An Information Theoretic Approach to Gonadotropin-Releasing Hormone (GnRH) Signalling:

ERK-mediated feedback loops control hormone sensing

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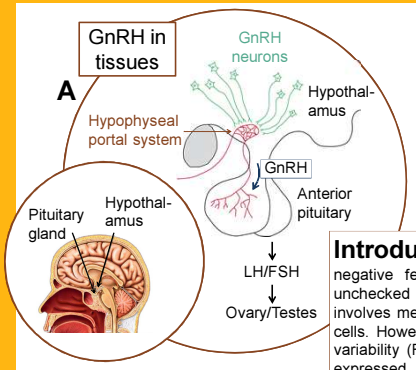
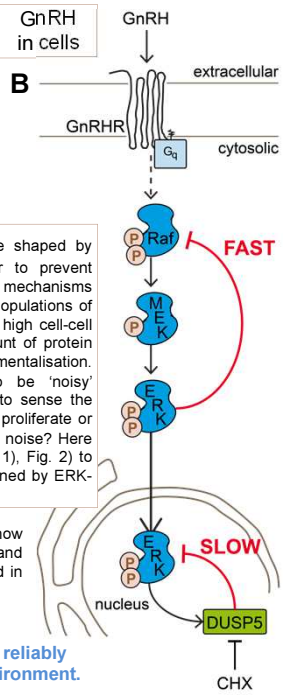


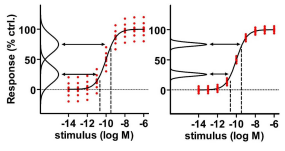
Figure 1: GnRH is a neuropeptide central in the control of reproduction. **(A)** It acts via GnRH receptors (GnRHR) on gonadotropes in the pituitary to control the synthesis and secretion of LH and FSH. **(B)** GnRHR activates $G_{q/11}$, which in turn trigger the Raf/MEK/ERK cascade. Signalling through this pathway is regulated by fast and slow ERK-mediated negative feedback.



Introduction

Intracellular signalling pathways are shaped by negative feedback loops that attenuate signal transfer to prevent unchecked hyperactivity. Most work on cell signalling mechanisms involves measurement of average responses from large populations of cells. However, single cell measurements typically exhibit high cell-cell variability (Fig. 1) due to intrinsic differences in the amount of protein expressed, amount activated and relative compartmentalisation. Signalling pathways can therefore be considered to be 'noisy' information channels. It is crucial that each cell is able to sense the environment and react appropriately (to survive or die, to proliferate or differentiate). How are cells able to distinguish signal from noise? Here we apply information theory (Mutual Information (MI) (ref. 1), Fig. 2) to GnRH sensing (Fig. 1) and explore how sensing is fine-tuned by ERK-mediated negative feedback loops.

Figure 2: Mutual Information (MI). Information transfer through a signalling pathway can be measured as the reliability with which an output (Z) can be used to infer the input (S). **MI** is a statistical measure of this which can be used to measure the reliability of hormone sensing.



MI is sensitive to both signal size and noise. Graphs (left) show concentration-response curves with identical potency and efficacy but with different cell-cell variation. MI is measured in bits.

$$I(Z;S) = H(Z) - H(Z|S)$$

1 bit of information indicates that the output can reliably distinguish between two different states of its environment.

Methods

HeLa cells were transduced with adenovirus (Ad) to express GnRHR together with, where indicated: GFP-ERK2-WT or K52R catalytically inactive mutant (following treatment with ERK1/2 siRNA to knock-down expression of endogenous ERK; ref. 2); or MYC-tagged Dual Specificity Phosphatase (DUSP)5-WT or mutant R53/54A under control of the Egr-1 promoter. Cells were stained with DAPI for identification of the nucleus and immunostained for dual phosphorylated (pp)ERK (Fig. 3).

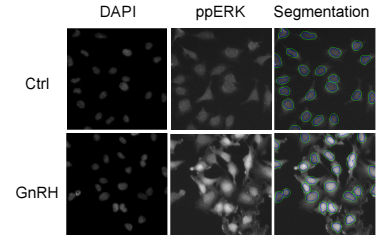


Figure 3: ERK activation assay. HeLa cells expressing GnRHR stimulated with 10^{-7}M GnRH for 5 mins and stained with DAPI and for ppERK (ref. 3).

Automated fluorescence microscopy was carried out using an IN Cell Analyzer 1000 high content imaging system. IN Cell software was used to quantify nuclear fluorescence of many (10^5-10^7) individual cells. These single cell measures were used to calculate population averaged responses as well as MI for specific input-output pairs.

Results

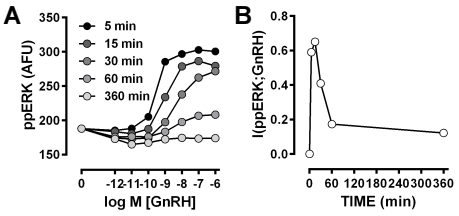


Figure 4: Quantifying GnRHR-mediated ERK signalling. Cells transduced with Ad-GnRHR were stimulated with GnRH for a range of doses and times, as indicated. **(A)** Population averaged responses to GnRH were rapid and transient. **(B)** The single cell data was then used to calculate information transfer in response to GnRH, measured by MI. The amount of information transferred also reached maximum rapidly and then declined. Notably, the MI maximum did not climb beyond 0.7 bits indicating poor sensing: under these conditions a single cell is unable to distinguish between even two states of its environment.

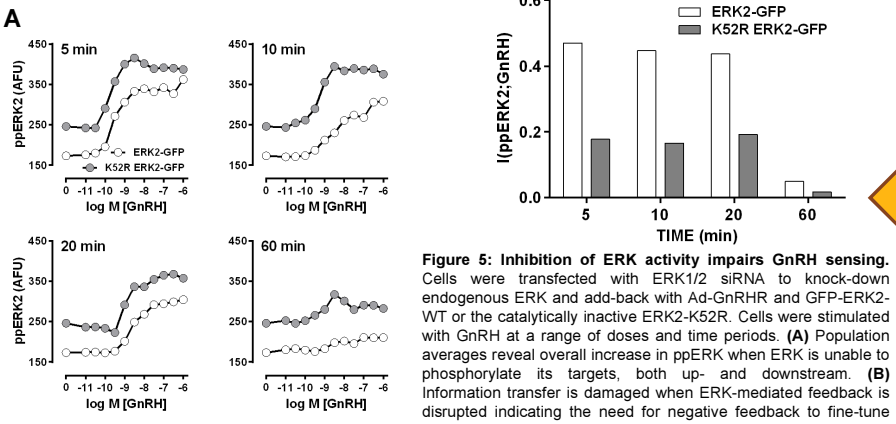


Figure 5: Inhibition of ERK activity impairs GnRH sensing. Cells were transfected with ERK1/2 siRNA to knock-down endogenous ERK and add-back with Ad-GnRHR and GFP-ERK2-WT or the catalytically inactive ERK2-K52R. Cells were stimulated with GnRH at a range of doses and time periods. **(A)** Population averages reveal overall increase in ppERK when ERK is unable to phosphorylate its targets, both up- and downstream. **(B)** Information transfer is damaged when ERK-mediated feedback is disrupted indicating the need for negative feedback to fine-tune hormone sensing.

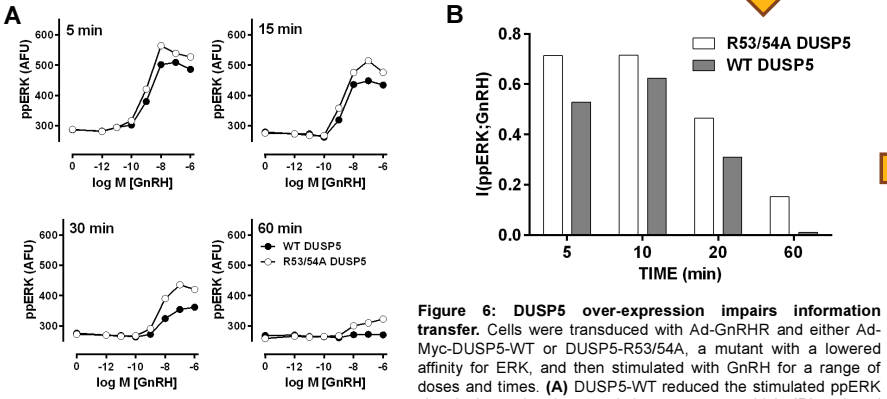


Figure 6: DUSP5 over-expression impairs information transfer. Cells were transduced with Ad-GnRHR and either Ad-Myc-DUSP5-WT or DUSP5-R53/54A, a mutant with a lowered affinity for ERK, and then stimulated with GnRH for a range of doses and times. **(A)** DUSP5-WT reduced the stimulated ppERK signal shown by the population averages, which **(B)** reduced information transfer from GnRH at all timepoints assessed.

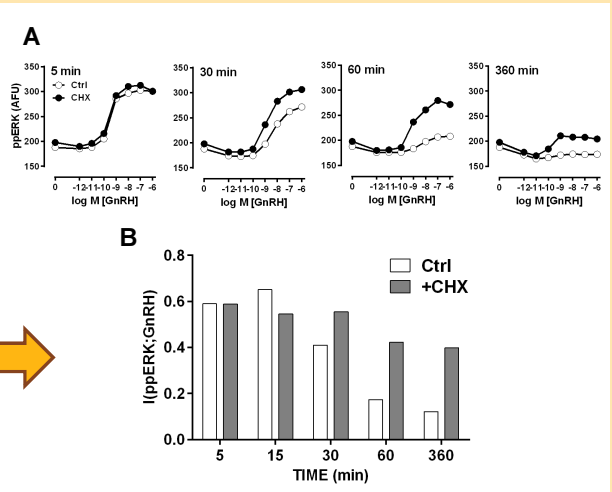


Figure 7: Protein synthesis inhibition can improve GnRH sensing. Endogenous nuclear-inducible DUSP expression is increased by stimuli which activate ERK, including GnRH in this system. Cells were transduced with Ad-GnRHR and treated with 30μM cycloheximide to prevent DUSP induction for 30 mins prior to stimulation with GnRH. **(A)** Cycloheximide increased the stimulated ppERK signal shown by the population averages, which **(B)** improved information transfer from GnRH. Despite this, MI values remained <1 bit.

References

1. Voliotis M *et al.* (2014) Proc.Natl.Acad.Sci. (USA) E326.
2. Caunt C *et al.* (2008) J Biol Chem 283: 26612.
3. Armstrong S *et al.* (2010) J Biol Chem 285: 24360.

Acknowledgements

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Conclusions

- GnRH signalling pathways can thought of as noisy communication channels.
- MI can be used to measure the reliability of hormone sensing via these channels.
- Reliability of hormone sensing is influenced by slow and fast ERK-mediated feedback loops.
- Single cells do not sense GnRH reliably (MI <1 bit).