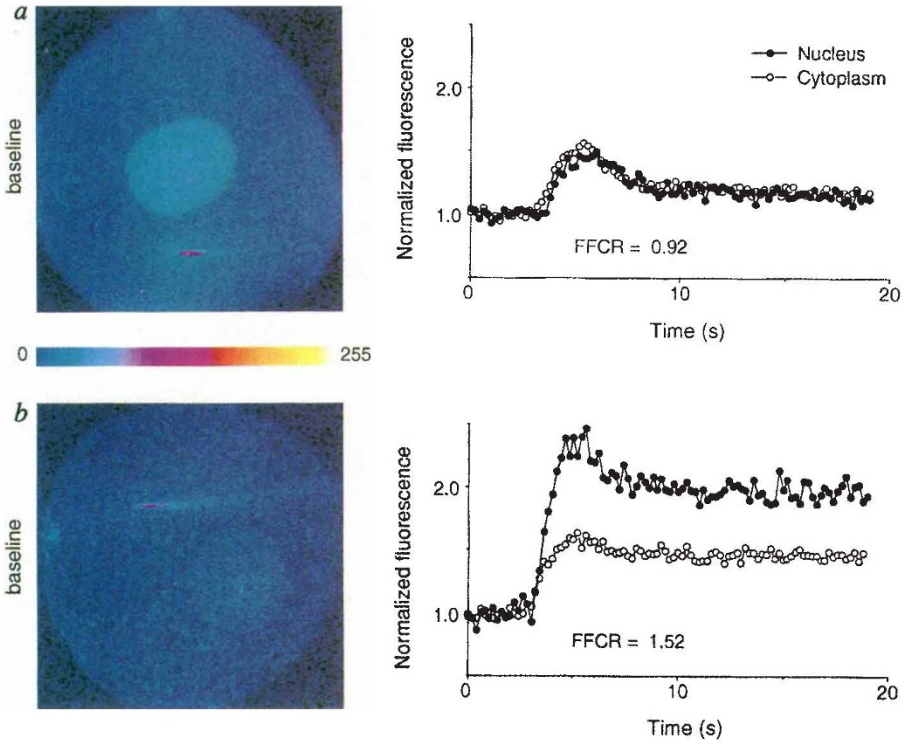

Baseline nuclear and cytoplasmic Fluo-3 fluorescence change ratios (FFCR) in neurons with large (*a*) and small (*b*) elevated Ca^{2+} signals after disrupting the cell membrane with a sharp micropipette (the pipette tip is the small bright spot in both confocal images). Normalizing fluorescence changes during depolarization to these baselines revealed a proportionally equivalent increase in nuclear and cytoplasmic signals for the neuron in *a*, and a 1.5-fold greater increase in nuclear signals for the neuron in *b*.

that the Ca^{2+} signals of the neurons studied by Al-Mohanna *et al.* were elevated at baseline as a consequence of the dye-loading technique.

Using sharp microelectrodes to inject Fluo-3 into DRG neurons, as did Al-Mohanna *et al.*, we detected apparent amplification of nuclear Ca^{2+} signals only in neurons minimally injured by the impalement. The figure shows normalized fluorescence (fluorescence change ratios[1]) in the nucleus and cytoplasm of two neurons that were impaled with sharp microelectrodes and allowed to fill with 1 mM Fluo-3 for up to 4 minutes (to minimize dye sequestration artefact[1]), then depolarized during confocal imaging. Both neurons maintained physiological resting potentials (less than −55 mV); however, the neuron shown in *a* in the figure took about 10 seconds to acquire its resting potential, indicating that there had been a slight injury upon impalement, whereas the neuron in *b* stabilized its resting potential immediately after penetration. After depolarization, the fractional fluorescence change ratio of this neuron indicated an increase in Ca^{2+} 1.5 times greater in the nucleus than in the cytoplasm. In contrast, the neuron in *a* responded as in the Al-Mohanna *et al.* report, with a somewhat reduced increase in nuclear Ca^{2+} fluorescence compared with the cytoplasm. The most obvious difference is that the neuron in *a* had proportionally higher nuclear fluorescence at baseline. Altogether, eight of the neurons we examined had elevated baseline signals and gave responses similar to that in *a* (no apparent amplification), and five neurons had moderate baseline signals and gave responses similar to that in *b* (apparent amplification).

Thus, in our opinion, the use of microelectrodes to introduce Ca^{2+} indicator dyes into neurons can cause problems for evaluating baseline nuclear Ca^{2+} signals, and we believe that the important question of whether nuclear Ca^{2+} amplification indeed occurs remains unanswered.

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AL-MOHANNA *ET AL.* REPLY — In our hands neither temporary cell impalement with a sharp micropipette nor patch clamping permanently raises the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). For instance, N1E-115 cells injected with Fura-2 by micropipette had an average $[Ca^{2+}]_i$ of 88 nM (ref. 10), whereas $[Ca^{2+}]_i$ in patch-clamped cells was 95 nM (ref. 11). Rand *et. al.* state that a bright nucleus in a cell injected with calcium-green or Fluo-3 indicates that $[Ca^{2+}]_i$ is high. This is not so: nuclei fluoresce more brightly because there is more dye there. Careful work from 1975 onwards[12] has shown that after equilibration the gross concentration of small polar solutes is higher in the nucleus, because the significant volume of the cytoplasm that lies within membrane-bound organelles is inaccessible to the solute. Records, such as that in *b* of Rand *et al.*'s figure, in which the nucleus is not brighter, must be in error, as are the $[Ca^{2+}]_i$ changes calculated from such records. There are two likely sources of error: one is uptake of dye into organelles, as discussed in our paper[1]; the other is incorrect correction for autofluorescence, as discussed by O'Malley[13].

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Male suckling

SIR — The fascinating report of mammary development and signs of secretory activity in male Dayak fruit bats by C.M. Francis *et al.* in Scientific Correspondence (*Nature* **367**, 691; 1994) is a splendid illustration of important questions being raised by investigation of whole animals in their environment. The question in this case is: do male fruit bats suckle their young? Further studies are essential because milk secretion without suckling would be a curious biological phenomenon in a wild mammalian population, but one that might be explained by phytoestrogens in these frugivorous bats. Suckling, and the physiological investment of lactation, by a male of any species as a normal part of its life history would be an important biological phenomenon.

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