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

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Introduction

Most work on cell signalling mechanisms involves measurement of average responses from large populations of cells. Single cell measurements typically exhibit high cell-cell variability (fig. 1) due to intrinsic differences in amount of protein expressed, amount activated and relative compartmentalisation. Signalling pathways can be considered to be 'noisy' information channels; information can be defined as the uncertainty about the environment that is removed by signalling. It is crucial that each cell is able to sense the environment and react appropriately (to survive or die, to proliferate or differentiate, to express or not express a particular gene). Here we apply information theory (Mutual Information (MI) (ref. 1), fig. 1) to GnRH sensing (fig. 2) and explore how this reliability might be regulated

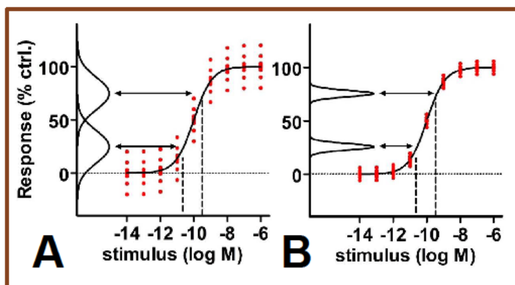

Fig.1: Mutual Information (MI). Concentration-response curves with identical potency and efficacy but with different cell-cell variation (red dots and frequency distribution plots) (A, B).

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 and can be used to measure the reliability of hormone sensing.

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

MI is sensitive to both signal size and noise. It is measured in bits, where 1 bit of information indicates that the output can reliably distinguish between two different states of the environment.

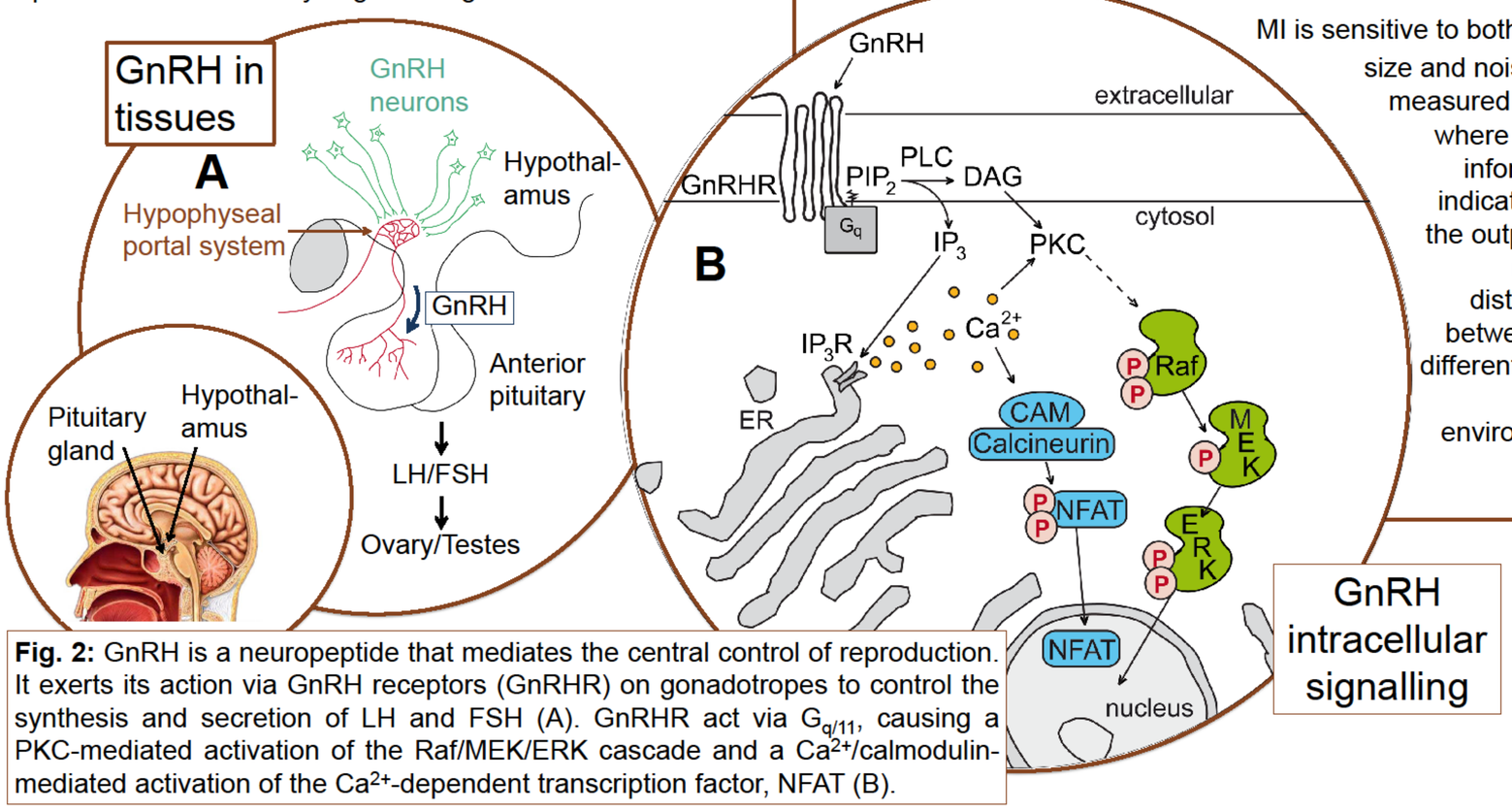

Fig. 2: GnRH is a neuropeptide that mediates the central control of reproduction. It exerts its action via GnRH receptors (GnRHR) on gonadotropes to control the synthesis and secretion of LH and FSH (A). GnRHR act via $G_{q/11}$, causing a PKC-mediated activation of the Raf/MEK/ERK cascade and a Ca^{2+}/calmodulin-mediated activation of the Ca^{2+}-dependent transcription factor, NFAT (B).

Methods

LβT2 (gonadotrope lineage) or HeLa cells were transduced with adenovirus to express NFAT1c-EFP, Egr1-zsGreen (transciptional reporter for ERK), NFAT-RE-asRED (transcriptional reporter for NFAT) or HA-GnRHR. Cells were stained with DAPI for identification of the nucleus immunostained for dual phosphorylated (pp)ERK. Whole cell ppERK levels were used as a readout for Raf/MEK/ERK activation (ref. 2) and the fraction of NFAT1c-EFP in the nucleus as a readout for Ca^{2+}/calmodulin activation (ref. 3).

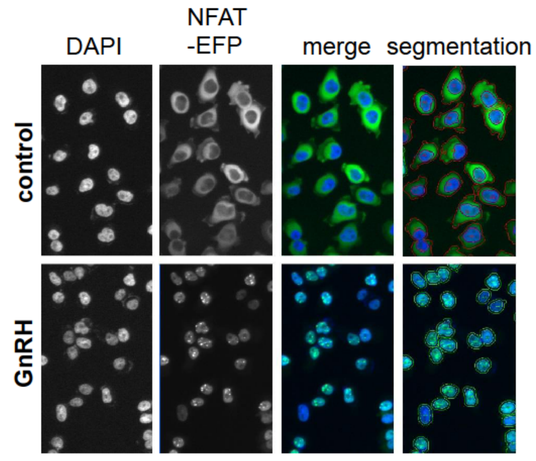

Fig. 3: NFAT-EFP nuclear translocation assay
HeLa cells co-expressing GnRHR and NFAT-EFP stimulated with 10^{-7}M GnRH.

Automated fluorescence microscopy was carried out using an IN Cell Analyzer 1000 high content imaging system. IN Cell software was used to quantify cytoplasmic and/or 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

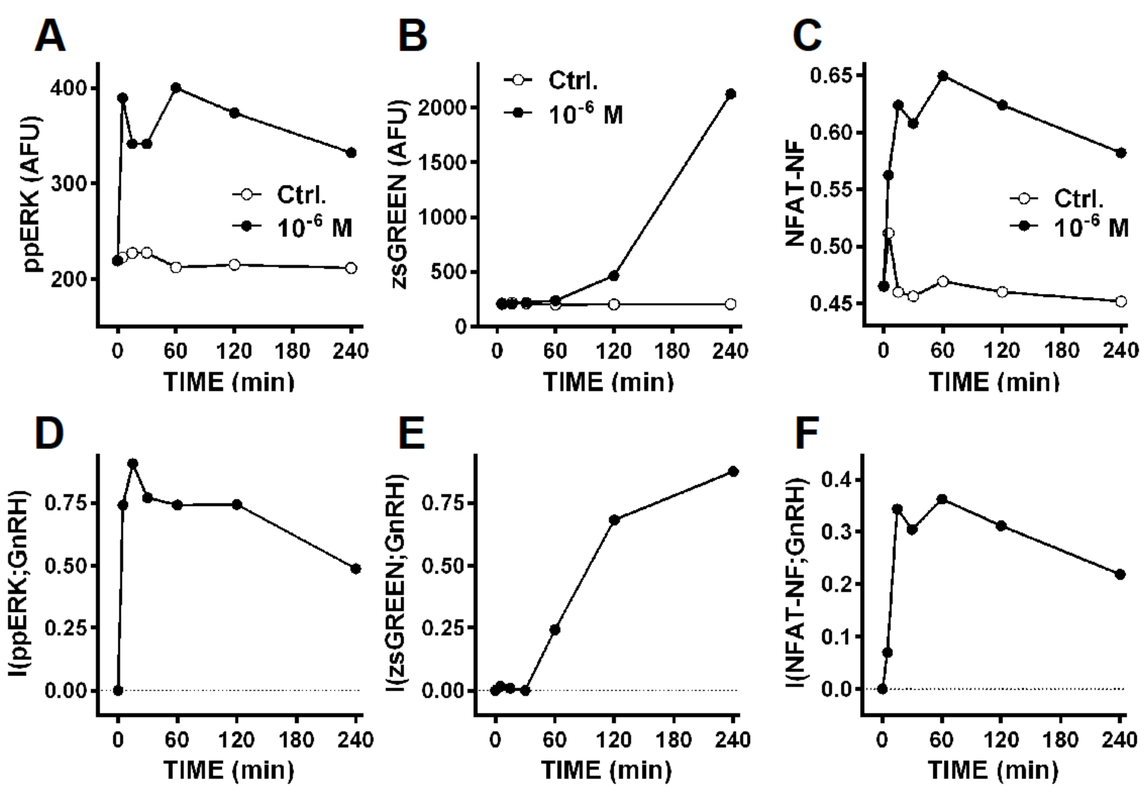

Fig. 4: Measuring MI for GnRH signalling. GnRH caused the expected concentration- and time-dependent increases in ppERK (A), NFAT1c-EFP nuclear fraction (C) and ERK-driven gene expression (B) in LβT2 cells. MI values between GnRH and these responses had similar time-courses to the population averages (D, E, F).

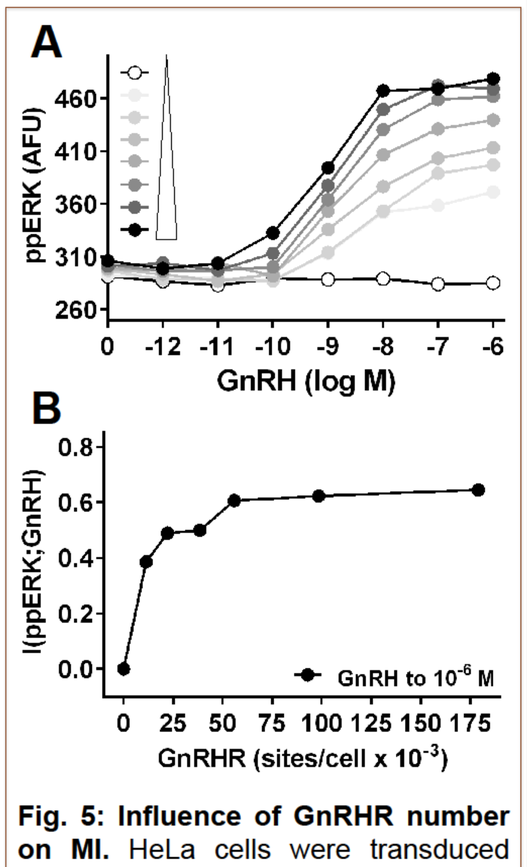

Fig. 5: Influence of GnRHR number on MI. HeLa cells were transduced with Ad-GnRHR at varied titre. GnRH caused a dose- and titre-dependent increase in ppERK (A). MI values increased as a function of GnRHR number (B).

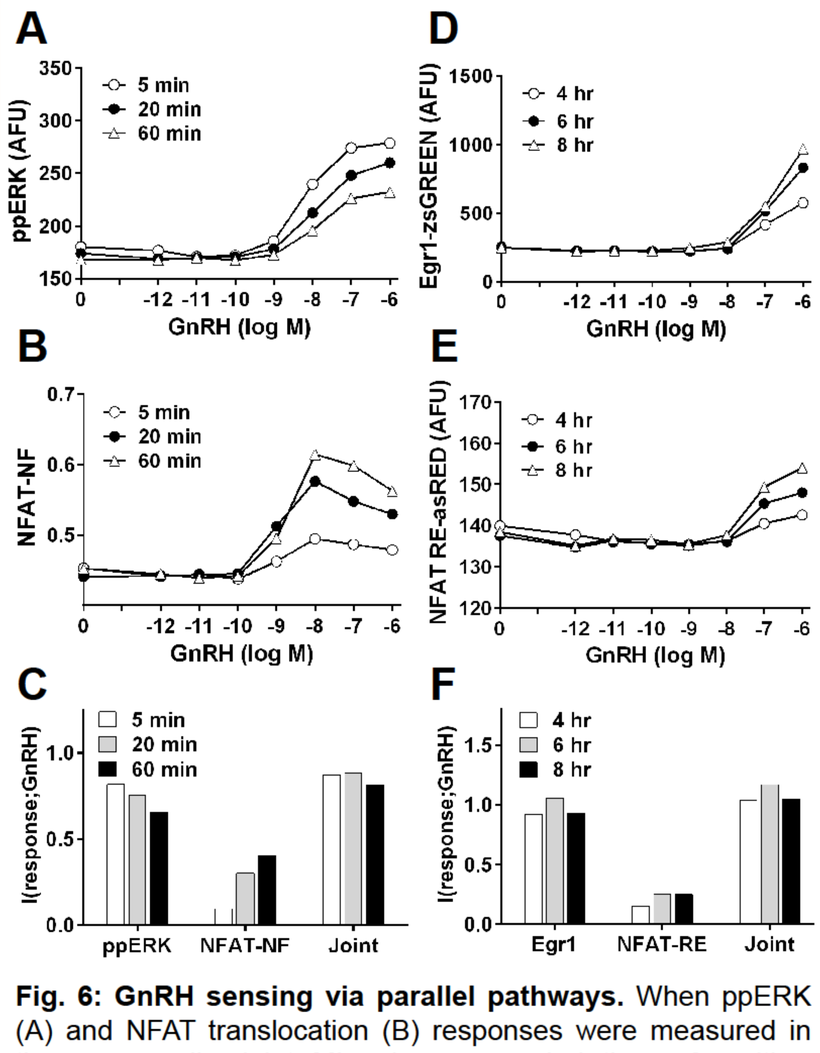

Fig. 6: GnRH sensing via parallel pathways. When ppERK (A) and NFAT translocation (B) responses were measured in the same cells, joint MI values exceeded those for either response alone (C). Similarly, when transcriptional responses were measured in the same cells (Egr1-zsGREEN (D), and NFAT-RE asRED (E)) joint MI values exceeded those for either response alone (F).

References

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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.
- Single pathways in single cells do not sense GnRH reliably (MI <1 bit).
- GnRH sensing is improved by increasing GnRHR number and by use of convergent pathways.

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