Measuring of information transfer via gonadotropin-releasing hormone receptors (GnRHR) shows a remarkable loss of information through signalling

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INTRODUCTION

Gonadotropin-releasing hormone (GnRH) is a pivotal hypothalamic hormone that controls reproductive function. It is secreted in pulses and acts via GnRHR on the pituitary gonadotrope causing a PKC-mediated activation of ERK and Ca^{2+}-mediated activation of Nuclear Factor of Activated T-cells (NFAT), both of which mediate GnRH effects on gonadotropin expression (Fig.1).

Conventional metrics that are used to study signal transduction pathways depend mainly on measuring the average responses from a large number of cells, despite cell to cell variability which often has physiological implications.

Information theoretic approaches are increasingly being used to study the fidelity of signalling pathways. It is an approach that considers the cell to cell heterogeneity and its impact on information transfer (1).

Here we use the mutual information (MI) between GnRH concentration and measured responses [I(response; GnRH)] as a measure of information transfer via GnRHR. MI is measured in Bits, with an MI of 1 Bit indicating a system that can unambiguously distinguish between two inputs (i.e. two GnRH concentrations).

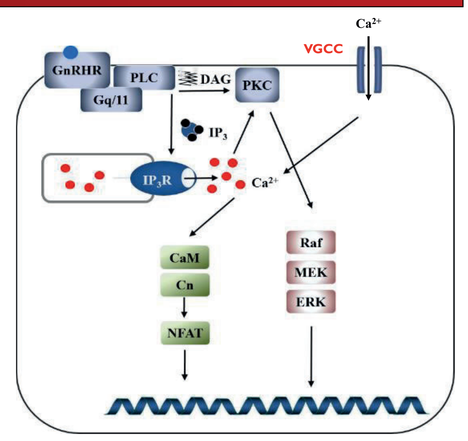

Fig 1. Diagram of GnRHR signalling to ERK and NFAT

METHODS

Automated fluorescence microscopy (InCell 2200) was used to quantify the effects of GnRH on nuclear translocation of NFAT and on cytoplasmic calcium [Ca^{2+}].

For live cell imaging: LßT2 cells (a murine gonadotrope-derived cell line) were transduced with Ad NFAT1c-EFP as a readout of Ca^{2+}/calmodulin activation and nuclei were stained (Hoechst), or loaded with Fluo-4 AM (a Ca^{2+} indicator). The cells were imaged in physiological salt solution buffer (PPS) at 37°C both before and during continuous stimulation with varied concentrations of GnRH.

Data analysis: InCell investigator software was used to analysis the images**.** A MatLab (R2017a) tracking algorithm was used to follow individual cell responses over time for MI calculation (1,2), using the following formula: **I(Z;S) = H(Z) -H(Z | S).**

RESULTS

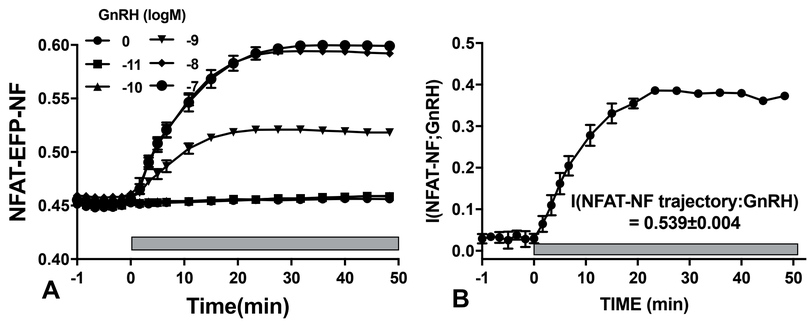

Figure 2. Sensing dynamics and live cell NFAT-EFP imaging in LβT2 cells. Ad NFAT1c-EFP transduced cells were stimulated with GnRH (from t=0). Images were acquired at the indicated times and a Matlab tracking algorithm was used to follow individual cell responses over time. **A.** Population average responses for all tracked cells, (means ± SEMs n=3, 69-320 cells). **B.** I(NFAT-NF; GnRH) values for the tracked cells at each time point, in addition to MI between GnRH and the translocation response taking single cell response trajectories into account.

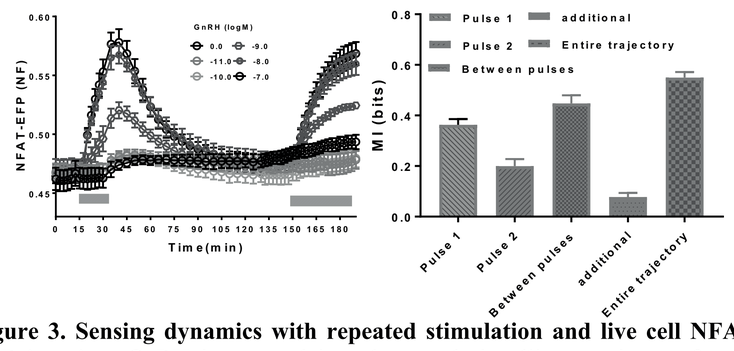

Figure 3. Sensing dynamics with repeated stimulation and live cell NFAT-EFP imaging. GnRH is secreted in pulses, and signalling continues after the GnRH pulse, therefore we measured the amount of information cells gain by sensing during and after the pulse**.** Here, Ad NFAT1c-EFP transduced cells were stimulated with GnRH for 15 min (first grey bar). Afterwards, cells were washed and then imaged for a further 2 h prior to repeating the GnRH stimulation (second grey bar) for 30 min. **A.** population average responses for all tracked cells (means ± SEMs n=4, 60-171 cells). **B.** MI values of 1st pulse (0.38±0.018 Bit), 2nd pulse (0.2±0.03 Bit), and between pulses (0.45±0.032). Little information was gained by sensing both pulses ~0.08 Bit. These values were increased to (0.54 ± 0.022 Bit) with trajectory consideration.

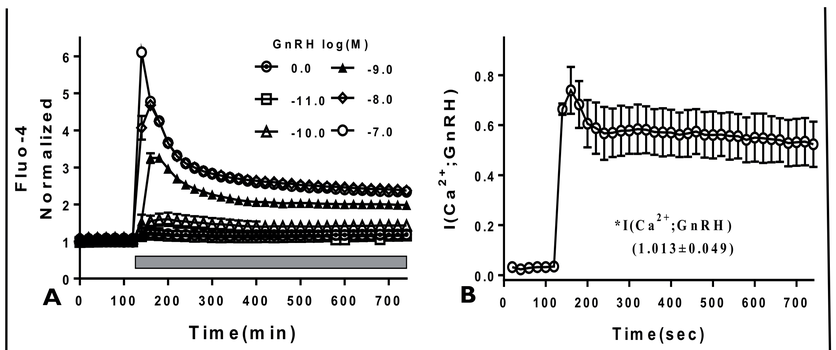

Figure 4. Live cell cytoplasmic calcium [Ca^{2+}] responses and sensing dynamics. LβT2 cells were incubated with Fluo-4 for 30 min, then washed prior to stimulation with GnRH for 10 min in PSS. Images were acquired every 20 sec, and a Matlab tracking algorithm was used to follow individual cell responses over time. **A .** population average responses for all tracked cells n=127-326 cell. **B.** I(Ca^{2+};GnRH) values for the tracked cells at each time point, (mean ± SEM, n=3), and MI value calculated taking trajectory into account (140, 160, 300 Sec).

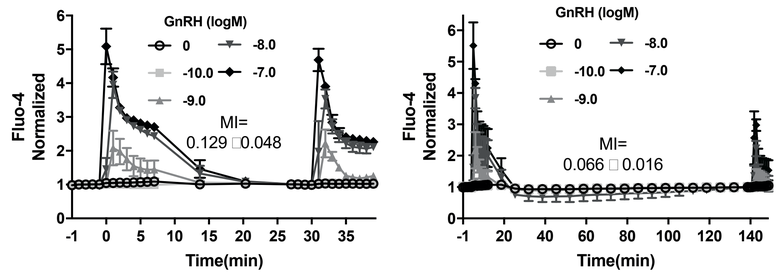

Figure 5. Sensing dynamics with repeated stimulation and live cell [Ca^{2+}] imaging. It has been hypothesised that if the response in the second pulse is predicted, cells gain little or no additional information (3). Thus, increasing the time between the pulses could cause cells to gain more information from a second pulse. Here, the time between pulses was varied from 10, 20, 60 to 90 min (Only 10 min (A) and 90 min (B) results are shown). Panels show the population average responses for all tracked cells (means ± SEMs, n=3). A Matlab tracking algorithm was used to follow individual cell responses over the time and to calculate the additional gained information from sensing a 2nd pulse. The MI values were comparable at all tested conditions and Two-way ANOVAs of the MI values between pulses revealed that varying the time between pulses is not a significant source of variation (P>0.05). Also, MI was increased to approximately 1Bit under all conditions with trajectory consideration.

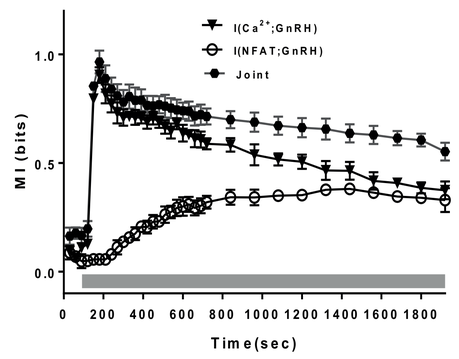

Figure 6. Sensing dynamics and live cell Rhod-3 in combination with NFAT-EFP in LβT2 cells. Ad NFAT1c-EFP transduced cells were loaded with Rhod-3, stimulated with GnRH and imaged every 45 sec in PSS imaging buffer. The figure shows the MI values for all tracked cells (means ± SEMs n=4, 110-300 cells) at each time point. The maximum MI values were 0.38±0.018Bit for NFAT-NF, 0.91±0.036Bit for Ca^{2+} and ~1Bit for joint pathways.

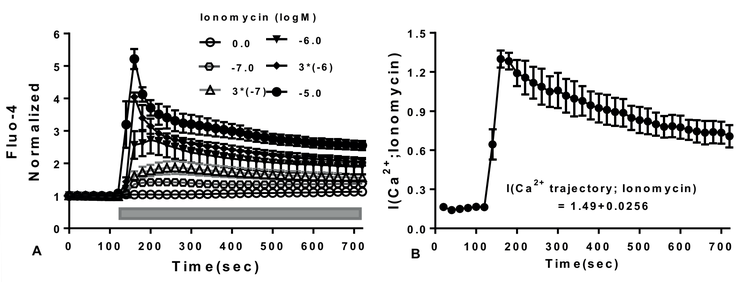

Figure 7. GnRHR-independent Ca^{2+} mobilisation. Results in Fig. 4 revealed that most information is lost early in the GnRH signalling cascade, prior to Ca^{2+} mobilisation. It is therefore likely that the transmitted information can be enhanced by using a stimulus that increases the [Ca^{2+}] in a GnRHR-independent manner (e.g. Ionomycin). Here, loaded Fluo-4 LβT2 cells were stimulated with 0 or 10^{-7}-10^{-5} M Ionomycin (providing cells with ~3Bit of information). **A.** Population average responses for all tracked cells (mean ± SEM, n=4, 130-320 cells). **B.** I(Ca^{2+}; Ionomycin) values for the tracked cells at each time point. The maximum MI value was 1.3±0.066 Bit, and this increased to 1.5±0.026 Bit with trajectory consideration. **Note that the MI values for cells stimulated with Ionomycin are higher than MI values in cells stimulated via GnRHR (compare with Fig. 4).**

CONCLUSION

LβT2 cells are unreliable sensors of GnRH concentration because a considerable amount of information is lost through signalling. Here, despite providing cells with an input of 3 Bits, less than 1 Bit was transferred to NFAT and Ca^{2+}.

Information transfer was increased by joint sensing, trajectory sensing and sensing repeated pulses. However, the increases were limited.

The additional information from sensing a 2nd pulse was low (~0.08Bits) and did not differ significantly with different inter-pulse intervals, indicating that the sources of cell to cell heterogeneity are relatively stable over the time course examined in this work.

Our data suggests that the loss of information through signalling occurs upstream of calcium.

REFERENCES

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