

Cocorocchio et al.:

Bitter Tastant Responses in the Amoeba *Dictyostelium* Correlate with Rat and Human Taste Assays

Supplementary Data

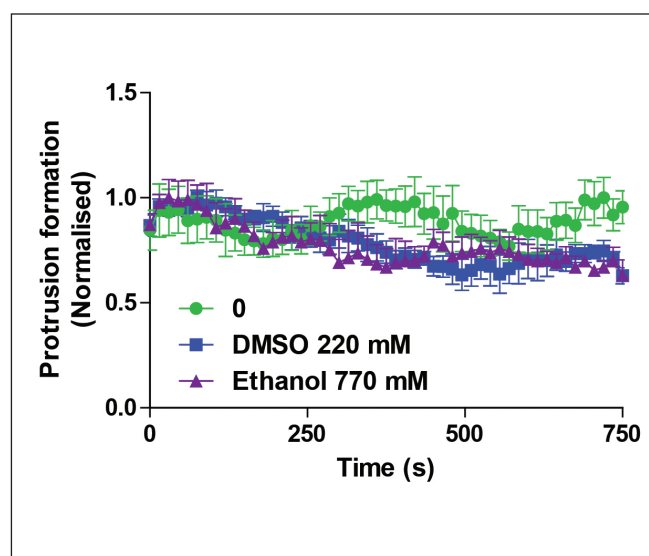


Fig. S1: Solvents do not affect *Dictyostelium* cell behavior
Addition of maximal concentrations of solvent: DMSO at 1.5% (220 mM) or ethanol at 4.5% (770 mM) did not significantly affect cell behavior. Data represent the number of protrusions formed (mean of at least three experimental repeats) after the compound addition (last 8 minutes). Analysis with one-way ANOVA showed no significant difference between control conditions and treatment with either solvent.

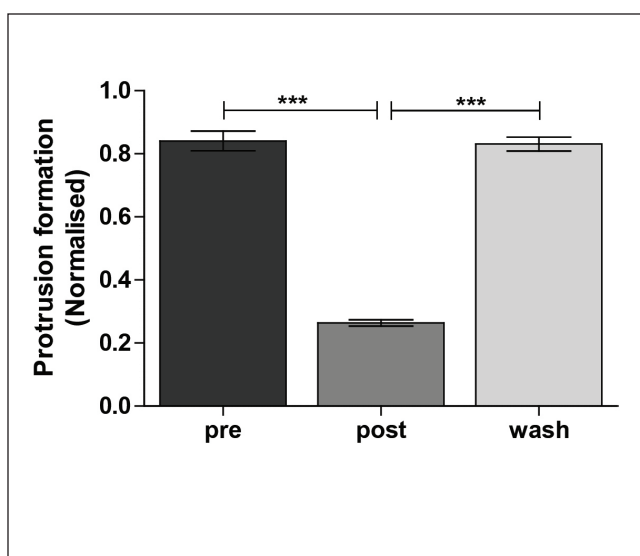


Fig. S2: Bitter tastant effects are reversible
After the addition of the bitter tastant (azelastine 0.5 mM), there is a strong inhibition of protrusion formation, with a full recovery of behavior following removal of the tastant (1 h post tastant addition, one-way ANOVA, $p < 0.001$ ***).



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<http://dx.doi.org/10.14573/altex.1509011s1>

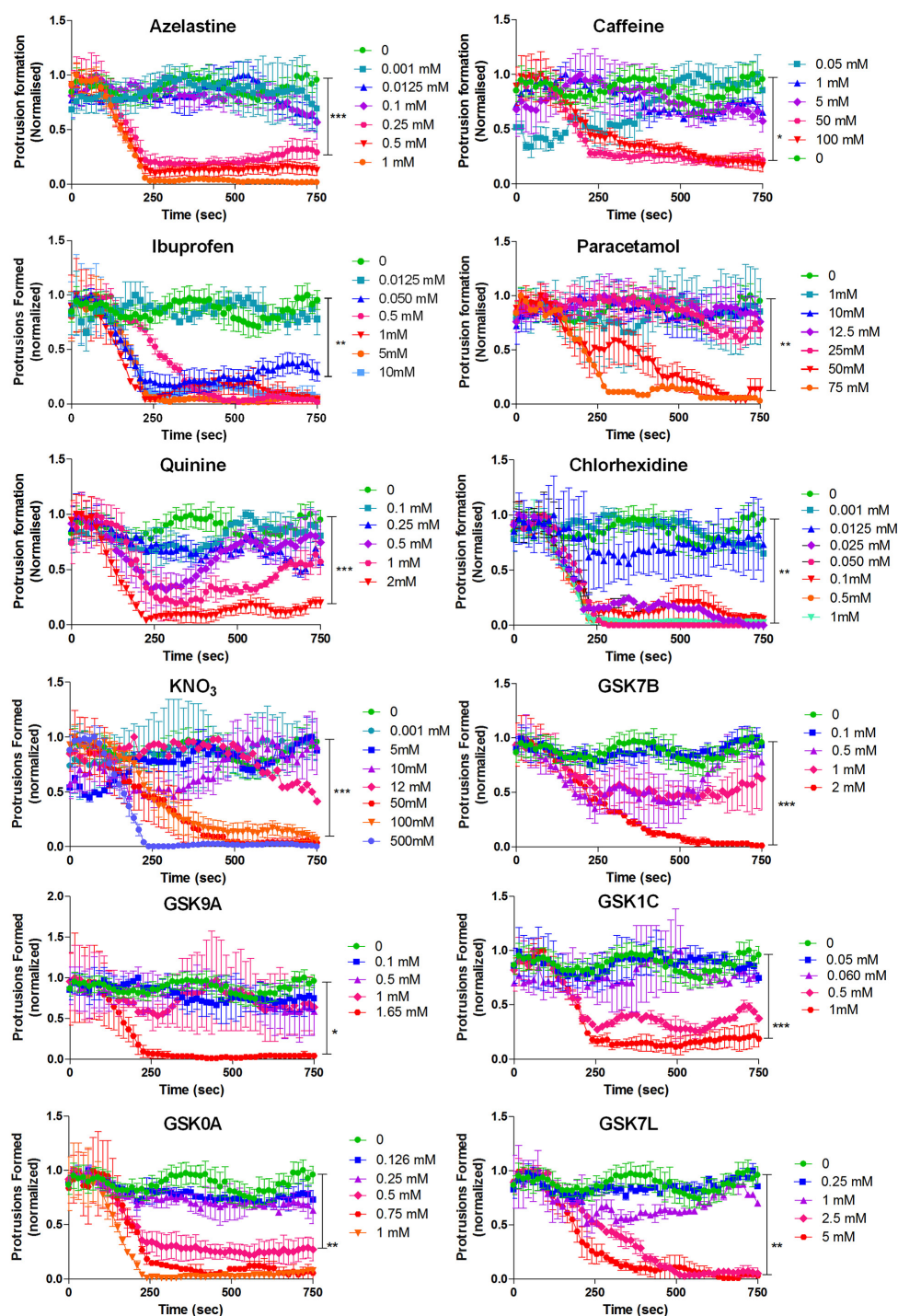


Fig. S3: Raw data of *Dictyostelium* response to standard bitter compounds

Time-dependent changes in *Dictyostelium* cell behavior (membrane protrusion) were recorded over a 15 minute period for triplicate experiments ($\pm$ SEM) at increasing concentrations of seven different compounds to assess their ability to inhibit cell behavior. The addition of different concentrations of each compound at 210 seconds caused a reduction in protrusion formation. Data is presented as normalized to control conditions. Analysis with one-way ANOVA showed significant changes after the treatment with: azelastine 0.25 mM ($p < 0.001$ ***); caffeine 5 mM ($p < 0.05$ *); chlorhexidine 0.025 mM ($p < 0.01$ **); ibuprofen 0.050 mM ($p < 0.01$ **); paracetamol 50 mM ($p < 0.01$ **); potassium nitrate 50 mM ($p < 0.001$ ***); quinine 2 mM ($p < 0.001$ ***); GSK7B 2 mM ($p < 0.001$ ***); GSK9A 1.65 mM ($p < 0.05$ *); GSK1C 1 mM ($p < 0.001$ ***); GSK0A 0.5 mM ($p < 0.01$ **); GSK7L 2.5 mM ($p < 0.01$ **).

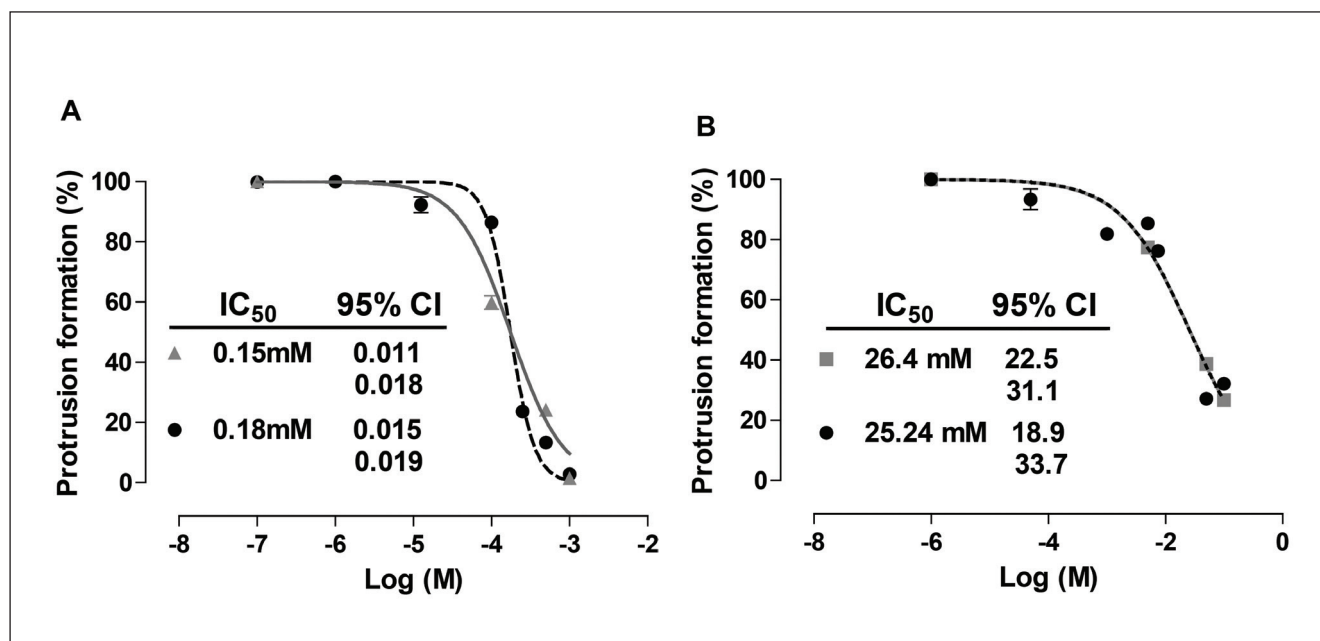


Fig. S4: Effects of bitter compounds are reproducible

To assess the reproducibility of the assay a set of repeat experiments were carried out one month after the initial experiments.

(A) Comparison of data obtained from the azelastine shows that IC₅₀ values are the same for both data sets. (B) The effects of caffeine show a similar response in both data sets.

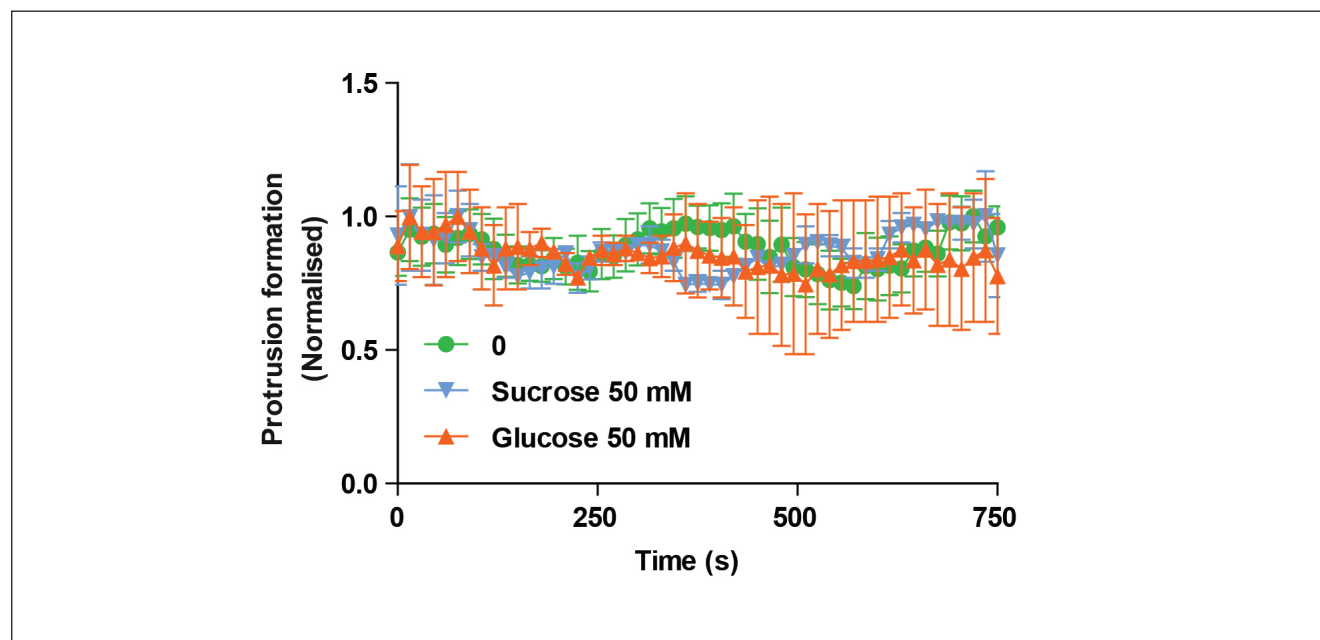
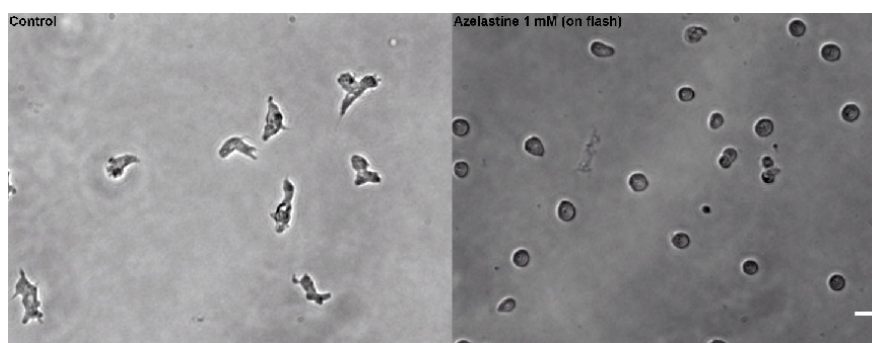


Fig. S5: Raw data of *Dictyostelium* response to non-bitter compounds, glucose and sucrose

Addition of glucose 50 mM and sucrose 50 mM did not affect cell behavior. Data represent the number of protrusions formed (mean of at least three experimental repeats) after the compound addition (last 8 min). Analysis with one-way ANOVA showed no significant difference between control conditions and treatment with either compound.

**Supplementary Movie: Bitter tastants regulate *Dictyostelium* cell behavior**

Wild type *Dictyostelium* cells were monitored by time-lapse photography to record cell behavior every fifteen seconds over a fifteen minute period. At 5 minutes (black frame) either solvent (Left) or bitter substance (Right) (azelastine, 1 mM) was added to cells and images were recorded for a further 10 minutes. Subsequent computer generated outlines enabled protrusion formation to be quantified. Bitter tastants gave rise to a loss in protrusion. View movie at <http://dx.doi.org/10.14573/altex.1509011s2>